VoxSanguinis
The International Journal of Transfusion Medicine
ISBT International Society of Blood Transfusion

Local Day
Donors and Donation

1A-01-01

STATUS OF BLOOD SAFETY AND AVAILABILITY IN THE WHO EASTERN MEDITERRANEAN REGION

YE Abdella[1], H Ashghar[2], J Yu[3], A Cheraghali[3], AA Pourfathollah[3] and CT Smit Sibinga[4]

[1]World Health Organization, Cairo, Egypt [2]WHO EMRO, Cairo, Egypt [3]WHO HQ, Geneva, Switzerland [4]IQM Consulting, Zuidhorn, The Netherlands

With the goal of ensuring universal access to safe blood and blood components, WHO has been at the forefront of the movement to improve blood safety and availability as mandated by successive World Health Assembly resolutions, the earliest dating from 1975, and Regional Committee resolution in 1987. However, many countries in the Eastern Mediterranean Region (EMR) still face major challenges in ensuring availability, safety, quality, accessibility, affordability, and clinical efficacy of blood and blood components. In addition, humanitarian emergencies and armed conflicts in many countries in the Region increased the demand for blood and blood components and made delivery of these lifesaving products challenging and complex.

Data on blood safety and availability was collected for the period 1 January to 31 December 2013 from countries in the EMR through a survey linked to the WHO Global Database on Blood Safety (GDBS). Data entry and analysis was performed using Microsoft Office Access (2010 version).

The findings indicate gaps in all areas of the key elements of a national blood system, including, leadership and governance, coordination and collaboration of national blood systems, provision of safe blood and blood components, patient blood management and clinical transfusion, and quality system and management. In 2013, about 7 million units of whole blood were collected. Blood donation rates ranged from 0.6 to 28.9 per 1,000 population. The proportion of voluntary non-remunerated blood donation (VNRD) was 65.8% and 34.7% of VNRD was from first-time donors. The survey showed that half of the countries in the Region have national blood transfusion services. However, despite the existence of these services and their vital role in the implementation of essential interventions for Reproductive, Maternal, Newborn and Child Health, delivery on Universal Health Coverage, and achievement of the 2012–2016 strategic public health priorities of the Region, overall, inadequate attention was given to blood transfusion services as an essential part of the national health system.

WHO Regional Office for the Eastern Mediterranean developed a 10 year (2016–2025) Regional Strategy for Blood Safety through a broad consultation with national blood transfusion service providers, regional and international organizations and experts working in the field. The strategy addresses the identified gaps from the GDBS with the aim of improving the health of nearly 660 million people in the 22 countries in the Region. It will guide countries to develop and strengthen national blood systems to ensure the continuity, sufficiency, sustainability and security of national supplies of safe and efficacious blood and blood components to meet their national need.

This strategy will play an important role to integrate blood transfusion services into the healthcare system as a crosscutting service and to support the implementation of WHO resolutions and Executive Board Decisions, namely, on the safety, quality and availability of blood and blood components (WHA28.72, WHA58.13, WHA63.12 and EM/RC34/12); on Guiding Principles on Human Cell, Tissue and Organ Transplantation (WHA63.22); on Principles for global consensus on the donation and management of blood, blood components and medical products of human origin (EB136.2); on Hepatitis (WHA67.6); on Sickle-cell anaemia (WHA59.20); and on Thalassemia and other haemoglobinopathies (EB118.R1).

1A-01-02

STRATEGIES TO IMPROVE BLOOD DONORS RECRUITMENT PROCESS

RH Alabdulrazza

Club 25 Kuwait, Salwa, Kuwait

Background: Recruitment and retention of Voluntary Non Remunerated Blood Donors (VNRBD) to maintain adequate blood supply remains a major challenge that faces blood services worldwide. Till 2006 the Kuwait Central blood bank KCBB used to rely largely on paid blood donors who contributed to 60% of donors the remaining were a mixture of replacement and volunteer donors. A decision was made in late November 2006 to stop paid donations and therefore alternative measures for recruiting voluntary donations had to be developed. At that time a huge media campaign was launched and has managed to trigger the change. Since then we were faced with new challenges to proceed with the success and to reach a 100% VNRBD system in Kuwait. It is important to know however that till date there is no national donor program in place in Kuwait and there is no national donor recruitment plan in place as well. To overcome this several strategies were developed utilizing the available low budget marketing tools including social media, traditional media and organizing public events. Additionally we also invested in the improvement of the existing tools including mobile blood drives and extending the KCBB capacities. To achieve our goals we had to involve volunteer groups and build long lasting partnerships with corporates, NGOs and expats communities in the country.

Aims: In this lecture we will describe the challenges we faced, our successes and our failures with suggestions on how to build National donor recruitment strategies in your own blood bank.

Clinical Aspects

1B-02-01

APPROPRIATENESS OF TRANSFUSION SERVICE (PATIENT APPROPRIATENESS)

I Sadek

Dalhousie University, Halifax, Canada

Appropriate blood transfusion practices ensure patient safety by eliminating the risk of unnecessary exposure to blood transfusions and its possible side effects. Inappropriate transfusions may also contribute to blood shortages and increase costs.

Choosing Wisely Canada is a campaign to help physicians and patients engage in healthy conversations about potentially unnecessary tests, treatments and procedures, and to help physicians and patients make smart and effective choices to ensure high-quality care. It started in spring of 2014 and continues to grow in Canada and internationally.

The Canadian Society for Transfusion Medicine (CSTM) has joined the Choosing Wisely Canada campaign in October 2014. CSTM was also able to put forward a total of ten choosing wisely recommendations that promote appropriateness in Transfusion Medicine. Since transfusion medicine is an integral part of many other clinical practices, other clinical societies have also put forward recommendations about appropriate transfusion. The CSTM recommendations include appropriate transfusion of blood products such as red blood cells, plasma and platelets as well as manufactured products such Prothrombin Complex Concentrates and Intravenous Immunoglobulin.

The presentation will focus on appropriate utilization of red blood cells and plasma. More recent literature suggests that at least 20% of red cell units transfused may be unnecessary in centres that routinely transfuse multiple red cell units simultaneously despite that a restrictive red cell transfusion strategy has been proven to be very effective in multiple randomized studies. We will present local experiences in transfusing one unit of red cell at a time and the benefits of implementing such a strategy.

In a similar manner, many studies have suggested that up to 45% of plasma transfusions are inappropriate. This presentation will focus on practical methods to promote a more appropriate plasma transfusion, increase education and enforce plasma transfusion guidelines. We will also explain the role of Prothrombin Complex Concentrate in warfarin reversal and appropriate plasma utilization.

1B-02-02

PLATELET REFRACTORINESS-CAUSE AND MANAGEMENT

JD Sweeney and C Nixon

Brown University, Providence, Rhode Island, United States of America

Background: Platelet transfusion therapy is an essential aspect of the supportive care of patients with transient hypoproliferative thrombocytopenia secondary to marrow disorders or chemotherapy. Platelet transfusion results in an immediate increment in the platelet count within 10 min which is less but generally sustained at 16–24 h. Platelet refractoriness is typically associated with a diminished or absent increment and may have several causes.

Aim: Describe approaches to the management of severely refractory patients.
Methods: Platelet refractoriness can be classified as immune or non-immune. Among the immune causes are alloantibodies to ABO, HLA class I or platelet specific antigens or rarely anti-platelet autoantibodies,. A recently postulated immune mechanism does not involve circulating allo or autoantibodies but rather CD8+ T cells mediated clearance via the perforin granzyme mechanism. The non immune causes are miscellaneous and involve recipient factors such as spleenomegally, various antibiotics, veno-occlusive disease, graft-versus-host disease and various infections. Refractoriness is typically measured using a corrected count increment (CCI) where the CCI is (Post platelet count -preplatelet count) × body surface area 9 m^2) ÷ platelet product yield ($\times 10^{11}$). A CCI <7,500 (platelet increment per 1 m^2 BSA per 10E11 platelets) indicates refractoriness.
Results: Patients exhibiting refractoriness should be examined for ABO incompatibility of platelets and screened for the presence of class I anti-HLA antibodies. Some patients show pan-reactivity, and providing a high grade HLA matched platelet product will be required to achieve an acceptable increment. Other patients show more limited reactivity and choosing a HLA matched product which lacks cognate antigens to which the patient has high titer antibodies can be equally successful. For example, a patient with a strong anti-A2 might achieve a satisfactory increment with HLA Class I A2 negative platelets. Some patients may have a strong platelet specific alloantibody and respond to platelets lacking the cognate antigen but this is mostly described in case reports. Some patients may have autoantibodies. This is a difficult diagnosis to make in practice. Use of intravenous immune globulin or thrombopoietin mimetics may be useful in these patients An alternative approach is to procure cross match compatible platelets in facilities which have this capability. Those patients in whom anti-HLA antibodies cannot be demonstrated are not known to benefit from HLA selected patients. They are typically managed by transfusing a standard platelet product but may benefit from very fresh platelets (<24 h), but these are not generally made available. Other approaches to prevent major bleeding events are to transfuse red cells to maintain a higher hematocrit (>33) or the use of antifibrinolytic therapy. Although theoretically intruiging, neither approach has been shown to be clinically effective.
Conclusions: Refractory patients should be evaluated primarily for anti-HLA antibodies and managed as above. Other tests are of more limited usefulness. Prophylactic Platelet transfusions should continue to be administered as per routine protocols but limited in general to a single product and excessive transfusions avoided.

1B-02-03
MASSIVE TRANSFUSION PROTOCOLS (MTP): HOW CAN WE STANDARDIZE OUR PRACTICE?
M Algora
Cleveland Clinic Abu Dhabi, Abu Dhabi, United Arab Emirates

Massive Transfusion is generally defined as transfusion requirements equaling one blood volume,-typically 8–10 units of PRBC in the average-sized adult-, over the course of 12–24 h, or half volume within 3 h. This definition has some shortcomings: we are defining the condition based on its treatment retrospectively. So, currently we need to recognize this condition as a function of bleeding rate (>150 ml/min) and standardize the practice for urgent support of those patients with life threatening hemorrhage.
This life-threatening situation can occur not only in trauma patients, but frequently related to obstetric hemorrhage, vascular rupture, gastrointestinal bleeding and any unexpected surgical blood emergency.
Trauma patients normally present with a triad of coagulopathy, acidosis and hypothermia that need rapid *hemostatic resuscitation,* while non-trauma patients (vascular emergencies, GI bleeding, and surgical hemorrhage) need hypovolemic *resuscitation*.
Experience with trauma patients in the battlefield has shown that driven replacement formula (1:1:1) and early transfusion of plasma (and in big amounts) has beneficial effects in outcome of severely injured patients. Subsequently those protocols were transferred to management of different situations of life-threating hemorrhage in civilians (for instance, in cases of aneurysm rupture), in which patients had not such an extreme injury with tissue thromboplastine highly involved. Therefore the point of discussion is whether hemotherapy based on driven formulas, with high consumption of plasma, should be strictly applied or blood support should be based upon test results. Converse to the theory of restrictive use of plasma, some publications suggest that Fresh Frozen Plasma in addition to providing clotting factors, can have an important role in promoting vascular stability through regulation of critical junction proteins in vascular endothelium. So the controversy is still open.
Opposite to the theoretical 'beneficial' effect on subendothelium, the reality is a high ratio of wastage of units with 1:1:1 formula, which can have important ethical, financial and inventory impact.
To coordinate the urgent support in massive hemorrhage situations, Transfusion Medicine Services need to implement a Massive Transfusion Protocol which is a set of instructions for clinicians, blood bank personnel, and laboratory staff intended to expedite delivery of needed blood products to patients undergoing life threatening hemorrhage. Transfusion Services have to lead MTP, defining essential aspects as:
Identification of patients: rules for patient identification. . .
Specimen: emergency teams should have procedures that ensure collection and properly identified specimen. . .
Selection of Blood (ABO and Rh): different scenarios according to known/unknown blood type in current specimen, serological findings, patient ABO history, what if significant irregular antibodies. . .
Blood issue: defining the unit age, issue multiple units, simplified paper work. . ..
Use of Blood warming devices: methods used for blood warming..
Intraoperative blood recovery: when possible use system for collection and reinfusion of lost blood. . .
Ethical aspects of Massive transfusion: consumption of inventory, disproportionate use of blood products and hospital resources. . .
Over transfusion can waste inventory and also result in alteration in recipient physiology due to storage blood characteristics caused by anticoagulant-preservative solution (citrate toxicity), by storage-related metabolic processes (potassium, acidosis) or by temperature requirements for blood products storage (hypothermia); and also over transfusion can cause acute pulmonary failure (overload or distress) and dilutional coagulopathy.
The implementation of a hospital MTP standardized practice will expedite the delivery of components, improve communication (intrafacility and with blood supplier) and provide better outcomes for patients.

1B-02-04
TRANSFUSION IN HEMATOPOIETIC STEM CELL TRANSPLANT PATIENTS
M Almohammadi
King Abdulaziz Medical City, National Guard, Jeddah, Saudi Arabia

Background: Hematopoietic stem cell transplant is considered a standard therapeutic measure for many hematologic neoplastic and non neoplastic disorders. Transfusion of blood components is to patient undergoing this measure is a challenge. Firstly because of the increased quantity needed by these patients and secondly due to the processing needed.
Aim: To give a brief overview on the subject of transfusion in hematopoietic stem cell transplant patients.

Quality Management/ Haemovigilance/TTI

1C-03-01
BLOOD SUPPLY EMERGENCY & DISASTER PLANNING
M Raouf
Dubai Health Authority, Dubai, United Arab Emirates

Introduction: During any disaster natural or manmade many people often suffer life threatening injuries that require the need for blood transfusions.
Aim: Blood supply emergency and disaster planning help blood centers, hospital blood banks, and transfusion services to be prepared for and respond to domestic disasters and acts of terrorism that affect the blood supply.
Material: Reviewing previous disasters and tragedies; countries have learned many lessons and have started planning properly for disaster management including training of concerned staff through emergencies drills to be ready for response.
Results: A 'disaster' includes any domestic disaster or act or terrorism that suddenly requires a much larger amount of blood than usual or temporarily restricts or eliminates a blood collectors ability to collect, test, process and distribute blood or temporarily restricts or prevents the local population from donating blood or restricts or prevents the

use of the available inventory of blood products requiring immediate replacement or resupply of the region's blood inventory from another region or creates a sudden influx of donors requiring accelerated drawing of blood to meet an emergent need elsewhere. 'The two primary lessons learned from the Sept. 11 disaster include the need to control collections in excess of medical need and the need to ensure that facilities maintain adequate inventories to prepare for disasters at all times in all locations'.

Conclusion: Disaster management cycle requires blood facilities to plan for pre disaster, disaster, response and post disaster and to have their facilities and staff trained on that. The Cycle include Mitigation: efforts are focused on making permanent changes to plant and property in order to reduce the overall exposure to various known hazards. Preparedness: focuses on areas of risk that can't be addressed through mitigation efforts alone. Emergencies are dynamic and complex events, and careful preparation efforts are needed to reduce the loss of life and property while sustaining business operations. Response: takes place during an emergency and typically involves time-sensitive action steps taken by the staff to protect life and property and to stabilize the organization (e.g. communication with the staff, evacuation procedures, response to customers and the media). And Recovery: efforts begin once the initial response actions have taken place. Efforts focus on restoring critical lifeline systems in order to resume and maintain business operations (e.g. communications, water, power, sewage).

1C-03-02

No abstract available.

1C-03-03

THE ENBTC (EGYPTIAN NATIONAL BLOOD TRANSFUSION CENTRE'S) JOURNEY TO AABB ACCREDITATION: STEPS AND CHALLENGES

A El Sayed

National Blood Transfusion Center, Giza, Egypt

Background: Accreditation is a voluntary method of quality assurance in which certification of competency is presented by an accredited certification body. While it is dependent on a set of rigorous protocols and research-based processes, it also involves evaluating an organization's effectiveness as a whole. Given the complexity of the process it was expected by the nbtc that the journey to accreditation would be long and have many challenges.

Aims: To illustrate the steps to aabb accreditation and the challenges faced.

Methods: The first step of NBTC AABB enrollment occurred in July 2010. It involved the reception of the self-study kit and tools, used to make gap analysis and evaluate the quality of the program and make improvements. The second step of self-assessment started in February 2011, using the self studies tools that allowed the NBTC to complete a comprehensive internal evaluation of both the business and service aspects of the NBTC and to submit self assessment documents. Starting December 2011 the AABB had several inquiries that needed to be addressed, in response to these inquiries a NBTC-study on HTLV prevalence was done among 5,000 random samples of NBTC-donors, only three samples were indeterminate, 4,997 were free for HTLV – a reply was submitted February 2013 and a variance request of standards for the serological testing of HTLV & WNV (for its low prevalence in NBTC-donors) & HBCAB (NBTC has introduced NAT testing). On-site assessment occurred, 1–3/9/2014, a team of AABB assessors visited the NBTC to view the NBTC operations, determined compliance with standards, and conducted a public information session – findings were reported to the AABB for final determination of accreditation status. Next was AABB review and decision, based on the formulation of a plan of correction previously submitted by the NBTC.

Results: Board review acceptance and AABB-accreditation was granted in 1/10/2014. However the process was nonetheless full of challenges as: the existence of financial constraints on the initial NBTC level that will need to be further addressed once the remaining RBTCs are included. At the start of the project it was aspired to accredit all the 24 RBTCs, regional- blood-transfusion-centres, but logistical difficulties hampered this. There were some variations from AABB specifications related to the testing algorithm: no testing done for anti HTLV I/LL, anti-HBC, this was substituted by HBV-DNA. Also no testing for WNV due to the very low prevalence in Egypt. A lack of any bacterial detection tests regarding platelet components and no SOP for microbial detection, as it was only performed on quality control samples. This was addressed by the implementation of bactec FX40 & bacterial cultures as a backup plan. ISBT-128 questionnaires were also a prerequisite that caused it issues regarding permission from the ICCBBA, which then approved a list of assigned codes.

Conclusion: AABB accreditation is a mark of distinction to any blood bank that works within a quality system. The inevitable challenges to accreditation, however many, can be overcome through handling obstacles with creativity and determination, while maintaining compliance and striving for reaccreditation.

1C-03-04

IS IT TIME TO START HEPATITIS E TESTING – DONOR CENTRE PERSPECTIVE

G Nasrallah[1], E Al Absi[1], N Ali[1], R Ghandour[2], S Taleb[1], L Hedaya[1], F Ali[1], M Sami[1] and A Husseini[2]

[1]Qatar University, Doha, Qatar [2]Birzeit University Palestine, Ramallah, Palestinian Territory

Background: HEV is a small RNA virus from the *Hepeviridae* family. Usually, HEV causes an acute self-limiting infection. However, it may cause serious hepatic infection in immunocompromised patients and pregnant women. Although fecal-oral is the known route of transmission, recently, transmission through blood transfusion was also documented. Thus, it remains uncertain whether there is a need for HEV testing prior transfusion. Since Qatar population consist of more than 80% of expatriates, it is important to estimate the seroprevalence and to compare between different nationalities residing in Qatar.

Although serological tests are important for estimating HEV disease prevalence, detecting HEV RNA donor blood by RT-PCR remains the gold standard method that should be used to prevent HEV transmission by transfusion. That is, due to the nature of immune response to HEV, the available serological tests fail to detect antibodies to HEV in donor blood during the very early infectious stage.

Aims:

1. To estimate the seroprevalence of anti-HEV antibodies (IgG and IgM) among healthy blood donors in Qatar.
2. To evaluate performance of five commercially available anti-HEV IgG immunoassay kits including ELISA kits from Wantai Biological Pharmacy, China; Diagnostic Automation, USA; Euroimmun; Germany, and an immunoblot kit Mikrogen Diagnostik; Germany.

Methodology: A total of 5,046 blood donor samples were collected from the blood donation center at Hamad Medical Corporation. Wantai HEV IgG and IgM ELISA kits were used to estimate seroprevalence, as these are the most common commercial kits used in the literature for epidemiological studies. All positive IgM specimens were tested for the presence of HEV RNA by RT-PCR kit (Mikrogen). For kit performance evaluation, the first 1,034 samples were tested by all of the five aforementioned immunoassay kits. True positive or negative statuses of the sample are defined based on any three or more concordant test results out of all five HEV kits used in this study.

Results: The mean age of the donors was 36.6 ± 9.4 years. Only 150 (3.0%) of the samples belong to females. Out of the 5,042 samples, 1,019 (20.2%) and 35 (0.7%) were tested positive for IgG and IgM, respectively. Interestingly, none of these IgM

Diagnostic Parameter	Wantai	Diagnostic Automation	MiKrogen	MP	EUR
Sensitivity (n=182)	96.70	99.45	97.80	87.91	53.85
Specificity (n=852)	99.41	96.71	98.71	99.06	98.94
Accuracy (n=1034)	98.94	97.20	98.55	97.10	91.01
Positive predictive value	97.24	86.60	94.18	95.24	91.59
Negative predictive value	99.30	99.88	99.53	97.46	90.94

Table 1. Evaluation of performance of five different immunoassay kits.

positive specimens belong to Qatari donors. The prevalence of anti-HEV IgG among non-Qataris nearly doubles that of Qataris (22.4% vs 11.4%, P < 0.05). A significant association was detected between IgG seroprevalence and age group (P < 0. 05). There was no significant association between gender and HEV seroprevalence (F 18% vs M 20.3%).

All of the 35 IgM positive samples were tested negative by the RT-PCR, suggesting that there is no correlation between IgM and RT-PCR results. The sensitivity, specificity, and predictive value results for the five different kits are summarized in Table 1. All kits demonstrated a similar and excellent overall specificity and accuracy ranging from 96.7–99.0%, 91.0–97.1%, respectively. However, for sensitivity, Diagnostic automation (99.4%), Mikrogen (99.4%), and Wantai (96.7%) demonstrated superior sensitivity over MP biomedical (87.9%), and Euroimmune (53.8%). Further, almost all of the kits showed excellent predictive values.

Conclusion: HEV seroprevalence among blood donor is high. Thus, we recommend adding HEV RT-PCR screening, particularly, in case of blood transfusion to immunocompromised patients or pregnant women. We also recommend using Wantai or Diagnostic automation kits for anti-HEV screening, as they are they are the more sensitive, less expensive and labor intensive than other kits.

Blood/Blood Components and Products

1D-04-01

AUTOMATION IN BLOOD PROCESSING

M El Ekiaby

Shabrawishi Hospital, Giza, Egypt

Background: In the past, transfusion therapy was largely dependent on the use of whole blood. While whole blood may still be used in certain limited circumstances, the main thrust of modern transfusion therapy is to use the specific component that is clinically indicated. Components are those therapeutic constituents of blood that can be prepared by centrifugation, filtration and freezing using conventional blood bank methodologies. The blood components prepared by blood transfusion centers include different forms of packed red blood cells, plasma and platelet concentrate.

Requirements for preparation of blood components: Choice of the blood bag configuration should match the targeted components to be produced. In addition to blood bags, balances, cooling centrifuges, plasma extractors, electric sealers and sterile docking machines are required.

Automation in blood processing: In order to reduce the laborious steps and standardize the quality of blood components several technologies have been developed to automate blood component preparation. The advantages of these technologies include reduction of a number of equipment, such as plasma extractors, electric sealers. This leads to reduction of workspace and consequently may increase production efficiency. It also reduces the work load for the operators since these automates usually include several steps like breaking of clamps, extraction of the blood components as well as sealing of the pilot lines between the blood bags. More over the automation will reduce the operator inter variability leading to production of more standardized blood components.

Automates for preparation of components from whole blood: Two types of automates are commercially available. The first type will depend on automation of separation of blood components from whole blood units after centrifugation in classical blood bank cooling centrifuges. They can usually process both top/top or top/bottom blood bags for separation of RBCs, platelet rich plasma (PRP), fresh plasma (FP) and buffy coat (BC). The other type of automate combines both centrifugation and separation of blood components from whole blood units, using a special medical device to which four or six whole blood units will be docked and can consequently produce four or six units of RBCs, PCs and FP.

Preparing your lab for automated blood processing: In order to efficiently transform your blood component lab to automated blood processing certain measures should be considered. You may need to know the throughput of such automates and the number of whole blood units prepared each day to decide on the number of automates needed. You will also need to have the required space as well as enough well trained staff that can use these automates. Maintenance programs are of prime importance to insure that you will not face frequent failures that may lead to reduction of your production capacity. In case of automate that will need related disposable system you need to make sure that the supply of such devices will be continuous to avoid work interruption.

Conclusion: Automation of blood component preparation from whole blood offers several advantages that include reduction of standard equipment, space, workload to lab staff as well as more standardized blood components.

1D-04-02

COMPARISON OF WHOLE BLOOD AND APHERESIS PLATELETS

JD Sweeney and C Nixon

Brown University, Providence, Rhode Island, United States of America

Background: The availability of allogeneic platelets for transfusion to severely thrombocytopenic patients is an important supportive therapy for patients with hematologic disorders, patients undergoing chemotherapy, bone marrow transplant recipients, trauma with massive transfusion and bleeding in association with cardiac surgery. Allogeneic platelets can be sourced from whole blood donations (WBD) or using apheresis technology (APH). Platelet concentrates can be manufactured from whole blood using the buffy coat or platelet rich plasma method; typically these are leukoreduced prestorage by filtration. An important consideration is whether to supply the community need for allogeneic platelets with apheresis platelets, whole blood derived platelets, or both but there has been an increasing trend towards apheresis platelets. This has largely been driven by the perception that apheresis platelets are a superior quality product, i.e. have intrinsically a higher potency (ability to produce better increments) and better safety (reduce risk of infectious disease transmission).

Aim: The aim is to compare these two products.

Methods: The frequently cited advantages for APH are as follows: (i) Less Bacterial Sepsis largely negated by bacterial screening or pathogen reduction; (ii) Fewer donor exposures which has a logical appeal but minimal clinical impact in practice; (iii) Better corrected count increments,but in the PLADO study this was minimal and not associated with a difference in clinical bleeding; (iv) Fewer allergic or non-hemolytic transfusion reactions – this is controversial and not seen in the TRAP study; (v) Larger available pool of HLA Typed Donors but crossmatching is an alternative; (vi) Fewer Donors to Screen for CMV which is negated by leukoreduction; (vii) Less outdating but this is a result of the preferential use of APH by technologists and apheresis outdating increases as relative use of APH increases; (viii) Elimination of post storage pooling but this is negated by prestorage pooling.

Results: The question of bacterial contamination rates was an important recent driver towards the use of APH in the U.S. However, with the availability of prestorage pooling and culture based testing, it is unclear whether this advantage currently exists, since contaminated products are removed from inventory. Hence, clinical sepsis, the only important measure, may be similar and a recent report from the American Red Cross shows similar or less rates of bacterial sepsis between product types. The associated donor exposure question is viral disease transmission. The argument is only relevant if the absolute residual transmission risk remains high; residual transmission rates per unit (USA) for HBV are approximately 1:350,000 and for HCV/HIV <1:1,000,000: hence, although the relative risk of whole blood pools remains, the absolute risk is near statistical zero.

The problem of TRALI associated with APH is a major disadvantage, and a report from the American Red Cross indicted a relative increased risk of 7.8 (CI, 2.5–24.8). Plasma mismatched hemolysis remains a rare complication of platelet transfusion and 2/3 of all such cases are associated with APH. APHs by definition come from a single donor; recent data shows that as many as 20% of such donors have defective platelet function, probably as a result of recent aspirin or NSAID ingestion which could compromise hemostatic effectiveness.

Conclusion: If pathogen reduction and use of platelet additive solutions become common practice, no clinical difference in outcomes would be expected between WDP and APH.

1D-04-03

PLASMA FRACTIONATION, THE MOROCCAN EXPERIENCE

K Lahjouji

National Blood Center, Rabat, Morocco

To ensure availability in plasma derived drugs (PDD), the National Center for blood transfusion and haematology of Morocco (CNTSH) signed a Plasma fractionation agreement of with LFB Bio medicaments. The first agreement was signed in 1999, renewed in 2006 and then in 2012. The signed contract provides all the terms: from the preparation of the fresh frozen plasma for fractionation by the CNTSH until the PDD reception in Morocco. The contract also provides the terms of pharmacovigilance,

hemovigilance, PDD legal registration, prices and terms of payment. On one hand, the CNTSH is committed to comply with the qualitative and quantitative aspects of the fresh frozen plasma, as described on the book of specification. On the other hand, the LFB Bio-médicaments agrees to manufacture PDD in accordance with the latest version of the good manufacturing practice and in accordance with the manufacturing processes and controls described in the marketing authorization agreement.

The plasma specification book is established with the texts in force, as transfusion practices and good manufacturing practices for PDD, it sets the specific requirements for plasma for fractionation and specifies the operational conditions on the origin, collection, freezing, storage and plasma controls. The fresh frozen plasma transport from Morocco to the France is organized by the LFB using specialized refrigerated trucks.

A control at the reception is performed at the LFB site and based on: Visual criteria, identification, immunohematological and screening for infectious diseases. In addition, quality audits are conducted yearly by the LFB auditors for the qualified regional blood transfusion centers (CRTS). Qualification for new CRTSs is undergone regularly on the CNTSH request in order to integrate them into the process of plasma fractionation. After each audit, a report is established by the LFB auditors and addressed to the CNTSH, an action plan for the correction of non-conformities is then established and sent to the LFB for follow up.

In 1999, date of signature of the cooperation, only two CRTSs have been qualified by the LFB, namely CRTS Rabat and Casablanca. In 2011, two additional CRTS were qualified (Marrakech and Oujda) and in 2014 the CRTS Fez was also qualified. The amount of plasma produced by the CRTS and sent to the LFB has registered a significant increase; it rose from 4,000 l in 1999 to 6,000 l in 2006, to 12,000 l in 2009 and to 14,000 l from 2014. PDD produced by the LFB for the CNTSH are: the human albumin 20% and 4%, coagulation factor VIII and IX and finally, the intra venous human immunoglobulin 5 and 10 g. These medicines are sent to Morocco by airplanes.

After controls PDD are released by the Moroccan authority and distributed by the CNTSH to hospitals and patients.

ISBT Academy Day Practical Approach to Donors and Donation/The Spectrum of Donors and Donation

2A-01-01

EFFECTIVELY RECRUITING AND RETAINING REGULAR VOLUNTARY BLOOD DONORS

RH Alabdulrazzak

Club 25 Kuwait, Salwa, Kuwait

Background: Kuwait, like any other country faces great challenges when it comes to recruiting and retaining volunteer blood donors. The Kuwait Central Blood Bank has managed over the past 10 years to achieve great success in its path towards achieving 100% Voluntary non remunerated blood donations particularly in recruiting whole blood donors. However, we were not equally successful when it comes to apheresis donors and rare blood groups donors, as these donors require special recruitment strategies to insure their long-term commitment. One of the main reasons for this is the lack of a national donor recruitment program and campaigns budgeting. Lacking a proper donor recruitment plan and structure has been a main obstacle in achieving further progresses in our donor recruitment effort.

Aims: To showcase the different tools that can be used in donor recruitment and retention, how to make the best out of them and how to choose the right tools that will help donor centers reach their desired goals.

Methods: Utilizing 10 years of donor recruitment data from the Kuwait Central Blood Bank and Club 25 Kuwait in an attempt to figure out the efficacy of the various strategies that were adopted to recruit various types of donors. We will try to look closely to understand what motivated blood donors to donate and what worked best.

Results: Over the past 10 years, the Kuwait Central Blood Bank has received around 700,000 blood donors. Various donor recruitment strategies were adopted including huge national media campaigns, mobile blood drives, traditional media interviews and advertisements, social media campaigns and partnering with youth volunteer groups. All of these tools have contributed to the success in recruiting volunteer whole blood donors and maintenance of adequate blood supplies. For apheresis donors and rare blood donors less tools were adopted and the success was much less.

Conclusion: Mobilizing the crowds and changing their perception and attitudes towards blood donation is a difficult mission. Therefore, It is important for donor centers in order to be efficient in their donor recruitment efforts to keep a close eye and evaluate their implemented strategies periodically. Strategies that worked 10 years ago might not be the best strategy for the new generation. Building the right donor recruitment team and adopting the proper strategy requires a deep understanding of the public interests. Always remember, convincing people to donate their blood is very similar to convincing a customer to purchase an item. To sell them your idea you need to use your marketing skills and tools and not your white coat and needles.

2A-01-02

OVERCOMING BLOOD SUPPLY CHALLENGES IN MULTI-ETHNIC DONOR AND PATIENT POPULATIONS: EXPERIENCE FROM MALAYSIA

WS Kam

National Blood Centre, Kuala Lumpur, Malaysia

The blood transfusion service in Malaysia began in the 1950s and has since evolved into the national transfusion medicine service today, based on voluntary non-remunerated donations as outlined in the national blood transfusion policy. A country of over 30 million, Malaysia is made up of heterogeneous multicultural, multi-ethnic donor and patient populations. The unique complexity of the donor and patient socio-ethnic demographics further compound the blood supply challenges faced by the national transfusion medicine service. These challenges become more pronounced during holidays, which are observed and celebrated differently among the donor and patient populations.

As a precious resource, the national transfusion medicine service relies on the good will, altruism and the generosity of the community. A sustainable nationally coordinated blood donation program, coupled with an efficient national blood inventory management and patient blood management ensure that the blood supply is adequate, accessible and capable of meeting the blood demand. Effort must be made to continuously promote donor recruitment and retention while actively engaging the community. These activities can be further enhanced from a better understanding of the donor psyche and behavior and of the transfusion needs, in the context of the pluralism and diversity which exist within the population. Fostering a close and meaningful relationship with blood donors and blood donation organizers as well as the education and promotion of blood donation serve as the foundation of the numerous strategies to overcome the blood supply challenges.

2A-01-03

UNDERSTANDING IRON DEPLETION AND OVERLOAD

F O'Brien and M Goldman

Canadian Blood Services, Ottawa, ON, Canada

Blood donor iron status is integral to suitability to donate blood. Normal iron stores in healthy men are about 1,000 mg, whereas in healthy females only about 300 mg. A whole blood donation depletes the donor of approximately 225–250 mg of iron. For females this means that often her iron stores are completely depleted after only one donation. Dietary absorption of iron can be up to 3.5–4 mg/day when iron stores are low, less when iron stores are higher. It takes 78–122 days for hemoglobin to return to 80% of baseline, and longer to recoup iron stores, hence to donate frequently many donors may require iron supplements.

Hemoglobin is routinely measured to determine donor eligibility. However, because iron stores will be largely depleted before hemoglobin concentrations drop, it is a late stage indicator of iron deficiency. Serum ferritin is correlated with total body iron, thus is a convenient marker of iron status. Recent studies have shown that iron depletion is common among blood donors. Some people, especially young females, have low iron stores on their first donation. However, low iron stores are correlated with higher frequency of blood donation in both females and males. In countries largely reliant on regular donors, up to 60% of donors may be iron deficient. Low iron stores are associated with a number of adverse outcomes such as fatigue, decreased physical endurance, and difficulty concentrating and learning. Low iron may be particularly detrimental among women of child bearing age and young donors due to the importance of iron in brain development.

Strategies to address low iron stores in blood donors include reducing donation frequency, providing donor education about iron, as well as testing donors for ferritin levels and notifying them of abnormal results, and/or providing iron supplements.

Although low iron stores will be usually related to diet and blood donation, it can also be a sign of other pathology. In certain donor populations it may be advisable that donors with abnormal results consult their family physician. The threshold hemoglobin for male donors can be raised. Ideally, deferral for low hemoglobin should be long enough to recoup body iron stores and iron supplements have been shown to be beneficial. Different strategies for females and males are physiologically justified.

Hemochromatosis, a condition of excess iron stores, is usually a genetic disorder, and is relatively common in certain European populations where up to 0.6% of people may be affected. If left untreated iron overload is associated with pathology such as heart and liver disease, and osteoarthritis. Treatment usually involves phlebotomy thus donating blood can be beneficial. Donors who have iron overload may be unaware of their condition, and iron supplements without ferritin testing could be detrimental.

Many blood services are performing studies and revising policies to better address iron status and safety of blood donation. Due to country specific factors such as the nutritional/health status of the population, variable reliance on repeat donation and operational issues, no one policy will suit all centers.

Practical Approach in Immunohaematology/ The Spectrum of Immunohaematology

2A-02-01

RED CELL ALLOANTIBODIES – CLINICAL SIGNIFICANCE OR NOISE

JL White

UK NEQAS (BTLP), Watford, United Kingdom

Antibodies directed to red cell antigens can cause significant intravascular and extravascular haemolysis, leading to mortality and morbidity in both transfusion and antenatal settings. For pre-transfusion testing, clinically significant antibodies can be defined as those that are capable of causing patient morbidity due to the accelerated destruction of a significant proportion of transfused red cells. The clinical significance of an antibody in the context of antenatal testing will also depend on the antibody's ability to cross the placenta and the stage of development at which the corresponding antigen is expressed on fetal red cells.

It is possible to predict the likely clinical significance of an antibody according to its characteristics, (e.g. specificity, thermal range, Ig class and subclass, ability to fix complement), and those of the corresponding antigen (e.g. density on red cell, presence on other tissues or in a soluble form). Whilst categorising clinical significance in this way is useful and forms the basis of most practical guidance, the clinical setting and the patient's individual immune response are also significant factors. It is worth remembering that there are many cases where antibodies have not 'obeyed the rules', and also that an antibody that is considered 'harmless' in one situation has the potential to cause harm in another. Haemovigilance schemes are a useful source of data in this respect.

The aim of antibody screening is to detect only antibodies that are likely to cause problems in the clinical setting for which the screening is designed, i.e. for pre-transfusion patient testing, antenatal testing or donor testing. The requirements for screening will be different as will the definition of a significant antibody in each of these situations. Detection of 'insignificant' antibodies creates unnecessary work along with potential delay in providing compatible blood for transfusion, or unnecessary repeat sampling/interventions in pregnancy. When selecting techniques for screening there is always a balance between sensitivity and specificity, i.e. the risk of missing something significant vs that of detecting too much 'noise'. The method (IAT, enzyme etc.), technology (tube, gel, solid phase etc.) and antigen profile of the screening cells selected can influence this balance.

For routine pre-transfusion and antenatal testing, a sensitive and validated indirect antiglobulin test (IAT) should be sufficient to detect most clinically significant antibodies, and the use of additional methods, e.g. enzymes, will increase the risk of detecting antibodies that are in the vast majority of cases not significant. It is not possible to design a system where non-significant antibodies are never detected, and further investigations to determine the specificity (if any) and thermal range of the antibody must be undertaken to allow decisions to be made on whether antigen negative blood is required for transfusion. Pan-reactive alloantibodies are often clinically significant and directed against a high incidence antigen or against multiple more common antigens, but may sometimes be shown to be clinically insignificant, e.g. complement related antibodies, in which case it is important that all clinically significant antibody specificities are excluded.

2A-02-02

INTRODUCING GENOTYPING INTO ROUTINE PRACTICE

JR Storry

Clinical Immunology & Transfusion Medicine, Lund, Sweden

The past decade has seen the introduction and implementation of PCR kits and more sophisticated platforms for blood group determination by molecular genotyping into many laboratories across the world. The technology is robust and the error rate low. There are many different applications but they divide primarily into three categories; (i) testing of samples from individual patients with the purpose of providing a more specific personalized care; (ii) testing samples from blood donors in order to provide a greater inventory of broadly phenotyped blood to meet the needs of patients with antibodies; (iii) determination of fetal RhD type from cell.-free DNA from maternal plasma. The third category remains restricted to more specialized laboratories but is becoming more mainstream as health services realize the cost-savings benefit of only administering prophylactic anti-D to those women who need it.

There are challenges to introducing genotyping. At the top of the list is education: many laboratories have introduced genotyping with personnel who have no or limited training in molecular techniques but a deep understanding of blood group antigens. This picture has changed in the past decade and today's clinical laboratory scientists are very knowledgeable about modern techniques but possibly have a more superficial understanding of blood group antigen complexity. We need to find a balance so that today's clinical scientists know how to interpret the molecular data. How should genotyping results be incorporated into current laboratory information systems, and thus made remunerable? This remains a challenge as long as genotyping is not considered to be phenotyping but I consider this testing to 'molecular phenotyping', while well-aware that very occasionally, the results might not reflect blood group antigen expression at the cell surface.

As with any laboratory technique, there need to be quality assurance programs in place. In the early years, this was achieved by a collegial exchange of DNA samples between laboratories, spearheaded by the ISBT in Europe and the CBGG in North America, but now the various quality assurance agencies have developed programs that permit all laboratories to fulfil external quality assurance requirements.

The advantages to such testing platforms are many but if one focusses on patient care alone, the benefits are clear. We can more rapidly identify suitable donors for patients requiring better matched blood; we can obtain phenotypes on transfusion-dependent patients and we can type foetuses to prevent maternal alloimunisation to RhD. Finally, the possibility to identify rare donors by molecular techniques has helped to provide blood in a more timely manner to patients in need. Mass genotyping has in some centres meant that red cell units that otherwise would have been frozen, with all the expense associated with freezing and then subsequent thawing, are now available as fresh liquid units. This has greater implications in patient care, from providing a better product to reducing hospital stay for patients that would otherwise have to wait for a blood transfusion.

2A-02-03

SOLVING DIFFICULT CASES IN AUTOIMMUNE HAEMOLYTIC ANAEMIA

L Castilho

University of Campinas, Campinas, Brazil

Autoimmune haemolytic anaemia (AIHA) is caused by the production of 'warm-' or 'cold-' reactive antibodies directed against antigens on the patient's red blood cells (RBCs). Autoantibody production can be triggered by disease, viral infection, or drugs; from breakdown in immune system tolerance to self-antigens; or from exposure to foreign antigens that induce antibodies that cross-react with self-RBC antigens. Autoantibody specificity is not always obvious because patient's antigen expression can be depressed when autoantibody is present. AIHA can be classified in

different types including warm AIHA (WAIHA), cold hemagglutinin disease (CHAD), mixed-type AIHA, paroxysmal cold hemoglobinuria (PCH) and drug-induced IHA. Warm AIHA (WAIHA), is the most common type of AHAI characterized by warm autoantibodies primarily IgG (rarely IgM or IgA) reacting best at 37°C. Most are directed against the Rh protein, and over 90% of cases have a positive DAT. The patient's plasma contains autoantibodies that appears as a panagglutinin and the biggest concern is that they mask the presence of alloantibodies. It is important to rule out, or identify, alloantibodies in the plasma/serum and identification of the autoantibody specificity is rarely useful. RBC components should be ABO and RhD compatible and lack antigens corresponding to any clinically relevant alloantibody (ies). The serologic work required to detect and identify underlying alloantibodies may include multiple autologous adsorptions and elutions and once a patient is transfused, alloantibody identification becomes more difficult, requiring allogeneic adsorptions. Transfusing the least incompatible blood is rarely of value and one common strategy is to provide extended phenotype matched blood but this approach is only applicable when the patient does not have a strongly positive DAT or has not been transfused. Molecular testing is being used as a valuable method to predict the extended red cell antigen profile in order to select antigen-negative RBCs for adsorption of autoantibodies when searching for underlying alloantibodies. Matching the genotype of donor and patient can also replace allo- and auto-adsorptions by providing the most highly matched blood to the patients with AIHA without heavy, costly, lengthy, and sample consuming adsorption procedures. However, adsorption methods may still be needed when the patient's genotype does not allow antigen-matched units selection. Considering that patients with AIHA become alloimmunized much more commonly than other patients, it is very important that efficient procedures for detecting underlying alloantibodies, although labor-intensive, must be used in pre-transfusion testing. Evidence of immune haemolysis, transfusion history and pregnancy, diagnosis and medications are important information's to direct the decision-making pathway for determining what further studies are necessary and how quickly they must be performed. A systematic approach is required to solve difficult cases of warm, cold and drug-induced autoantibodies and to optimize the process in order to provide appropriate transfusion support for patients with AIHA.

Practical Approach to Organisation and Quality/The Spectrum of Organisation and Quality

2B-03-01

MANAGING HAEMOVIGILANCE AT HOSPITAL AND NATIONAL LEVEL

S Hindawi

KAUH, Jeddah, Saudi Arabia

Background: The Haemovigilance system is an adverse event monitoring system established at the beginning of the last decade (1990s) in Europe. It then became a crucial part in quality system of blood transfusion in developed countries. However, there have been difficulties in taking farther steps in the direction of Haemovigilance in developing countries up until now due to a number of challenges.

Haemovigilance is a safety concept refers to the use of a measurement system to record unwanted outcomes of transfusion chain. It involves a continuous surveillance of all the procedures in the transfusion chain from the blood donor to the recipient of the blood components, in order to improve the safety of both.

Aim: The intention is to collect and evaluate information on unexpected or undesirable events. The aim is to prevent the risk and/or to reduce the severity of such events.

Method: In each country a clear plan and proposal should be submitted to an official local body responsible of transfusion services in the country to get approval and support. The proposal should include aim and objective of having such system, a written policy and standard to be followed, working group or committee to be responsible for the process of implementation, funds to cover system requirement and the running cost of the system. The decision must be taken to apply the haemovigilance system as voluntary or mandatory process according to the need of the country. At national level, the ministry of health (MOH) or equivalent body should ensure that bidirectional traceability is maintained from donor to patient and vice versa. In addition they should ensure that mechanisms are in place for data collection, monitoring, analysis, reporting, evaluation and assessment.

The haemovigilance system should be set up in each facility where blood donation and transfusion are performed. It is critical that activities in haemovigilance are coordinated; hence the system needs effective leadership and governance. Effective haemovigilance system relies upon reporting of adverse events, and analysis of what went wrong and why (root cause analysis). It is therefore essential that a haemovigilance system operates in a non-punitive environment and that reporting should be confidential and anonymous. Actions should be taken to identify weaknesses and deficiencies though corrective and preventive actions (CAPA). This system will provide hospital with evidence-based knowledge for improvement of safety and quality of blood transfusion services in the hospital.

Summary: Haemovigilance should be part of quality management systems of blood centres and health care institutions; and should result in improved policies, procedures and practices in the blood transfusion chain. Training, education and increase awareness among workers in blood transfusion facilities will facilitate the development and establishment of the haemovigilance system in all developing countries.

2B-03-02

WHAT DOES YOUR DATA TELL YOU? HOW TRANSFUSION CHAIN DATA CAN SUPPORT MANAGERIAL DECISION MAKING

MP Janssen

UMC, Utrecht, The Netherlands

In recent years there has been an increasing focus on the collection and utilization of data. Expanding digitization of information combined with the proliferation of multi-channel processes and transactions has resulted in a data deluge. As a consequence, big data has been promoted as being a holy grail, the answer to many of our problems. Despite the fact that there are numerous examples where data do provide valuable insights, in the end data can only contribute those problems for which the right information is captured within the data. Conceptually, the only way in which data can be used to create value is by: (i) providing new insights through revealing or highlighting existing patterns or associations; (ii) creating a reference for interpretation of new (or alternative) data; (iii) providing insight in the variability of measured parameters; (iv) identifying clusters with similar characteristics.

Data is essential for quality monitoring and systematic process improvement. Any quality control or process improvement effort therefore starts with the collection of data about the (current) status of a process. Many methods and standards for interpreting data and process improvement have been developed and applied in the past. Nonetheless, the increasing digitization of our society and the associated capabilities for expanding data collection now opens up completely new opportunities for both data collection and interpretation. The backside of such opportunities is that there are various pitfalls to be noted when trying to analyse these data. In literature such pitfalls are commonly divided in three categories: (i) Biases occurring in the data collected; (ii) Errors in the methodology applied; (iii) Faulty interpretation of results.

The first item refers to systematic errors in the data. For instance, if the data only cover a particular part of a study population, any conclusions drawn from the data might not be applicable to the whole population. Errors in methodology might refer to the application of a statistical method for which the underlying assumptions are not valid. Related to the interpretation of results, the most common error is confusing correlation with causation, but interpreting a statistical assessment in itself may even be challenge for non-experts.

Adequate collection and analysis of data may provide a solid handle for management. It may not only highlight the significance of a problem, but in addition may allow quantification of the size of improvements achievable and provide guidance as to how potential improvements are best achieved. A number of examples from within the blood supply (predicting donor population demographics, interpreting infections in donor centres and predicting future red blood cell demand) will be provided with a discussion on how these contribute to management decision making.

2B-03-03

AIMING FOR QUALITY AND ACCREDITATION IN THE HOSPITAL BLOOD BANK

P Flanagan

New Zealand Blood Service, Auckland, New Zealand

The primary goal of a hospital blood bank will be to ensure the supply of accurate test results and appropriate blood products for patients at the right time and place. Implementation of a quality system will help assure these outcomes and accreditation of the laboratory will provide external evidence that effective systems are in place.

Haemovigilance systems continue to demonstrate the capacity for error in the transfusion chain. This chain starts with the decision to transfuse a patient and ends with the completion of the transfusion and assessment of its benefit. The hospital blood bank plays a critical role in the process not only as a provider of laboratory services but also as the natural focus for evaluation of the complete transfusion chain.

There are a number of different types of quality systems that can be applied in the hospital blood bank setting. These can either be based on medical laboratory systems (ISO 15189) or on principles of Good Manufacturing Practice (GMP). Different countries apply different requirements. For example in the European Union hospital Blood Banks should comply with Good Practice guidelines based on the principles of GMP whereas in Australasia medical laboratory accreditation based on ISO 15189 is the norm.

Guidelines and Standards on transfusion laboratory practice are produced by a number of organisations. Examples include the British Committee for Standards in Haematology (http://www.bcshguidelines.com/), AABB Standards and the Australian and New Zealand Society of Blood Transfusion (http://anzsbt.org.au/publications/index.cfm). These identify the basic technical requirements that must be in place. They should cover the pre-analytic (specimen acceptance requirements, analytic (reagents and test procedures) and post analytic (reporting) components of blood bank work. The technical requirements identify what must be achieved and the quality system requirements identify the processes that will need to be in place to ensure that this occurs.

Increasingly hospitals can also be accredited and this process can involve assessment of criteria relevant to transfusion practice. This type of process tends to focus on transfusion practice in clinical areas of the hospital. Hospital accreditation systems aim to demonstrate that health care organizations to accurately assess their level of performance in relation to established standards and to implement ways to continuously improve. Areas of focus might involve review of patient identification systems and sample labelling error rates and evidence of active review of the appropriateness of transfusion.

Health care activities are all prone to the risks associated with human error. Quality systems can assist in reducing the impact arising from such events and assist organisations to improve their practices over time. Information on the types and frequency of error can help identify priorities for practice improvement and assist in appropriate allocation of resources. An effective quality system should assure that expected outcomes are achieved and that, in the context of the hospital blood bank, improve the likelihood that transfusion is provided safely to those patients who will benefit from it.

Practical Approach to Transfusion Therapy/The Spectrum of Transfusion Therapy

2B-04-01

MANAGING THE PATIENT WITH HEMOGLOBINOPATHY AND MULTIPLE RED CELL ANTIBODIES

A Matteocci

A. O. S. Camillo – Forlanini, Rome, Italy

The main supportive treatment for hemoglobinopathies is repetitive red blood cell (RBC) transfusions but this may lead to development of RBC alloimmunization which represents a critical and clinically relevant barrier. This complication is especially common among sickle cell anaemia (SSA) patients and it depends on multiple clinical and biological factors such as sex, number of transfusions and age at which therapy begins. Recent studies suggest that an increased risk may also be related to the chronic inflammatory status and polymorphisms of the *HLA II* class and *CD81* genes. Furthermore, one of the greatest risk factors for alloimmunization is the antigen disparity between donor and recipient. This is prevalent with European donors and African recipients because of different blood group phenotypes. Various authors have reported that from 5.2% to 30% of thalassaemia patients and from 18% to as much as 47% of SSA patients to be affected. These are markedly higher rates than in other multiply transfused populations, but there are much lower rates in countries where there is a greater racial homogeneity. Over 2/3 of all alloantibodies have specificities against Rh and Kell blood group systems. RBC alloimmunization can cause delayed haemolytic transfusion reactions (DHTR) with different risks of morbidity and mortality in SSA patients. These patients can also develop hyperhemolysis syndrome (HS) which is characterized by the destruction of both the donor's and the recipient's RBCs. The presence of single or multiple allo and autoantibodies increases the complexity of the serologic work-up to find compatible products and demands highly skilled investigation by different panels, elutions, adsorptions, etc, and this delays transfusion therapy. In addition, RBC alloantibody evanescence, e.g. anti-Jk^a, and the lack of serologic identification, carries a risk of severe and life-threatening hemolytic transfusion reactions after the re-exposure to the immunizing antigen. There is no universal agreement on the most appropriate method for selecting RBC units for chronically transfused patients with hemoglobin disorders. In addition to ABO and RhD matching, some institutions have called as a prevention measure, for C, c, E, e, and K antigens matching procedures which have demonstrated significant reduction in RBC alloimmunization. Despite Rh matching, Rh antibodies may occur in patients whose RBCs express variant antigens undetected through routine serologic examination. Furthermore, prophylactic matching should be extended to include other blood group systems such as MNS, Duffy and Kidd as well and RBC genotyping may improve a precise matching of *RH* alleles and be a valuable support in the event of multiple RBC antibodies. This must be carried out using high-throughput platforms with large-scale genotyping based on DNA analysis targeting single nucleotide polymorphisms (SNP) associated with non-ABO blood group antigens. The greater the level of matching, the closer one comes to the 'perfect match' which is an innovative strategy adopted to ensure the effectiveness and safety of transfusion therapy at an affordable cost. Therefore, the molecular non-ABO extended matching may actually help in the management and mitigation of clinical risks associated with RBC alloimmunization which still remains a major challenge to chronic transfusion therapy in patients with hemoglobinopathies.

2B-04-02

No abstract available.

2B-04-03

ALLOANTIGENS ON NEUTROPHILS AND PLATELETS IN MULTI-ETHNIC POPULATIONS

S Santoso[1,2,3]

[1]*Justus Liebig University Giessen, Giessen, Germany* [2]*Institute for Clinical Immunology and Transfusion Medicine, Justus Liebig University, Giessen, Germany* [3]*Guangzhou Blood Center, Guangzhou, China*

Neutrophils and platelets express membrane glycoproteins (GPs) on the cell surface. In humans, some of these GPs have been found to be encoded by two or more allelic isoforms that differ by single nucleotide polymorphisms (SNPs). This nucleotide exchanges result in amino acid substitutions leading to the formation new alloantigen determinants which can be recognized by our immune system.

Immunization against human neutrophil antigen (HPA) and human platelet antigen (HPA) can lead to the production of neutrophil and platelet alloantibodies responsible for the pathomechanism alloimmune mediated neutropenia, TRALI (transfusion related acute lung injury) and alloimmune thrombocytopenia (platelet transfusion refractoriness, neonatal alloimmune thrombocytopenia), respectively.

In the last two decades, genotyping analyses have been conducted to determine the risk of alloimmunization against HPAs and HNAs. The frequencies of some HPAs and HNAs differ among different populations suggesting different clinical impacts. Indeed, recent studies showed difference clinical relevance of alloantibody specificities among Caucasian and Asian populations. The identification of platelet and neutrophil alloantibodies is mandatory for the accurate diagnosis and treatments of immune mediated neutropenia thrombocytopenia. Several methods for the detection of platelet and

neutrophil antibody detection are available. All these techniques, however, have some limitations which need urgently to be improved in the near future.
Several studies in the past indicated that the amount of platelet and neutrophil alloantibodies did not always correlate with the clinical pictures; a phenomenon which complicates the clinicians to define good treatment strategy. Recently, several evidences indicated that the nature of alloantibodies (e.g. glycosylation) and alloantigens (e.g. heterogeneity of alloantigenic determinants, their expression on other blood cells and endothelial cells) play also important roles on the pathomechanism of alloimmune mediated disorders.
In this lecture, the clinical important of different HNAs and HPAs among different populations will be underlined. In this context, the current problems and challenge of antigen and antibody detection will be discussed. Scientific results gained from recent *in vitro* as well as from *in vivo* studies will be presented to illustrate the possible translation from bench-to-bedside in the future.

Practical Approach to TTID/ The Spectrum of TTID

2C-05-01

THE LATEST IN HEPATITIS B AND C

M Satake

Japanese Red Cross, Tokyo, Japan

The risk of transfusion-transmitted (TT) HBV infection largely derives from two sources of infectious blood in terms of the infection status of blood donors; namely, those within the window period (WP) and those with occult HBV infection (OBI). Because of the long doubling time of HBV in peripheral blood, only NAT with very high sensitivity can effectively detect WP-derived blood. However, a theoretical residual risk remains even with the implementation of individual donation (ID) NAT. OBI-derived blood often has a very low viral load that ID NAT cannot detect. However, detection can be achieved with seroreactivity to HBcAg, although a considerable proportion of non-infectious blood would also be disqualified with screening for HBcAb. The adequacy of implementing HBcAb screening into the blood program thus invariably depends on the security of the blood supply in the area in question.
Increasing sensitivity to HBV has resulted in the emergence of a clear relationship between component infectivity and total viral load infused, leading to the expectation that future occurrences of TT-HBV will be confined to the transfusion of fresh frozen plasma or platelet concentrate with a large plasma volume.
The marked advances in NAT technology, which theoretically offer a broader coverage over HBsAg screening, are leading to questions of whether HBsAg screening will be continued. Before ending HBsAg screening, we need to be certain that HBsAg-positive, NAT-negative blood is noninfectious. The Japanese Red Cross Blood Center has detected several such blood donations every year. However, after excluding non-specific HBsAg reactions, blood with HBsAg-only-positive reactions are invariably HBcAb-positive. We currently have no reason to resist cancelling HBsAg screening, but continued care is warranted for this issue.
With the decreasing trend in the frequency of TT-HBV, there may be an increasing tendency for HBV reactivation to be misdiagnosed as TT-HBV. HBV reactivation occurs in patients with past HBV infection who are receiving immunosuppressive molecularly targeted regimens, and is usually fatal in the absence of very early treatment. Because such patients often receive blood transfusion during the course of treatment, the possibility exists that hepatitis B in such patients may be diagnosed as TT-HBV.
Screening for HCV using serology (HCVAb) and mini-pool NAT has become established as a standard HCV screening strategy. Because the doubling time of HCV is very short, even mini-pool NAT can efficiently detect WP-derived blood. In fact, cases of TT-HCV have been uncommon since the introduction of this strategy. A decreasing trend is also being seen in the number of NAT-only positive donation or new infections in Japan. Interestingly such new infections have been detected over a wide range of donor ages in women as well as men. No decreasing trend is being seen in the number of suspected TT-HCV cases reported from physicians in Japan. Almost all could be excluded from the possibility of TT-infection based on detailed analyses of patient pre- and post-transfusion samples and repository samples from donors, raising the high likelihood of nosocomial HCV infection.

2C-05-02

GLOBAL CHALLENGES OF MANAGING MALARIA RISK

K Owusu-Ofori[1], O O'Shirley[2]and I Bates[3]

[1]Kwame Nkrumah University of Science and Technology, Kumasi, Ghana [2]Komfo Anokye Teaching Hospital, Kumasi, Ghana [3]Liverpool School of Tropical Medicine, Liverpool, United Kingdom

Malaria is a protozoan disease that is transmitted by the *Anopheles* mosquito. It can however be transmitted by blood transfusion if the blood donor is parasitaemic. Of the five species of *Plasmodium* that causes malaria, *P. falciparum* causes the most severe form of malaria. Nearly half of the world's population is at risk of Malaria. Mortality due to malaria has reduced by 48% from 839,000 deaths in 2000 to 38 deaths in 2015.
Two main approaches used to control malaria are vector control and antimalarial drug use. The vector control aims to reduce transmission of parasites from humans to mosquitoes and back to humans and the interventions include indoor residual spraying (IRS) and insecticide treated nets (ITNs). Antimalarial drug use involves both suppression of blood stage infection in humans (chemoprevention) and prompt diagnosis and treatment (case management).
Despite these interventions malaria morbidity and mortality remains high. Some of the challenges have evolved from the interventions while others may be inherent with parasite. The complex life cycle of the plasmodium parasite and the different stages it undergoes both in the mosquito and human requires a multifaceted approach to reduce or eliminate the burden of malaria. For example the search for a malaria vaccine has proved elusive. A vaccine for malaria has always been considered as a cost-effective tool in the midst of increasing spread of resistance strains of plasmodium falciparum and spread of strains of *Anopheles gambiae* that are resistant to pyrethroids. However till date, no effective malaria vaccine has been produced. The most advanced vaccine RTS, S did not achieve the expected efficacy during the phase III trials and it is unclear how this vaccine will be deployed for use.
Lessons learned over years show that we cannot be complacent and rely on a small number of tools or interventions such as ITNs, IRS and Artemisinin Combination Therapy (ACT) for malaria treatment. In addition, there is the need to identify gaps and harmonise institutions with a common goal of eliminating the risk of malaria.
Parasitaemia in blood donors is a gap that needs critical evaluation. There are no evidence-based policies to guide how healthy donors in malaria endemic regions should be handled. Depending on the region, up to 50% of blood donors are parasitaemic. The presence of parasitaemia in blood donors represents a risk for the transmission of malaria by transfusion as well as serving as a reservoir for environmental transmission. The method for the detection of parasitaemia in donors represents a challenge since the highly sensitive PCR is not widely available as a screening tool. One potential intervention to reduce transmission of malaria by transfusion is the use of pathogen inactivation systems, which does not require prior use of screening test. There is the need for institutions such as National Blood Services and Malaria Control programmes to collaborate and establish joint interventions that reduce the malaria risk. Such collaborations between all stake holders including academia, policy makers, funders, governments and international organisations may help in overcoming some challenges in managing malaria risks.

2C-05-03

APPROACHES FOR THE ASSESSMENT OF NOVEL PATHOGENS

S Stramer

American Red Cross, Gaithersburg, Maryland, United States of America

Background: Blood safety has relied on many tools to determine the appropriate measures to prevent or limit disease transmission. The primary approach has been vigilance and careful surveillance followed by assessments of risk and preparedness in the event that an assessment determines that an invention is needed. Efficacy of the intervention, once implemented, is necessary to determine if modifications of the intervention are required.
Aims: The strategy or algorithm mentioned above has been repeated numerous times during history and applies to agents that are emerging, re-emerging, or increasing in geographic distribution, increasing in pathogenicity or those agents associated with increased patient susceptibility. We will examine multiple methods and review how these have been used to ensure blood safety.
Methods: Vigilance is required for monitoring known infectious disease agents in the event that policy changes result in the alteration of the efficacy of currently employed interventions. This also applies if changes occur to the agents themselves or the characteristics of the donor or recipient population. Testing donated blood for markers of infectious disease has played a major role in establishing and maintaining blood safety. However, testing methods are not always immediately available

and in the future more proactive approaches may turn out to have a greater spectrum of success or cost-benefit (e.g., pathogen inactivation in that such an approach is not agent specific). However, limitations with these strategies may exist, such as not being readily available or economically feasible. Results: New challenges that have faced the international blood community within the past several years include: coronaviruses, Ebola virus, hepatitis E virus, dengue viruses, chikungunya virus and most recently Zika virus. While the general approach to each has been similar; that is, assessing and evaluating the need for an intervention, followed by evaluating those interventions that may be available, and implementing as necessary, major differences in approaches have occurred. The appropriate mix of interventions resulting in an algorithm to ensure safety depends on local epidemiology, infrastructure and economic considerations. Strategies to ensure the safety of blood components have varied from: stopping collections when geographically feasible and importing blood; relying on donor symptoms by questioning or physical examination to identify risk; travel deferrals or other risk-based deferrals; product quarantine while awaiting donor call-back to clear the components for release; and testing and pathogen inactivation.

Summary/Conclusions: Blood systems worldwide must be ready to adapt to the emergence of new agents or changes in existing agents or changes in their population's epidemiology. Blood systems must be able to respond to the availability of new diagnostic technologies, have access to new methods to inactivate blood components, and respond to rapid shifts in economic conditions and public expectations to accommodate the ever-changing landscape of infectious disease and blood safety.

Practical Approach in Clinical Transfusion/The Spectrum of Clinical Transfusion

2C-06-01

MANAGING BLEEDING EMERGENCIES

S Allard

Consultant Haematologist, Barts Health NHS Trust & NHS Blood and Transplant, London, United Kingdom

Whilst there is much focus on practice recommendations for bleeding in trauma patients, the management of bleeding in other clinical settings (e.g. gastrointestinal, obstetrical or surgical) also needs specific attention. Management of major haemorrhage in any setting requires a multidisciplinary approach with good communication. The coagulopathy of bleeding is related to loss of blood, consumption of coagulation factors, activation of fibrinolysis and haemodilution by resuscitation fluids. Hypothermia, acidosis and hypocalcaemia will further worsen coagulation. It is important to monitor haemostatic changes to guide use of blood components after initial resuscitation until bleeding ceases.

The Clinical Randomization of Antifibrinolytics in Significant Haemorrhage (CRASH-2) study, a randomized controlled trial (RCT) of tranexamic (TA) vs placebo in trauma showed that death in the first 4 weeks was reduced by 9% with TA which was also shown to be safe with no increase of thrombotic events. The benefit was greatest the earlier that TA was given after injury with possibility of harm if given >3 h after injury.

Preliminary data has shown that TA may reduce blood loss in PPH but the quality of evidence is poor. The WOMAN (World Maternal AntifibriNolytic) study, a double blind randomized study of TA vs placebo recruiting ~ 20,000 women is due to report in 2016.

Fresh Frozen Plasma (FFP) has been the component of choice to manage coagulopathy of bleeding, but there is little high quality data to inform optimal replacement of coagulation factors. The recent PROPPR (Pragmatic, Randomized Optimal Platelet and Plasma Ratios) randomized clinical trial in trauma patients with massive blood loss reported that there was no difference in overall survival between early administration of plasma, platelets and red blood cells in a 1:1:1 ratio compared to 1:1:2. However in additional analyses, more patients in the 1:1:1 group were reported to achieve 'anatomic' haemostasis and fewer may have experienced death due to exsanguination by 24 h. The relative contribution of platelets or plasma to the resuscitation outcomes could not be defined in this study.

FFP should be given in the initial resuscitation process in at least a 1:2 unit ratio with red cells. However, in traumatic bleeding, consideration should be given to transfuse plasma: red blood cells initially in a 1:1 ratio. Further transfusion administration should be guided by results of conventional laboratory-based (PT, APTT, fibrinogen) or near-patient tests (e.g. TEG/ROTEM), if part of a clinical trial, to evaluate their utility. Fibrinogen supplementation should be given if fibrinogen levels are <1.5 g/l. In major obstetric haemorrhage, consideration given to the early use of fibrinogen supplementation when fibrinogen levels are <2.0 g/l and there is ongoing bleeding. Platelet transfusion should be given as one adult therapeutic dose (one apheresis pack or four pooled units) when the platelet count falls below 50×10^9/l.

There is a need to conduct high quality research to guide optimum blood and component usage in many patient groups in addition to trauma such as post-partum, GI or vascular bleeding, including studies on cost effectiveness.

2C-06-02

No abstract available.

2C-06-03

INVESTIGATION AND MANAGEMENT OF NON INFECTIOUS ADVERSE TRANSFUSION REACTIONS

G Clarke

Canadian Blood Services, Edmonton, Canada

Appropriate management of non infectious adverse transfusion reactions begins with recognition that a change in clinical status during or following a transfusion may represent an adverse event. Appropriate monitoring of patients during transfusion and explicit training of staff to recognize the signs and symptoms of adverse transfusion reactions is the key to diagnosis and to management. As some reactions may occur in the hours following transfusion, patient education and instruction on reporting relevant symptoms is also important. The typical symptoms that herald the onset of a transfusion related adverse event include: fever, rash, shock and respiratory distress. Hemoglobinuria may also be a presenting feature. Early signs or symptoms may reflect more than one type of reaction.

All transfusionists must be aware of the steps in acute management of a suspected adverse transfusion reaction. For those events that occur while the transfusion is ongoing, stopping the infusion and maintaining intravenous access is the important first step. Rapid evaluation of the patient's vital signs, a bedside check of the unit and patient identification, as well as assessment of the appearance of the blood component, are the next steps. Supportive care based on the patient's signs and symptoms must follow while additional laboratory and clinical investigations are initiated. In most cases of transfusion related adverse events a 'post transfusion' blood sample should be evaluated for the possibility of serological incompatibility. In addition most moderate and severe reactions would be investigated with a blood count, renal and liver function tests and assessment of urine for hemoglobin. Other specific investigations depend on the presenting features and initial serologic findings.

Based on the clinical, laboratory and or imaging studies, most transfusion related adverse events can be classified into one of the categories of acute transfusion reactions. These include acute hemolytic transfusion reactions, febrile non hemolytic, allergic, anaphylactoid, septic, circulatory overload, hypotension, and transfusion related acute lung injury. Transfusion associated graft-versus-host disease and post transfusion purpura can be considered in some circumstances and delayed hemolytic transfusion reactions may be seen in the days to week following a transfusion.

This diagnostic classification is important in optimizing acute management and may also contribute to decisions about component selection for subsequent transfusion.

Reporting of adverse transfusion events is also an important part of management. Reporting to the hospital blood bank assists with diagnosis and decisions regarding future blood component therapy. The hospital transfusion committee may monitor transfusion reaction rates and trends as a quality indicator that can be used to change practices. The blood supplier must be notified of all reactions which may be attributable to a particular donor or donor unit, especially if recall or quarantine of associated blood components may be necessary. Regional or national hemovigilance programs may require notification and can contribute to changes in standard practices to address common or serious adverse transfusion events and in early recognition of uncommon complications.

Practical Approach to Blood Components and Supply Management/The Spectrum of Blood Components

2D-07-01

AUTOMATION IN BLOOD PROCESSING

M El Ekiaby

Shabrawishi Hospital, Giza, Egypt

Background: In the past, transfusion therapy was largely dependent on the use of whole blood. While whole blood may still be used in certain limited circumstances, the main thrust of modern transfusion therapy is to use the specific component that is clinically indicated. Components are those therapeutic constituents of blood that can be prepared by centrifugation, filtration and freezing using conventional blood bank methodologies. The blood components prepared by blood transfusion centers include different forms of packed red blood cells, plasma and platelet concentrate.

Requirements for preparation of blood components: Choice of the blood bag configuration should match the targeted components to be produced. In addition to blood bags, balances, cooling centrifuges, plasma extractors, electric sealers and sterile docking machines are required.

Automation in blood processing: In order to reduce the laborious steps and standardize the quality of blood components several technologies have been developed to automate blood component preparation. The advantages of these technologies include reduction of a number of equipment, such as plasma extractors, electric sealers. This leads to reduction of workspace and consequently may increase production efficiency. It also reduces the work load for the operators since these automates usually include several steps like breaking of clamps, extraction of the blood components as well as sealing of the pilot lines between the blood bags. More over the automation will reduce the operator inter variability leading to production of more standardized blood components.

Automates for preparation of components from whole blood: Two types of automates are commercially available. The first type will depend on automation of separation of blood components from whole blood units after centrifugation in classical blood bank cooling centrifuges. They can usually process both top/top or top/bottom blood bags for separation of RBCs, platelet rich plasma (PRP), fresh plasma (FP) and buffy coat (BC). The other type of automate combines both centrifugation and separation of blood components from whole blood units, using a special medical device to which four or six whole blood units will be docked and can consequently produce four or six units of RBCs, PCs and FP.

Preparing your lab for automated blood processing: In order to efficiently transform your blood component lab to automated blood processing certain measures should be considered. You may need to know the throughput of such automates and the number of whole blood units prepared each day to decide on the number of automates needed. You will also need to have the required space as well as enough well trained staff that can use these automates. Maintenance programs are of prime importance to insure that you will not face frequent failures that may lead to reduction of your production capacity. In case of automate that will need related disposable system you need to make sure that the supply of such devices will be continuous to avoid work interruption.

Conclusion: Automation of blood component preparation from whole blood offers several advantages that include reduction of standard equipment, space, workload to lab staff as well as more standardized blood components.

2D-07-02

APPROACHES TO ASSESSING AND MINIMISING BLOOD WASTAGE IN THE HOSPITAL AND BLOOD SUPPLY CHAIN

M Yates

Cranfield University, Bedford, United Kingdom

Despite the scale of blood usage worldwide, blood remains a scarce and precious resource. As with any perishable product careful management of inventories to minimise wastage is crucial. However, due to the nature of the supply of blood, wastage is not only an economic issue but has a normative societal impact. As every unit wasted, squanders the time and effort of the human donor. Blood inventory management across the supply chain is therefore a trade-off; ensuring 100% availability of all blood products at all times whilst minimising wastage.

The most prevalent and important cause of blood wastage from an inventory management perspective, is due to time expiry of units at the end of their shelf life. Visibility of wastage levels is crucial, without this it is impossible to control and minimise them. Therefore, wastage must be measured and not only at an overall measure level, visibility of wastage by blood product and blood group is essential. Useful Key Performance Indicators (KPIs) for this purpose are Waste as a Percentage of Issues (WAPI) and Waste as a percentage of Average Stock Level (WAASL).

Hospitals are at the front line of blood use and are the location where much blood is wasted. Inventory management practises in hospital transfusion laboratories are critical. Much of the extant literature in this area posits that good management of hospital blood inventories is due to sophisticated inventory models and algorithms. However, recent research has found that in the UK at least this is not the case and that good management practises are much more important. The drivers for low wastage and good inventory management practise relate to: simple inventory policies and transparent inventories coupled with a strict focus on freshness and remaining shelf life, human resources and internal collaboration within the hospital.

Blood supply chain management is much more than managing wastage in hospitals. Proper management of the supply chain as a whole can lead to significant reductions in wastage of blood. Recent research has found that by taking a size based approach to managing blood supply; significant wastage reductions can be achieved. For example, demand variability of the most prevalent blood groups is much lower than for smaller groups; in fact it is relatively predictable. These groups can and should be manged according to lean principles with less inventory held. This not only leads to reductions in wastage, but to a freeing up of management time to focus on smaller more variable groups. In addition, it is known that small hospitals waste more than their larger counterparts, in part due to economies of scale. The sharing of stock between hospitals, where small hospitals move units which are close to their expiry dates to larger hospitals which have a greater chance of using the unit, has been found to be an effective means of reducing wastage across the supply chain.

2D-07-03

THE MEASUREMENT OF BLOOD COMPONENT QUALITY

D Devine

Canadian Blood Services, Vancouver, British Columbia, Canada

The assurance of blood component quality is achieved by testing a fraction of the components produced in order to establish whether they meet predetermined values. Quality control tests are an example of well-established tests that provide information on the product process control. Other kinds of tests may be used to assess aspects of blood component quality; these are often less well-developed than routine quality control tests. This presentation will explore what can be learned about blood component quality from these non-traditional measurements.

Red cell concentrates: Quality assessments of red cell concentrates focus most simply on the amount of hemoglobin in the unit, its hematocrit and the level of hemolysis at the end of the storage period. Concern over the potential detrimental effects of long storage periods spurred studies of red cell concentrates that used a broader panel of tests that better evaluated the metabolic capacity of the cells, their membrane integrity and oxygen carrying capacity. Appreciation of the role of rapid clearance of transfused RBCs with abnormal morphology has led to the use of tests that attempt to predict splenic clearance of stored RBCs. Measurements of the cells themselves comprise the majority of quality tests for RBCs, but recent appreciation of the role of microvesicles that accumulate in stored blood products has led to their quantitation being a more common quality metric.

Platelet concentrates: The assessment of effective process control in platelet concentrate production does not directly measure platelet efficacy; the standard tests are count, pH and sterility, none of which indicate that the platelets are functional. Thus, additional measures that focus on platelet function can be made to assess quality. These range from tests that assess the activation state of platelets to measures of platelet responsiveness to physiological stimuli. Most of these tests are performed without the presence of shear or endothelial cells and thus are incomplete assessments of platelet efficacy.

Plasma: Quality assessment of plasma does not generally parallel its clinical uses. The choice of measures, while most commonly a direct test of coagulation factor function, is directed at labile coagulation factors such as factor VIII which can be present in very low levels and still maintain normal hemostasis. The application of measures that more fully assess coagulation factor function is in its infancy and most assessments simply report the activity levels of a list of coagulation factors.

For most of the measurements described above, there is little evidence that they correlate strongly with the clinical efficacy of a transfusion. This has led to many of the tests being performed in panels in order to provide multiple avenues of assessment of a single sample. As more experience is gained with these measures and others in development, some will be abandoned and others will become used routinely.

Practical Approach to Cell Therapy/The Spectrum of Cell Therapy

2D-08-01

SUPPORTING BLOOD NEEDS IN PATIENTS UNDERGOING HEMATOPOIETIC CELL TRANSPLANTATION

JP Miller

National Marrow Donor Program, Minneapolis, Minnesota, United States of America

Over the past several decades, there has been an ever-increasing number of hematopoietic cell transplants (HCT) performed for patients with malignant and non-malignant blood disorders, with 20,000 unrelated allogeneic transplants performed worldwide in 2015. In support of patients needing a HCT from an unrelated donor, there are over 27 million HLA typed donors on international registries and over 700,000 umbilical cord blood units are available worldwide.

Transplantation is a clinical journey for both the donor and the patient. Blood centers and transfusion services may be involved in many of the steps along the way. They may help recruit donors to registries and evaluate eligibility and suitability for donors who match a specific patient. Transfusion services may also collect PBSC or autologous blood for marrow donors.

HCT recipients have unique transfusion needs both pre- and post-transplant that need to be carefully managed by transplant teams in partnership with the transfusion service. Patients may present to hematology clinic severely anemic, thrombocytopenic and/or neutropenic related to their underlying blood disorder. They will need transfusions of blood products in order to get them to transplant.

Following transplant, multiple RBC and platelet transfusions may be required especially until engraftment occurs. Alloimmunization to RBC or platelet antigens may develop making RBC or platelet matching challenging and platelet refractoriness may develop. Rarely, hemolysis related to donor or 'passenger' lymphocyte syndrome may occur.

Often there is an ABO mismatch between the donor and the recipient. While the number of RBC in the HCT products may be reduced by RBC depletion methods, considerable incompatible RBC and plasma may be infused and transfusion reactions, including hemolysis, may occur. The patient will ultimately have the donor's blood type, but immediately post transplant, blood will need to be carefully selected for major and minor ABO compatibility of donor and recipient RBC.

Both myeloablative and nonmyeloablative preparative regimens are extremely immunosuppressive, so a CMV negative patient should receive CMV-safe blood to prevent primary CMV infection. Irradiated blood products should be provided to minimize the risk of transfusion-associated graft-versus-host disease, which is more severe than transplant-associated GVHD and is almost universally fatal.

The use of growth factors such as G-CSF and GM-CSF seem to be more effective in the autologous transplant setting, and are not routinely used prophylactically in allogenic HCT recipients, although they may be used in patients with delayed engraftment or marrow suppression secondary to infection or drug therapy. The use of erythropoietin may be effective in patients with prolonged anemia post-transplant.

In summary, patients undergoing HCT require considerable blood component support before and after transplantation. Collaboration between the transfusion and clinical transplant services is essential to effective manage their transfusion needs. Exciting advances cellular therapies for patients with blood disorders such as virus-specific and leukemic-antigen specific cytotoxic T lymphocytes have been developed to treat post-transplant infectious disease and relapse. Such advances will improve patient outcomes for many years to come.

2D-08-02

HLA TYPING IN DIVERSE POPULATIONS

R Holdsworth

Red Cross Blood Service, West Melbourne, Victoria, Australia

The Human Leucocyte Antigens (HLA) genes are part of the Major Histocompatibility Complex (MHC) and are the most polymorphic gene complex in the human genome. The HLA genes are clustered into six groups (loci) with many forms of the genes (alleles) at each locus. HLA genes are co dominantly inherited from each parent in 'linked strings' or haplotypes and the hyperpolymorphism of the HLA alleles generates thousands of HLA (haplo) types. Currently over 18,000 HLA alleles have been described with 2000+ new alleles identified each year.

The HLA molecules play a critical role in our immune defence and the extreme diversity ensures a robust immune response. Over many generations, HLA types are retained through immune advantage which may be the basis of the high frequency of some HLA alleles in specific regions.

Further restriction of HLA types can be found in populations due to language, religious or geographical influences. Pre the 20th century, mass population relocation was uncommon however travel for trade and exploration has contributed to movement of HLA genes over many centuries.

HLA typing with the extreme polymorphism in the HLA genes is very complex. HLA typing requires DNA based testing methods to identify the hundreds of nucleotide differences throughout the introns and exons of the HLA genes for each loci/allele. The level of resolution of the HLA result depends on the type of assay used. Assay design and testing can be time-consuming and manufacturers may use 'SMART' software with population reference data to estimate the most likely HLA alleles in the allele assignment. Determination of common HLA alleles from large stem cell registry data sets has been used, however population specific data may not transfer to the HLA types of other populations. Other methods have utilised haplotype data for HLA typing assignment but the identification of haplotypes by inference rather than direct sequencing of alleles may generate ambiguous results.

Next Generation Sequencing (NGS) assays are designed to detect the entire nucleotide sequence of the HLA gene and accurately assign HLA alleles without ambiguity. However NGS can be time consuming and low throughput with large amounts of data generated, much of which may not be clinically significant, e.g. intron sequence variation. NGS will identify many novel alleles as the HLA allele reference data base, IMGT/HLA, does not yet have HLA sequence data for all HLA alleles.

Diverse populations will have a wide range of HLA types and this can lead to challenges in assigning HLA alleles. Calculating HLA alleles in diverse populations may not be accurate when using population data from other populations as a basis.

2D-08-03

No abstract available.

Parallel Sessions Scientific Programme Transfusion Medicine I

3A-S01-01

EVIDENCE-BASED PATIENT BOOD MANAGEMENT GUIDELINES FOR NEONATAL AND PAEDIATRIC PATIENTS

G Crighton

Haematology Society of Australia and New Zealand, Sydney, New South Wales, Australia

Introduction/Aims: The National Blood Authority (NBA), Australia has funded and managed the development of evidence-based Patient Blood Management (PBM) Guidelines, comprising six modules: Critical Bleeding/Massive Transfusion (2011), Perioperative (2012), Medical (2012), Critical Care (2012), Obstetric/Maternity (2015) and Neonatal/Paediatrics (2016). A national strategy for implementation has been developed by the NBA with a series of tools and activities to support health departments and health providers in their uptake of these guidelines.

Module 6 – Neonatal and Paediatrics aimed to systematically review and critically appraise the literature with respect to PBM in neonatal and paediatric patients.

Methods: The module was developed by collaboration between professional partners in systematic review and guideline authorship and a Clinical/Consumer Reference Group (CRG) comprising experts from clinical colleges and societies; in the areas of fetal-maternal medicine, neonatology, paediatric haematology, paediatric anaesthesia, intensive care, cardiac surgery, oncology, haemoglobinopathies, nursing and PBM, in addition to consumer and indigenous representatives.

Systematic review questions were formulated for the target population 'neonatal and paediatric patients' and included: the effect of red blood cell (RBC) and other blood component transfusion on patient outcomes, the effect of non-transfusion measures to increase haemoglobin on patient outcomes and the effect of strategies to minimize blood loss and/or reduce RBC transfusion.

A comprehensive search of electronic databases and clinical trials registries was conducted for the years 1995 to 2014 (inclusive).

Three types of guidance were developed; Recommendations were developed where a question had been critically appraised and evidence was found to guide practice, and Practice Points were developed by CRG consensus where insufficient evidence was found. Expert Opinion Points were formulated based on CRG consensus for other areas that were considered important in neonatal and paediatric PBM but outside the scope of the systematic review.
Module 6 has been peer reviewed, and independently assessed according to the Appraisal of Guidelines for Research & Evaluation II. The Module's recommendations were approved by the National Health and Medical Research Council on 21 March 2016.
Results: Twelve Recommendations for or against the use of a number of interventions in neonatal and, or, paediatric patients were developed. These included RBC transfusion in critically ill patients and sickle cell disease (SCD), erythropoiesis-stimulating agents in preterm infants, hydroxyurea in SCD, fresh frozen plasma based primes and recombinant factor VIIa in cardiac surgery, intravenous immunoglobulin for rhesus disease of the newborn, prevention of hypothermia and anti-fibrinolytics in surgical patients.
In addition 40 Practice Points and 37 Expert Opinion Points provide further guidance.
Conclusions: These guidelines will aid decisions on whether to transfuse a neonate or child in the context of specific patient circumstances, and the full range of other available treatments, balancing the evidence for efficacy and improved clinical outcome against the potential risk.
Many areas were identified with insufficient evidence to make recommendations. Furthermore, despite the potential for long-term consequences of neonatal and paediatric interventions, most studies only addressed short-term outcomes, which were often insufficient to determine overall risks and benefits. The evidence gaps identified provide a focus for future research.

3A-S01-02

PATIENT BLOOD MANAGEMENT IN SURGERY – RESULTS OF A UK NATIONAL COMPARATIVE AUDIT IN 2015

S Allard[1], K Pendry[1], T Richards[2], J Grant-Casey[3], D Highton[4], D Lowe[5] and On behalf of the National Comparative Audit project Group

[1]NHS Blood & Transplant, London, United Kingdom [2]University College London Division of Surgery, London, United Kingdom [3]NHS Blood and Transplant, Oxford, United Kingdom [4]Neurocritical Care, National Hospital for Neurology and Surgery, London, United Kingdom [5]Astraglobe Limited, Congleton, United Kingdom

Background: Patient Blood Management (PBM) is a program of quality improvement in transfusion practice with a patient-centred approach to anaemia management, minimisation of blood loss and transfusion only when appropriate.
Aims: We undertook a large national comparative audit in the UK to assess application of PBM measures in a range of surgical procedures associated with a high transfusion usage.
Methods: Hospitals in the UK were asked to collect data on consecutive patients undergoing surgery over a 3 month period in 2015 who received transfusion. The procedures include orthopaedic, cardiac, colorectal, urological, gynaecological and vascular surgery. Patients with a fractured neck of femur were also included. Based on current practice recommendations and clinical guidelines a series of PBM algorithms were developed and designed as audit standards.
Results: Data was received from 190 sites for 3,897 patients over a 3 month period. All patients received at least one red cell transfusion during the surgical episode (~8,500 red cell units transfused). Practice was assessed against 11 PBM standards, in the pre-operative (5) operative (2) and post-operative care settings (4).
Pre-operative anaemia was present in half of patients but often identified late with only 53% patients having a haemoglobin level tested at least 14 days pre-operatively despite an average of 42 days between listing for elective surgery and operation. Overall 46% of patients had attempts at pre-operative anaemia management (PBM1). Transfusion in the pre-operative setting was rarely performed appropriately against given standards (PBM 2, 12%; PBM 3, 2%; PBM 4, 28%).
At operation most patients received one PBM measure prior to transfusion (PBM 6, 83%) but rarely all those PBM measures recommended (PBM 7, 16%). In particular there was variation in the use of Tranexamic acid. Post-operative transfusion was not often performed within recommendations (PBM 8, 24%) with a single unit policy adopted only in a third of cases (PBM 9, 38%). Post-operatively, in those who received a transfusion, most patients had received one of the proposed PBM measures (PBM 10, 85%) but only 8% of cases had received all recommended PBM measures (PBM 11).
Conclusions: This large audit highlights the need to develop a standard of practice in surgical PBM to promote appropriate use of transfusion in surgery. Certain aspects of PBM are low cost and can be readily implemented such as the use of Tranexamic acid. Mechanisms to identify, investigate and manage preoperative anaemia and a single unit transfusion policy need to be developed and implemented to reduce unnecessary transfusion in surgery. Improvement in PBM practice to help ensure appropriate use of transfusion and alternatives, where available, will benefit patients and reduce healthcare costs for hospitals.

3A-S01-03

RIDING THE FERROUS WHEEL: QUALITY PREOPERATIVE ANAEMIA ASSESSMENT AND MANAGEMENT

AL Harper, L Bielby, C Akers, B Glazebrook, P Beard and C Hogan

Australian Red Cross Blood Service, West Melbourne, Victoria, Australia

Background: Undiagnosed anaemia is common in the surgical setting and is associated with increased perioperative morbidity and mortality and longer hospital stays (*Module 2 – Perioperative*). The Australian National Blood Authority's PBM guidelines Module 2 outlines the components of quality preoperative anaemia assessment and management, and provides a template for this. Blood Matters performed an audit of the assessment and management of reversible anaemia prior to surgery, and impacts for patients undergoing elective surgical procedures.
Aims: This audit aimed to: determine processes in place within health services to assess anaemia in the elective preoperative patient explore the quality of preoperative assessment
Methods: The audit was performed in two parts. Part A audited processes for assessing and managing preoperative anaemia. Part B assessed practice through retrospective audit of elective surgery patients.
Results: Of 56 participating health services, 18 (32%) stated that they had a preoperative haemoglobin optimisation pathway; 13 (72%) reported that this included a preoperative haemoglobin assessment and optimisation template.
Data were received for 1,142 patient episodes, of these 1,057 (93%) were reported to have been assessed for anaemia preoperatively; however, in fact very few had a quality assessment as defined by PBM Module 2 (84, 8%). Only 335 (32%) patients were assessed >28 days prior to surgery, and 268 (25%) of patients had a documented ferritin level. Additional testing related to chronic disease was undertaken in only 145 (14%) patients.
Health services greatly under-reported the number of anaemic patients (117, 11%) when compared with the number defined as anaemic by PBM Module 2 (212, 20%). This represents a missed opportunity to assess and treat patients who are at risk of anaemia.
Treatment for anaemia was instigated in 56 of 212 (26%) patients. Despite evidence that 31 patients were iron deficient, and a further 93 possibly iron deficient, only 44 patients received iron therapy prior to their surgery. Eighteen received a red cell transfusion, and in nine patients this was the only treatment. The closer the surgery was to assessment date, the more likely the patient was to receive a red cell transfusion, and less likely to receive iron therapy.
Where treatment was instigated only 37 (66%) patients were re-assessed for response prior to surgery, with only five patients (9%) showing resolution of anaemia.
Average length of stay ranged from 8 days for non-anaemic, non-transfused patients; to 17 days for transfused anaemic patients who had no treatment for their anaemia preoperatively.
Conclusions: Although health services reported 93% (n = 1,057) of patients were screened preoperatively for anaemia, very few patients had a quality assessment as outlined by PBM Module 2, by at least ensuring haemoglobin and ferritin results are available at time of assessment, and assessed in a timeframe allowing for intervention, if appropriate. This is a concern considering 20% (n = 212) of audit patients presented for surgery with anaemia as defined by the PBM Module 2. There is more work to be undertaken to ensure elective surgery patients proceed to surgery with optimised red cell mass.

3A-S01-04

PATIENT BLOOD MANAGEMENT INITIATIVES ON A GLOBAL LEVEL THE RESULTS OF A SURVEY ON PATIENT BLOOD MANAGEMENT (PBM) BY THE ISBT WORKING PARTY ON CLINICAL TRANSFUSION

C So-Osman[1], DE van der Wal[2] and S Allard[3]

[1]Sanquin Blood Bank, Leiden, The Netherlands [2]ISBT Central Office, Amsterdam, The Netherlands [3]Barts Health NHS Trust and NHS Blood and Transplant, London, United Kingdom

Background and objectives: Patient Blood Management (PBM) is an evidence-based, multidisciplinary approach aimed at optimising the care of patients who might need transfusion. It puts the patient at the heart of decisions made around blood transfusion, promoting appropriate use of blood and blood components and timely use of

alternatives where available. The ISBT Working Party on Clinical Transfusion aimed to assess the level of PBM activities within hospitals around the world.
Methods: In 2014, a survey was sent to all ISBT members (~n = 1,600), who were asked to provide general data regarding Patient Blood Management activities and implementation of protocols within their hospitals. If the ISBT member was not involved in PBM activities or could not provide hospital information the survey was ended. Hospitals were categorized in university hospitals, large, general, medium-sized and small rural hospitals. Its population type and yearly blood use were collected (categorical), and it was asked whether PBM protocols were implemented. Data were collected electronically by a personalized Survey tool (Survey Monkey), which was provided by the ISBT Central Office.
Results: A total of 277 ISBT members responded (17.3% of all ISBT members), of which the majority 211 (76.17%) were involved in PBM. These 277 members finalized the survey. The majority of responders categorised themselves as 'medical transfusion specialists' in either hospital (41%) or blood centre (19.5%)' and were from six different WHO regions of which the majority (n = 122; 44.04%) were from Europe and the Western Pacific (including Australia/New Zealand). Most responders worked in either university (43.5%) or large general hospitals (20.5%). Most hospitals had trauma care, ICU and haemato/oncology wards and half had organ transplant patients. 89.83% of the hospitals used transfusion guidelines of which 58.93% were national and 39.88% local hospital based guidelines respectively. Protocols used included transfusion threshold for RBCs, platelets and plasma (87.42%), major haemorrhage (76.73%), single unit transfusion policy (40.88%) (mostly university and large general hospitals) and managing coagulo-pathy (55.97%). 68.64% of hospitals used an outpatient pre-operative screening policy. 47.5% used a protocol to diagnose pre-operative anaemia. 44.35% used IV iron to correct for anaemia before surgery and 21.49% used erythropoietin (mostly in university and large general hospitals). 62.73% utilized autologous blood transfusions, of which 48.46% predonation and 70.68% used intra – or post-operative cell salvage. Tranexamic acid was used in the perioperative setting in 62.5%.
Summary/Conclusions: The results of this survey provide an overview of PBM initiatives in many countries. The majority of responses were from large and university hospitals with many indicating involvement in PBM activities. Limitations of the study could be the presence of a selection bias, due to the low response rate. One of the strengths of this study is the global character of the survey, although 44% of all responses came from high-income countries.

3A-S01-05
ELEARNING TO IMPROVE PATIENT BLOOD MANAGEMENT AND CLINICAL TRANSFUSION PRACTICE: DOES IT MAKE A DIFFERENCE?

DM Peterson[1], T Verrall[1], A Thomson[1], A Catherwood[1], L English[1], T Clark[1], B Cook[1], L Lower[1], S Ireland[2], R Allden[2] and S Russell[3]

[1]*BloodSafe eLearning Australia, North Adelaide, South Australia, Australia*
[2]*Department of Health South Australia, Adelaide, South Australia, Australia*
[3]*National Blood Authority, Canberra, Australian Capital Territory, Australia*

Background: BloodSafe eLearning Australia (BEA) (www.bloodsafelearning.org.au) is an education program that commenced in 2007 for Australian healthcare professionals. There are currently 16 courses available that are aligned with national priorities in the blood sector. These include:
Transfusion practice courses – Clinical Transfusion Practice, Collecting Blood Specimens and Transporting Blood
Patient blood management (PBM) courses – Principles of Patient Blood Management, Critical Bleeding, Perioperative, Critical Care, Postpartum Haemorrhage, Iron Deficiency Anaemia and Medical Specialties: cancer, cardiac, chronic kidney disease, chronic transfusion and gastrointestinal
Courses are interactive and include case studies, videos, and best-practice tips. Successful completion of a multiple-choice assessment is required for learners to gain a certificate.
Funding is provided by the National Blood Authority on behalf of all Australian Governments and courses are freely available.
Aim: To evaluate whether the BEA program was meeting its objectives and whether individual courses provide knowledge of patient blood management and safe clinical transfusion practice that help to improve patient outcomes.
Method: Evaluation of the program has been undertaken using stakeholder workshops, interviews and meetings for quality assurance and improvement and to inform decision making regarding future directions. Evaluation of individual courses is provided by users after completion of a course to identify improvements and changes they intend to make to their work practices.
Results: Analysis of user completion data shows that the BloodSafe eLearning program has more than:

- 360,000 registered learners
- 500,000 course completions
- 15,000 course completions per month on average.

Analysis of stakeholder workshops and interviews shows that the program: provides credible, consistent education across Australia; is cost effective and reduces duplication; is 'best-practice' elearning that is readily accessible; allows institutions to focus on practical aspects of transfusion education; results in change to clinical practice; and supports the broader implementation of a blood management strategy in Australia.
User evaluation shows that the courses have a positive impact on knowledge and practice improvement with 89% of respondents stating that they had gained additional knowledge of transfusion practice, processes and/or policy.
More than 80% of respondents state that they will make changes to their clinical practice to improve patient outcomes and safety. Changes that have been identified include: blood ordering practices in stable, non-bleeding patients; assessment and treatment of anaemia prior to elective surgery; modification of transfusion assessment criteria to include more than just haemoglobin level; and implementation of transfusion protocols for massive transfusion and PPH.
Recommendations and areas for further development include: targeting of students to provide foundational knowledge; promotion and marketing to specific user target groups; better support for the use of a blended-learning approach with 'face-to-face' resources that can be used by hospital educators; and development of courses related to other blood components including fresh frozen plasma and coagulation factor replacements.
Conclusion: The BloodSafe eLearning Australia program has provided learning and development to a large number of health professionals. Evaluation demonstrates that these courses provide users with a consistent and reliable knowledge base and helps to identify opportunities to improve practice and patient outcomes.

3A-S01-06
ELECTRICALLY CARDIO-METERING INTRAOPERATIVE AUTOLOGOUS DONATION

J Narula, U Kiran, P Kapoor, M Choudhury, P Rajashekar and UK Chowdhary
All India Institute of Medical Sciences, Delhi, India

Background: Intra-operative autologous donation (IAD) besides its proven role in blood conservation, decreases the circulatory overload being handled by the ventricles in volume loaded patients with pulmonary hypertension (PH) secondary to left heart disease (LHD). Bioimpedance based electrical cardiometry (EC) has recently shown to provide accurate measurements of cardiac output (CO), thoracic fluid content (TFC) and volume shifts, estimation of which is of prime importance in patients with critical pre-operative state.
Aim: To evaluate the effect of IAD induced volume shifts on hemodynamics and lung gaseous exchange using EC in patients with pulmonary artery hypertension secondary to left heart disease.
Methods: Prospective randomized controlled trial conducted in 50 patients scheduled to undergo heart valve replacement. IAD performed in the test group was simultaneously replaced with 1:1 colloid. TFC, hemodynamic and EC data were recorded at T1-baseline (post induction) and T2-20 min post-IAD.
Results: Withdrawal of 15% of blood volume in the IAD group caused a significant reduction in TFC −10.1% (−15.0 to −6.1), right atrial pressure −23% (−26.6 to −17.6), mean arterial pressure −12.6% (−22.2 to −3.8), airway pressures; [peak −6.2% (−11.7 to −2.8) and mean −15.4% (−25.0 to −8.3)] and oxygenation index (OI) −10.34%(−16.4 to −4.8). Linear regression analysis showed good correlation between the amount of autologous blood removed and percentage change in TFC, RAP, peak and mean airway pressures and OI.
Conclusions: Our study shows that therapeutic benefits derived from IAD include decongestion of volume loaded patients, decrease in intrathoracic fluid and improvement in gas exchange. EC tracks beat-to-beat fluid and hemodynamic fluctuations during IAD and can help to execute an early patient specific goal directed therapy allowing for its safe implementation in high risk patients with PH-LHD.

Donor Recruitment

3A-S02-01

HOW CAN WE ENCOURAGE OUR VOLUNTARY NON-REMUNERATED DONORS TO DONATE MORE FREQUENTLY?

TE Davison[1], B Masser[2] and C Chapman[2]

[1]Australian Red Cross Blood Service, Melbourne, Victoria, Australia [2]The University of Queensland, Brisbane, Queensland, Australia

Background: Retained donors are generally considered to be safer and more cost effective than new donors, and yet increases in demand and natural attrition of the donor panel are typically countered through increased recruitment efforts. An alternative approach would be to increase the supply of blood products from existing voluntary non-remunerated donors by persuading them to donate more frequently. Analyses suggest that this heightened frequency would have the additional benefit of improved long-term retention of those donors. Many donors are medically eligible to donate more frequently than they currently do. For example, in Australia in 2015, the median whole blood donation rate across all donors was 25%, the O Neg rate 50%, and the plasma rate 12%, of the rates medically allowed. However, to date, how to increase the frequency of donation by our voluntary non-remunerated donors is relatively unexplored in donor research.

Aims: The aim of this review is to draw on the existing empirical literature and to draw insights from allied literatures to identify ways in which blood collection agencies may be able to actively encourage existing donors of blood products to donate more frequently.

Method: A review of the existing blood donation literature focused on frequency of donation was undertaken. In addition, suggestions for future directions for increasing donation frequency were gained from the allied literatures on charitable giving and behavior change.

Results: While the existing blood donation literature has identified personal (e.g., age, personality traits) and structural features (e.g., time constraints) associated with donation frequency, little research in this area has identified ways that blood collection agencies can interact with their donors to increase their frequency of donation, aside from simple requests to donate more, or different products. Suggestions from the broader literature on charitable giving and behavior change suggests that a variety of techniques may be useful in increasing donation frequency. Possibilities around targeted approaches designed to incrementally build frequency and engender longer term commitment will be presented. Further, additional approaches based on re-structuring donor loyalty schemes and strategic use of descriptive and injunctive norms will also be discussed.

Conclusion: Although increasing the frequency of donation by voluntary non-remunerated donors would provide benefit both in terms of immediate supply and long term retention, relatively little is known about how to effectively achieve this. This review paper draws on the extant blood donor research literature and allied literatures to identify a range of possible techniques that blood collection agencies can use to optimize the frequency of donations obtained.

3A-S02-02

THE IMPACT OF SOCIOECONOMIC FACTORS ON DONATION FREQUENCY

SU Christensen[1], MS Petersen[2], OB Pedersen[3], KS Burgdorf[4], E Sørensen[4], KR Nielsen[5], MT Bruun[6], K Rostgaard[7], H Hjalgrim[7], H Ullum[4] and C Erikstrup[2]

[1]Aarhus University Hospital, Aarhus N, Denmark [2]Department of Clinical Immunology, Aarhus University Hospital, Aarhus, Denmark [3]Department of Clinical Immunology, Naestved Hospital, Naestved, Denmark [4]Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitale, Copenhagen, Denmark [5]Department of Clinical Immunology, Aalborg University Hospital, Aalborg, Denmark [6]Department of Clinical Immunology, Odense University Hospital, Odense, Denmark [7]Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark

Background: Blood transfusions are essential in modern healthcare and the blood supply relies on active donors. Though the use of red cells is declining, there is an increasing demand for plasma-derived products. To meet this shift in demand, more plasma donors are needed.

Aim: To explore how socioeconomic factors affect donation frequency to optimize recruitment of donors who are willing to donate frequently.

Methods: Danish blood donors who donated whole blood at least once in 2008 were selected from the *Scandinavian Donations and Transfusions Database* (SCANDAT). The donors were divided into three groups according to donation frequency during the following 3 years (Low: 1–3 donations, Intermediate: 4–8 donations, High: more than eight donations). Socioeconomic variables were assessed through Danish registers and predictors for donation frequency were explored by multinomial logistic regression, stratified for sex and adjusted for age and blood type. Results were presented as Odds Ratios (ORs), i.e. ratios of odds of becoming low-/high-frequency donor respectively compared with intermediate-frequency donor.

Results: Generally, men donated more often than women. Income (deciles), education level, area of occupation, and employment status were associated with donation frequency.

Among men, low-frequency donors were rarer if personal income was above the 3rd decile, compared with income in the lowest decile. However, men who had completed education at bachelor or master level were rarely high-frequency donors (Bachelor: OR = 0.87, Confidence Interval (CI) 95%: 0.81–0.94; Master: OR = 0.84, CI 95%: 0.77–0.91), compared with men who completed elementary school as their highest level of education.

Men who were not employed in the public sector were rarely low-frequency donors. Furthermore, men employed within Trade and Transportation and Business Service were more often high-frequency donors (OR = 1.16, CI 95%: 1.09–1.22 and OR = 1.29, CI 95%: 1.13–1.47, respectively). Men who had retired were more often low frequency donors (OR = 1.37, CI 95%: 1.19–1.57), compared to basic level wage earners.

The same tendencies were found among women. Women with personal incomes in 7th to 9th decile were more often high-frequency donors, than women with personal incomes in the lowest decile. However, high education was associated with a lower likelihood of being high-frequency donors (Bachelor: OR = 0.74, CI 95%: 0.68–0.81; Master: OR = 0.80, CI 95%: 0.70–0.91). Employment in Trade & Transportation was also predictive for becoming high-frequency donor among women (OR = 1.13, CI 95%: 1.05–1.21).

		Women		Men	
		Low vs. intermediate	High vs. intermediate	Low vs. intermediate	High vs. intermediate
	Age	0.976*** [0.974,0.978]	1.031*** [1.028,1.034]	0.997*** [0.995,0.999]	1.018*** [1.016,1.020]
Income decile Base income is 1st decile	2. income	0.972 [0.905,1.044]	0.988 [0.866,1.128]	0.960 [0.870,1.060]	1.028 [0.916,1.154]
	3. income	1.022 [0.944,1.107]	1.001 [0.871,1.150]	0.945 [0.847,1.054]	1.093 [0.969,1.234]
	4. income	0.981 [0.902,1.068]	1.054 [0.913,1.216]	0.837** [0.749,0.935]	1.130* [1.002,1.274]
	5. income	0.967 [0.886,1.055]	1.075 [0.928,1.245]	0.772*** [0.692,0.862]	1.036 [0.920,1.167]
	6. income	0.938 [0.856,1.027]	1.134 [0.977,1.316]	0.778*** [0.697,0.869]	1.097 [0.975,1.234]
	7. income	0.901* [0.820,0.990]	1.218* [1.048,1.417]	0.748*** [0.671,0.835]	1.032 [0.918,1.161]
	8. income	0.922 [0.836,1.016]	1.321*** [1.133,1.541]	0.765*** [0.686,0.853]	1.067 [0.949,1.200]
	9. income	0.924 [0.834,1.025]	1.250** [1.065,1.468]	0.779*** [0.699,0.868]	0.982 [0.873,1.104]
	10. income	1.070 [0.955,1.199]	1.171 [0.981,1.397]	0.859** [0.771,0.957]	0.907 [0.806,1.020]
Highest level of completed education Base is elementary school	High school	0.887*** [0.838,0.939]	0.923 [0.838,1.017]	0.906** [0.844,0.972]	0.920* [0.854,0.992]
	Vocational education	0.949* [0.902,0.998]	0.961 [0.898,1.029]	0.889*** [0.843,0.937]	0.997 [0.947,1.048]
	Short education	0.928 [0.859,1.003]	1.014 [0.915,1.124]	0.882** [0.817,0.953]	0.972 [0.904,1.046]
	Bachelor level education	1.004 [0.947,1.065]	0.741*** [0.679,0.809]	0.913* [0.852,0.979]	0.872*** [0.814,0.935]
	Master level education	1.134** [1.043,1.234]	0.796*** [0.699,0.906]	0.925 [0.851,1.006]	0.839*** [0.771,0.912]
	PhD	1.136 [0.843,1.529]	1.185 [0.785,1.787]	0.865 [0.698,1.073]	0.764* [0.611,0.954]
Area of occupation Base is public sector	Farming, forestry and fishery	0.754* [0.604,0.942]	0.914 [0.671,1.243]	0.719*** [0.617,0.837]	1.105 [0.963,1.268]
	Manufacturing	0.898*** [0.849,0.951]	0.914* [0.842,0.992]	0.924** [0.873,0.978]	0.944* [0.892,0.998]
	Construction	0.814** [0.699,0.947]	0.943 [0.768,1.159]	0.888** [0.824,0.956]	1.043 [0.972,1.120]
	Trade and transportation	0.881*** [0.840,0.923]	1.130*** [1.053,1.213]	0.898*** [0.848,0.952]	1.157*** [1.094,1.224]
	Information and communication	0.973 [0.887,1.066]	0.968 [0.841,1.114]	0.838*** [0.770,0.912]	1.081 [0.996,1.173]
	Finance and insurance	0.882*** [0.820,0.948]	0.934 [0.844,1.032]	0.827*** [0.759,0.902]	0.978 [0.900,1.064]
	Real estate	0.872 [0.743,1.024]	0.936 [0.742,1.181]	0.965 [0.833,1.118]	1.286*** [1.126,1.468]
	Business Service	0.968 [0.915,1.023]	1.065 [0.978,1.159]	0.935* [0.876,0.999]	1.061 [0.993,1.135]
	Culture and other services	0.918* [0.848,0.992]	1.050 [0.936,1.178]	0.880* [0.792,0.979]	1.086 [0.980,1.202]
Employment status Base is basis level wage earner	Self-employed	1.184** [1.055,1.330]	1.015 [0.864,1.192]	1.003 [0.915,1.100]	0.813*** [0.743,0.890]
	Chief executive	1.020 [0.889,1.172]	0.966 [0.805,1.160]	0.999 [0.902,1.107]	0.885* [0.802,0.976]
	Wage earner, high level	1.140*** [1.069,1.216]	0.990 [0.897,1.094]	0.976 [0.912,1.044]	0.974 [0.911,1.041]
	Wage earner, intermediate level	1.102*** [1.053,1.153]	1.021 [0.957,1.090]	1.012 [0.956,1.072]	1.013 [0.959,1.069]
	Wage earner, level not specified	1.079** [1.023,1.138]	1.075 [0.994,1.161]	1.019 [0.966,1.075]	0.988 [0.939,1.039]
	Apprentice	1.000 [0.916,1.091]	1.040 [0.867,1.248]	0.930 [0.822,1.052]	0.981 [0.843,1.142]
	Unemployed or on leave	1.552*** [1.297,1.857]	0.810 [0.582,1.128]	1.350* [1.060,1.720]	1.127 [0.872,1.454]
	Retired	1.939*** [1.699,2.212]	1.093 [0.911,1.313]	1.366*** [1.186,1.574]	0.934 [0.810,1.077]
	Social Security	1.663*** [1.330,2.079]	0.929 [0.613,1.407]	1.459* [1.075,1.980]	1.073 [0.751,1.533]
	Foreign origin	1.361*** [1.223,1.514]	0.879 [0.728,1.062]	1.383*** [1.226,1.559]	0.880 [0.765,1.012]
	N	82 708		85 014	

Exponentiated coefficients; 95% confidence intervals in brackets, * p<0.05, ** p<0.01, *** p<0.001

Caption 1: Results

Women who were not working were more often low-frequency donors compared to basic level wage earners, evidenced by unemployment (OR = 1.55, CI 95%: 1.30–1.86), retirement (OR = 1.94, CI 95%: 1.70–2.21) and social security (OR = 1.66, CI 95%: 1.33–2.08).
Furthermore, donors of foreign origin were more often low-frequency donors compared with donors of Danish origin (OR = 1.36 CI 95%: 1.22–1.51, OR = 1.38, CI 95%: 1.23–1.56 for women and men respectively).
Summary/Conclusions: We found that donors who were either unemployed, retired or on social security were more often low frequency donors. This was also the case for donors of foreign origin. Donation frequency varied less by the remaining socio-demographic predictors considered; income, education and occupation.

3A-S02-03

KNOWLEDGE, AWARENESS, ATTITUDES AND CONCERNS OF VOLUNTARY NON REMUNERATED BLOOD DONORS REGARDING BLOOD DONATION, IN COLOMBO, SRI LANKA

A Samaranayaka, M Krishnapillai and AJML Jayasekara
National Blood Center, Colombo, Sri Lanka

Background: From 2013, all blood donations in Sri Lanka are strictly from voluntary, non remunerated donors and the practice of paid or replacement donors are now a history. Though there are various researches that assessed the knowledge of the donors, only very few studies are carried out on their concerns regarding blood donation. It is critical to address such concerns of donors and obtain their satisfaction, especially for a country like Sri Lanka which totally depends on non remunerated donors to maintain the adequate blood supply and the regular donor pool. This study mainly targets their concerns and whether medical teams have addressed them.
Objectives: The objectives were to assess the level of knowledge, awareness and the attitude of voluntary donors towards blood donation and to identify their main concerns as well as to learn whether they were addressed by the medical staff.
Method: A cross sectional descriptive study was conducted at six different mobile blood donation campaigns around Colombo, among 500 voluntary donors who were eligible to donate blood. Data was collected from self administered structured questionnaire and statistically analyzed with the PSPP software.
Results: Among the 500 donors, 372 (74.40%) were males while 128 (25.60%) were females. Mean age of the study population was 31.6 years. Among them, 73.4% (367) had scored their knowledge above the average calculated for the study population. The commonest restricting factor among the first time donors (214; 42.80%) was the inability to find a suitable location or time to donate (75, 30.37%) followed by the fear of needles or reactions (60; 28.03%). Interest in helping others was the most frequent (394; 78.8%) motivating factor among all the donors. Most common concern among donors during donation process was long waiting time (182; 36.40%) followed by poor organization (85; 17.20%) of donation campaigns and 56 (11.20%) of them had concerns about the pre donation counseling done by the medical officers, and only 3% (16) were concerned about the poor attitude of other medical staff. Among the entire donors 7.2% (36) thought that their questions were not properly answered by the medical officers. The most common misconception among the donors was the weight gain following blood donation (132; 26.4%).
Conclusion: About two third of the study population are male donors. Most of the voluntary donors were motivated by the interest in helping save the lives while unable to find a suitable place and time to donate was the commonest restricting factor. Compared to the other neighboring developing countries, donors had relatively better knowledge about blood donation although the misconception of gaining weight following blood donation was observed among some donors. Small percentage of the donors had concerns about long waiting time and the poor organization of the donation campaigns. Even though only a few donors had complaints about the medical officers and other medical staff, addressing such concerns will further improve the quality of the service.

3A-S02-04

PUBLIC AWARENESS ON BLOOD DONATION, UNITED ARAB EMIRATES

M Raouf[1], M Mahmood[2], H El Najar[2], S Emelee[3] and S Amer[2]
[1]Dubai Blood Donation Center, Dubai, United Arab Emirates [2]MOH, Sharjah, United Arab Emirates [3]College of Dentistry Sharjah University, Sharjah, United Arab Emirates

Background: Blood transfusion is an essential part of patient care. Countries should intensify their efforts to raise public awareness about blood donation to ensure availability of blood and sustainability of their national blood donation program.
Aim: To assess public knowledge and attitude toward blood donation and help us planning the required awareness and motivation programs about blood donation aiming to create a wide base of healthy, regular blood donor population.
Methods: A questionnaire has been developed for this study; composed of introduction, objectives and three parts of questions; tick boxes used for answers. Under part A (tell us about your self):there are 12 questions; under part B (Participants Level of Knowledge regarding Blood donation) there are 25 questions and under part C (Participants attitudes, believes and perceptions toward blood donation) there are five questions. 992 person has been included in this study, our target was blood donors, students (colleges), non blood donors general population. Data was collected & analyzed with assigned significance value of $P < 0.05$.
Results: Under part A: males constitutes 50.6% and females 49.4% of the studied group. 31.3% of participants were <18 years of age and 60.5% were between 18 and 44 years of age. Concerning educational level: 31.9% were having secondary school, 57.4% were university graduates. 26.2% did not know their blood group and 30.4% have donated blood before. 59% knows the location of blood donation center in their area. Concerning part B of questions: 21.4% knows the correct accepted age range for donation, 17.3% knows the correct interval for whole blood donations, 27.8% knows the amount of blood collected in each time, 44% answered with 'I don't know' concerning eligibility after visiting a dentists, 67% responses with yes for question about blood donation could transmit infection to the donor,and 19% answered with 'I don't Know'. For knowledge about World Blood Donor Day: 30.3% answered 'I don't Know' and 38.9% answered with 'NO'. 73% answered with 'NO' concerning question: dose blood donation cause anemia. Concerning the number of lives saved by each donation: 20.6% answered three lives and 22.9% with four lives. For benefits of blood donation: 44.3% answered correctly and 15.1 with 'NO'. For part C of questions: 30.3% stated that donating blood means helping others and 9.8% donate blood to do laboratory tests. What can stop you from donation; 20.6% answered 'Busy' and 19.2% answered with 'No body asked me to do so'. For the question about do you donate blood if you receive a call from blood bank? 30.6% stated that they will when they are free and 21.3% will donate if there is an emergency.
Conclusions: This study shows that there is poor knowledge and misconception about blood donation among public. Awareness and educational programs are required by different means like through students syllabus;media educational and entertainments programs, World blood donor day and assigning a day for awareness on blood donation.

3A-S02-05

BARRIERS TO RBC DONATION FOR SUBJECTS OF SUB-SAHARAN ETHNICITY

A Berzuini, B Foglieni, M Spreafico and D Prati
Ospedale Manzoni Lecco, Lecco, Italy

Background: A substantial increase of persons affected by Sickle Cell Disease (SCD) is expected in European countries, due to migratory flows from Sub-Saharan (SSA) and other endemic areas. Blood transfusion is the essential treatment for people affected by SCD but, due to an intrinsic susceptibility, at least 1/3 of them develop red blood cell (RBC) alloimmunization, which is also favoured by the peculiar antigen patterns found in SSA. For these reasons SCD patients should receive fully matched blood starting from early childhood, and the only way to reach this objective is to include in the donor pool persons of the same ethnicity, who are also at risk of having congenital red cell and haemoglobin (Hb) defects. The conditions are potentially harmful for blood recipient due to increased risk of hemolysis and/or veno- occlusive crises after transfusion.
Aims: The aim of this study was to assess the prevalence of RBC and Hb defects in an apparently healthy population of first generation foreign citizens native of SSA areas.

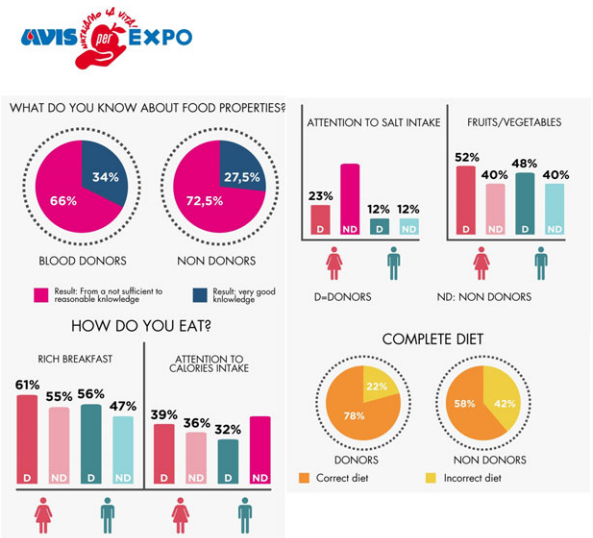
Methods: In March 2014 we started a 24-months program for the recruitment of foreign citizens to become blood donors in the area of Lecco, Italy. Of 450 potentially eligible persons, 175 (65 f, 110 m), gave their informed consent to undergo clinical and behavioural pre donation assessments according to the European regulation, as well as to the glucose-6-phosphate dehydrogenase (G6PDH) concentration and Hb electrophoresis.
Results: G6PDH concentration could be determined in 169 persons, allowing us to identify 55 (33%) with reduced enzyme concentration, including 25 (15%) with severe deficiency (i.e. <5 u/gHb). Despite the X-linked transmission, 8% of females had severe deficiency. Hb electrophoresis could be assessed in 173 persons. The overall prevalence of Hb variants was 32%: 28 subjects (21% f, 12% m), were carriers of HbS; 19 (11%) had beta thalassemia trait, and eight (5%) were HbC carriers. Coexpression of G6PDH deficiency and HbS was found in 5% of subjects.
Conclusions: Despite the clinicians expectations of an increased availability of donors from SSA to provide a better matched blood supply for SSD patients, a substantial proportion of candidate donors will be carrier of RBC and/or Hb abnormalities, representing a major contraindication to RBC transfusion.

3A-S02-06

AVIS AND THE PROMOTION OF HEALTHY LIFESTYLES

V Saturni[1], A Poli[2] and F Marangoni[2]
[1]*AVIS Nazionale, Milano, Italy* [2]*Nutrition Foundation of Italy, Milan, Italy*

Background: Avis (Italian Voluntary Blood Donors Association) was founded in 1927 with the aim of granting access to whole blood, labile blood components and plasma-derived medicinal products coming from regular, voluntary, non-remunerated, responsible, associated donors.
Aims: In the recent years, AVIS has strengthen its engagement in promoting wealthy and positive behaviors as essential elements in a blood donor's lifestyle.
In 2015 the city of Milan hosted the universal exposition entitled 'Feeding the planet, energy for life'. Inspired by the main topic of this event, the project called 'AVIS for EXPO. We feed life!' was launched with the aim of encouraging people – in particolar volunteers, families, children and youngsters – to adopt a proper nutrition and a healthy lifestyle. Promoters of this project were AVIS Nazionale, the Italian Volunteer Blood Donors Association, Avis Regionale Lombardia, Avis Provinciale di Milano, Avis Comunale di Milano, the Nutrition Foundation of Italy, Uisp, IFBDO – International Federation of Blood Donor Organizations and its International Youth Committee, in cooperation with the Padan Technology Park.
Methods: Within the framework of this project, AVIS launched an online survey which intended to understand people's eating habits and their attitude to physical activity, focusing on what they know about food and how they eat. Another aim of this research was to understand whether blood donors were more conscious of good dietary practices rather than non donors.



Graph 1.

Results: The study was based on 16.170 questionnaires (22% of which were answered by non donors and 78% by blood donors). The latter ones proved to have a better knowledge about food and proved to pay more attention to physical wellbeing. Female blood donors tend to avoid caloric food more than male donors (39% vs 31%) and more than women who do not donate.
Blood donors also practice sport and physical activities more than others: this study proves indeed that they walk, run and cycle more regularly.
For more data, see the attached infographics.
Conclusions: These positive results show how blood donors care for safe and correct lifestyles in their everyday actions. They can also be seen as a goal achieved by AVIS through its campaigns and projects that have been recently focused on the prevention of HIV and other infectious diseases. This is a core topic that reflects AVIS' aim to promote health as a main value for everyone.

Blood Processing

3A-S05-01

No abstract available.

3A-S05-02

EFFECTIVE INACTIVATION OF THE NON-ENVELOPED FELINE CALICIVIRUS WITH AMUSTALINE/GSH IN RED BLOOD CELLS

F Santa Maria[1], A Laughhunn[1], R Lenhoff[1] and A Stassinopoulos[2]
[1]*Microbiology Department, Cerus Corporation, Concord, California, United States of America* [2]*Global Scientific Affairs, Cerus Corporation, Concord, California, United States of America*

Background: The INTERCEPT™ Blood System for Red Blood Cells (RBC) uses the small molecule amustaline to form covalent crosslinks within nucleic acids of leukocytes and contaminating pathogens to prevent replication. The process includes addition of 0.2 mM amustaline and 20 mM glutathione (GSH) and an 18–24 h incubation at RT. Although inactivation is complete after 3 h, the additional incubation ensures complete decomposition of amustaline. A final centrifugation and exchange of the supernatant with additive solution (SAG-M) provides pathogen-reduced RBC for transfusion. The objective of this study was to evaluate the inactivation of feline calicivirus (FCV) with the INTERCEPT Blood System[1] for RBC prepared in Optisol (AS-5). FCV is a positive-sense, single-stranded RNA virus in the genus Vesivirus within the family Caliciviridae and serves as a model for small, non-enveloped viruses, such as hepatitis E virus.
Methods: For each experiment, a single RBC unit was spiked with FCV to a final concentration of ~$10^{8.0}$ pfu/ml and treated with amustaline. The spiked RBC units were mixed with GSH and control samples (Control T = 0) were taken to determine pre-amustaline titers. Each unit was then dosed with amustaline and test samples (Test T = 3) were removed to determine levels of inactivation. Control and Test samples were serially diluted and inoculated onto CrFK cells. The plates were incubated

Feline Calicivirus Inactivation in Red Blood Cells

Replicate	Log Titers (pfu/mL)		Log Reduction per mL
	Control T=0	Test T=3	
1	6.0	<0.2	>5.8
2	6.1	<0.2	>5.9
3	5.9	<0.2	>5.7
Mean±SD	**6.0±0.1**	**<0.2±0.0**	**>5.8±0.1**

Table 1.

for 3 days at 37°C, stained with crystal violet and the plaques enumerated. Log reduction was calculated as the difference between the mean titer in pre-amustaline samples and the mean titer in the 3 h post-amustaline samples.
Results: Robust inactivation of FCV was achieved as summarized in Table 1.
Conclusion: The non-enveloped feline calicivirus was inactivated to the limit of detection in RBC after treatment with GSH and amustaline. Inactivation of >5.8 log of FCV was achieved in the CrFK infectivity model.
[1]The INTERCEPT Blood System for RBC is not approved for use

3A-S05-03

EVALUATION OF THE MIRASOL PATHOGEN INACTIVATION SYSTEM AS AN ALTERNATIVE TO BACTERIAL SCREENING OF PLATELET COMPONENTS

CP McDonald, J Allen, T Pitt, G Orr, D Sawicka, R De Ritis and S Robbins
NHS Blood and Transplant, London, United Kingdom

Background: The interventions of improved donor arm disinfection, diversion and bacterial screening of platelet components have been implemented by NHSBT to improve the safety of the blood supply. In 2011, bacterial screening was implemented by NHSBT and in screening over 1 million donations, there has been only one transmission to a recipient, but three near miss cases were reported (2011–2016). Prior to the implementation of bacterial screening in the reporting period 2006–2010, there were seven transmissions with 13 patients affected, three fatalities and three near misses reported from platelet components. NHSBT is evaluating pathogen inactivation (PI) systems as an alternative to bacterial screening.
Aim: To determine the effectiveness of the Mirasol PI system against key organisms from transfusion reactions or isolates obtained from screening of platelet components.
Method: Ten bacterial species were spiked independently in pooled platelet components suspended in platelet additive solution. The bacterial species were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus bovis*, *Streptococcus dysgalactiae*, *Streptococcus mitis*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Listeria monocytogenes* and *Serratia marcescens*.
A pooled and split method was used for each organism tested. Twelve day 1 platelet bags were used for each bacterial species. Spiking concentrations were 10^{-1}, 10^{3}, 10^{4} and 10^{5} cfu/ml. Three bags were used for each concentration tested with one as a spiked, but PI untreated control. Bacteria were enumerated and enrichment culture performed immediately after spiking, prior to treatment (2 h post spiking), immediately after treatment, and at day 7 of shelf life.
Results: At the 10^{-1} cfu/ml concentration, all organisms were inactivated and positive controls yielded growth in the range of 10^{4} and 10^{9} cfu/ml at day 7, with the exception of *S. dysgalactiae* which was not detected at any time point. At the 10^{3} cfu/ml concentration immediately post treatment, no organisms were detected by plate count. Growth was obtained with all organisms at day 7 in the range of 10^{5} and 10^{9} cfu/ml, with the exception of *S. pneumoniae* which was completely inactivated. At 10^{4} cfu/ml, breakthrough was obtained with all organisms with the exception of *S. pneumoniae* which was completely inactivated. Day 7 growth at the 10^{4} cfu/ml spiking concentration ranged from 10^{5} and 10^{9} cfu/ml. Breakthrough occurred at the 10^{5} concentration with all organisms in all replicates with the exception of one replicate of *S. pneumoniae*. The final concentration at day 7 was in the range of 10^{3}–10^{9} cfu/ml.
Conclusion: The Mirasol System inactivated all organisms at low concentration levels. Use of the system may be more appropriate nearer to the point of donation. The 10^{3} concentration test indicates the inappropriateness of logarithmic reduction data obtained immediately after treatment for the determination of the effectiveness of PI systems, as no growth was obtained immediately after treatment by plate count, but significant levels were obtained at day 7. NHSBT intends to further evaluate the Mirasol System to determine the most appropriate time to PI treat platelet units and the breakpoint concentrations of key bacterial species at which the system is unable to inactivate.

3A-S05-04

EVALUATION OF THE CERUS INTERCEPT SYSTEM AS AN ALTERNATIVE TO BACTERIAL SCREENING OF PLATELET COMPONENTS

J Allen, T Pitt, K Aplin, J Ball, G Orr, S Robbins and C McDonald
NHS Blood and Transplant, London, United Kingdom

Background: NHSBT has implemented the interventions of improved donor arm disinfection, diversion and bacterial screening to reduce the risk of bacterial transmission by transfusion. The NHSBT bacterial screening system of platelet components reduced the number of transmissions from seven (13 patients affected) with three fatalities and three near misses in the period prior to introduction, 2006–2010, to one transmission (one patient) and three near misses in screening over 1 million donations (2011–2016). NHSBT is evaluating pathogen inactivation (PI) systems as a potential alternative to bacterial screening.
Aim: To determine the effectiveness of the Cerus Intercept System against key organisms from transfusion reactions or isolates obtained from screening of platelet components at concentrations that are appropriate at the time NHSBT can currently operationally PI treat platelet components.
Method: Ten bacterial species were spiked independently in pooled platelet components suspended in platelet additive solution. The bacterial species were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus bovis*, *Streptococcus dysgalactiae*, *Streptococcus mitis*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Listeria monocytogenes* and *Serratia marcescens*.
A pooled and split method was used for each organism tested. Nine day 1 platelet bags were used for each bacterial species. Spiking concentrations were 10^{-1}, 10^{3} and 10^{5} cfu/ml. Three bags were used for each concentration tested with one as a spiked untreated control. Bacteria were enumerated and enrichment culture performed immediately after spiking, prior to treatment (2 h post spiking), immediately after treatment and at day 7 of shelf life. Failure at the 10^{5} level would result in a repeat using the same method at concentrations of 10^{3}, 10^{4} and 10^{5} cfu/ml.
Results: Inactivation at all levels was obtained with all bacterial species with the exception of *S. marcescens*, which broke through at 10^{5} cfu/ml concentration in both replicates tested with a final concentration at day 7 of 10^{9} and 10^{10} cfu/ml. On repeat in triplicate, breakthrough was obtained in one replicate at 10^{5} and 10^{4} cfu/ml. At day 7, the bacterial counts for these replicates were 10^{9} and 10^{10} cfu/ml respectively.
Conclusion: Intercept offers a potential alternative to bacterial screening. NHSBT will undertake further evaluation on this PI system to determine the most appropriate time to PI treat platelet units, the breakpoint concentrations of key bacterial species at which Intercept is unable to inactivate and the effectiveness of the system on platelet components suspended in plasma.

3A-S05-05

IN VITRO CELL QUALITY OF REVEOS-DERIVED POOLED PLATELETS IN PLASMA AFTER TREATMENT WITH THE MIRASOL PATHOGEN REDUCTION TECHNOLOGY SYSTEM

ME Shipps, LM Rouse, KM Colagrosso and CR Kruk
Terumo BCT, Lakewood, Colorado, United States of America

Background: The Mirasol Pathogen Reduction Technology (PRT) System for Platelets in Plasma combines riboflavin (vitamin B2) and UV energy to inactivate pathogens in platelet products. Platelet concentrates in plasma treated with the Mirasol PRT System have been well characterized in numerous studies. However, no studies have yet to explore the cell quality of whole blood platelet concentrates derived from pooled Reveos 3 Component (3C) Individual Platelet Units (IPUs) that have been treated with the Mirasol PRT System.
Aim: The objective of this study is to evaluate *in vitro* platelet cell quality data from Mirasol-treated platelets in plasma that were derived from Reveos-processed whole blood.
Methods: Groups of five type matched Whole Blood units were collected in CPD with collection parameters set to target a collection volume of 450 ml. Pooled platelet concentrates of five IPUs produced on the Reveos System were obtained according to the manufacturer's instructions, utilizing a Reveos 3C process targeting a 55 ml IPU product volume. IPUs were produced on the day of collection, then rested and agitated overnight in the platelet incubator prior to pooling the next day. Post pooling, the platelet concentrates were rested for a minimum of 2 h prior to being treated with the Mirasol PRT System for platelets according to a defined protocol. Several *in vitro* cell quality parameters including swirl, blood gas analysis, glycolytic

Parameter	Day 5-Mean Value (N=10)
Lactate Production Rate mmol/10^{12} platelets/hr	0.096 ± 0.02
Glucose Consumption Rate mmol/10^{12} platelets/hr	0.039 ± 0.01
$pH_{22°C}$	7.22 ± 0.04
Swirl	3 ± 0.63

Table 1. pH 22°C on Day 5 of storage

Pooled Platelet Concentrates In Plasma	$pH_{22°C}$ Day 5
1	7.23
2	7.22
3	7.21
4	7.24
5	7.31
6	7.16
7	7.20
8	ND*
9	7.23
10	7.17

*$pH_{22°C}$ Day 6=7.13

Table 2. Platelet cell quality parameters on day 5 of storage at 22°C

metabolism, cell count, and platelet activation were measured on the day of treatment (Day 1) and on Day 4 and 5 of storage.
Results: n = 10 Reveos-derived, Mirasol-treated pooled platelet concentrates in plasma were included in this study. All 10 units met the CoE Guidelines (18th edition) for $pH_{22°C}$ >6.4 at day 5 of storage (Table 1). Additionally, all other cell quality parameters indicated good platelet quality through the 5-day storage period (Table 2).
Conclusion: Mirasol-treated platelets in plasma derived from Reveos-processed whole blood exhibit good *in vitro* cell quality profiles and fulfill the CoE pH requirement. The results of this study demonstrate that the application of Mirasol treatment to Reveos-processed platelet pools in plasma produces a platelet product with acceptable *in vitro* platelet cell quality characteristics.

Plenary Session I: New Insights in Mechanisms and Consequences of Haemolysis

PL1-01

MECHANISMS OF HEME TOXICITY IN HEMOLYSIS AND PROTECTION BY THE HEME-BINDING PROTEIN, HEMOPEXIN

A Smith

School of Biological Sciences, University of Missouri-KC, Kansas City, Missouri, United States of America

Heme (iron-protoporphyrin IX) is vital to aerobic life. However, heme is potentially toxic because the chemical reactions of heme that occur in the presence of oxygen can destroy most biological molecules. By binding heme tightly in a manner that limits its chemical activity, hemopexin protects all cells from heme toxicity. Furthermore, hemopexin transports heme into cells via receptor-mediated endocytosis for catabolism and iron conservation; and normally, recycles intact analogously to transferrin. A surprisingly large number of disease states characterized by hemolysis or complicated by hemolysis (*e.g.* sickle cell disease, sepsis, hemolytic anemias, viral hemorrhagic fevers) lack targeted therapies. Novel treatments currently being developed include replenishment therapies with hemopexin infusions or hepatic gene therapy to express hemopexin. Due to the rapid loss of haptoglobin-hemoglobin complexes after hemolysis of ~5 ml of blood, the hemopexin system is a vital protection for all cells to bind heme released from hemoglobin during hemolysis. Our first objective is to characterize the key protective mechanisms that hemopexin instigates. Our second objective is to model how intracellular heme toxicity develops. Our third objective is to evaluate the clinical importance of the hemopexin system in protecting against extracellular heme-activated pathology in intravascular and extra-vascular hemolysis.
The main findings are that endocytosis of heme-hemopexin co-regulates the safe trafficking of three redox active metals through subcellular compartments and, compared with the response to 'free' heme, uniquely activates several signaling pathways and transcription factors that induce the production of anti-oxidant enzymes and molecules. Data from historical studies in humans reveal the importance of the hemopexin system, even in haptoglobin-replete conditions. Additional data from recent studies of patients who received modified hemoglobin-based blood substitutes or had HIV infection showed deficiencies in hemoglobin clearance by the haptoglobin system with consequences for hemopexin. In conclusion, mounting evidence from patient longitudinal studies supports that impairments of heme and hemoglobin clearance are often unrecognized. There is a need not only to appropriately collect and store blood and plasma samples for analyses but also to reconsider the standard parameters of the hemolysis index to provide information on hemoglobin and heme clearance, respectively, allowing a better prediction of the likelihood of the development of heme toxicity. In conclusion, knowledge of the biology of hemopexin has yielded a novel therapeutic and provided an impetus and means for improving diagnosis of hemolytic diseases and conditions.

PL1-02

PATHOPHYSIOLOGY OF EXTRACELLULAR HEMOGLOBIN: USE OF ANIMAL MODELS TO TRANSLATE MOLECULAR MECHANISMS INTO CLINICAL SIGNIFICANCE

M Gram

Lund University, Lund, Sweden

Background: Hemoglobin is the major oxygen-carrying system of the blood, but has many potentially dangerous side effects due to oxidation and reduction reactions of the heme-bound iron and oxygen. While encapsulated within the red blood cells, the hemoglobin are kept separated from the rest of the body and an arsenal of protective mechanisms inhibit the side-reactions. However, Hb is a very abundant molecule, in fact, the concentration in blood is around 150 g/l and an adult human body consequently contains almost one kg of the protein. Thus, significant amounts escape from the red blood cells under healthy conditions and massive amounts can be released during pathological conditions involving hemolysis. Therefore, a number of defense mechanisms have evolved to counteract the threat of extracellular hemoglobin to exposed tissues.

Many pathological conditions, diseases and iatrogenic conditions, such as hemolytic anemias, transfusion-induced intravascular hemolysis, preeclampsia, intraventricular hemorrhage, sickle cell disease and infusion of artificial blood, involve abnormal levels of hemolysis and extracellular hemoglobin. Although they often have vastly different etiology, they share many symptoms and clinical sequelae as a consequence of the Hb-induced damage.

Aim/Summary: It is the objective of this presentation to give an overview of the pathophysiological mechanisms of extracellular hemoglobin and its metabolites. Furthermore, it will highlight the use of animal models in advancing the understanding of these mechanisms and how to utilize the knowledge in development of new and better pharmaceutical therapies.

PL1-03

COMPLICATIONS FOLLOWING RED BLOOD CELL TRANSFUSION – HYPERHAEMOLYSIS

SE Robinson

Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom

Hyperhaemolysis is a severe, potentially life threatening complication of transfusion. Characterised by haemolysis affecting the recipient's own red cells in addition to the transfused red cells that results in a decrease in the haemoglobin below the pre transfusion level and may be associated with a reticulocytopenia. It may be triggered by a new red cell antibody, but frequently no new red cell antibody is identified.

Although predominantly described in patients with sickle cell disease (SCD), it is also described in patients with other haemoglobinopathies and in patients with other haematological and non-haematological diagnoses.

There is a growing body of literature since 1980 including both case reports and case series. In addition since 2009 hyperhaemolysis has been included in the UK Serious Hazards of Transfusion (SHOT) reporting scheme. As such our understanding of the pathophysiology of hyperhaemolysis and how we manage this life threatening delayed haemolytic transfusion reaction sub-type is advancing.

Guy's and St Thomas' NHS Foundation Trust and the Evelina Children's Hospital located in South East London provide care for more than 1,000 patients with SCD, more than 100 patients are on a regular transfusion regimen and many more receive transfusion in an acute setting. Since 2009 more than 10 cases of hyperhaemolysis have been identified. This case series will be described alongside local management strategy, review of the literature and available SHOT reports to date.

In patient's with SCD the mainstay of treatment is steroids and immunoglobulin with the addition of haematinic and erythropoietin support. Further transfusion has been associated with increasing haemolysis and worsening anaemia, and should be avoided if possible. In cases where there is very rapid haemolysis and critical anaemia, additional transfusion will be required and this should be preceded by immunoglobulin and steroids. More recently there have been reports of the use of rituximab in the acute setting and prophylactically and the use of salvage treatment with eculizumab a human monoclonal antibody that inhibits terminal complement activation. Hyperhaemolysis can recur with subsequent transfusions and as such affects decision making regarding future transfusions.

Educating patients with a history of hyperhaemolysis regarding informing future clinicians who may care for them of their transfusion history supported by the development of shared care systems to identify patients with alloantibodies and or hyperhaemolysis to the transfusion laboratory team is a necessary safety initiative. In addition work is required to develop national and international registries to determine the true incidence of hyperhaemolysis, better understand the pathophysiology, identify markers to predict which patients are at risk and inform management guidelines and future trials.

Apheresis and Haemovigilace

3B-S06-01

WHERE DOES ALL THE ALBUMIN GO? AN AUDIT OF HUMAN ALBUMIN SOLUTION USAGE POST IMPLEMENTATION DEMAND MANAGEMENT PROGRAMME

M Yazdani[1], A Retter[1], T Maggs[2], M Robson[1], C Reid[3], P Holmes[1], T Garood[1] and S Robinson[1]

[1]Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom [2]Viapath, London, United Kingdom [3]Evelina Children's Hospital, London, United Kingdom

Background: There is no formal UK guidance governing the use of Human Albumin Solution (HAS) and in many clinical situations there is limited evidence and alternatives may exist. Widespread use of HAS, combined with restrictions on the use of UK derived plasma have led to high cost and shortages. A severe shortage in the supply of HAS in 2015 prompted the development of a Trust Demand Management Programme. This aimed to restrict use to the most appropriate indications and consider alternative treatment when possible to ensure adequate stocks remain. Indications were allocated to a colour coded category according to evidence available similar to the Department of Health Immunoglobulin Demand Management Programme and for plasma exchange indications a locally modified version of the American Society for Apheresis (ASFA) categories were used. The colour coding and modified ASFA categories are now used to demand manage HAS usage. Post implementation 6 months of audit data is now available.

Aims: To audit HAS usage from July 2015 to December 2015 at Guy's and St Thomas' NHS Foundation Trust (GSTFT) and Evelina London Children's Hospital (ELCH).

Methods: The data was complied from the electronic patient record HAS order form, laboratory information system and medical notes and analysed in Excel. Data included age, weight, gender, HAS concentration, indication and volume infused.

Results: The results are summarised in Table 1.

A total of 1,303.1 l of HAS were used in 1,139 infusions. 737 (64.7%) infusions were 20% HAS, accounting for 175.7 l (13.5%) in 263 patients. Indications for 20% HAS were red (53.9%, 94.7 l) e.g. acute lung injury and severe respiratory failure, hepatorenal syndrome and paracentesis, blue (26.5%, 46.5 l) e.g. severe sepsis and septic shock and grey (19.6%, 34.5 l) e.g. volume resuscitation for hypovolaemia.

The 4.5 and 5% HAS infusions accounted for the remaining 1,127.4 l (86.5%). 1,102.3 l (97.8%) were used for plasma exchange (Table 1); 941.4 l (85.4%) ASFA category I, 93.7 l (8.5%) category II, 25.5 l (2.3%) category IV, 41.7 l (3.8%) for indications not specified according to ASFA. 25.1 l (2.2%) were used for a Grey indication (volume resuscitation for hypovolaemia).

Summary: This data outlines the volume of HAS used at GSTFT and ELCH according to indications. Implementation of a demand management programme enables verification of appropriate use and provides information to progress demand management. The majority of HAS indications were appropriate during this time. Plasma exchange accounted for 84.6% of total HAS usage and will be the focus of further demand management strategies at GSTFT. However 67.2 l (5.2%) (£5,162.6) were used for ASFA category IV (inappropriate) and other indications not specified according to ASFA (potentially inappropriate) and in addition represent a potential financial saving. The demand management programme whilst aiming to promote

Table 1 – Summary of Results (HAS Infusions at GSTFT July-December 2015)

HAS Conc	Indications			Sessions	Volume (ml)	Patients	Male	Female	Mean Age (Years)	Mean Weight (Kg)
20%	Acute lung injury and severe respiratory failure			65	15600	27	13	14	50.6	76.6
	Hepato-renal syndrome			105	21700	27	18	9	54.2	77.1
	Paracentesis			150	57400	50	28	22	57.7	73.6
	Childhood nephrotic syndrome			78	10400	6	4	2	8.7	33.8
	Severe Sepsis and septic shock			163	32600	60	36	24	60.1	76.8
	Spontaneous bacterial peritonitis			13	3500	4	2	2	63.0	95.5
	Volume resuscitation for hypovolemia			163	34497	83	43	40	55.3	71.5
	Total			737	175697	263	148	115	54.7	73.6
			Red	320	94700					
			Blue	254	46500					
			Grey	163	34497					
4.5/5%	Plasma Exchange			352	1102250	33	18	15	31.9	61.5
		Post Transplant FSGS	ASFA-I	148	485950	3	2	1	33.0	62.2
		FSGS	ASFA-I	12	28000	1	1	0	9.0	29.7
		Renal Transplant Rejection (Humoral)	ASFA-I	106	343700	11	4	7	33.4	68.3
		Renal Transplant Desensitisation (ABOi/HLAi)	ASFA-I	19	63000	9	6	3	49.2	89.0
		Familial Hypertiptaemia	ASFA-I	18	18700	1	0	1	5.0	36.5
		Anti GBM Disease	ASFA-I	1	2000	1	1	0	71.0	70.0
		Neuromyelitis optica	ASFA-II	13	44500	2	0	2	36.5	64.9
		CIDP	ASFA-II	11	39000	1	1	0	62.0	80.4
		Presumed catastrophic APLS/systemic vasculitis	ASFA-II	1	5000	1	1	0	48.0	51.0
		Guillaine Barre syndrome	ASFA-II	3	5200	1	0	1	1.0	12.1
		Lupus like Glomerulonephritis	ASFA-IV	7	25500	1	1	0	6.0	25.9
		Rapidly Progressive Crescentic Glomerulonephritis	-	8	24200	1	1	0	9.0	36.8
		Faciobrachial Dystonic Seizures - Cause?	-	5	17500	1	1	0	56.0	97.0
	Volume resuscitation for hypovolemia			50	25122	19	14	5	32.3	60.2
	Total			402	1127372	52	31	21	32.0	61.3
	Grand Total			*1139*	*1303069*	*218*	*119*	*99*	*43.7*	*67.4*

Table 1. Summary of Results HAS Infusions at GSTFT July-December 2015

best transfusion practice also ensures a tool to manage future shortages according to indication and available supply. Data from other centres is necessary to compare usage and may also guide manufacturers regarding UK requirements.

3B-S06-02

COST EFFECTIVENESS IN TREATING GUILLAIN BARRE SYNDROME PATIENTS WITH INTRAVENOUS IMMUNOGLOBULIN AS COMPARED TO THERAPEUTIC PLASMA EXCHANGE: A PROSPECTIVE RANDOMIZED CONTROLLED TRIAL

A Maheshwari, RR Sharma, R Hans, N Sharma, M Modi, S Prinja and N Marwaha
PGIMER, Chandigarh, India

Background: Therapeutic Plasma Exchange (TPE) and Intravenous Immunoglobulin (IVIG) are first-line treatments for Guillain Barre Syndrome (GBS), but there is currently much debate over the cost effectiveness of these two treatments. Further, it becomes a major concern in countries with lower GDP (Gross Domestic Product) per capita, as treatment cost is also an important determining factor in planning treatment strategies.

Aims: To assess the incremental cost effectiveness for treating Guillain Barre Syndrome patients with IVIG as compared to TPE.

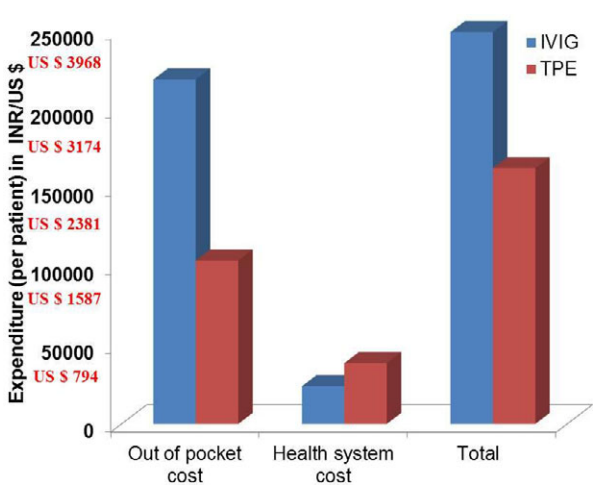
Methods: This study was a prospective randomized controlled trial in which we included 40 GBS patients with a GBS disability score grade 4 or 5, within 4 weeks of onset of flaccid limb weakness according to the Ausbury criteria. Patients were followed up for 12 weeks in total. Patients were treated with 3–6 TPE procedures over a period of 7–14 days. In each procedure around 1 to 1.5 plasma volume was exchanged using replacement fluid of 4% Human serum albumin. IVIG was given as 400 mg/kg/day for 5 consecutive days. A social perspective was also taken into account to determine the cost effectiveness.

Results: Out of 40 patients, 24 were part of the IVIG group and 16 were in the TPE group. Mean duration of hospital stay was 15.33 ± 13.89 in the IVIG group and 17.88 ± 10.13 in the TPE group. No statistically significant differences were observed in the GBS Disability scores, Overall Neuropathy Limitations Scale and MRC Sum Scale during overall treatment course in both treatment groups ($P > 0.05$). Only onset of significant recovery rate was faster in IVIG group (2 weeks) as compared to the TPE group (4 weeks; $P < 0.05$). Health system cost of treatment in the Emergency ward was Rs 19,762 (304 US $) per patient. Out of pocket cost for the IVIG group was: Rs 2,19,247 (3,373 US $) and for the TPE group: Rs 1,04,070 (1,601 US $). Incremental cost effectiveness ratio (ICER) of treatment with IVIG as compared to TPE was Rs 2,61,766 (4,027 US $) in total. This implies that the incremental cost of treating GBS patients with IVIG as compared to TPE is Rs 2,61,766 (4,027 US $) per unit reduction in GBS disability score. With the GDP per capita of Rs 68,757 (1,057 US $) in India the treatment of GBS patients with IVIG is not a cost effective option as ICER is 3.8 times of the GDP per capita. According to WHO-CHOICE it is recommended that an ICER value of more than three times GDP per capita doesn't considered a cost effective treatment option in planning treatment strategies.

Conclusion: In terms of ICER the treatment of GBS with IVIG does not appear to be a cost effective option in the Indian region and TPE appears to be the better option for cost-constraint countries.

GBS Disability Score	IVIG (n=24)		TPE (n=16)		p value
	Mean	SD	Mean	SD	
GBS-DS at day 1 (of treatment)	4.17	0.38	4.25	0.45	0.53
GBS-DS at day 7	4.13	0.68	4.38	0.72	0.27
GBS-DS at day 14	3.79	0.88	4.00	0.97	0.49
GBS-DS at week 4	3.04	1.20	3.56	1.31	0.20
GBS-DS at week 12	1.75	1.45	2.38	1.67	0.22

Caption 1. GBS Disability Score showing comparable recovery pattern



Caption 2. Unit cost comparison for IVIG and TPE treatment in GBS patients

3B-S06-03

FLOW CYTOMETRIC PLATELET CROSSMATCHING APPROACH FOR SELECTION OF PLATELETS FOR TRANSFUSION IN HEMATOPOIETIC STEM CELL TRANSPLANT PATIENTS

N Sharma[1], SB Rajadhaksha[1], S Ojha[2], MG Kamble[2], N Palumaru[2] and N Khattry[1]
[1]Tata Memorial Hospital, Mumbai, India [2]ACTREC-TMC, Navi-Mumbai, India

Background: Blood component support during Hematopoietic Stem Cell Transplant (HSCT) is specific and specialized. During the peritransplant phase patients alloimmunized to platelets pose challenges in transfusion management.

Aims: (i) To find out incidence of alloimmunization. (ii) To find out effect of alloimmunization on Corrected Count Increment (CCI) after providing crossmatched platelets. (iii) To study response of providing crossmatched platelets vs non crossmatched platelets in order to develop an approach for better selection of platelets for transfusion in HSCT patients.

Methods: The study was performed on 32 randomly selected patients who underwent HSCT during June to December 2015. Panel reactive antibody (PRA) estimation (FLOWPRA Screening Test kit, One Lambda Inc.) was done on all patients. However only 22 patients (52 transfusions) could be evaluated for studying response to crossmatched platelets, as 10 patients were excluded (nine did not meet inclusion criteria and one required plasma reduced platelet transfusions in view of anaphylaxis to previous transfusion). All patients were provided with apheresis platelets and crossmatch was performed by flow cytometry using FITC labelled anti-human IgG+IgA+IgM antibody. The median fluorescent channel shift by the patient's sample was compared with that of negative control and sample showing low Mean Channel Number (MCN) value was considered as crossmatch compatible unit amongst tested platelet units. Evaluation of platelet transfusion response was done by calculating CCI at 1 and 18–24 h post transfusion. One hour CCI >7,500 and 24 h CCI >5,000 was considered as good response.

Results: Incidence of alloimmunization was found to be 37.5% (15.63% moderate and 21.88% high; Table 1). Good response in 1 h CCI was observed in 33.33% of crossmatched transfusions in alloimmunized patients whereas in non alloimmunized patients it was observed in 65% transfusions ($P = 0.348$). Overall good response to crossmatched platelets at 1 and 24 h post transfusion was observed in 58% events whereas for uncrossmatched it was 50% at 1 h ($P = 0.578$) and 39% at 24 h ($P = 0.165$) post transfusion (Table 2). In presence of clinical factors, providing crossmatched platelets resulted in good response in 47% & 60% of transfusions at 1 and 24 h respectively whereas in the absence of clinical factors it was 72% and 55%

Table 1: Incidence of HLA allo-sensitization in HSCT patients

PRA	HLA CLASS I (n)	Percent of patients
1-10% (Low)	20	62.5%
11-50% (Moderate)	5	15.63%
51-100% (High)	7	21.88%

Table 2. Effect of providing crossmatched platelets on transfusion outcome

	CCI		CROSSMATCHED PLATELETS		UNCROSSMATCHED PLATELETS		p VALUE
AT	CCI ($x10^3$)	Transfusion response	Number	%	Number	%	
1 hour	>7.5	GOOD	15	57.69	13	50	0.578* (Not significant)
	<=7.5	POOR	11	42.31	13	50	
24 hour	>4.5	GOOD	15	57.69	10	38.46	0.165* (Not significant)
	<=4.5	POOR	11	42.31	16	61.54	

*p value was calculated by Chi-square test

respectively. The difference was not significant (1 h CCI P = 0.441 and 24 h CCI P = 0.781).

Conclusion: The difference in CCI with flow cytometric crossmatched platelets in alloimmunized vs non alloimmunized patients was not significant. Flow cytometric crossmatched platelets were not shown to have any benefit over uncrossmatched platelets in HSCT patients. These findings can probably be attributed to the unique characteristics of study population comprising only of HSCT patients who are immunosuppressed following myeloablative conditioning and are under posttransplant GVHD prophylaxis. Also, absence of alloimmunization in majority of transfused patients (17/22) could be the reason for adequate increments even with uncrossmatched platelets. Since our sample size was small, further study with larger sample size may establish more conclusive evidence.

3B-S06-04

TEN YEARS OF HAEMOVIGILANCE: A SYSTEMATIC REVIEW OF PATIENT SAFETY DATA IN NEW SOUTH WALES (NSW), AUSTRALIA

S Francis, A Thomson and A Dalli

NSW Clinical Excellence Commission, Sydney, Australia

Background: NSW public hospitals use a centralised incident reporting platform for all incidents and near misses, the Incident Information Management System (IIMS). Since 2008, NSW has contributed to the National Haemovigilance Program by performing a targeted analysis of transfusion related adverse events as reported in IIMS. De-identified data is extracted and then reviewed by a clinical expert working group for classification, severity and content.

Approximately 12% of the data extracted from IIMS is compliant with the required national reporting program. Our program determined that the amount and richness of the data that has previoulsy been unreported, and only minimally analysed could provide valuable learning opportunities, as well as opportunities to feedback to those reporting and trending incidents at a local level.

Aim: Our aim was to collate, categorise, assess trends and apply a thematic analysis of 10 years, 2005–2015, of all incidents identified as either a primary or secondary blood and blood product incident type.

Results: Data extracted was reveiwed for relevancy and a total of 17,679 were included for analysis.

Entered incidents are rated for outcome severity at the local level using a Severity Assessment Code (SAC). There are four SAC ratings, ranging from SAC1 (extreme risk) to SAC4 (low risk).

Less than 3% of blood product related incidents notified were rated as SAC1 or SAC2, indicating that the vast majority of incidents resulted in little or no harm to the patient. All SAC1 incidents are subject to a thorough investigation known as a root cause analysis (RCA), to determine causality and identify opportunities to make our services safer.

Entered incidents were further categorised as an actual or near miss error in the process or activity related to blood and blood products (incident), or a physiological response to the blood or blood product administered (complication).

There were 15,893 incidents, and 1,786 complications reported, however the proportion of complications:incident reports has been steadily increasing over time, with a mean of 4% between 2005 and 2009, increasing to 13% between 2010 and 2015.

Accounting for expected annual fluctuations, the number of all notifications has been statistically stable over time and consistent with the rate of all clinical incident notifications during this period. There was a statistically significant increase in specific incident types including wastage and labelling/patient identification errors. Reported complications such as FNHTR and allergic reactions also increased significantly.

Summary/Conclusion: As a result of our analysis, a specifc project targeting the improvement in the rate and quality of reporting of all transfusion related near miss and actual incidents and complications has commenced. Further work on the initial identification and management of complications, as well as resources for senior clinicans responsible for external reporting and patient follow up is also in development phase.

Importantly, the context of how we collect haemovigilance data in NSW must considered in all analysis. The NSW IIMS is a volunatry incident reporting system and caution is required when analysising and reporting data. The increasing reporting should be a considered as part of a strong and growing safety culture and will continue to be fostered by the Blood Watch Program.

3B-S06-05

ISTARE DATA 2006–2014 ON RECIPIENTS: VARIATION BETWEEN COUNTRIES IN THE INCIDENCE AND IMPUTABILITY OF TRANSFUSION REACTIONS

C Politis[1], C Richardson[2], J Wiersum-Osselton[3], P Robillard[4], MA Escoval[5], P Renaudier[6], JC Faber[7] and E Wood[8]

[1]Hellenic CDC, Athens, Greece [2]Panteion University department of Statistics, Athens, Greece [3]TRIP National Hemovigilance and Biovigilance Office, Leiden, The Netherlands Antilles [4]HEMA, Montreal, Quebec, Canada [5]Centro de Sangue e da Transplantacao, Lisbon, Portugal [6]Agence Regionale de Sante, Nancy, France [7]Lux Consul Trans., Banglamoung, Thailand [8]Department of Epidemiology and Preventive Medichine Monash University, Melbourn, Victoria, Australia

Background: ISTARE, the International Haemovigilance Network's online database, aims to unify the collection and sharing of information with a view to harmonizing best practices for haemovigilance systems around the world and to allow benchmarking. It incorporates the surveillance of all adverse reactions (ARs) of definite, probable or possible imputability to transfusion of blood components, irrespective of severity.

This study examines the overall incidence of ARs and the grading of their imputability to transfusion and variation between countries over the whole period of the epidemiological surveillance.

Methods: Aggregate data on ARs are recorded by blood component, type of reaction, severity and imputability to transfusion, using internationally agreed standard definitions. Data are submitted confidentially. Rates and ratios of ARs are automatically provided for analysis of national data and charts are produced comparing international data for 1 or more years.

Results: From 2006 to 2014, 149 national sets of annual aggregated data were received from 28 countries (36% outside Europe), referring to 171.3 million blood components issued. The incidence of all ARs was 87 per 100,000 components issued, of which 25% were severe. The most common ARs were allergic (34%) FNHTR (33%) and DSHR (14%). Of 495 deaths (0.28 per 100,000 blood components issued), 55.1% were due to the three ARs related to the respiratory system: transfusion-associated circulatory overload (TACO, 24.5%) transfusion-associated acute lung injury (TRALI, 17.6%) and transfusion-associated dyspnoea (TAD, 13%). Acute hemolytic transfusion reactions (AHTR, 7.2%) were also among the more common causes of death. The distribution of the incidence of ARs by country showed significant variation.

Data on imputability reported for 148,337 ARs were 23% definite, 41% probable and 36% possible. The distribution of the three categories of imputability varied considerably between countries. However, it is noteworthy that a group of seven countries – all EU Member States and thus sharing a common approach for definition of serious ARs – provided relatively homogeneous data on imputability. Existing differences between countries and haemovigilance systems regarding transfusion and reporting practices, and in the grading of imputability and severity, may be attributed to variable compliance with international standard definitions. The extent of under-reporting of ARs may also help to account for this diversity.

Conclusion: In its 9 years of operation, ISTARE has developed into a well-established platform for international discussion of the incidences of transfusion reactions of all levels of severity and imputability. ISTARE may play a significant role in ameliorating the existing substantial variation that exists between countries.

3B-S06-06

TRANSFUSION PRACTICE AND TRANSFUSION-RELATED ADVERSE REACTIONS IN THROMBOTIC MICROANGIOPATHIES: AUSTRALIAN & NEW ZEALAND THROMBOTIC THROMBOCYTOPENIC PURPURA/THROMBOTIC MICROANGIOPATHIES REGISTRY DATA

L Kivivali[1], N Waters[1], L Fox[1], P Blombery[2], Z McQuilten[1], E Wood[1] and S Cohney[1]

[1]Monash University, Melbourne, Victoria, Australia [2]Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia

Background: Patients with thrombotic thrombocytopenic purpura (TTP) and other thrombotic microangiopathies (TMAs) often require substantial transfusion support. Adverse reactions (ARs) during or after plasma exchange (PEx) may occur. However, no Australian data on frequency or severity of these ARs are available.
Aims: To describe current transfusion practice and transfusion-related ARs in patients from the Australian and New Zealand (ANZ) TTP/TMA Registry.
Methods: The ANZ TTP/TMA Registry was established in 2009 and prospectively collects information on demographics, diagnosis, management and outcomes patients with TMAs. Forty-two large hospitals participate, with 258 patients of all types of TMAs enrolled to April 2016. Data from 108 patients with confirmed diagnosis of TTP or atypical haemolytic uraemic syndrome (aHUS) were analysed. The remaining patients include other TMAs and cases with insufficient information to confirm a diagnosis (e.g. missing ADAMTS13 levels).
Results: Patient demographics and results are shown in Table 1. PEx was provided to most TTP and aHUS patients. Just under 20% of TTP and aHUS patients received plasma infusions, suggesting some delay in commencing PEx. Patients not receiving PEx included three aHUS patients receiving plasma infusion only, one TTP patient responding to prednisolone alone, two (1 each TTP and aHUS) were associated with illicit intravenous oxycontin which spontaneously resolved, one aHUS patient administered eculizumab promptly after diagnosis, and two TTP patients for whom there was insufficient data. TTP patients underwent longer PEx treatment than aHUS patients; with one TTP patient receiving PEx on 187 days. Approximately equal numbers of TTP patients received either cryodepleted plasma (CDP) or fresh frozen plasma (FFP), with slightly more receiving a mixture of both; FFP was the most common replacement fluid for patients with aHUS undergoing PEx. TTP patients received a median of almost 62 l of plasma (range 8–578 l), almost four times as much as aHUS patients, with one patient receiving 578 l. Time to commencement of PEx was longer in aHUS patients, and where detail was provided, appeared to be due to patient transfer or diagnostic uncertainty. ARs during or following PEx were recorded for 16 TTP and eight aHUS patients, of which five were severe. ARs for one patient were life-threatening, requiring steroids, antihistamines and admission to intensive care for 4 days. Two other patients suffered anaphylactic ARs requiring adrenaline but subsequent PEx were tolerated with steroid and antihistamine premedication. Another patient suffered transfusion-associated circulatory overload.
Conclusion: Blood product use in patients with TMAs is high, especially in patients with TTP. ARs were reported in one quarter of patients during or following PEx (likely still, however, to be under-reported), five of which were life-threatening. Further exploration of consequences of these reactions and measures to limit PEx-related ARs is warranted. There was variation in type of PEx fluid used. Availability of national Australian guidelines for the management of patients with TTP and other TMA may be helpful to harmonise practice. TTP/TMA Registry findings describing current 'real world' practice are an important point of reference for future clinical and research activities.

Table 1. TTP and aHUS transfusion data from the Australia and New Zealand TTP/TMA Registry

	TTP (n=72)	aHUS (n=36)
Female	54 (75%)	23 (64%)
Age (median) years	41 (range 19 – 79)	31 (range 0 – 68)
PEx	69 (96%)	29 (81%)
Plasma infusion given	13 (18%)	7 (19%)
Days of PEx/infusion (median)	13.5 (range 2 – 187)	7 (range 1 – 58)
Exchange fluid reported to registry (remaining fluid usage unknown)	CDP: 19 (28%) FFP: 22 (32%) Mixture: 27 (39%)	CDP: 0 FFP: 15 (52%) Mixture: 7 (24%)
Total exchange volume (median)	61.5L (range 8L – 578L)	17.5L (range 3.5L – 232L)
Time to PEx (median hours)	8.5h (range 2h – 96h)	19.5h (0 – 2400h)
Of patients receiving PEx, adverse reaction reported	16 (23%)	8 (27%)

Donor Health

3B-S07-01

HEALTH EFFECTS OF BLOOD DONATION AND IRON DEPLETION

H Ullum[1], E Sørensen[1], AS Rigas[1], OB Pedersen[2], H Hjalgrim[3] and C Erikstrup[4]

[1]Copenhagen University Hospital, Copenhagen, Denmark [2]Naestved Sygehus, Næstved, Denmark [3]Statens Serum Institut, Copenhagen, Denmark [4]Aarhus University Hospital, Aarhus, Denmark

Background: Blood transfusion service is an integrated part of modern health care systems. Donation of blood is therefore both frequent and essential and must be safe for the donor. Short term risks include needle injuries and fainting. Long term risks include iron depletion to which women in childbearing age are especially vulnerable. Iron depletion, if severe, leads to anemia, whereas milder forms have been associated with tiredness and neurocognitive impairment in off-sping.
Aims: We want to estimate the risk of iron deficiency, to identify predictors of iron deficiency and to explore clinical consequences of iron deficiency.
Methods: The Danish Blood Donor Study (DBDS) was initiated in 2010 and has now included more than 107,000 participants. The study includes measurements of ferritin, DNA for the testing of genetic risk markers, a plasma repository, and questionnaire data to determine lifestyle associated risk factors (meat intake, alcohol intake, iron supplementation) as well as self-reported health (Short Form 12). In The Scandinavian Donation and Transfusion Database (SCANDAT) we studied mortality of 1,182,495 Swedish and Danish blood donors followed for 9,526,627 person years.
Results: In DBDS we found a high risk of iron depletion (ferritin < 15 ng/ml) among frequent blood donors. The risk was highest among premenopausal women (38%), followed by postmenopausal women (26%) and men (10%). Recorded dietary factors had limited effect on risk of iron depletion. The genetic markers (*HFE*: rs1800562, rs179945 and *TMPRSS6*; rs855791) affect iron levels and may together with other markers be included in future risk stratification. We found that ferritin was the strongest predictor of future decline in hemoglobin. However, iron depletion without anemia did not influence self-perceived physical and mental health. In SCANDAT we estimated that mortality risk was markedly lower among high frequency donors compared to low frequency donors. A statistical adjustment for an internally estimated healthy donor effect reduced the association between high donation frequency and low mortality risk.
Summary/Conclusions: Iron deficiency is common among blood donors. The strongest risk factors are high donation frequency, female sex and, among women, childbearing age. Dietary factors and genetic constitution may also be used in prediction of iron depletion. Iron depleted donors are at risk of a subsequent decline in hemoglobin, however, their self-reported health is not inferior to that of iron replete donors. Although the improved survival among high frequency donors may be caused by a healthy donor effect the data strongly contradicts severe negative effects on survival. Future studies need to focus on more specific health risks (restless leg syndrome, tiredness, concentration problems) and on adverse effects in pregnancy. Blood banks may consider providing targeted iron supplementation. It is still unknown whether blood donation is beneficial for the health of the donor or whether iron depletion poses a significant risk for their future health.

3B-S07-02

A EUROPEAN SURVEY ON DONOR DEFERRAL FOR ALLERGY: RATIONALE AND INITIAL RESULTS OF A TWO-TIME SURVEY IN 36 COUNTRIES

O Garraud[1], A Haddad[2], C Martin[3], Z Anouty[2], M Bagge Hansen[4], G Rautmann[5], S MacLennan[6] and R Norda[7]

[1]INTS, Paris, France [2]Department of Clinical Pathology and Blood Banking, Sacre Coeur Hospital, Beirut, Lebanon [3]Department of General Medical Practice, University Hospital of Saint-Etienne, Saint-Etienne, France [4]Rigshospitalet, Copenhagen, Denmark [5]European Directorate for the Quality of Medicines, the Council of Europe, Strasbourg, France [6]National Blood Service, Leeds, United Kingdom [7]Akademiska Sjukhuset, Uppsala, Sweden

Background: A survey on Allergy and consideration of blood establishments (BEs) for donor/donation acceptance or deferral criteria was conducted under the aegis of the European Committee (Partial Agreement) on Blood Transfusion (CD-P-TS) of the Council of Europe. The initial purpose of the survey was to evaluate practices in different countries/BEs regarding mitigation of any risk related to allergy both in

donors and recipients of the donated components; the secondary purpose was to draft a recommendation to be submitted to the CD-P-TS if needed.

Materials and methods: A survey was prepared and disseminated to representatives of CD-P-TS members states and observers, and other BEs in the Mediterranean region who are neither members nor observers to the CD-P-TS but have implemented French, Belgian, Dutch or German regulatory requirements applied to BEs. It consisted of two consecutive questionnaires. Responses to the first questionnaire indicated that the questions posed were not precise enough as left room for interpretation and the same BE attitude could be quoted differently by respondents. The second questionnaire was presented as a diagram, with decision tree pathways to be circled by responders.

Results: The questionnaire was sent to 43 BE representatives with, to date 25 responses from 31 CD-P-TS members, six from six CD-P-TS observers, and four from six Mediterranean observers (81.39% success rate). Among the 35 replies recorded, based on responses, we were able to stratify into six groups: No policy (3/35; all non CD-P-TS members); donor deferral when documented severe allergy only (22/35); donor deferral when symptomatic allergy (9/35); donor deferral when any type of allergy being reported (3/35); donor deferral when local allergy to anaesthetics, disinfectants or drug being reported (6/35); donor not excluded (1/35) for allergy issues. The rationale behind the policy in most BEs is to protect both donors (26/35 recorded answers) and blood recipients (27/35) even thought the concern of antigen or antibody transfer in the recipient is not consensual (respectively 17/35 and 23/35 admit the transfer).

3B-S07-03

GENETIC FACTORS INFLUENCING HAEMOGLOBIN IN BLOOD DONORS: RESULTS FROM THE DANISH BLOOD DONOR STUDY

E Sorensen[1], AS Rigas[1], LW Thørner[1], KS Burgdorf[1], OB Pedersen[2], SR Kotzé[3], C Erikstrup[3], MS Petersen[3] and H Ullum[1]

[1]Copenhagen University Hospital, Copenhagen, Denmark [2]Naestved Hospital, Naestved, Denmark [3]Aarhus University Hospital, Aarhus V, Denmark

Background: Low haemoglobin (Hb) due to iron deficiency is a common cause of blood donor deferral. Several studies have identified genetic variants associated with Hb or other markers of iron levels. Recently, we found that three specific single-nucleotide polymorphisms (SNPs) had an effect on the ferritin level of blood donors, but not consistently on the risk of iron deficiency (Sørensen et al. Transfusion 2016).

Aims: The current study is part of an effort to establish a panel of SNPs suitable for predicting the risk of low Hb or iron levels. Thus, we investigated the effect of the same set of SNPs on haemoglobin levels on 14,122 Danish blood donors. Knowledge of variants associated with susceptibility to low Hb level and/or iron deficiency could allow individualised bleeding intervals according to genetic risk.

Methods: Samples were collected from Danish blood donors participating in the Danish Blood Donor Study (DBDS). Hb was measured on all donors. DNA was extracted and analysed by LGC Genomics Extraction and Genotyping Service (Hoddesdon Herts, United Kingdom). Six SNPs in four genes were investigated. The studied genes and SNPs were: (a) *TMPRSS6,* encoding Transmembrane protease serine six involved in the regulation of hepcidin: rs855791; (b) *HFE,* associated with hemochromatosis: rs1800562 and rs1799945; (c) *BTBD9*, encoding BTB/POZ domain-containing protein nine associated with restless legs syndrome: rs9357271; (d) *TF,* encoding Transferrin: rs2280673 and rs1830084. For each SNP the effect on Hb levels was assessed by multivariable linear regression analysis. Results are presented as regression coefficients. Multivariable logistic regression analysis was used to assess each SNP as a risk factor for low Hb levels (<8.4 mM for men; <7.8 mM for women).

Results: Results from the linear and logistic regression analyses are shown in the Table 1. The multiple linear regression analysis showed a significant association of the SNPs in *HFE* and in *TMPRSS6* with Hb levels using an additive genetic model. Specifically, the G-allele in rs1800562 and the C-allele of rs1799945 in *HFE* both correlated negatively with Hb levels. The same alleles were associated with an increased risk of low Hb among men, whereas only rs1799945 increased the risk of low Hb among women. In addition, the T-allele of *TMPRSS6* rs855791 was negatively associated with Hb levels in both sexes. This allele increased the risk of low Hb among women, but not among men. None of the genetic variants in *TF* and *BTBD9* showed any association with Hb levels.

Summary/Conclusions: Our study showed that the investigated SNPs in *HFE* and *TMPRSS6* had a significant effect on Hb levels. In addition, all three SNPs in *HFE* and *TMPRSS6* affected the risk of low Hb among either men or women or both. Thus, the SNPs had a more profound impact on Hb than on ferritin levels, for which we previously only found increased risk of iron deficiency associated with the *TMPRSS6* SNP in men only. Our data support that genetic testing may be useful in a future assay for personalised bleeding intervals according to genetic risk.

Table: Impact of specific single nucleotide polymorphisms (SNPs) on haemoglobin levels (Hb) and on the risk of low Hb*

		Men				Women			
Multiple linear regression analysis; outcome: Hb									
Gene	SNP	Coef.	p value	CI		Coef.	p value	CI	
HFE	rs1799945	-0.057	<0.001	-0.08	-0.03	-0.043	<0.001	-0.07	-0.02
	rs1800562	-0.097	<0.001	-0.13	-0.06	-0.061	<0.001	-0.09	-0.03
TMPRSS6	rs855791	-0.039	<0.001	-0.05	-0.02	-0.046	<0.001	-0.06	-0.03
Logistic regression analyses; outcome: Low Hb**									
Gene	SNP	OR	p value	CI		OR	p value	CI	
HFE	rs1799945	1.39	0.006	1.10	1.75	1.22	0.013	1.04	1.43
	rs1800562	1.48	0.023	1.06	2.08	1.16	0.190	0.93	1.45
TMPRSS6	rs855791	1.01	0.940	0.87	1.16	1.18	0.002	1.06	1.32

*Data shown for selected SNPs only; **Men <8.4 mmol/L, women <7.8 mmol/L

3B-S07-04

COMBINED CELL INDEX (CCI) IN THE ASSESSMENT OF IRON STORES IN BLOOD DONORS

T Vuk, J Bingulac-Popovic, T Ocic and I Jukic

Croatian Institute of Transfusion Medicine, Zagreb, Croatia

Background: Iron deficiency is common and important consequence of frequent blood donations, especially in female donors. Ferritin measurement is reliable method for estimating iron stores in blood donors. The sensitivity of red blood cell (RBC) parameters of complete blood count (CBC) in detecting non-anemic iron deficiency is significantly lower. Consequently, there were several attempts to increase the detection sensitivity by combining these parameters in different indices. In 2007, Frank Boulton proposed the application of the CCI (Combined Cell Index) in the assessment of iron stores in blood donors (Boulton, Transfusion Medicine, 2007). This index combines MCV, MCH and RDW and is calculated according to the formula: RDW $\times$ 10^4 $\times$ MCV^{-1} $\times$ MCH^{-1}.

Aims: The aim of the study was to assess the appropriateness of using CCI in the assessment of iron stores in blood donors, as well as its position in relation to other RBC parameters.

Methods: The study was conducted at Croatian Institute of Transfusion Medicine in Zagreb. The study involved whole blood donors (792 women and 1,084 men), accepted for blood donation, including the requirement of minimum haemoglobin level (125 g/l for women and 135 g/l for men), estimated by copper sulphate method. Serum ferritin was determined on Cobas c311 analyzer (Roche Diagnostics, USA), and CBC using Cell-Dyn Ruby (Abbott Diagnostics, USA) haematology analyzer. We have analyzed the correlation between the RBC parameters (RBC, Hgb, MCV, HCT, MCH, MCHC, RDW) and CCI with the serum ferritin. For six parameters with the highest level of correlation relative to ferritin (Hgb, MCV, MCH, MCHC, RDW and CCI) the diagnostic efficacy in the detection of iron depletion (ferritin <12 μg/l) was assessed using ROC analysis with calculation of AUC (Area Under the Curve). Sensitivity and specificity was calculated for the optimal cut-off values (obtained by Youden index).

Results: CCI showed the highest degree of correlation with ferritin ($r = -0.373$ for men and $r = -0.590$ for women) and highest AUC (0.961 for men and 0.864 for women) in evaluating the diagnostic value of different tests for the detection of depleted iron stores. Using the cut-off value of 52.6 for men and 50.6 for women, corresponding Youden index was highest for CCI in both sexes (0.851 for men and 0.612 for women). The sensitivity and specificity of CCI in the population of male donors (0.941 and 0.910, respectively) was higher in comparison to female donors (0.851 and 0.761, respectively). With the cut-off value of 135 g/l for men and 125 g/l for women, the sensitivity of haemoglobin was only 0.177 and 0.228, respectively.

Summary/Conclusions: The results of the study confirm satisfactory diagnostic value of CCI in detecting depleted iron stores in blood donors. The use of CCI could be particularly useful to blood establishments analyzing CBC in all blood donors and measuring ferritin level depending on the results obtained. By adapting blood bank computer software it is possible to achieve the transfer of the results from the haematology analyzer, calculate the CCI and create decision algorithms on further actions.

3B-S07-05

FREQUENCY OF LIFE THREATENING ALLERGIES AMONG BLOOD DONORS

F O'Brien, K Gilmore and M Goldman

Canadian Blood Services, Ottawa, Ontario, Canada

Background: There have been rare cases of passive transfer of food specific hypersensitivity from donors to recipients leading to anaphylactic reactions. In Canada in 2006 a plasma recipient developed an anaphylactic reaction after eating peanut butter 2 days post- transfusion; the donor had a history of severe peanut allergy. In a 2008 donor survey, 40% of donors stated they have allergies; 7.7% stated that they had severe allergies. Recently a recipient developed anaphylactic reactions to salmon and peanuts in the days post-platelet transfusion, one of the donors was found to have a history of severe food allergies.
Aims: To determine if additional screening questions could identify a subset of donors at higher risk of donating blood that puts recipients at risk of hypersensitivity to allergens.
Methods: In October, 2015 as part of a quarterly on-line satisfaction survey, 6,233 blood donors were randomly selected from all regions of Canada except the province of Quebec. All donors had donated within the previous 3 months. Donors were invited by email to participate and provided a link to the on-line survey. They were asked if they had severe (possibly life threatening) allergies. Donors responding affirmatively were asked to select causes and symptoms from lists, and were also asked if they carry and have used a self-injecting epinephrine pen. Weighting factors were applied to the data to ensure regional representativeness. Frequencies of responses were tallied.
Results: There were 1,559 donors who completed the on-line questionnaire (25% response rate). Of these, 5% (82) stated that they had a possibly life threatening allergy. Common causes (more than one response was possible) were medications (44%), peanut or other nut allergies (27%), shellfish or other fish (10%), other food allergies (23%), and insect stings (14%). Common symptoms (more than one response was possible) were swelling (61%), difficulty breathing (53%), itching (53%) and rash (47%). One third of respondents stating that they have life threatening allergies carry an epi-pen all or most of the time, with 17% of these reporting ever using it.
Summary/Conclusions: Self-reported serious allergies are common. Severe food allergies are reported in about 2.7% of donors, correcting for donors with multiple food allergies. Symptoms reported are consistent with severe reactions. A smaller segment of donors carry and have used a self-injecting epinephrine pen, however this is in part related to the allergen (not necessary for medication use) and not to the severity of the reactions. It is difficult to identify a particularly high risk subset of donors without leading to significant deferral of many safe donors.

Young Investigators Scientific Session

3B-S08-01

EFFICIENT THERAPEUTIC FUNCTION ON AUTOIMMUNE DISEASE MODEL: EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE) MICE BY SELECTIVELY INDUCED AND EXPANDED POLYCLONAL HUMAN CD8+ REGULATORY T CELLS

J Sun, YM Yang, XN Huo, XY Jiang, RF Xie, L Gao, YM Zhu, HH Fan and J Yang

Shanghai Blood Center, Shanghai, China

Background: Autoimmune disease (AID) is an immune system disease which develops with slow pace and would cause serious destruction to the human body. It is widely believed that AID is caused by the fact that T cells recognize self antigens and escape normal immune regulation of the body. Although clinical therapy using chemical drugs has a certain positive effect, it also increases the risk of tumors and infections as a side effect. In recent years, research shows that regulatory T cells in AID patients are deficient or disfunctional and transfusing regulatory T cell has an efficient therapeutic function on AID models. However, due to the limited number of natural Treg cells, amplification of Treg cells *in vitro* for adoptive therapy is needed. Unfortunately, the expanded CD4+ Foxp3+ Tregs could transform into effect T cells easily in inflammation. Several approaches for inducing Ag-specific CD8+ Tregs have been reported, but there is currently no reliable protocol for the *ex vivo* inducing and large-scale expansion of human polyclonal CD8+ Foxp3+ Tregs.

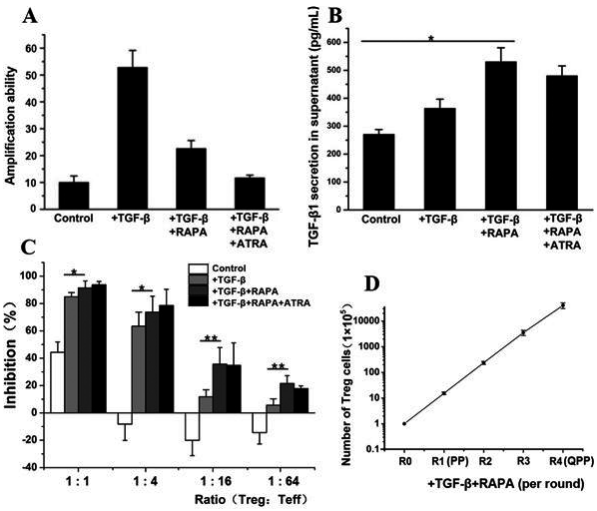


Figure 1. Comparing the induction of CD8+ Treg under different inducing conditions and amplification ability of selected Treg (induced with TGF-β+rapamycin.

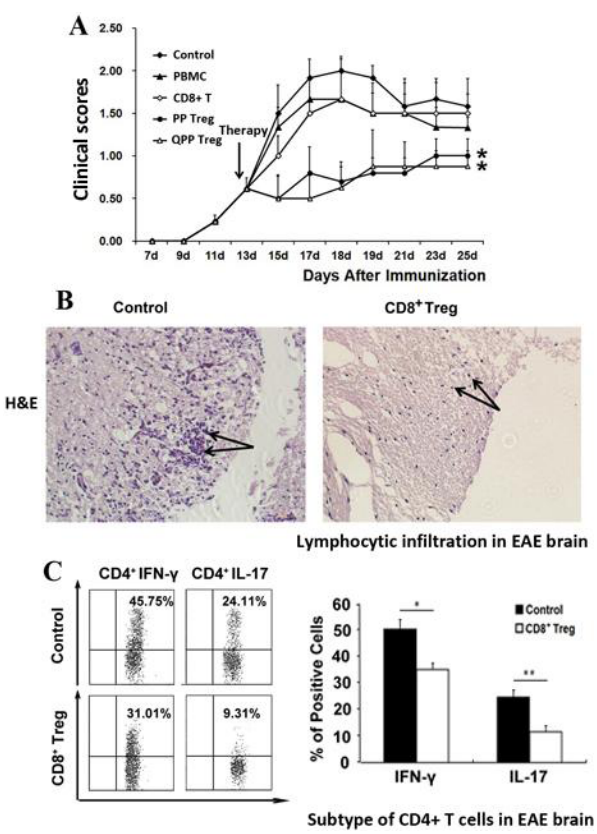


Figure 2. Therapeutic function on EAE mice by *ex vivo* induced and expanded CD8+ Tregs.

Aims: In this study, we compare different inducing conditions on CD8+ Tregs development in order to select an effective method to induce and amplify polyclonal CD8+ Treg cells *in vitro*. We also investigate their therapeutic function on autoimmune disease model: experimental autoimmune encephalomyelitis (EAE) mice.

Methods: Human CD8+ T lymphocytes (isolated from PBMCs) were cultured with anti-CD3/28 beads and IL-2, plus TGF-β1 or TGF-β1 + rapamycin (RAPA) or TGF-β1 + rapamycin+all-trans retinoic acid (ATRA) to induce CD8+ Tregs *in vitro*. The selectively induced CD8+ Tregs were amplified for another three rounds of re-stimulation. The first and the fourth round Tregs (PP and QPP) were transfused into EAE mice. The features and functionalities of the expanded CD8+ Tregs were investigated and EAE mouse model was induced with MOG_{33-55} as an AID model.
Results: CD8+ Foxp3+ Treg could be efficiently induced and expanded (10,000 times at least) *in vitro* and has an efficient therapeutic function on EAE mice. Through comprehensive consideration of amplification ability, secretion of inflammatory cytokines, expression of Foxp3 and suppression capacity, we found TGF-β1 + rapamycin were the most appropriate induced condition. The obtained CD8+ Tregs (TGF-β1 + rapamycin induced) expressed high level of Foxp3 (up to 90%), CD25, and CD103, secreted small amount of IL-2, IL-10 and TGF-β, did not secret IL-17A, and were stable in inflammatory conditions which were different from the plastic characteristic of expanded CD4+ Tregs in inflammation. The expanded Tregs adopted vigorous suppression function on CD4+ CD25− effect T cells (mainly dependent on cell contact) and anergy ability *in vitro*. In EAE mice, both PP and QPP (the first round and the fourth round) CD8+ Foxp3+ Tregs treatments could significantly alleviate the severity of disease. The disease scores and lymphocytic infiltration in mice brain were reduced after adoptive transfusing with CD8+ Treg. Moreover, CD8+ Treg treatment can significantly down-regulate both IFN-γ+ CD4+ T cells and IL17A+ CD4+ T cells in lymphocytic infiltration, revealing the fact that through this way the damage on brain tissues can be reduced.
Conclusion: The results revealed that human CD8+ Treg cell could be selectively induced and expanded *in vitro* and such cell has an effective therapeutic function on EAE mice. This research can provide a novel cell for efficient cell therapy on autoimmune disease and improve the utilization of blood components.

3B-S08-02

ROLE OF FCG-RECEPTORS IN IGG-ANTIBODY MEDIATED RED BLOOD CELL CLEARANCE AND IMMUNE SUPPRESSION

TC Stegmann[1], HR Waterman[2], A Bentlage[1], L Kapp[2], S Lissenberg[1], R Plomp[3], M Wuhrer[3], RBM Schasfoort[4], G Vidarsson[1], E van der Schoot[1] and JC Zimring[2]
[1]*Sanquin, Amsterdam, The Netherlands* [2]*Bloodworks Northwest, Seattle, Washington, United States of America* [3]*LUMC, Leiden, The Netherlands* [4]*Universiteit Twente, Enschede, The Netherlands*

Background: Alloantibodies against red blood cells (RBC) mediate rapid clearance of RBC, causing hemolytic transfusion reactions and hemolytic disease of the newborn. Passive administration of allo-antibodies against RBC can prevent humoral immune response, a phenomenon exploited in Rh-immunoprophylaxis. It is generally thought that Rh-Ig induces rapid clearance of circulating fetal D-positive RBC, by binding to activating IgG-Fc receptors (FcgR), preventing recognition of these cells by the immune system. However, how the different FcgRs are involved in RBC clearance and/or antibody-mediated immune suppression is not known.
Aim: To determine which FcγR mediate RBC clearance and promote immune suppression in a mice.
Methods: Anti-KEL1, V-gene matched, mouse IgG1, IgG2a, IgG2b and IgG3 were engineered as well as IgG1 and IgG2a with low core-fucosylation. Wild-type (WT) mice or mice lacking the common FcγR-signaling chain (Fc-g-chain$^{-/-}$) were passively immunized with these anti-KEL1 IgG antibodies followed by transfusion with DiI-labeled mouse-RBC expressing human KEL1, mixed with DiO-labeled WT-RBC. The survival of the incompatible KEL1 RBCs was determined as a function of survival of compatible WT RBCs using the DiI:DiO ratio in each animal. Mice received a booster transfusion at day 14 and to measure anti-KEL1 specific immune responses, sera were collected at day 6 and 21 post RBC transfusion. Specific anti-KEL IgG and IgM antibodies were measured by flowcytometry. Binding affinity of all IgGs used in this study to all FcgRs was measured by SPR. This SPR measures the interaction of monomeric IgG with FcγR and therefore does not necessarily reflect the *in-vivo* situation where FcgR interact with IgG-coated RBCs. Closer modelling of the *in-vivo* situation was achieved with our new cellular-SPR-imaging (cSPRi) technique.
Results: Clearance was not observed in IgG3 immunized mice nor in any of the Fcg-chain$^{-/-}$ mice, indicating that activating FcγR are indispensable for clearance. IgG2b caused only limited clearance while IgG1 and IgG2a both induced strong and similar clearance. IgG1 and IgG2a with decreased fucosylation induced less RBC clearance, whereas hypofucosylation did not affect the affinity of monomeric IgG1 to any FcγR, and even increased the affinity of IgG2a for FcγRIV. However, these affinities do not reflect the binding strength between FcγR and RBC-bound IgG. In particular, deposition of IgG on the surface of RBCs was found to have profound effects on the interaction with FcγRIIb. Upon opsonization, IgG2a bound the inhibitory FcγRIIb only after hypofucosylation, which also enhanced the avidity of IgG1 for FcγRIIb. Altogether our findings support a model in which activating FcγRIII is the predominant receptor for clearance of anti-KEL1-opsonized RBC, and FcγRIIb inhibits this clearance. Next immune responses following passive immunization were studied. While IgG1 induced immune suppression, IgG2a was immune enhancing. This immune-enhancement by IgG2a dissipated completely in Fc-γ-chain$^{-/-}$ mice, suggesting that this effect is mediated by the activating FcγR, while immune-suppression is mediated by FcγRIIb.
Conclusion: In mice RBC are cleared mainly via FcγRIII while interaction with FcγRIIb inhibits clearance. Antibody-mediated RBC clearance does not predict the immunomodulatory effect. Subtle changes in IgG-subclass composition and glycosylation may affect RBC clearance and immunomodulating capacity of IgG antibodies. These findings may have direct implications for the design of recombinant anti-D IgG for immunoprophylaxis.

3B-S08-03

RED BLOOD CELL METABOLIC RESPONSES DURING BLOOD BANK STORAGE UNDER MILD AND ACUTE HYPOXIA

A D'Alessandro[1], T Nemkov[1], RC Hill[1], KC Hansen[1] and Y Tatsuro[2]
[1]*University of Colorado Denver, Aurora, Colorado, United States of America* [2]*New Health Sciences Inc, Cambridge, Massachusetts, United States of America*

Background: Despite reassuring evidence from randomized clinical trials supporting the safety and efficacy of red blood cell (RBC) transfusion, erythrocytes stored under blood bank conditions undergo a significant number of biochemical and morphology alterations, collectively referred to as the storage lesion. While the clinical significance of the storage lesion is still matter of debate, hypoxic/anaerobic storage of RBCs has been proposed to mitigate the storage lesion. Deoxygenation of packed RBCs decreases stored RBC hemolysis, morphological lesions, osmotic fragility and alterations to the cytoskeletal proteome. Anaerobic storage of RBCs resulted in beneficial effects on energy metabolism, likely promoted by the oxygen-dependent metabolic modulation phenomenon, which involves competitive binding of deoxyhemoglobin and rate limiting glycolytic enzymes to the N-terminal cytosolic domain of band 3. Recently, we demonstrated that alkalinization induced by anaerobiosis only explains part of the beneficial advantages in terms of energy metabolism in hypoxic stored RBCs, while promotion of energy metabolism through increased glycolytic fluxes may compromise the antioxidant potential of hypoxic RBCs.
Aims: Deoxygenation promotes energy metabolism of stored RBCs, but SO2% windows that maximize such benefits have not been determined. Beneficial effects are anticipated in response to exposure of packed RBCs to mild/acute hypoxia in a dose dependent fashion. Tracing experiments with $^{13}C_{1,2,3}$-glucose will reveal whether hypoxic conditions entirely compromise RBC antioxidant capacity by promoting a complete shutdown of the pentose phosphate pathway or rather prevent oxidative stress through the activation of unanticipated pathways.
Methods: A paired study (n = 4) was designed to investigate the effects of % O_2 on log4 leukoreduced stored RBC metabolism. Units were stored either under control conditions (~60% SO_2), hyperoxia (+90% SO_2) or mild to acute hypoxia (20, 10, 5, and <3% SO_2). $^{13}C_{1,2,3}$-glucose was spiked into the Additive Solution three to monitor metabolic fluxes through UHPLC-MS metabolomics on a weekly basis until storage day 42 and redox proteomics at day 2 and 42.
Results: Omics technologies, in particular proteomics and metabolomics have contributed significant advancements in our understanding of the storage lesion. Here, metabolomics and proteomics technologies were adopted to investigate RBC storage under mild/acute hypoxic conditions. Glucose oxidation through glycolysis was promoted in a hypoxic-dose-dependent fashion, resulting in the preservation of higher than normoxic control levels of 2,3-DPG and ATP throughout storage. Tracing experiments with $^{13}C_{1,2,3}$-glucose showed that activation of the NADPH-generating pentose phosphate pathway was proportional to oxygen saturation levels, and it was highest at +90% SO_2, and lowest, but still significantly active at <3% SO_2. Consistently, anaerobic RBCs had higher NADPH/NADP+ ratios, decreased hemolysis, methemoglobin and irreversible hemoglobin oxidation at functional His93 and Cys94 of beta hemoglobin. In addition, increased total GSH and GSH/GSSG ratios, and decreased supernatant urate oxidation are suggestive of suggestive of decreased oxidative stress and increased total antioxidant capacity in hypoxic/anaerobic RBCs. Tracing experiments indicate that GSH synthesis, an ATP-dependent process, was inversely proportional to SO_2 in a dose-dependent fashion
Conclusions: Hypoxia ameliorates the energy and oxidative metabolic lesion in a dose-dependent fashion, with an optimal window within 10–5% SO_2.

3B-S08-04

A COMPARISON OF ADDITIVE SOLUTIONS (SAG-M, AS-3 & ESOL-5) FOR STORAGE OF THAWED DEGLYCEROLISED RED CELLS

K Winter, RG Webb, L Johnson and DC Marks

Australian Red Cross Blood Service, Sydney, Australia

Background: The Australian Red Cross Blood Service has been routinely freezing and deglycerolising red cells for several decades to preserve rare phenotypes and for anti-RhD immunisation. However, deglycerolised cells are resuspended in SAG-M, which limits the shelf-life to 24 h due to high haemolysis. Use of an alternative additive solution, such as AS-3 or ESOL-5 may allow an extension of this shelf-life.
Aim: The aim of this study was to compare the quality of deglycerolised red cells stored in additive solutions, SAG-M, AS-3 and ESOL-5, to extend the shelf-life beyond 24 h.
Methods: Three ABO/RhD-matched, leukocyte-depleted red cells in SAG-M (day 7 post-collection) were pooled and split for a three-arm study (n = 9 replicates). A sample of the pooled red cells was taken as a pre-freeze baseline measurement. The red cells were glycerolised with approximately 40% glycerol, using an ACP-215 cell washer and frozen at −80°C. For each replicate, three matched components were thawed and deglycerolised using the ACP-215 cell washer, then resuspended in equivalent volumes (30 or 40 ml depending on unit size) of either SAG-M, AS-3 or ESOL-5. Units were stored at 2–6°C and sampled on day 0, 1, 3, 7, 10 and 14, or until haemolysis exceeded 0.8%. Samples were tested using a range of *in vitro* assays. A two-way repeated measures ANOVA was performed to compare data from deglycerolised red cells in each of the three-arms on day 0, 1 and 3. Due to high haemolysis in the SAG-M group, sampling was not continued and therefore a second two-way repeated measures ANOVA was performed comparing data for AS-3 and ESOL-5 on day 0, 1, 3, 7, 10 and 14. A $P < 0.05$ was considered significant.
Results: There were no significant differences in the pre-freeze parameters of the red cells in the three-arms. Red cells stored in SAG-M exceeded the upper limit of haemolysis (<0.8%) on day 3 post-thaw, compared to day 10 for red cells in AS-3 and day 14 for red cells in ESOL-5. Post-thaw, there were statistically significant differences in MCHC ($P = 0.0264$), pH ($P < 0.0001$), potassium concentration ($P = 0.0155$) and glucose concentration ($P < 0.0001$) between the three resuspension solutions. Further, the haematocrit ($P = 0.004$), MCHC ($P = 0.0006$), pH ($P = 0.0012$) and glucose concentration ($P < 0.0001$) were different between the AS-3 and ESOL-5 groups. Interestingly, the 2,3-DPG concentrations were not significantly different between the groups, possibly due to the age of the red cells prior to freezing. Red cells resuspended in ESOL-5 maintained a higher concentration of ATP than AS-3 red cells on days 10 and 14 post-thaw.
Summary/Conclusions: Alternative additive solutions, AS-3 and ESOL-5, could allow extension of the shelf-life for deglycerolised red cells to between 10 and 14 days. In particular, lower haemolysis and LDH concentrations, together with a higher ATP concentration in the red cells stored in ESOL-5 may allow extension of the post-thaw shelf-life to 14 days.

Additive	SAG-M		AS-3				ESOL-5			
Day post-thaw	0	3	0	3	7	14	0	3	7	14
Haemolysis (%)	0.2 ± 0.1*	0.7 ± 0.3*†	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.7 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.2
2,3-DPG (μmol/g Hb)	4.7 ± 2.8	0.9 ± 0.8	4.8 ± 2.8	0.6 ± 0.7	0.0 ± 0.0	0.0 ± 0.0	4.5 ± 2.6	0.9 ± 0.9	0.1 ± 0.2	0.0 ± 0.1
ATP (μmol/g Hb)	5.0 ± 0.4	4.4 ± 0.5	4.9 ± 0.6	4.1 ± 0.5	3.2 ± 0.5†	2.4 ± 0.4†	4.8 ± 0.7	4.2 ± 0.5	3.8 ± 0.5	3.1 ± 0.5

* p-value < 0.05 compared to AS-3
† p-value < 0.05 compared to ESOL-5

Caption 1. Post-thaw characteristics

3B-S08-05

PREVALENCE AND IMPACT OF ANTI-CYTOKINE AUTOANTIBODIES IN HEALTHY BLOOD DONORS – RESULTS FROM THE DANISH BLOOD DONOR STUDY

J Hjorth von Stemann, M Bagge Hansen, H Ullum, L Wegner Thoerner and A Stribolt Rigas

Copenhagen University Hospital, Copenhagen, Denmark

Background: Natural cytokine-specific auto-antibodies (c-aAb) are novel players in terms of immune dysfunction, and their presence in blood and capacity for neutralization of cytokine function are well established. Such cytokine neutralization may result in lacunar immune deficiencies, and several c-aAb specific for pro-inflammatory cytokines correlate with increased susceptibility to opportunistic infections. C-aAb are found in the plasma of both patients and healthy donors, in donor-derived IgG pools, and may be transferred through transfusions or induced through cytokine therapy. The precise etiology of c-aAb etiology remains undefined.
Aims: We aim to characterize the prevalence of c-aAb in healthy blood donors, to identify predictors of elevated c-aAb levels and to determine the impact of c-aAb on blood donor and patient health.
Methods: C-aAb levels were detected using a validated assay for the Luminex 100 platform. Briefly, plasma samples from the Danish Blood Donor Study were incubated with cytokine-conjugated MagPlex beads (Luminex corp.), followed by addition of PE-tagged anti-human secondary antibody. Signal specificity was validated by signal displacement through pre-incubation of samples with excess free cytokine, or pre-incubation of cytokine-conjugated beads with excess cytokine-specific antibody. Following c-aAb screening, linear and logistic regression analyses were used to investigate c-aAb correlations to epidemiological and biochemical parameters. Blood donors with highly elevated C-aAb will be further monitored across consecutive donations. C-aAb-mediated functional cytokine neutralization was investigated by correlating c-aAb levels with donor self-perceived health, prescription history and C-reactive protein (CRP).
Results: Because blood donors represent a selected healthy population c-aAb did not correlate with overt pathology; however, we observed several indications of a possible c-aAb-mediated functional impact. High levels of IL-6 specific c-aAb correlated with threefold increased odds of undetectable levels of CRP ($P = 0.005$), an inflammation maker known to be induced by IL-6. IL-1α specific c-aAb correlated with significantly reduced scores of self-perceived physical health for the youngest donors (age = 18–39 years, $P = 0.001$). C-aAb specific to anti-inflammatory IL-10 were correlated with reduced likelihood of several antibiotic prescriptions ($P = 0.02$–0.05). Advancing age was a common predictor of IL-1α, IL-6, IL-10 and GM-CSF c-aAb ($P = 0.001$–0.007), and IL-1α and IL-10 were associated with men ($P = 0.006$–0.025).
Together these data suggest a varied set of predictors and functional impact for individual c-aAb in healthy donors.
Conclusions: Cytokine-specific autoantibodies are a common feature in healthy Danish blood donors, and may reach levels that impact cytokine function. This makes them a possible factor in the development of transfusion-related immunomodulation, and a relevant component of screenings of immunodeficient patients.

3B-S08-06

ACUTE NORMOVOLEMIC HEMODILUTION IN MAJOR ORTHOPAEDIC SURGERY

N Bansal, G Kaur, S Garg and S Gombar

Government Medical College and Hospital, Chandigarh, India

Background: Allogeneic blood transfusion is associated with the risk of transmission of infectious agents, transfusion related immunomodulation among others. With increasing concerns about the newer emerging pathogens, alternatives to allogeneic blood transfusion are being explored and acute normovolemic hemodilution (ANH) is one of them.
Aims: 1. To determine the safety and efficacy of ANH in patients undergoing total hip replacement (THR)/total knee replacement (TKR).
2. To determine effect of ANH on allogeneic blood requirement, clinical outcome and length of hospital stay in patients undergoing THR/TKR.
Methods: A prospective interventional study was conducted from March 2014 till September 2015 in patients undergoing TKR/THR. A total of 50 patients were assigned to ANH group (n = 25) or control group (n = 25). Patients aged 18–65 years, having American Society of Anesthesiologists physical status I/II, with a preoperative hemoglobin of more than 12 g/dl were included in the study. Hemodilution was performed in patients of ANH group with ringer lactate as the replacement fluid. Patient vitals were constantly monitored during phlebotomy and during operation. Blood sample was withdrawn preoperatively, after completion of hemodilution, after completion of surgery, 24 h after completion of surgery and at discharge from hospital and evaluated for hemoglobin, hematocrit, platelet count, coagulogram (PT, PTI, aPTT, INR). Patients were followed up till discharge from hospital.
Results and discussion: Hemoglobin levels decreased from 10.4 ± 0.7 g/dl at completion of surgery to 10.0 ± 0.7 g/dl, 24 h after completion of surgery in the ANH group which was less as compared to control group in which hemoglobin levels decreased from 11.1 ± 0.6 g/dl at completion of surgery to 10.0 ± 0.6 g/dl, 24 h after completion of surgery, as shown in Table 1. This can be attributed to higher postoperative bleeding in control group as compared to ANH group. All parameters

PARAMETER	ANH Group	Control Group	P value
Allogeneic Blood Transfusion (number of patients)	4	15	0.001
Post operative complication (number of patients)	7	16	0.01
Length of hospital stay (days)	10±3	12±3	0.06

Table 1. Transfusion and patient outcomes

	Hemoglobin (g/dL)		
Stage	ANH group	Control group	p-value
Pre-operative	12.7±0.7	12.6±0.6	0.56
After completion of hemodilution	11.6±0.4	-	-
After completion of surgery	10.4±0.7	11.1±0.6	0.0002
24 hours after completion of surgery	10.0±0.7	10.0±0.6	0.72
At discharge	10.8±0.6	11.1±0.5	0.17

Table 2. Changes in mean hemoglobin of the patients

of coagulogram, although within normal limits, were more deranged in control group as compared to ANH group ($P < 0.05$), 24 h after completion of surgery. As fresh clotting factors are returned at wound closure in ANH there appears to be better replenishment of clotting factors in ANH group. ANH did not produce any adverse effects on systolic BP (122 ± 6 vs 121 ± 7 mm Hg, $P = 0.38$), diastolic BP (81 ± 4 vs 79 ± 5 mm Hg, $P = 0.10$), heart rate (76 ± 6 vs 78 ± 7, $P = 0.30$) or oxygen saturation (98.6 ± 0.8 vs $98.5 \pm 1.1\%$, $P = 0.91$), neither during phlebotomy, and neither during operation. Allogeneic blood requirement was significantly less in ANH group as compared to control group (4 vs 15, $P = 0.001$). Postoperative complications were significantly lower in ANH group as compared to control group (7 vs 16, $P = 0.01$). This can be attributed to avoidance of immunomodulatory effect of allogeneic blood transfusion as a result of ANH. Length of hospital stay was 10 ± 3 days in ANH group as compared to 12 ± 3 days in control group. Although it was statistically not significant ($P = 0.06$), but even reduction of 2 days, significantly reduces cost of treatment for the patient and also increases the bed turnover rate of hospital.

Conclusions: ANH is a safe, efficacious and cost effective procedure, with decreased allogeneic blood requirement and decreased post operative infection rate in patients undergoing THR/TKR. It is of increased value in developing countries like India where chances of transfusion transmitted infections are still high because of lack of nucleic acid testing in majority of blood banks, and where there is high demand of blood and strain on the blood bank inventory.

Platelets and HLA

3B-S09-01

ACID-TREATMENT CREATES HLA-DEFICIENT PLATELETS THAT REMAIN FULLY FUNCTIONAL BUT ESCAPE IMMUNE DESTRUCTION BY HLA ANTIBODIES

S Meinke[1], C Karlström[1,2], A Mörtberg[2], P Sandgren[3], E Refsum[2], I Douagi[1], A Wikman[2,3] and P Höglund[1,3]

[1]Department of Medicine Huddinge, Karolinska Institutet [2]Department of Laboratory Medicine, Karolinska Institutet [3]Clinical Immunology and Transfusions Medicine, Karolinska University Hospital

Platelet transfusion refractoriness, the repeated failure to achieve an adequate post-transfusion platelet count increment, represent a challenge in platelet transfusion management. When refractoriness is immune-mediated, antibodies against HLA class I antigens are the most common cause. Such antibodies bind to HLA class I on the surface of the transfused platelets and mediate their rapid clearance from the circulation. Activation of the complement system and phagocytosis by Fc receptors-expressing cells are supposed to be the main mechanisms of antibody-mediated platelet destruction.

Refractoriness of immunized patients can be overcome by transfusion of HLA-matched donor platelets. A retrospetive anlaysis of HLA-matched platelet transfusions in our clinic between 2007 and 2015 suggests that as a group, matched transfusions gave a better average response than random donor platelets, but the variation was large and approxiately 25 % of all transfusions failed to raise the platelet count. Moreover, the complexity of the HLA system makes it difficult to find matched platelets for all patients within a limited donor pool, and less than 20 % of all transfusions in our cohort represented a complete match.

A different approach to overcome platelet refractoriness caused by HLA antibodies may be to create platelets that lack HLA molecules on their surface. A short treatment at low pH (pH 2.9) removes the bound peptide and the associated β2-microglobulin, leaving a denatured heavy chain on the cell surface. Contradictory results have been published regarding the efficacy of HLA removal on platelets and the function of acid-treated platelets. It has also not been shown whether acid treatment protects platelets from complement activation and phagocytosis in the presence of anti-HLA antibodies.

We confirmed that a short acid treatment indeed removed between 70 and 90 % of the native HLA class I complexes from the platelet surface. Acid-treated platelets showed an increased expression of activation markers, but still responded to stimulation with further up-regulation reaching similar expression levels as stimulated untreated platelets. The ability of acid-treated platelets to aggregate in response to different stimulating agents was comparable to untreated platelets. Furthermore, acid-treated platelets were protected from complement lysis in the presence of anti-HLA antibodies and anti-HLA antibody-mediated phagocytosis was clearly reduced. Our data suggest that acid-treated platelets may become an option for transfusion to HLA-immunized patients, as a complement or an alternative to HLA-matched donors.

3B-S09-02

DENGUE VIRUS REPLICATION IN PLATELETS

ELG Pryzdial, MR Sutherland and AY Simon

Canadian Blood Services/University of British Columbia, Vancouver, British Columbia, Canada

Background: Dengue virus (DENV) infections cause more than 100 million cases of febrile illness annually. Although most are self-dissipating and excruciating, approximately 2 million of these will further escalate to life-threatening hemorrhagic fever or shock syndrome. More than 200 million high titer infections are asymptomatic and a risk to blood systems even in non-endemic areas due to global travel. Transfusion transmission is well documented.

A hallmark of both mild and severe forms of DENV pathology is thrombocytopenia. To begin to understand the mechanism of reduced platelet count, virus-induced shape change and apototic transitions have been documented. Further evidence for a DENV-platelet interaction comes from observations of semi-purified DENV in the platelet pellet after centrifugation and virus-like particles reported in platelets of an infected patient. These findings imply direct virus-platelet binding with the possibility of cell entry. Interestingly, platelets contain the necessary sub-cellular organelles for translating an RNA template. Like all members of the Flavivirus genus (e.g. Zika virus and West Nile virus), DENV has a positive-sense single-stranded RNA (ssRNA) genome. Therefore we hypothesized that DENV engages specific receptors on the platelet surface and is permissive to entry and production of virus progeny.

Aims: To quantify direct DENV-platelet binding, identify the DENV receptors on platelets, and examine the replication of DENV by purified platelets and platelet concentrates.

Methods: Highly purified DENV serotype 2 binding to purified platelets was measured by following the trypsin-releasable virus genome copy number remaining associated with the platelet pellet after centrifugation using quantitative reverse transcription-PCR (qRT-PCR). Western blots, qRT-PCR and classic cytolytic virus plaque forming assays on vero cell mono layers were conducted to follow translation of a virus-encoded protein (NS1), replication of the viral genome and production of infectious progeny, respectively. Immunoinhibiton assays were conducted to determine the presence of putative DENV receptors. Additionally, virus was spiked into donor-derived, leukoreduced platelet concentrates (PCs) produced at the Canadian Blood Services-affiliated, Network Centre for Applied Development and stored according to SOP. Infectious DENV or DENV RNA was detected using cytolytic plaque assays or quantitative qRT-PCR over storage, respectively.

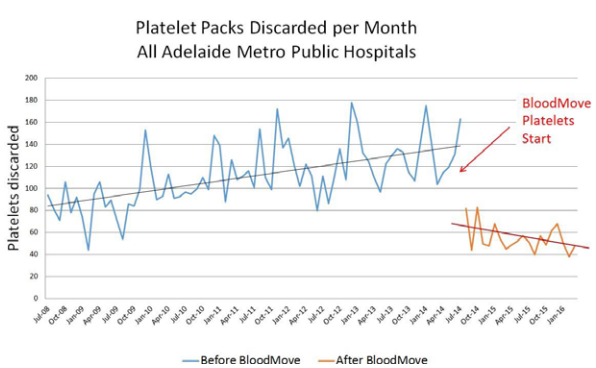
Results: Approximately 800 DENV specifically bound per platelet using a DC-SIGN and heparan sulfate proteoglycan co-receptor system. NS1 and new copies of virus genomic RNA were generated in the presence of viable platelets. When leukoreduced PCs were inoculated with purified DENV at a titer consistent with asymptomatic donors (~10^5 infectious units/ml), replication of viral genome by as much as 10-fold was also observed. For both purified platelets and PCs, viable DENV underwent spontaneous logarithmic decay. Using a protein translation inhibitor, cycloheximide, new virus progeny was confirmed, which off-set the rate of intrinsic DENV decay for both purified platelets and PCs.
Conclusions: Platelets directly bind DENV, permit entry and produce infectious virus. This finding adds to our understanding of thrombocytopenia during DENV infection. While DENV persisted through the duration of PC storage, the identification of logarithmic decay draws attention to the possibility that older units may be less infectious.

3B-S09-03

A SUCCESSFUL AND SUSTAINED MULTI-SITE PLATELET WASTAGE MINIMISATION PROGRAM

R Tocchetti[1], D Roxby[1], R Sinha[2] and S Ireland[2]
[1]SA Pathology, Adelaide, Australia [2]SA Health, Adelaide, Australia

Background: Blood product wastage minimisation is a stewardship obligation for all transfusion laboratories and hospitals and requires conscientious efforts to achieve this goal. Platelet inventory management and wastage minimisation forms part of this stewardship and poses significant challenges due to the limited product shelf life.
In Australia, government health services purchase blood products from the sole Australian blood supplier, the Australian Red Cross Blood Service (ARCBS) which is a non-government organisation.
Aim: For the financial year 2012/13, the South Australian platelet wastage rate was 17% which equated to an approximate cost of AUD 900,000. The South Australian BloodMove Platelets Project aim was to develop a sustainable city-wide multi-site process to significantly decrease platelet outdating and associated costs.
Method: The project commenced in August 2014 after initial planning, preparation and education and was followed by a staged implementation, consisting of the:
Routine transfer of near expiry platelets from low use hospital sites to high use sites
Establishment of a metropolitan-wide common Day 5 platelet inventory to be used by all sites prior to using available fresher platelets or ordering from the ARCBS
Results: Within the first month of operation, the Project achieved a significant drop in the platelet wastage rate for metropolitan public hospitals from a typical 21.5% in July 2014 to 10.7% in August 2014. This decrease has been sustained for 20 months, post Project with a discard rate of 7.6% [95% Confidence Interval (CI) 7.1–8.3]. The average number of platelets discarded per month post Project is 55 compared to 130 prior to the project representing a decrease in discard of 57%. The overall reduction in discarded platelets is shown in the graph.
Other key performance indicators of the Project were the median number of days with zero discards per month which increased to from pre Project of 3 [Interquartile range (IQR) 1–4] to post Project of 10.5 (IQR) 9–13 and the median number of units discarded per day deceased from 4 (2–7) pre Project to 1 (0–3) post Project.



Graph 1. Platelet Packs Discarded per Month – All Adelaide Metro Public Hospitals

Summary and Conclusions: The transfer of near expiry platelets from low use sites to high use sites proved beneficial. This practice whilst maintaining standby stocks at the low use sites for emergency situations also resulted in wastage rates approaching zero.
The sourcing of platelets for elective transfusion from the multi-site Day 5 inventory prior to using available fresher platelets or ordering from the ARCBS resulted in significant wastage reduction. This practice was also extended to ABO cross group issues, i.e. pooled or low titre apheresis Day 5 group O platelets were issued to non-group O patients instead of using fresher ABO specific platelets.
Through detailed planning, education, collaboration and support, the BloodMove Platelets Project has been extremely successful, resulting in significant and sustained reduction of platelet wastage and financial savings across all public metropolitan Adelaide hospitals.

3B-S09-04

LEUKOCYTE CYTOKINES DOMINATE OVER PLATELET CYTOKINES OVERTIME IN NON-LEUKOREDUCED PLATELET COMPONENTS

O Garraud[1], C Aloui[2], T Chakroun[2], J Fagan[3], S Jemni Yacoub[4], F Cognasse[3] and S Laradi[3]
[1]INTS, Paris, France [2]University of Lyon-Saint-Etienne, Saint-Etienne, France [3]EFS Rhone-Alpes-Auvergne, Saint-Etienne, France [4]Centre régional de transfusion sanguine, CHU Farhat Hached, Sousse, Tunisia

Background: Leukoreduction of blood components 'of which platelet components' is strongly encouraged but not yet universal, especially in countries presenting with medium level income such as Tunisia.
Aims: As both leukocytes and platelets secrete copious amounts of pro-inflammatory cytokines/chemokines under various conditions and during storage, we aimed at investigating the potential of their respective secretory programs in platelet components obtained from Tunisian donors.
Methods: A total of 158 non-leukoreduced Standard Platelet Concentrate (SPC) prepared from whole Blood donation using the PRP method were analysed. For each SPC two samples were taken aseptically (one at the day of preparation: D0 and one at the day of delivery) and tested for characteristic biological response modifiers (BRMs) of leukocytes (IL-1β, IL-8), platelets (sCD62P, sCD40L) and both cell types (TNF-a, RANTES) in the presence or absence of thrombin stimulation and at different shelf life times (Day 0 to 5). BRMs were assayed using ELISA and Luminex technologies.
Results: Leukocyte- and platelet-associated BRMs appeared in clearly distinct profiles both at the onset (Day 0) and termination (Day 5) of the observation period but shifted during the intermediate preservation periods to invert their respective importance; in fact, the profiles were likely merged and indistinguishable on days 2–3. The leukocyte-derived BRMs largely dominate over platelet-derived ones and further alter the BRM platelet secretion program.
Conclusion: Herein we have showed that in non-leukoreduced PC, the leukocyte-derived BRMs largely dominate over platelet-derived ones and further alter the BRM platelet secretion program. Thus, to prevent BRMs related transfusion adverse events, we recommend to do not transfuse non-leukoreduced PC aged more than 3 days particularly for critical ill patients.

Malaria

3B-S10-01

PREVENTION OF TRANSFUSION TRANSMITTED MALARIA

S Owusu-Ofori[1], JP Allain[2] and AK Owusu-Ofori[3]
[1]Komfo Anokye Teaching Hospital, Kumasi, Ghana [2]University of Cambridge, Cambridge, United Kingdom [3]School of medical Science, Kwame Krumah University of Science & Technology, Kumasi, Ghana

Transfusion transmitted malaria (TTM) occurs when the plasmodium found in transfusion recipient and the blood donor are genotypically identical. All five species of Plasmodium including *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi* are known to cause TTM. Transfusion transmission of Plasmodium has been estimated between 14% and 28% in regions, where >50% of the population carries the parasite. Most recipients of blood transfusions living in malaria-endemic areas are

semi-immune to malaria. Malaria transmitted via blood transfusions to nonimmune recipients can be rapidly fatal. Young infants in endemic areas without repeated exposure to the parasite may be regarded as non-immune recipients thus susceptible to TTM. The presentation and clinical severity of transfusion-transmitted malaria may differ between malaria endemic countries and non-endemic countries. Some elements critical to outcomes in recipients include parasite load transfused, patient anti-*Plasmodium* titre pre-transfusion, percent clearance of parasites and level of anti-*Plasmodium* humoral immune response.

Several strategies have been tried including Donor deferral, Screening, Prophylaxis and presumptive treatment and now Pathogen inactivation. Transfusion services in non-endemic countries use travel history and serologic tests to identify donors at risk of transmitting malaria. In the absence of an FDA licensed test for donor screening in the USA; deferral of donors who have had a malaria infection or possible exposure risk to malaria are measures to prevent TTM. This is elicited with a donor questionnaire to accurately identify donors with a potential to transmit malaria and hence deferred for varying periods of time.

WHO recommends that donated blood be tested for malaria 'where appropriate and possible' but the stark reality is an absence of practicable, affordable and suitably sensitive screening tests for endemic countries. Such countries facing perennial blood shortages; with introduction of testing could eliminate and drastically reduce potential donor numbers thus affect the donor base negatively and compounding situations. Malaria microscopy, frequently used and referred to as gold standard in SSA for screening blood has limited sensitivity. Testing using PCR though efficient is cost-inhibiting to most malaria endemic countries. Combinations of malaria antigen and antibody Elisa tests with improved sensitivities and specificities may facilitate early detection of acute infections.

Practice across SSA shows no uniformity with respect to screening; whilst some countries have no policy for preventing TTM; others screen blood by microscopy. Blood safety policy statements from WHO advocate for presumptive antimalarial treatment of transfusion recipients in malaria endemic countries. Thus not at tandem with treatment guidelines for malaria programs which require parasitological confirmation before antimalarial administration.

Improving safety of units without compromising blood supply in high disease burden areas may be achieved with an extracorporeal technology employed in a randomised controlled trial resulting in an 87% reduction in TTM incidence. Countries with high risks for TTM who inadvertently have high transfusion rates among young children and women may seek to benefit from applying this technology to eliminate substantially the residual risk of transfusion transmissible infections including malaria.

3B-S10-02

AN EPIDEMIOLOGICAL STUDY OF MALARIAL INFECTION IN HONG KONG BLOOD DONOR POPULATION

SWM Lam and WC Tsoi

Hong Kong Red Cross Blood Transfusion Service, Hong Kong, China

Background: Malaria can be transmitted through transfusion of cellular blood components. In Hong Kong, the current strategy to mitigate the risk of transfusion transmitted malaria (TTM) is to defer prospective donors with past history of malaria and those with geographic exposure risk such as recent travel to or residence in malaria endemic areas defined by World Health Organisation. Although Hong Kong is a non-endemic area, being surrounded by endemic countries, presence of the vector for parasite transmission, increasing trend of immigration and popularity of international travel impose risk of TTM. There are also concerns of 'semi-immune' donors who have persistent low-level yet infective parasitaemia due to frequent exposure to malaria parasites. They are usually originated from previous residence in endemic areas but without indicated recent travel history.

Aims: (i) Using enzyme immunoassay to detect anti-malarial antibodies and determine the prevalence of malaria infection in Hong Kong blood donor populations with and without geographic exposure risk; (ii) To determine the parasitaemia rate in antibody repeatedly reactive samples.

Methods: A total of 441 samples collected from deferred donors with potential malaria risk and 299 samples from eligible donors without the risk were assayed for anti-malarial antibody (Malaria EIA, Newmarket, UK) during the period from October 2008 to March 2012. Initial reactive samples were subjected to repeat testing. Fisher's exact test was applied to test for difference between the two groups of data; $P < 0.05$ was considered significant. Nested polymerase chain reaction (PCR) was performed on repeat reactive samples to detect the presence of genus and species specific malarial DNA according to established methods described elsewhere (Momar N, et al., J Clin Microbiol. 2004; 42:2694–2700; Singh B, et al., Am J Trop Med Hyg. 1999; 60:687–92).

Results: Antibody reactive rates for donor with geographic exposure risk and without the risk were found to be 2.49% (0.93–4.05% at 95% CI) and 0% (0–0.91% at 95% CI) and the difference was statistically significant ($P = 0.0039$). Confirmed malarial DNA positive rate for donors with repeat reactive malarial antibody was 0% (0–0.37% at 95% CI).

Conclusions: This study demonstrated the ineffectiveness of the current donor screening criteria in that only 2.49% of those donors with history of travel to or residence in endemic areas (WHO definition) were repeat reactive by enzyme immunoassay; none were PCR positive, indicating past but not active infection. A high proportion of individuals with such geographic exposure risk, but otherwise no laboratory evidence of past or active malarial infections, were then deferred for blood donation. On the other hand, evidence of past malarial infection was significantly commoner found in those with travel or residence history than those without. Hong Kong is not endemic for malarial infection. From an operational point of view, travel-based deferrals provide a precautionary measure to prevent donation by asymptomatic donors who may carry the Plasmodium parasites when striking a balance between maintaining blood provision sufficiency and protecting blood safety.

3B-S10-03

PREVALANCE OF PLASMODIUM INFECTION IN BLOOD DONORS IN CHINESE NON-ENDEMIC AREA

SH Zhu, SJ Zhu, H Lin, L Shao, YC Liu, CY Huang and J Sun

Jiangsu Province Blood Center, Nanjing, China

Background: Addressing risk of imported malaria is complicated by four human species of Plasmodium. Most areas of China are non-endemic for plasmodium now. But there were many workers exported their labor in endemic area, e.g. Africa with long-term exposure and travelers migrated frequently, so the number of reported imported *Plasmodium vivax* (*P. vivax*) infection increased in recent years in Jiangsu, east of China. Some of them infected plasmodium and developed malaria or became asymptomatic. They would be blood donors to donate their blood which concealed infected history in healthy consultation. Due to limitations of screening assays, the risk of transfusion-transmitted plasmodium was higher and it happened occasionally.

Aims: To investigate the prevalence of plasmodium infection in blood donors in Jiangsu,east of China.

Methods: Eligible blood donors were selected whose samples were negative by routine screening in Jiangsu Province Blood Center, China, corresponding to the period from April to July 2015. All plasma samples from 704 eligible donors were tested for malarial antibodies using an enzyme immunoassay kit (Pan malaria antibody CELISA, CeLLabs, Australia) as recommended by the manufacturer. Nucleic acids of EIA reactive samples were extracted from 0.5 ml of whole blood using a DNA blood mini kit (Qiagen, German) according to the manufacturer's instructions. A genus-specific primer pair and four species-specific primer pairs (targeting *P. falciparum, P. vivax, P. malariae,* and *P. ovale*) for nested PCR assay were designed based on the Plasmodium small-subunit ribosomal RNA (ssrRNA) genes and nest- PCR was performed.

Results: From 704 selected donors, 44 originated from foreign countries, 270 were migrant workers from other provinces, 206 were university students from tropical or subtropical zone of China and 184 were local citizens. There were 1.14% (8/704) reactive samples of anti-malaria were found and they were four males and four females. Four of them were migrant workers, two was local citizens, one was foreign university student from Africa and one worked in a foreign company. One local citizen was repeat donor and others were first donors. No plasmodium was detected in red cells using microscopy. None was DNA positive.

Conclusion: Plasmodium infection prevalence appears to be high in the east of China although there was no nucleic acid. Now it can not be routinely screened plasmodium in blood donors due to the high cost of reagents. So healthy consultation is very important and education should be strengthened, especially in migrant workers.

3B-S10-04

THE DARC SIDE OF RETICULOCYTES: THE PLASMODIUM VIVAX DUFFY BINDING PROTEIN DOMAIN IN DARC IS EXPOSED IN IMMATURE RETICULOCYTES AND CONVERTS TO A CLOSED STATE UPON MATURATION TO ERYTHROCYTES

E van den Akker[1], E Ovchynnikova[1], AEH Bentlage[1], N Salinas[2], F Aglialoro[1], M von Lindern[1] and N Tolia[2]

[1]Sanquin, Amsterdam, The Netherlands [2]Washington University, St. Louis, Missouri, United States of America

Background: *Plasmodium vivax* (*P. vivax*) is the second most prevalent parasite species causing malaria in humans and exclusively infects reticulocytes. Reticulocyte infection is facilitated by *P. vivax* Duffy binding protein (DBP), which utilizes Duffy antigen chemokine receptor, DARC, as an entry point. Previous research suggests that this preference for reticulocytes was not a result of higher DARC expression or binding of DBP to DARC on the total population of reticulocytes, making P.vivax preference to reticulocytes unclear. Aims: Reticulocytes express CD71 and have residual RNA that can be detected by Thiazole orange (TO) staining, both markers are gradually lost during reticulocyte maturation. Taken this into consideration we hypothesize a small population of immature reticulocytes may display increased association with P.vivax DBP potentially explaining the preference of P.vivax for a specific reticulocyte population.
Methods: Reticulocytes were enriched from human peripheral blood from healthy volunteers after written consent by continuous percoll gradient. FACS was used to further delineate the mature and immature reticulocyte populations using CD71 and TO. Surface plasmon resonance was used to quantify associations of antibodies and recombinant P.vivax proteins and red cell populations. SDS-PAGE and Western blotting was used to asses expression levels of DARC and other membrane proteins.
Results: D71/TO double staining of peripheral blood reveals four distinct reticulocyte populations. These are with increasing maturity: (i) CD71high/TOhigh (<1%) (ii) CD71low/TOhigh (4%), (iii) CD71−/TOhigh (30%), and (iv) CD71−/TOlow (70%). Binding of Duffy antibodies recognizing the DBP binding pocket as well as DBP itself to CD71high/TOhigh reticulocytes was significantly higher compared to other reticulocyte populations. Interestingly, the expression of DARC did not change significantly during reticulocyte maturation. In addition, surface plasmon resonance experiments using DBP coated chips confirmed increased reticulocyte binding compared to erythrocytes. Of note, anti-Fy3 binding to reticulocytes and erythrocytes was comparable.
Summary/Conclusion: The data suggests an increased epitope exposure of membrane proteins and in particular Duffy epitopes in immature reticulocytes which is probably a key to the preferential binding of DBP to immature reticulocytes and a potential mechanism underlying the preferential infection of reticulocyte subset by *P. vivax*.

3B-S10-05

INCIDENCE OF ACUTE HEPATITIS E INFECTION IN BLOOD DONORS IN THE WESTERN CAPE, SOUTH AFRICA

T Lopes[1], TG Maponga[1], C Pistorius[2], R Cable[2], W Preiser[1] and MI Andersson[1]

[1]University of Stellenbosch, Cape Town, South Africa [2]WPBTS, Cape Town, South Africa

Background: Hepatitis E virus (HEV) is the most common cause of acute viral hepatitis worldwide. There are four different HEV genotypes. Genotypes 1 and 2 are associated with faecal oral transmission associated with contaminated water sources, whilst genotypes 3 and 4 are associated with foodborne zoonotic transmission. HEV genotype 3 may cause chronic liver infection, sometimes resulting in cirrhosis and liver failure. This HEV genotype is known to be transmitted in blood and blood products. An appreciation of the importance of HEV as a transfusion transmitted infection is increasing. Some countries have introduced screening for HEV. Little is known about the risk of acute HEV transmission in South Africa.
Aims: To assess the incidence of acute HEV infection in blood donors in the Western Cape, South Africa and to determine the risk factors associated with past infection.
Methods: From September 2014 to November 2015 we randomly selected 15,003 donor samples from clinics across the Western Cape for HEV testing. Using a commercial qualitative transcription-mediated amplification (TMA) assay with a limit of detection (LOD) of 7.89 IU/ml per individual donation (Procleix HEV assay, Grifols Diagnostic Solutions Inc., Spain) on the Panther system (Hologic Inc., USA) all samples were tested. Following consent a further 250 donors from 25 donor clinics in Cape Town completed an HEV risk questionnaire. This group were tested for anti-HEV IgG using a Wantai ELISA assay (Fortress Diagnostics, UK) and for HEV RNA as 25 plasma minipools of 10 donations per pool, using an in-house real-time reverse transcriptase quantitative PCR (RT-PCR) assay with a LOD being 3000 IU/ml per pool.
Results: The sampled donor population mean age was 42 years and there was an even proportion of male and female donors consistent with WPBTS populations. In only 1 of 15,003 donations was HEV RNA detected, giving an incidence of 0,007% [95%CI, 0.00–0.02]. Phylogenetic analysis showed the donor sample clustered with HEV genotype 3e. The HEV seroprevalence of 250 selected donors was 42.2% (106/250) [95%CI, 36.08–48.32]. None of the 25 minipools tested positive for HEV RNA. Data on risk factors for positive serology will be presented.
Summary: This study shows a high prevalence of past HEV infection in donor samples from the Western Cape, South Africa. However the incidence of acute infection at donation was shown to be low. The identified genotype 3 in the positive donor suggests that the risk of transmission exists. Since HEV seroprevalence increases with age, and most donors are >50 years it may be that the majority of donors have already been infected and are therefore immune to infection by the time that they donate blood. The risk factors associated with HEV infection in our setting are possibly from zoonotic and waterborne routes of transmission. Further investigations are needed to improve our understanding of HEV incidence and the risk factors associated with HEV infection in resource limited settings.

Transfusion Practitioner Forum – Patient Safety

3B-S11-01

PATIENT SAFETY: YOU ONLY NEED A PENCIL, PAPER, PEOPLE AND SOME TIME

R Deelen

IJsselland Hospital, Capelle a/d IJssel, The Netherlands

Background: Since 2008 a safety management system (SMS) is mandatory in all Dutch hospitals. The goal is to guarantee a safe environment for the patient. The basic requirements of a SMS are outlined in the Netherlands Technical Agreement 8009. Part of this system is analysing risks in healthcare practice. Risk analysis is done retrospectively using root cause analysis, and prospectively using HFMEA/SAFER methods. Healthcare failure mode and effects analysis (HFMEA) originally developed in the United States was adapted to suit Dutch hospitals in 2006, and is called Scenario analysis of failure modes SAFER. This structured approach is easy to use, and even for a non-specialist a valuable tool.
Steps taken: In 2014 preparations were made to analyse the risk of the transfusion chain in our hospital. We defined the starting and endpoint of the process as: 'indications for use of blood products until the patient leaves the hospital after receiving a transfusion'.
The transfusion chain is complex, even in a medium-sized hospital. For this project the transfusion process was split up in six manageable parts, and flowcharts were developed for each part.
We formed a small team consisting of a Transfusion Practitioner and a quality officer. The chair of the transfusion committee, a clinical chemist became project leader, and promoted it as the coolest project of 2015.
We used a modified version of HFMEA/SAFER to be more efficient, and prepared much of the work outside the meetings, resulting in only three meetings rather than six to map each sub-process. With this mapping information, and the support from our project leader we gained management support throughout the hospital, and started asking co-workers to join the meetings.
The original flowcharts were discussed at the beginning of the first meeting, adjusted if necessary, and effective tool to guide discussion. The min of the meetings were used afterwards to fill the HFMEA/SAFER worksheets and write an overall report. Again it was possible to cover all steps of the HFMEA/SAFER method in three meetings for each sub-process.
Conclusion: A Risk analysis is a helpful tool to collect information about all parts of the transfusion chain in your hospital. For our purposes using an adjusting HFMEA method proved to be an efficient way to analyse risk. Involving a multidisciplinary team helped to create awareness, and objectify risks.
The HFMEA/SAFER method presented some challenges: it is time consuming, and difficult to objectify the probability of occurrence for each of the potential causes. It's difficult to prove that all risks were covered as ratings were made using the

experience and expertise of those who attended the meetings. A flowchart agreed by all members of the multidisciplinary team was a good way to start the risk analysis. If you lead the discussions it is essential to have knowledge of the transfusion chain. And don't hide behind a computer during the meetings. Write everything down and transfer it later to the worksheets, and check them at the next meeting.

Netherlands Technical Agreement 8009, 'Safety management system for hospitals and organizations which administer hospital care' (NTA 8009). Retrieved from website may 18 2016, www.vmszorg.nl
SAFER; ZonMW, UMC-Utrecht, De Maastro Clinic en TU Eindhoven (Habraken, Reijnders, Schaaf van der, Leistikow et. al., 2006).
VMS Veiligheidsprogramma; Praktijkgids Prospectieve risico-inventarisatie, 2009.

3B-S11-02

HOW THE TRANSFUSION PRACTITIONER ROLE CAN INVOLVE PATIENTS IN THE TRANSFUSION PROCESS

RL Moss

NHSBT, London, United Kingdom

Involving patients in all aspects of their medical and nursing care should be standard practice within any healthcare system. Gaining informed consent from patients for medical interventions is a legal requirement. Consent (where possible) must be obtained before treatment; the risks, benefits and alternatives (where appropriate) must be explained.

The 'Expert Patient' is a term used for some patients who have chronic conditions (such as a haematological or renal disease). Being an 'Expert Patient' not only helps them to live with their disease better but ensures they understand all therapies relevant to their condition. Although patients with short-term conditions are less likely to become 'expert', all patients have access to a great deal of information through the Internet. They can all research their condition and gain a better understanding of the treatments required (including blood transfusion).

Blood transfusion has often been regarded as a part of the patient's overall treatment whether undergoing chemotherapy or having a surgical procedure. Historically separate consent was not sought for blood transfusion. However it is now regarded best practice to seek informal consent from the patient, ensuring understanding of the risks, benefits and alternatives to transfusion. It is also expected that a discussion will take place with the patient if transfusion occurred unexpectedly and pre-transfusion informed consent was not possible (e.g. in trauma).

Within the limited literature available on patient information and consent it is apparent that consent for blood transfusion is variable. However there have been recent initiatives to standardise the process such as the SaBTO (Advisory Committee on the Safety of Blood, Tissues and Organs) recommendations in the UK, and it is an integral part of all established Patient Blood Management programmes in the USA and Australia.

To improve patient involvement in the transfusion process, it is important to ensure those who are discussing transfusion therapy include the rationale for transfusion, the benefits, risks and alternatives. The consenter must have the relevant transfusion knowledge and information to give to the patient. This is achieved through a programme of training and education and making this process integral to all patient care. Training of clinical staff can vary from locally-led teaching sessions to undertaking an established national programme such as the 'Learn Blood Transfusion' e-learning in the UK.

It is also necessary to have a process in place that triggers and records these discussions so they become part of the patient's transfusion pathway. There are a number of possible processes that can be developed into a policy including a paper-based consent sticker, patient information booklets, a decision-to-transfuse programme on an IT system or clear documentation in the patient's record.

The Transfusion Practitioner has a critical role in this by bringing all the required elements together for clinical staff, in order to make patients part of the transfusion process.

3B-S11-03

THE USE OF TECHNOLOGY IN PATIENT SAFETY

AS Dhesi

NHS Blood and Transplant, London, United Kingdom

Hospitals are under increasing pressure to become more efficient, whilst improving patient safety. Technology is advancing at an increasing rate, embracing and adopting health technology in transfusion will add real value by providing cost savings and releasing time to care. Two key areas in transfusion where technology is used in patient safety are the decision to transfuse and the administration process.

The UK Serious Hazards of Transfusion (SHOT) Haemovigilance scheme (2014) noted that 61% of cases reported could have been detected at the final key bedside administration step. End-to-End electronic blood tracking systems (sample taking, blood collection and administration) have been shown to reduce incompatible transfusions. With these systems the user is supported to ensure the correct patient is identified and prompted to go through key steps in the transfusion process.

National and large regional audits consistently show that 15–20% of blood component transfusions are not compliant with national guidelines. The potential impact of this being unnecessary costs, increased risk to patients and increased likelihood of blood shortages. The decision to transfuse is a key step in reducing the level of inappropriate use. However, for doctors whose primary remit is not transfusion, obtaining the information to answer these questions can be difficult. Decision support systems (DSS) provide the user with patient specific treatment recommendations and/or alerts, by comparing the indication for transfusion with test results and guidelines at the time of prescribing. The user therefore does not need to spend time searching for policies.

- The benefits of the above systems include:
- Improved transfusion safety – fewer errors;
- Reduced inappropriate use of blood and inventory – cost savings;
- Improved compliance with policy and regulatory requirements;
- Release of staff time – greater time to care; and
- Access to 'big data'.

These systems however require a large capital investment with ongoing training and running costs from each organisation. Return will be dependent on the levels of both blood usage and inappropriate use.

The majority of adults in the UK own a smartphone (cell phone) and over the past few years there has been a notable increase in ownership in emerging and developing nations. Surveys in Australia and the UK have shown 80–90% of Healthcare Professionals/junior doctors own a smartphone and the majority use it during clinical practice. These devices provide an opportunity for use in transfusion (e.g. a DSS app based on national guidelines) at a lower cost compared to the above systems. Studies have already shown that apps are more accessible than online guidelines and that they improve policy adherence and user knowledge.

Technology can make transfusion safer but it can also introduce new errors and risks into the system. Regulation, privacy and other legal aspects would need to be addressed. Technology cannot replace clinical skills. The Transfusion Practitioner can play a critical role in technology development and roll out. They have invaluable knowledge, connections with experts and end users and can ensure development is related to an effective PBM program with the patient's benefits in mind.

Exciting Developments in Platelets

3D-S11-01

PLATELETS FOR REGENERATIVE MEDICINE: WHAT ABOUT EVIDENCE?

K Schallmoser

Paracelsus Medical University of Salzburg, Salzburg, Austria

Platelets contain a plethora of potent substances in their specific granules essential not only for primary hemostasis but also endothelial integrity, host defense, wound healing and tissue repair. In the case of vascular injury these factors are released due to platelet activation, contributing to a local inflammatory reaction and finally regeneration of damaged tissue.

In the 1950s first efforts started to use the liquid tissue blood for enhancing coagulation and wound healing by successful development of fibrin glue. Since then various protocols for the preparation of platelet rich plasma, platelet rich fibrin and platelet gels have been established to utilize platelet-derived growth factors for tissue regeneration. Currently, more than 200 clinical studies are registered (www.clinicaltrials.gov), extensively testing the benefits of platelet rich formulations for orthopedic/surgical, rheumatic and dermatologic conditions. Furthermore, also platelet derived extracellular vesicles are tested for their potential support of neuroregeneration and post-ischemic revascularization.

Another important contribution of platelets for tissue regeneration is reflected by the efficient implementation of human platelet lysate (HPL) as source of growth factors

and nutrients *in vitro* in pre-clinical cell propagation, representing a human blood-derived raw material as substitute for fetal bovine serum.
However, due to a lack of standardization of platelet separation techniques, study protocols and outcome measures, already existing clinical data are difficult to interpret and to compare. Predictive preclinical models are urgently needed to develop optimized platelet preparation protocols for defined clinical conditions. Prospective randomized clinical studies will determine applicability and therapeutic efficacy enabling selective utilization of platelet rich products for differential indications.

3D-S11-02

CURRENT METHODS TO MANUFACTURE HUMAN PLATELET LYSATES AND POSSIBLE TRENDS IN PRODUCT SAFETY AND STANDARDISATION

T Burnouf

Taipei Medical University, Taipei City, Taiwan

Human platelet lysate (HPL) is a new blood-derived product obtained by the lysis of platelet concentrates (PC). The lysis process releases a fluid rich in growth factors, cytokines, and other bioactive molecules that can be used in various fields of regenerative medicine. In cell therapy applications, HPL is quickly emerging as a serious alternative to fetal bovine serum (FBS) as 'gold standard' growth factor supplement for clinical-grade *ex vivo* propagation of human cells, in particular mesenchymal stromal cells (MSC), for transplants. The advantages of HPL over FBS include (a) the absence of risks of immunological reactions possibly associated with xenoproteins from bovine source, and (b) a careful control of pathogen risks through human blood donor's screening and donation testing. Numerous *in vitro* studies have now clearly established that MSCs expanded in the presence of HPL exhibit improved proliferation behavior and decreased population doubling time compared to FBS-supplemented media, and maintain characteristic immunophenotype, typical differentiation capacity, and T-cell immunosuppression effect. Several methods have been developed to prepare platelet lysates for cell expansion. They include freeze-thaw cycles or sonication to fragment platelets, activation by thrombin or calcium chloride for platelet degranulation, or membrane dissolution by solvent/detergent. These steps are typically followed by centrifugation and filtrations to clarify the material and remove cell debris, prior to freezing for storage. Heparin anticoagulant addition is needed to avoid subsequent gelification of growth medium supplemented with HPL containing fibrinogen during cell culture. Although these modes of preparation can generally sustain expansion and differentiation of MSCs from various origins, variations are nevertheless observed, which may be explained by the mode of preparation of HPL and/or the characteristics of the individual starting PC raw material. Pooling of 40–50 therapeutic-grade PCs from blood establishments is essential to HPL standardisation as it minimises the inherent variability of individual donations and ensures uniform characteristics of starting PC material, e.g. in platelet count and residual leucocytes and plasma. Pooled allogeneic 'off-the-shelf' HPLs are currently produced by some blood establishments, or made available by commercial suppliers. The raw materials are fresh or expired PCs prepared from anticoagulated whole blood (buffy coat or 'PRP' method) or by apheresis following standard blood collection practices, and generally suspended in plasma. Processing typically involves three freeze-thaw cycles or CaCl2 activation of individual donations, followed by pooling, centrifugation, 0.2 μm filtration, and freezing prior to quality control and release. Further developments in HPL safety and standardisation can be foreseen in the near future. Those may encompass (a) the increase in HPL pool size, to further improve standardisation and cost-effectiveness, and (b) the implementation of dedicated viral/pathogen reduction treatments on starting individual PC material and/or HPL pools, as done over the last 20 years in the plasma fractionation industry. The prospect of the development of specialised cell therapy or regenerative medicine products obtained by HPL fractionation is also possible. In conclusion, scientific and medical rationales support that HPL prepared under controlled standardised conditions from therapeutic-grade PC, should become the gold standard supplement for human cell propagation and cell therapy.

3D-S11-03

3D SILK BONE MARROW MODEL FOR PLATELET PRODUCTION

A Balduini[1], CA Di Buduo[1], A Pecci[1], CL Balduini[1] and DL Kaplan[2]

[1]IRCCS San Matteo Foundation, University of Pavia, Pavia, Italy [2]Tufts University, Medford, Massachusetts, United States of America

Bone marrow is a complex tissue protected by bones and dedicated to the production of all blood cells. Bone marrow failure is the result of diseases, trauma, or cancer treatments, leading to ineffective hematopoiesis and the consequent necessity for blood transfusions. In particular, millions of platelets, specialized cells that participate in haemostatic and inflammatory functions, are transfused each year worlwide, but their supply is still limited. Platelets are produced by megakaryocytes, by extending long filaments, called proplatelets, directly into the bloodstream. Bone marrow structure and extracellular matrix composition together with soluble factors (e.g.; Thrombopoietin) are key regulators of megakaryopoiesis by supporting cell differentiation and platelet release. Despite this knowledge, little is known about the mechanisms involved in platelet production. To address all these needs, here we present the use of natural silk fibroin as a biocompatible and non thrombogenic material to engineer a 3D bone marrow system to house megakaryocytes for functional platelet production *ex vivo*. Using primary human megakaryocytes platelet generation was recorded inside this model in response to variations in surface topography, stiffness, co-culture with endothelial cells and shear forces. A critical feature of the system was the possibility to control silk binding of cytokines, extracellular matrix components and endothelial-derived proteins. Millions of human platelets were produced and showed to aggregate and participate to thrombus formation. Further, using adult hematopoietic progenitor cells our system demonstrated the ability to reproduce alterations observed in diseased states. Specifically, inherited thrombocytopenia are a heterogeneous group of rare disorders that occurs when a patient suffers from an abnormally low peripheral blood platelet count due to genetic mutations in genes relevant for control of megakaryocyte function. A great effort has been spent to generate drugs able to increase platelet production in these patients, without evoking an immune response. Among these Eltrombopag, a small non-peptide TPO bio-mimetic, currently approved by many regulatory agencies worldwide for increasing platelet count in subjects affected by different forms of thrombocytopenia. Despite the clinical practice, the mechanisms underlying Eltrombopag impact on megakaryocyte function are unknown. Thus, taking advantage of our silk model, we also investigated the effects of Eltrombopag on platelet production. Results demonstrated that Eltrombopag promotes normal *in vitro* megakaryocyte differentiation and platelet production, with a significant dose-dependent increment. Of note, Eltrombopag induced the activation of AKT and ERK1/2, two singling molecules that together have been demonstrated to be crucial for the regulation of physiologic platelet production. Finally, megakaryocyte cultured in the 3D silk bone marrow system showed similar platelet release number and function in thrombopoietin or Eltrombopag-treated samples.
The broader impact of this work offers a versatile new laboratory tool, reproducing key features of the physiological bone marrow environment, for studying physiologic and pathologic thrombopoiesis, as well as provide an high quality tissue system for production of functional platelets for drug testing.

Thrombosis and Haemostasis

3D-S12-01

ENGINEERED RBCS AS ANTIBODY CARRIERS FOR SYSTEMIC DELIVERY

NJ Huang, NJ Huang, N Pishesha and H Lodish

Whitehead Institute, Cambridge, Massachusetts, United States of America

Utilizing red cells as drug delivery platforms has been extensively exploited. Hypotonic dialysis and antibody affinity binding are the two most used approaches to load small molecules on erythrocytes. However, these methods either damage the erythrocyte membrane or produce unstable loaded-cargo. To overcome these problems, engineering red cells by gene modification which makes covalent binding of small peptide on erythrocyte is investigated. Here, we conjugated heterodimers of VHH (variable domains of camelid heavy chain-only) antibodies anti-botulunum toxin A (BoNT/A) or B with glycophorin A or Kell protein and expressed these chimera proteins on mouse and human erythrocyte surface. We demonstrated these engineered erythrocytes have normal differentiation characters such as enucleation rate, cell size and cell markers toward maturation. Moreover, we showed murine and

human red cells carry anti-botulinum A antibodies instead of anti-botulinum B can neutralize BoNT/A *ex vivo*. The specific neutralization effect is observed by reduced amount of cleavage of neuron synaptosomal-associated protein-25 (SNAP-25) in neuron lysates when co-incubated the anti-botulinum A VHH-expressing red cells with neuron cells and botulinum A. The neutralization effect has been further shown in the protection from lethality both in mice carrying the engineered blood cells by transplantation and in mice transfused with these engineered bloods. The study demonstrates a new avenue of producing drug delivering red cells by genetic modifying plasma membrane proteins on red cells and this approach can be used to produce stable cargo loaded red cells without affecting normal erythropoiesis.

3D-S12-02

ROLE OF RED BLOOD CELLS IN HEMOSTASIS AND THROMBOSIS

J Weisel

University of Pennsylvania, Philadelphia, Pennsylvania, United States of America

Background: Until recently it has been assumed that red blood cells (RBCs) play mainly a passive role in hemostasis and thrombosis, but now some studies have uncovered several novel roles for them in both physiological and pathological processes. The best-known effects of RBCs in clotting *in vivo* are rheological, involving laminar shearing plus aggregation and deformability of RBCs. In addition, RBCs have specific receptors for platelets and fibrin(ogen) that may be involved in thrombosis.

Aims: To investigate the structure and properties of contracting human and mouse model blood clots and thrombi taken from patients, including the RBCs trapped in the platelet-fibrin network during contraction (retraction), and determine the consequences for hemostasis and thrombosis.

Methods: The structure of contracting clots was visualized by confocal microscopy and scanning electron microscopy, and their mechanical properties were measured by rheometry. The kinetics of contraction was characterized by a novel optical tracking method. Venous and arterial thrombi and pulmonary emboli were collected from patients and their composition was determined. In a mouse model of hemophilia, vein segments containing clots were harvested and processed for scanning electron microscopy.

Results: Contracted blood clots develop a remarkable structure, with a meshwork of fibrin and platelet aggregates on the exterior of the clot and a close-packed, tessellated array of compressed polyhedral erythrocytes, named polyhedrocytes, within. Platelets (with their cytoskeletal motility proteins) and fibrin(ogen) (as the substrate bridging platelets for contraction) are required to generate the forces necessary to segregate platelets/fibrin from RBCs and to compress these cells into a tightly packed array. The structure and properties of contracted clots and the kinetics of contraction vary depending on the relative amounts of platelets, fibrinogen and RBCs and the conditions of clotting. Polyhedrocytes have also been observed in human arterial and especially venous thrombi, and pulmonary emboli, taken from patients. Moreover, the kinetics of contraction was found to be different in patients with sickle cell disease, ischemic stroke, and deep vein thrombosis. Such experiments suggest that the extent of clot contraction and the prevalence of polyhedrocytes may be associated with thrombosis and could be a marker of prothrombotic conditions. Consistent with this hypothesis, both the stiffness of RBCs and the extent to which they form a procoagulant surface to generate thrombin through exposure of phosphatidylserine appear to play an important role. In an experimental model, clots formed after puncture of the mouse saphenous vein are composed primarily of polyhedrocytes, suggesting that they may be important in venous hemostasis. This conclusion is reinforced by studies of hemophilic wound clots, where bleeding is associated with lower fibrin and polyhedrocyte content, which is increased by administration of Factor VIII.

Summary/Conclusions: These results demonstrate how contracted clots form an impermeable barrier important for hemostasis and wound healing and to restore flow past obstructive thrombi. On the other hand, they also help explain how fibrinolysis is greatly retarded after clots contract, as a result of these striking structural and functional changes, rendering thrombolysis less effective in aging thrombi. In summary, RBCs may perform a dual role, both helping to stem bleeding but at the same time contributing to thrombosis in a variety of ways, including when the tightly packed array of RBCs in a contracted clot blocks blood flow and resists dissolution.

3D-S12-03

INHIBITION OF SP1 BY MIRNA-331-3P CAUSES GLYCOSYLTRANSFERASE A REPRESSION AND MAY EXPLAIN CERTAIN WEAK A (A^X0^{1V})

R Kronstein-Wiedemann, N Kronstein and T Tonn

Medical Faculty Carl Gustav Carus, TU-Dresden, Dresden, Germany

Background: The molecular genetic basis of the AB0 system has been known since 1990. More than 100 AB0 subgroup-related variations were detected in the coding region of glycosyltransferases, consisting of seven exons, which may be causative for a weak blood group antigen A or B expression. Most variation in expression is explained genetically by mutations that reduce transferase activity. The majority are associated with missense or frameshift/stop codons. Only a small minority have not yet been explained, in the absence of analysis of regulatory promotor or enhancer regions. We found that miRNAs play a critical role in the regulation of AB0 blood group antigen. Here we show that the effects of miR-331-3p could be mediated by the inhibition of transcription factor SP1, which in turn is not able to bind to the promotor of the AB0 gene, thus downregulating blood group A antigen expression.

Methods: By distinct complementary approaches, including gene array analysis and overexpression of glycosyltransferase specific micro-RNAs (miRs) in primary hematopoietic stem cells, we identified that miR-331-3p and -1908-5p directly target glycosyltransferase A and B. Using microRNA target prediction tools we also identified Sp1 as a potential target gene for miR-331-3p. Furthermore two of the three binding sites for SP1 in the 5′UTR of glycosyltransferases are in addition also binding sites for miR-1908-5p. Therefore we treated hematopoietic stem cells with different concentrations of an inhibitor of SP1 (mithramycine A) or a stimulator of SP1 (insulin) and analyzed blood group A expression by flow cytometry and ID-Card gel method.

Result: Overexpression of miR-331-3p and -1908-5p in hematopoietic stem cells (HSCs) leads to a 30–50% reduction of blood group A antigens per cell in differentiated RBCs. Furthermore miR-331-3p and -1908-5p were enhanced in red blood cells of A_{weak} variants. Sequencing of the 3'UTR of six A_{weak} variants revealed the presence of more miRNA binding sites for miR-1908-5p compared to normal controls. Inhibition of SP1 also leads to 40–70% (n = 3) reduction of blood group A antigen per RBC, in case of blood group A_2 even up to 90% (n = 1). In a complementary approach stimulation of SP1 results in an increase of blood group A antigen per RBC by about 40–70% at day 8 of differentiation.

Conclusion: Glycosyltransferase A and B expression is regulated by miR's 331-3p and 1908-5p, by inhibition of the transcription factor SP1. This new concept of microRNAs as regulators of blood group glycosyltransferase expression may provide an explanation of the molecular basis for A^X0^{1V} weak blood group phenotypes. We assume that increased expression of miR-1908-5p and -331-3p in A_X variants may lead to downregulation of transcription factor Sp1 and to a competition of miR-1908-5p with Sp1 for binding sites in the promotor region of the AB0 gene which in turn results in further gene repression.

3D-S12-04

NOVEL MUTATION IN GTB FLEXIBLE LOOP AFFECTS B ANTIGEN EXPRESSION

XH Cai

Ruijin Hospital, Shanghai, China

Background: Glycosyltransferases (GT) A and B are responsible for the synthesis of A and B antigen in ABO blood group. High resolution structures of GTA and GTB revealed the internal flexible loop (amino acids 179–194) is involved in the multistage process of substrate binding. However, the mechanism of ABO subgroup with mutations in the loop has not been extensively explored.

Aims: By *in vitro* eukaryotic system, we evaluated the B antigen expression associated with a novel B subgroup mutation located in the loop.

Methods: Serologic investigations including serum B transferases activity assay were performed in a Chinese individual and his family members with standard methods. Saliva of ABx members was determined for water-soluble ABH antigens by agglutination inhibition tests. DNA sequences of all seven exons and exon-intron boundaries of ABO gene were analyzed using genomic DNA by polymerase chain reaction (PCR) and sequencing. Flow cytometric analysis of B antigen expression on RBCs and HeLa cells transfected with mutant plasmid were also performed, as well as B transferases activity assay in cell supernatant. Cell agglutination intensity was compared by direct microscopy after adding anti-B antibody to the Hela-Bx cells.

Results: ABx phenotypes were detected in the proband and his father by serologic typing. The ABx samples displayed Bw-like patterns by flow cytometric analysis using anti-B antibody, consistence with the serologic typing. Plasma from both ABx

samples in this study showed almost no B-transferase activities (titer: <1) and the soluble B antigen level in saliva of the proband and his father was obviously lower (both of the titers:64) than that of B secretor (tite:2). A novel allele *Bx11* (538C>T; R180C) was identified by DNA sequencing and sequencing after cloning in the father and son. The B antigen remarkably declined on cell surface upon ectopic expression of *Bx11* cDNAs in the Hela cells, and B transferases activity significantly reduced (titer: 32) in cell supernatants compared with the wild tpye B control (titer: 1024). Decreased cell agglutination was also observed by direct microscopy, which mimicking the weak agglutination of RBCs detected in ABx father and son.
Conclusions: We report for the first time that 538C>T mutation represents a new molecular basis for the B_x blood subgroup. Amino acid residue Arg180, which is located on the edge of internal disordered loop, may play a critical role for the shift of GTB's three conformations when the enzyme catalyses the synthesis of B antigen.

Sequencing Technologies

3D-S14-01

NEXT-GENERATION SEQUENCING FOR BLOOD GROUP GENOTYPING

Y Fichou, C Le Maréchal and C Férec
Inserm UMR1078 – EFS, Brest, France

For many years molecular study of blood group genes has been implemented in immunohematology laboratories. It has now become a routine strategy that has naturally taken advantage of the major advances in the available technologies for molecular analyses. From the simple PCR-derived tools that allow low- to high-throughput studies, next-generation sequencing (NGS), known as second-generation technologies in its current format, has emerged as the method of choice to explore and to decipher the human genome for the past 10 years. This advent has resulted in an extensive increase of the amount of genetic data available in a single experiment. NGS technology has undoubtedly contributed to tremendous improvements in both fundamental and clinical research, thanks to its ability to analyze regions ranging from targeted genes up to the whole genome in several individuals at the same time. For the very recent years a few pilot studies using various commercial platforms have paved the way towards blood group genotyping by NGS in both transfusion and obstetrical medicine. Advantages and limitations have been reported, as well as future improvements have been clearly identified, for example to circumvent the issue related to gene homology. In parallel high-throughput platforms using genotyping technologies have shown their potency in mass-scale donor genotyping. Outcomes have proven useful and potent for donor/patient cross matching. Third-generation sequencing, which is interestingly characterized by the production of long reads that have been shown to cover up to several dozen kilobases, has also become available in the meantime. This novel technology shows great promises for clinical applications for the very next future, most importantly by resolving the challenging issue of characterizing gene conversion events occurring in genes of the *RH* and *MNS* blood group genes. By considering recent and future advances in these technologies and their related bioinformatics resources it is tempting to suggest that a routine, NGS-based strategy will be soon available for blood group genotyping with accuracy at low price. It remains to be evaluated by clinicians whether this strategy is to be carried out and to define its area of applications.

3D-S14-02

NEXT-GENERATION SEQUENCING WITH AUTOMATIC ANALYSIS IS CREDIBLE TO PREDICT BLOOD GROUP GENOTYPE AND TO SOLVE COMPLEX CASES WITH NOVEL ALLELE FINDINGS

PC Wu[1], YH Lin[2], PL Chen[3], CY Chen[4], MH Chen[1], L Li[5], SC Pai[1] and YS Hung[1]
[1]Taipei Blood Center, Taiwan Blood Services Foundation, Taipei, Taiwan [2]Institute of Genomics and Proteomics NTU, Taipei, Taiwan [3]Department of Medical Genetics NTUH, Taipei, Taiwan [4]Department of Bio-Industrial Mechatronics Engineering NTU, Taipei, Taiwan [5]Taiwan Blood Services Foundation, Taipei, Taiwan

Background: Full extended and accurate blood group antigen typing is essential in transfusion medicine to reduce alloimmunization. Serology is commonly used but with many limitations addressed. Molecular techniques can be applied to identify genotypes and to solve serology complications. With the advances in Next-Generation Sequencing (NGS) and bioinformatics analysis, NGS is now the ideal platform for high-throughput, accurate, extensive and rapid blood group genotyping.
Aim: To establish NGS workflow with automatic analysis pipeline for fast and accurate genotype determination and prediction of blood group phenotype.
Methods: Genotyping: A customized probe-based capture panel targeting 23 genes associated with 15 clinically relevant blood group systems including ABO, RH, MNS, DUFFY and KIDD were tested. All targeted genes contained entire length including exons, introns, untranslated regions and promoters. The enriched DNA was sequenced using Illunmina MiSeq platform which generated 300 bp, paired-end reads. After bioinformatics analysis, automatic genotyping was performed by in house scripts utilizing databases including NCBI-dbRBC and RhusesBase. Genotype prediction software developed by Italian team -BOOGIE was used to compare with the results of our analysis pipeline.
Phenotyping: RBC antigen serology typing with Bio-rad ID system for ABO, RH, KEL, MNS, FY, JK, LE, LU, DIA and in house anti-sera for Mia with gel test. All samples underwent irregular antibody screening and identification. Also ABO subtyping with ad/el or saliva testing, weak D testing was applied when necessary.
Results: The average coverage was 178 folds, with 95% of coding sequence coverage >30×.
We found concordance between genotype with phenotype among all 42 samples tested with one discrepant phenotype result in Mia, which was later found interfered by autoantibody. Using NGS with bioinformatics analysis we are able to identify 10 samples with accurate blood group genotyping with phasing that would not be possible in SNP assay, including one novel variant not previously reported. An ABO subtype sample with weak B phenotype carries a normal O02 and a B101 allele with a novel variant at *ABO*:c.905A>G, p.Asp302Gly, in silico prediction gives probable damaging to the transcript. Two samples with a novel splicing site variant in *SLC14A1*:c.342-1G>A, result in possible nonsense mediated decay with no antigen expression from the allele. Other findings include copy number variation of one weak D sample give DV type 4 (Rh23) with deletion including exon 5 of *RHD* and structure variation of hybrid *GYP (B-A-B)* GP.Mur of Mia in five samples. BOOGIE gives incompatible results especially in ABO genotype prediction, where some A102/B101 or B101/O02 were misclassified as Ax02/Ax02, Ax02/O02 or O65.
Conclusion: A customized NGS panel for blood group system prediction was established. Multiple samples could be tested in a single run. Our results showed precise predictions for all the most clinically relevant blood system. We established automatic analysis pipeline combining open source bioinformatics tools and in house developed scripts which allow us to detect antigen determine SNPs, potential CNVs and also SV with gene hybridization and also novel SNVs for possible gene expression alteration. We are confident to validate NGS genotype result with high concordance with phenotype data and utilize it to solve complex cases. Analysis pipeline should be targeted and include regional specific or high prevalence alleles coupled with worldwide data to give accurate prediction.

3D-S14-03

BLOOD GROUP REFERENCE NEXT GENERATION SEQUENCING – IMPORTANCE OF REFERENCE BLOOD GROUP ALLELES TO CORRECT ASSIGNMENT OF BLOOD GROUP GENOTYPE

N Avent, M Altayar, A Halawani, T Madgett and M Kiernan
Plymouth University, Plymouth, United Kingdom

Background: Blood group genotyping (BGG) has emerged as a front line tool in the management of blood donors and patients and has proven superiority over serological approaches. Most commercially available BGG platforms have the absolute requirement of previous knowledge of the blood group defining SNP, and thus require routine updating. Direct sequence analysis has the obvious advantage that this is not the case. Several reports have described the application of next generation sequencing to BGG (NGS-BGG), but here we provide the most comprehensive analysis of fully sequenced blood group alleles to date.
Aims: To provide reference tools for NGS-BGG analysis as they emerge to replace array based approaches, and to explore the complexity of blood group active genes by their direct analysis and comparison to the public human geneome sequence.
Methods: We have devised next generation sequencing protocols based on long-range PCR (LR-PCR) amplification of the blood group genes *ABO, RHD, RHCE, KEL, JK and FY*. The LR-PCR products were fragmented and used to create libraries for analysis on a Life Technologies Ion-Torrent NGS machine.
Results: The complete coding sequence including introns, exons and flanking regions are amplified and sequenced fully. Extensive bioinformatic analysis by comparison of the sequences to the human genome database releases 19 and 38 (hg19 and hg38) reveals that the public databases are combinations of blood group

genotypes, that must be first deciphered to provide reference sequences for all major blood group alleles. Once these reference sequences have been defined, then subsequent sequencing can alignto them to predict blood group phenotype.
We will present data from the above mentioned systems to illustrate the requirement for a reference sequencing approach that will be critical for the correct implementation of Next Generation Sequencing Blood Group Genotyping (NGS-BGG). In the *JK* system, we have completely sequenced 67 individuals most of which have known Jk phenotypes. Our analysis reveals over 40 SNPs that closely correlate with the presence of *JK*A*, *JK*B* and *JK*Aweak* alleles, a number of which have complete concordance. In all instances the defined genotype matched the phenotype. We have discovered multiple new JK alleles, several of which are hybrid *JK*A/JK*B* genes, whilst others appear to have undergone gene conversion events. We have also defined a number of *JK*Awe*k specific SNPs, and have shown that these alleles represent almost one third of all Jk (a+b−) phenotype samples tested. We also show that a purported JKnull allele at the intron 8/exon nine splice boundary has no effect on splicing and expresses a normal Jk phenotype.
Similar analyses have been performed on the *ABO, RHD, RHCE, KE*L and *FY* genes and selected data from these highly complicated genotypes will be presented, including the description of multiple new alleles. in particular, analysis of the *RHD* gene has revealed several intronic SNPs in complete concordance with the *RH* haplotype cDE, providing an easy method to define it. This enables differentiation between CDe/cDE homozygous *RHD* samples and those that are hemizygous. When coupled with the digital droplet *RH* zygosity method we have developed (Sillence et al., this meeting) rapid methods for *RHD* zygosity testing are now available.
Summary: NGS-BGG will transform the entire BGG field, and will give huge insight into the evolution and complexity of human blood groups. It is not hindered by the requirement of previous knowledge of defined SNPs.

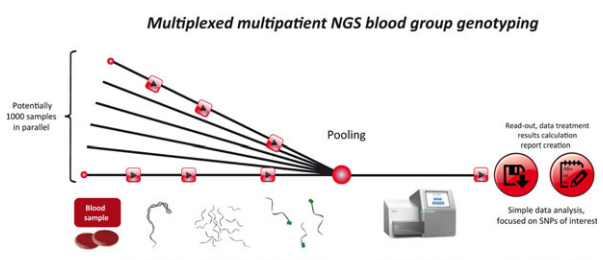
3D-S14-04

MASSIVELY PARALLEL AND MULTIPLEX BLOOD GROUP GENOTYPING USING NEXT-GEN-SEQUENCING

B Corgier[1], SA Boccoz[1], MMR Gontard[1], M Guinier[2], J Fouret[2], M Roche[2], J Lachuer[3], C Legras-Lachuer[2] and CA Marquette[4]

[1]AXO Science, Villeurbanne, France [2]ViroScan3D, Trévoux, France [3]ProfileXpert, SFR-Est, CNRS UMR-S3453 – INSERM US7, Lyon, France [4]Univ Lyon, Université Lyon1, CNRS, INSA, CPE-Lyon, ICBMS, UMR 5246, Villeurbanne, France

Background: Thirty-five blood group systems are listed by the International Society of Blood Transfusion (ISBT). They contain more than three hundred antigens and most of these antigens result from a single nucleotide polymorphism (SNP). Blood group typing is conventionally carried out by serology. However, this technique has some limitations and cannot respond to the growing demand of blood products typed for a large number of rare antigens. Once molecular basis of these Red Blood Cell systems are known, molecular biology methods can be implemented in immunohematology laboratories.
Aims: Here we describe a blood group genotyping assay directly from whole blood samples using Next-Generation-Sequencing (NGS). The developed method allows the simultaneous identification of 15 SNPs associated with blood group systems of 95 patients at once, leading to the determination of extended genotypes.
Method: After an automated DNA extraction, targets are amplified by multiplex polymerase chain reaction (PCRm). Two panels have been developed. Panel 1 includes *KEL*01*, *KEL*02*, *KEL*03*, *KEL*04*; *JK*01*, *JK*02*; *GYPA*01*, *GYPA*02*, *GYPB*03*, *GYPB*04*; *FY*01*, *FY*02*; *FY*02N.01* (*FY*Fy*) and *FY*X*. Panel 2 contains *YT*01*, *YT*02*; *CO*01*, *CO*02*; *LU*01*, *LU*02*; *DI*01*, *DI*02; DO*01*, *DO*02*, *DO*02.-04* (Hy-), *DO*01.-05* (Jo(a−)). For each sample, both panels are pooled and library is generated from amplicons and then sequenced using MiSeq (Illumina).



Caption 1. Multiplexed multipatient NGS blood group genotyping

Results: In a single experiment, 95 blood donor samples have been sequenced on the genes of interest. Amongst the 1,425 targeted SNPs, 1,420 were identified by sequencing, reflecting a coverage of 99.65%. The obtained data show a very good correlation (99% for all SNPs) with other blood group typing methods (genotyping or serology). Depending on the couple of alleles analyzed, correlations vary between 97.12% and 100%.
Conclusions: The use of NGS for blood group genotyping supplements serological and molecular techniques and, in the near future, could replace it with complete and fast results acquisition. The power of NGS technology is here advantageously employed to read a large amount of SNPs at the same time on many patients, in a single read. Potentially, 1,000 patient samples could be analyzed in a single read, each for 15 SNPs, allowing the identification of 15,000 SNPs. The resulting contraction of cost enables here to be used as an accessible IVD test service for blood banks.

3D-S14-05

EVALUATION OF TARGETED EXOME SEQUENCING FOR 28 BLOOD GROUP SYSTEMS, INCLUDING THE HOMOLOGOUS GENE SYSTEMS, FOR COMPREHENSIVE BLOOD GROUP GENOTYPING

CA Hyland, EM Schoeman, T Powley, B Wilson, J Martin, YW Liew, GH Lopez, G Millard, EC McGowan, H O'Brien and RL Flower

Australian Red Cross Blood Service, Brisbane, Australia

Background: Human blood group antigens are of significance in transfusion medicine because patients who have made antibodies to red cell antigens are at risk of being affected by haemolytic transfusion reactions following transfusion of incompatible blood. The Australian Red Cross Blood Service currently uses a SNP-array based genotyping platform within the Red Cell Reference Laboratory to complement serology studies to resolve complex blood group cases. SNP-array genotyping platforms extend blood group phenotyping but do not cover all blood group systems or cover the range of blood group antigens found in a diverse population.
Aims: This study aimed to show that Massively Parallel Sequencing (MPS), with a targeted exome strategy, extends and provides accurate blood group genotyping and correctly genotypes blood group variants within homologous gene systems such as RH and MNS.
Methods: A panel of 28 donor samples, genotyped by SNP array, were analysed using the TruSight™ One Sequencing Panel and MiSeq® platform. The panel included 12 samples that contribute to reagent typing panels and two laboratory controls; nine samples comprising *RHD* gene variants; four gene variants representing *GYP (B-A-B)* hybrids in the MNS system; and 1 *GYPC* (Gerbich type) gene variant. The CLC Genomics Workbench was used for reference alignment and variant analysis. Genes for 28 blood group systems, GATA1 and KLF1 were analysed. Copy number variation (CNV) analysis characterised complex structural variants. Our primary resource for tertiary data analysis to identify variants associated with blood group alleles and to predict the associated phenotype was the International Society of Blood Transfusion (ISBT) website. Additional resources included the Blood Group Antigen Facts Book, the BGMUT website and RHD RhesusBase.
Results: The average sequencing depth per target region was 66.2 ± 39.8. Each sample harboured an average of 43 ± 9 variants of which an average of 10 ± 3 (quality score ≥30) was used for genotyping. For all samples there was 100% concordance between predicted phenotype based on MPS variant analysis and that based on SNP genotyping. In addition variant analysis accurately called the sequences that defined the *GYP (B-A-B)* variants. Variant analysis combined with CNV accurately defined all *RH* and *GYPC* variants. CNV allowed accurate determination of the *RHCE*C* allele and *RHD* zygosity. Hybrid *RHD*D-CE-D* alleles were correctly defined but we were unable to confidently distinguish between a deletion and a rearrangement.
Summary/Conclusions: This targeted exome sequencing strategy extends the range of blood group genotypes compared to SNP platforms. This single test format includes detection of complex MNS hybrid cases and, with CNV analysis, defined complex *RH* hybrid genes along with the *RHCE*C* allele hitherto difficult to resolve by variant detection alone. For this study, we were dependent on well-curated public databases to define the genotypes. Even so inappropriate data file formats and a lack of key pieces of information (e.g. SNP IDs and chromosomal coordinates) prohibited full automation of the Bioinformatics process, necessitating manual curation. Notwithstanding these limitations the approach evaluated in this study is suitable as an adjunct to serology in resolving complex blood group cases in a red cell reference laboratory setting.

Risk Models

3D-S15-01

RISK-BASED DECISION MAKING FOR BLOOD SAFETY

D Devine

Canadian Blood Services, Vancouver, Canada

Blood collectors have a strong focus on blood safety, but when faced with the need to act on evolving information, may reach very different conclusions and plans. This lack of consistency in approaches to blood safety has resulted in unbalanced risk management practices across the industry. Lessons from other industries with respect to risk management have been used by the Alliance of Blood Operators to develop a framework and a set of tools for undertaking risk-based decision making. While the focus is on blood safety, honing risk-based decision making skills can be applied to many aspects of blood centre operations.

Decisions about blood safety are made in the context of emerging risks, evolving technology, societal issues, and economic realities. These decisions are aimed at managing the risks of blood donation, blood transfusion, and other aspects of blood system policy.

The intent of the risk-based decision-making framework is to help organizations and individuals identify, assess, act on, and communicate risk in a manner suited to each situation. The framework helps to organise and simplify the decision-making process by breaking it down into a series of logical and manageable steps.

The framework's objectives are to (i) optimise the safety of the blood supply while recognising that elimination of all risk is not possible; (ii) allocate resources in proportion to the magnitude and seriousness of the risk and the effectiveness of the interventions to reduce risk; and (iii) assess and incorporate the social, economic, and ethical factors that may affect decisions about risk.

Risk management policy foundations are background elements that express the values, priorities, and policies of an organisation in relation to risk management. They guide the decision-making process and must be in place before the process begins. Policy foundations include risk management principles, risk communication and stakeholder participation, assessment principles and risk tolerability.

The steps undertaken to make a decision are (i) preparation, (ii) problem formulation, (iii) participation strategy, (iv) assessment, (v) evaluation and (vi) decision. This presentation will take the participants through the framework and its tools by using case studies.

All of the RBDM tools are available on line at https://www.allianceofbloodoperators.org/abo-resources/risk-based-decision-making/rbdm-framework.aspx

3D-S15-02

ESTIMATED RISK OF INFECTED BLOOD PRODUCTS FROM DUTCH DONORS RETURNING FROM WEST NILE VIRUS AREAS IN EUROPE

RW Lieshout-Krikke[1], K Aranko[2] and MP Janssen[1]

[1]Sanquin, Amsterdam, The Netherlands [2]European Blood Alliance, Amsterdam, The Netherlands

Background: The occurrence of one or more human autochthonous cases of West Nile Virus (WNV) infection in an area triggers blood safety measures for affected and non-affected countries in Europe. Dutch donors who travelled to a WNV affected area are deferred for 28 days. The European Up-Front Risk Assessment Tool (EUFRAT) is designed to analyse emerging infectious disease (EID) outbreaks and to estimate the associated risk for transfusion recipients. This web-based tool, initially developed in 2011 for the European Centre of Disease Control (ECDC) and updated in 2015, is a ready-to-use application which provides stepwise estimates of the risk for transfusion recipients of being exposed to contaminated blood products. When specifying travel characteristics, the tool can also estimate the risk from donors returning from a region where an EID outbreak occurred.

Aims: To estimate the risk of WNV infected blood products from Dutch donors returning from WNV affected areas in France or Italy in 2015.

Methods: One case of human WNV infection in the Gard department (France) and 49 human cases in Northern-Italy were reported by ECDC in 2015. The risk of infected products from donors who travelled to WNV affected areas in France and Italy was calculated using EUFRAT. Details on travel behaviour were obtained from a study of Dutch donors (Lieshout, Transfusion, 2015). The following assumptions were made:

- Donors have the same probability of acquiring infection as other individuals;
- Travelling donors have the same exposure as the resident population;
- There is no effect of the infection on donation behaviour;
- The infections are evenly distributed over the outbreak period;

Description	Department Gard (France)	Northern-Italy
Number of infected cases in the region	1	49
Population of the outbreak region	740,660	9,231,108
Visiting donors per day	7.8	47.9
Number of donors exposed	932	5747
Duration of stay during the visit (days)	10	10.1
Total number of donors in the Netherlands (excluding plasmapheresis donor for fractionation and solvent/detergent plasma)	277,762	
Duration of epidemic	22 June till 15 November 2015	
Proportion of undetected cases	80%	
Duration of infectivity of infected individuals	6 days	
Latent period before becoming infectious	1.5 days	
Proportion of chronic disease cases	0%	
Products manufactured in 2015	435,405 red blood cells 294,940 buffy coats 3,625 apheresis platelets 1,162 plasma units	
Estimated number of infected products per year without WNV testing (years between invents)	8.64E-06 (115,740 years)	2.09E-04 (4,785 years)
Estimated number of infected products per year with MP16 WNV-NAT testing (years between invents)	1.21E-06 (0.8 million years)	2.92E-05 (34,247 years)
Estimated number of infected products per year with ID WNV-NAT testing (years between invents)	3.46E-07 (2 million years)	8.34E-06 (1.2 million years)

Table 1 Estimated number of infected products using the EUFRAT tool

Table 1. Estimated number of infected products using the EUFRAT tool

- The sensitivity of WNV NAT testing in minipools of 16 donations (MP16) and for individual donations (ID) are presumed to be 86% and 96% respectively.

Results: The parameters used for the EUFRAT and calculations are described in Table 1. Without testing the estimated number of infected products per year are 8.64E-06 and 2.09E-04 products when Dutch donors are not deferred for donation after travel to WNV affected areas in France or Italy, i.e. one infection in 115,740 and 4,785 years in the Netherlands respectively. The estimated number of infected products is reduced between 7 and 24 times after WNV NAT testing, depending on the test sensitivity.

Summary/Conclusions: The EUFRAT calculations clearly show that the risk of infected blood products of donations from donors who travelled to WNV affected areas in France or Italy is very low. For non-affected areas the risk is dependent on the exposure of donors whilst travelling abroad. One may question the necessity of blood safety measures for travelling donors who visited areas where the transmission risk of WNV to humans is relatively low.

3D-S15-03

RESIDUAL RISK OF HIV, HCV AND HBV AT CANADIAN BLOOD SERVICES

F O'Brien, QL Yi, W Fan, V Scalia and MA Fearon

Canadian Blood Services, Ottawa, Ontario, Canada

Background: In addition to donor education and deferral of high-risk donors, testing blood donations reduces transfusion transmitted infections. In 2011, nucleic acid testing (NAT) was upgraded from Roche COBAS AmpliScreen assay for HIV-1 and HCV (24 unit minipools) to Roche Cobas TaqScreen MPX for HIV, HCV and HBV (6 unit minipools).

Aims: To estimate window periods and risk-day equivalents for the Roche Cobas TaqScreen MPX assay.

To estimate current residual risk (2012–2014).

Methods: The 50% limit of detection for multiplex was estimated by Probit analysis using analytic sensitivity data for each virus from the package insert. Window period risk-day equivalents were modeled based on a published method taking into account lower risk early in the period with low viral concentrations and increasing probability of NAT detecting viremic donations as viral concentrations increase later in the period. All allogeneic donations made to Canadian Blood Services between January 1, 2012 and December 31, 2014 were included. Donors with at least two donations over 3 years were included for assessment of incidence. Incidence was the number of positive donations divided by the corresponding person-years of observation. Ninety-five percent confidence intervals were calculated by the exact method based on the Poisson distribution assumption. The HBV incidence rate was adjusted for transient viremia. Incidence in first-time donations was estimated by multiplying the repeat-donor incidence rate by a conversion factor of 3.2. The residual risk of transfusion-transmitted infection was calculated as the product of the incidence rate and the window period risk-day equivalents point estimates. Confidence intervals were estimated using a Monte-Carlo simulation to include the confidence interval

for the incidence rate and the confidence interval of the window period risk-day equivalents estimate.
Results: The window period risk-day equivalents are somewhat shorter than those calculated with the assumption of uniform infectivity over the full window period: HIV 6.1 (4.4–7.8) vs 8.0 (6.9–9.2) days; HCV 2.9 (2.1–3.8) vs 4.1 (3.6–4.6); HBV 18.8 (13.3–24.3) vs 22.4 (18.3–26.5). There were 2,579,980 allogeneic donations included in the analysis. The incidence of HIV was 0.28 (0.04–1.03) per 100,000 person-years, HCV 1.0 (0.04–2.06) and HBV 0.26 (0.01–1.37). Residual risk of HIV was 1 per 21.4 (111.1, 6.0) million donations, HCV 1 per 12.6 (33.3, 5.9) million donations and HBV 1 per 7.5 (50,1.5) million donations.
Summary/Conclusions: Our updated residual risk estimates reflect improvement in sensitivity of NAT, mostly due to a smaller minipool size (6 vs 24 units) but are not significantly lower than previous years after taking into account uncertainty around the incidence and risk-day equivalents. MPX was implemented largely for operational reasons, and these results highlight that in countries such as Canada that already have very low incidence, residual risk has become so low that any safety benefit of further shortening the window period is below the threshold to quantify.

3D-S15-04

UTILITY OF ALGORITHM IN CONFIRMING NAT/SERO YIELD CASES

K Bains
P D Hinduja National Hospital & MRC, Mumbai, India

Background: Since 2006, NAT was implemented in India in conjunction with Enzyme Immunoassay (EIA) to reduce the risk of Transfusion Transmitted Infections (TTIs) and consequently to provide safer blood units. In December 2011, an algorithm was proposed for NAT users India which we have been following in our institution to standardize the testing pattern in case of NAT yield and sero yield cases. NAT has been implemented in Hinduja Hospital Mumbai, India since March 2009. Donors were screened for HIV-1/HBV/HCV by NAT assay using the algorithm. Recently there have been reports showing prevalence of OBI (Occult HepB Infection) in the donor population in different parts of the world. OBI (HBV) refers to presence of HBV-DNA in the absence of HBsAg. OBI may reactivate and cause acute hepatitis in immuno-compromised patients receiving chemotherapy.
Aims: To confirm NAT/Sero yield using algorithm and to determine OBI frequency in donor population.
Methods: A total of 73,014 blood donor samples were tested by EIA (Axsym and Architect, Abott) for detection of HIV-Ag&tAb, HCV-Ab and HBsAg. Each donor was also tested by ID-NAT assay using Transcription Mediated Amplification (Procleix Ultrio Plus assay, Grifols). All reactive donors by NAT were subjected to discriminatory assay for detection of specific virus. NAT yield and sero yield cases were reported using algorithm. Additional biochemical (LFTs), serological [anti HBsAg, anti HBc (total), HBeAg, anti HBeAg] and molecular tests (HBV PCR) were performed in all HBV positive cases on discriminatory assay. In this study, we are also presenting an interesting case of HIV-HBV coinfection.
Results: Out of 73,014 donors screened, 26 were NAT yield and all these were found to be cases of HBV infection on discriminatory testing. Liver function tests of all donors were found to be within normal limits. 24/26 NAT yield donors were positive for anti HbsAg indicating occult hep-B infection (OBI), 17/26 for anti HBeAg and all were negative for HbeAg. 2/26 were window period infection. Seven NAT yield samples have been tested for HBV-PCR till date in which two samples showed viral load of 140 & 151 IU/ml whereas in five viral load was not more than 30 IU/ml. Results are still awaited for rest of the samples. The risk of TTIs in our setup when extrapolated from the NAT yield is 1:2,808 donor units tested. An interesting case was reported which was HIV positive on ELISA and NAT positive but on discriminatory assay it showed HBV positivity. Rapid test for HIV was performed showing positive results for HIV-II. To confirm western blot assay and PCR assay for viral load were conducted for HIV-I which came out negative. In addition, same sample was sent to National Institute of Virology (NIV) for HIV-II western blot assay, which confirmed HIV-II positivity. After this the case was finally reported as HIV-II with HBV coinfection (HBV NAT yield).
Conclusion: NAT yield 26/73,014 were confirmed using algorithm for Indian NAT users. 24/26 were OBI cases. The study reveals that blood donors with OBI are not uncommon in India. This highlights number of unique issues with OBI i.e. whether the HBV DNA positive donor samples represent ongoing viral replication that can eventually progress to clinically significant infection in the host or whether a risk of transmission exists.

3D-S15-05

DONOR SCREENING FOR TRANSFUSION-TRANSMISSIBLE INFECTIONS – A GLOBAL PERSPECTIVE

JGA Vincini and LM Cabuang
NRL, Fitzroy, Australia

Background: NRL has unique insight into global donor screening for transfusion-transmissible infectious diseases (TTID) through provision of quality assurance programs to the largest global donor screening network of any international provider of quality assurance services. This network incorporates all six continents and uses diagnostic devices ranging from high-throughput fully automated instruments such as Abbott PRISM and Grifols TIGRIS, to simple rapid test devices such as particle agglutination or immunochromatographic tests, reliant on manual setup and interpretation. Schemes provided by NRL specifically designed for blood and tissue donor testing sites allow an assessment of performance comparatively of these technologies.
Aims: A review the 2015 Donor Screening Serology and NAT scheme data to identify tests test technologies used and the proportion of erroneous results reported per technology type.
Methods: MMBS4320 2015 EQAS consisted of three 20-member panels. Each of these three panels was manufactured independently throughout the calendar year prior to distribution to, and eventual testing by, participants. Each sample in the panel contained pooled/unpooled normal human plasma (NHP) that was positive for HBsAg, anti-HIV, anti-HCV and/or anti-HBc total or confirmed negative for the presence of all of these markers. In the event where pooling was necessary, the pooled candidate samples were of identical serological profile to ensure the pooled sample matched the single candidates. Once manufactured, the panels were stored at 2–8°C until shipment to participants. Shipments were sent at ambient temperature as per NRL validated procedure. NATA4315 2015 EQAS consisted of three 15-member panels. All three panels were manufactured at the same time and distributed frozen on 'dry ice' to be stored by the participants until the appropriate testing period. The final samples were tested using NRL testing algorithms to confirm the samples performed as expected. These test results were used as reference results for the scheme to evaluate participation test results and assay interpretations. Test results and assay interpretations were collated using OASYS (Oneworld Accuracy, Canada) and analysed at NRL post-Test Event.
Results: Serology: A total of 36,807 test results were generated by 120 participants using 135 different test devices. Of these, 26 were chemilluminescence tests, 68 were enzyme immunoassays (EIA), 30 were immunochromatographic tests (ICT), two were particle agglutination assays (PAA) and nine were immunoblot tests.
NAT: A total of 15,787 test results were generated by 133 participants using 20 different test devices. Fourteen of the 20 test devices were provided by two manufacturers: Roche and Grifols.
Conclusions: Relatively few erroneous results were observed in NATA4315, consistent with the scheme being dominated by fully automated test platforms. MMBS4320 on the other hand demonstrated many examples of erroneous test results, many of which were attributable to a manual element to testing, particularly EIA, ICT and PAA where manual preparation or interpretation influences performance of these tests directly. The ramifications of erroneous results can differ greatly in their potential consequences. The consequence of a false-reactive test result may lead to unnecessary disposal of a blood donation and potential deferral of a blood donor and requirement for unnecessary costly repeat and further confirmatory testing. False reactive screening results in effect do not pose a threat to blood safety, but do add to the economics of providing a lean blood screening service. A false negative result, however, does pose a serious threat to blood safety through transfusion-transmitted infection.

Plenary Session II: Jean Julliard Prize

PL2-01

No abstract available.

PL2-02

BIG DATA IN TRANSFUSION MEDICINE – TOWARDS A DATABASE DRIVEN APPROACH TO ENSURING THE LONG-TERM HEALTH OF BOTH BLOOD DONORS AND TRANSFUSED PATIENTS

G Edgren

Karolinska Institutet, Stockholm, Sweden

Thanks to the hard work of researchers, clinicians and regulators, transfusion safety has seen continuous improvement in the past three decades. This can perhaps best be exemplified with the virtual removal of transfusion transmitted infections such as HIV and the hepatitis viruses from the blood supplies of most Western countries. Meanwhile, with the gradually decreased importance of the known transfusion transmitted infections, there is a growing apprehensiveness of non-infectious deleterious effects transfusions, or of the transfusion transmission of atypical infectious agents such as prions. It was in the wake of these perceived threats that we created the first version of the Scandinavian donations and transfusions (SCANDAT) database in 2005. At its inception, it encompassed all available computerized information on blood donations and transfusions in both Sweden and Denmark from the late 1960s until 2002. The database, which from the beginning provided up to 34 years of follow-up on 1.1 million blood donors and 1.3 million transfused patients, was originally created to study the occurrence of cancer among both blood donors and transfused patients, as well as the possibility that cancer could be transmitted from blood donors to their recipients. However, upon realizing the novelty and usefulness of this large-scale hemovigilance tool, its use was quickly expanded to incorporate also other health outcomes. Over the years since SCANDAT database was first described, it has resulted in the publication of nearly 30 papers covering many aspects of the wider concept of transfusion safety, such as donor health, health effects of blood donation, transfusion associated risks, and risk of disease transmission from donors to recipients. This work has been challenging as it has required the development of new statistical and epidemiological approaches for, the circumventing of legal hurdles, as well as the management of complex and large-scale administrative data. Overall, however, our work serves to ensure the health of both blood donors and transfused patients, with the ultimate aim of improving transfusion safety.

Blood Cells

4B-S16-01

EVIDENCE AGAINST A ROLE FOR RED CELL CLEARANCE IN ANTIBODY-MEDIATED PREVENTION OF ERYTHROCYTE ALLOIMMUNIZATION

AH Lazarus

The Canadian Blood Services, Toronto, Ontario, Canada

Anti-D has long been used in the successful prevention of hemolytic disease of the fetus and newborn. Despite this clinical success, it's mechanism of effect is not well understood. The leading hypothesis has been that anti-D suppresses erythrocyte immunization by the rapid removal of antigen positive cells before immunization can occur. We will revisit some of the older literature that was in conflict with this hypothesis and present new work in a mouse model of allogeneic erythrocyte immunization which demonstrates that complete suppression can occur with antibodies that do not mediate red cell clearance. We will also present recent data suggesting that Fc receptors are not necessary for preventing erythrocyte immunization.

4B-S16-02

ANTIBODY-MEDIATED PHAGOCYTOSIS OF RED BLOOD CELLS BY NEUTROPHILS IN THE HUMAN SPLEEN

SM Meinderts[1], PA Oldenborg[2], B Beuger[1], J Johansson[2], TW Kuijpers[3], M de Haas[1], TK van den Berg[1] and R van Bruggen[1]

[1]Sanquin Blood Bank, Amsterdam, The Netherlands [2]Umeå University, Umeå, Sweden [3]Emma Children's Hospital, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

Background: Homeostatic red blood cell (RBC) clearance is known to occur by erythophagocytosis in the spleen, and it is presumed to be primarily executed by red pulp macrophages. It can be further promoted by IgG opsonization of RBCs, a condition that can occur as a consequence of auto- or allo-antibody formation. Allo-antibodies can be formed after repeated transfusion while auto-antibodies can be formed during pathological conditions, such as autoimmune hemolytic anemia.
Aim: Investigate IgG-mediated RBC clearance by phagocytes of the human spleen.
Methods: To measure erythrophagocytosis *in vitro* in an unbiased fashion we used a highly specific method in which we incubate magnetically labelled RBCs with human splenocytes. The erythrophagocytic fraction of the spleen cells was isolated using MACS and analysis of the phagocytic cells was performed by flow cytometry. Furthermore, isolated blood neutrophils were used to study the requirements for neutrophil erythrophagocytosis. For a similar analysis *in vivo* in mice we injected PKH26-labeled IgG-opsonized RBCs intravenously into recipient wild-type mice and erythrophagocytosis was measured in splenocyte cell suspensions using flow cytometry.
Results: Surprisingly, we found that besides macrophages also neutrophils from the spleen are able to take up IgG coated RBCs. Erythrophagocytosis by neutrophils was strongly dependent on the degree of opsonization of the RBC. Additionally, the process was greatly enhanced by blocking the 'don't eat me' signal CD47 on the opsonized RBCs, which binds SIRPα, a myeloid inhibitory receptor that restricts phagocytosis. Finally, priming of neutrophils by inflammatory stimuli further increases the magnitude of erythrophagocytosis.
Conclusion: Collectively, our data suggest that whereas homeostatic clearance of RBC in the spleen may involve red pulp macrophages, splenic neutrophils can also contribute significantly to this process, particularly when RBC are opsonized by antibodies. Erythrophagocytosis by neutrophils can further be potentiated by inflammatory mediators and is counter-balanced by CD47-SIRPα interactions. This indicates a hereto unanticipated contribution of neutrophils in RBC phagocytosis which may contribute to the clearance of RBCs, especially under pathological conditions such as allo- or auto-immunization.

4B-S16-03

ABO HDFN – ARE WE DOING RIGHT? EXPERIENCE FROM INDIA

S Das[1], S Shastry[2] and P Baliga[1]

[1]Kasturba Medical College, Manipal, Udupi, India [2]Kasturba Medical College, Manipal University, Manipal, Udupi, India

Background: ABO incompatibility is the most common cause of Hemolytic Disease of Fetus and Newborn (HDFN) in developed countries, after the advent of routine immunoprophylaxis with RhIg. There is not much published literature on the true incidence and clinical significance of ABO HDFN in a developing country like India.
Aim: To assess the frequency of ABO HDFN and to establish the relationship of maternal IgG antibody titres and neonatal direct antiglobulin test (DAT) with the severity of HDFN.
Material and methods: A prospective study was carried out from October 2013 to May 2015 at our tertiary care center from south India. ABO cell and serum grouping

Table 1: Distribution of ABO blood group among the mothers and the newborns

	Mother					
Baby	**A**	**B**	**AB**	**O**	**Oh**	**Total**
A	229	***83 (9.4%)****	30	***372 (41.9%)****	***1 (0.1%)****	715
B	***54 (6.1%)****	303	39	***355 (40%)****	-	751
AB	43	53	16	***19 (2%)****	-	131
O	122	190	6	940	***1 (0.1%)****	1259
Total	448	629	91	1686	2	2856

(* - Group specific Percentage ABO Incompatiblity)

were carried out on the mother and newborn using column agglutination technology (Ortho AutoVue). Direct Coomb's test (DAT) was carried out on the neonatal red cells. Elution studies were performed to confirm the specificity of the antibody in DAT positive ones. ABO HDFN was diagnosed after ruling out all the other possible causes leading to HDFN. Antibody quantification by double dilution on the pre-treated maternal serum with dithiothreitol, using the corresponding antigen to the antibody was determined for the mothers whose newborn had a positive DAT. Neonatal outcomes were followed up.

Results: A total of 2,856 antenatal mothers and their newborns were reviewed. The frequency of ABO incompatibility was seen with 31.06% cases (n = 887). The distribution of the mothers and newborn ABO blood group is shown in (Table 1). ABO incompatible pregnancies were highlighted.

The incidence of newborn with a positive DAT was 12.3% (n = 108). The positive predictive value of DAT for clinical manifestation of jaundice in 35.2%.

High titers of maternal antenatal IgG anti-A and anti-B have been associated with ABO HDFN. The maternal antibody titre of 1:64 was noted to be significant in this study. A positive linear relationship was illustrated between the levels of maternal IgG antibody titer and the strength of the DAT. The onset of clinical jaundice in DAT positive newborns was early and needed phototherapy for a longer period.

Conclusion: In the present study, ABO incompatibility with a positive DAT showed an early rise in serum bilirubin levels and exhibited strong positive correlation with maternal antibody titers. Such newborns should be monitored for neonatal hyperbilirubinemia at an early stage.

4B-S16-04

DIAGNOSTIC ACCURACY OF LABORATORY MONITORING IN K-IMMUNIZED PREGNANCIES

YM Slootweg[1], ITM Lindenburg[1], JM Koelewijn[2], IL van Kamp[1], D Oepkes[1] and M de Haas[2]

[1]Leiden University Medical Center, Leiden, The Netherlands [2]Department of Immunohematology Diagnostics, Sanquin Diagnostic services, Amsterdam, The Netherlands

Background: K antibodies are second to RhD antibodies in importance as cause of severe hemolytic disease of fetus and newborn (HDFN). K antibodies cause hemolysis of fetal erythrocytes, but also inhibit the fetal erythropoiesis. Laboratory monitoring of K-immunized pregnancies by repeated measurement of antibody titers and the biological activity of the antibodies with the Antibody Dependent Cellular Cytotoxicity (ADCC) test has the purpose to select pregnancies at increased risk for development of fetal anemia. High-risk pregnancies need intensive clinical monitoring with ultrasound-based judgement of fetal anemia. In the literature, various critical titers are proposed for anti-K, varying from two to eight. The cut-offs of the laboratory test results for optimal selection of high-risk K-immunized pregnancies are still unknown. In The Netherlands, a titer of two and a threshold of 30% for the ADCC test is used.

Aim: The aim of this study was to assess the diagnostic accuracy of the ADCC test and antibody titer to select K-immunized pregnancies, at increased risk for severe HDFN.

Methods: Laboratory and clinical data of all pregnancies diagnosed with anti-K and an K-positive fetus were collected retrospectively for the period January 1999 until April 2015. Laboratory data were collected from the laboratory information system of the national reference center for laboratory monitoring in pregnancy of Sanquin Diagnostic Services. Clinical monitoring was collected at the national reference center for monitoring and treatment of maternal alloimmunisation, Leiden University Medical Center (LUMC), or from midwives and physicians.

Results: In 16 years, there were 1,023 K-immunized pregnancies, 126 pregnant women with an K-positive child were followed. From 121 cases all data were collected, outcome data of five cases were missing. Severe HDFN (need for IUT) occurred in 62 pregnancies, moderate HDFN (need for exchange or top-up transfusion, only) occurred in seven pregnancies and in 49 pregnancies there was no HDFN or only phototherapy was given. The first measurement of titer and ADCC activity was in week 14 (Interquartile range 25–75% 13–18 weeks). The titer showed a higher diagnostic accuracy with an AUC of 84% for the highest titer during pregnancy, compared with the highest ADCC test result with an AUC of 80%. Linear regression revealed that the titer and ADCC did not change significantly during pregnancy when the titer and ADCC were compared with the two foregoing measurements. The AUC of the first measured titer was 85%. The optimal cut-off point for the titer was 4. The ADCC test showed an optimal cut off a value of <10% and was therefore not informative. If a titer of four was used, only one case with the outcome of severe HDFN was missed. In this pregnancy, severe HDFN was detected late in pregnancy without previous laboratory monitoring.

Conclusion: To select pregnancies with an increased risk for anti-K-mediated severe HDFN, determination of the anti-K titer early in pregnancy is sufficient to select pregnancies at increased risk for severe HDFN. The optimal cut-off value is a titer of 4. Measurement of biological activity of the K antibodies with the ADCC test does not increase the diagnostic accuracy of laboratory monitoring.

4B-S16-05

THE FIRST REPORT OF ANTI-DHA (ANTI-GE8) IMPLICATED IN SEVERE ANEMIA OF THE FETUS AND NEWBORN IN TWO CONSECUTIVE PREGNANCIES

C Lomas-Francis[1], A Burgos[1], MG Dombourian[2], K Houston[2], S Vege[1], C Melland[3], A Deering[3], KE Capocelli[2] and CM Westhoff[1]

[1]New York Blood Center, Long Island City, New York, United States of America [2]Children's Hospital Colorado, Aurora, Colorado, United States of America [3]Bonfils Blood Center, Denver, Colorado, United States of America

Background: A 34-year old Caucasian woman had a history of anemia of the fetus and newborn (AFN) from her first pregnancy. This fetus was diagnosed with anemia and hydrops at 30 weeks gestation, transfused at 31 weeks, and delivered at 33 weeks. During her second pregnancy her fetus had severe anemia and hydrops at 27 weeks gestation. By week 36 the fetus had received four intrauterine transfusions. Labor was induced at 37 weeks gestation. Both babies had reticulocyte counts of <1% at birth. The second baby had an initial bilirubin level of 3.9 ng/ml peaking at 8.7 ng/ml on day 4 of life. Phototherapy was not required. An antibody (titer 8 at week 31) to an unidentified low-prevalence antigen expressed on the fetal RBCs, inherited from the father, specifically anti-M^g was suspected from initial testing of the patient's plasma.

Aims: A serological and molecular investigation was undertaken to identify the antibody and the low-prevalence target antigen.

Methods: Standard hemagglutination methods were used. Genomic DNA was isolated from WBCs of the parent's samples and from buccal swabs from child 1 and child 2. *GE* exons were amplified and sequenced.

Results: The patient's plasma, when tested against RBCs from the baby's father, was reactive in direct testing in saline at room temperature and reacted 3+ by LISS or PEG IAT. The previously suspected anti-M^g was confirmed but the father's RBCs were M^g-negative The unidentified antigen expressed on the father's RBCs and detected by the patient's plasma was sensitive to papain and trypsin but resistant to alpha-chymotrypsin or dithiothreitol (DTT) treatment, a pattern of reactivity unique to several MNS and Gerbich system antigens. The severe fetal anemia suggested the target antigen was on a molecule expressed early in erythropoiesis, e.g., Kell or glycophorin C and D. Sequencing of the father's *GE* showed heterozygosity for c.40C>T in exon 1 encoding a p.Leu14Phe (*GE*01/*01.08*) associated with expression of the low-prevalence Gerbich antigen Dh^a carried on glycophorin C. Serological testing confirmed the father's RBCs to be Dh(a+). The patient's plasma reacted with three examples of Dh(a+) RBCs, including RBCs from the original Dh(a+) proband. DNA testing of buccal swabs from the children showed both were heterozygous for c.40C>T and predicted to be Dh(a+). No changes were found in the mother's *GE* gene.

Conclusion: We report the first example of severe anemia of the fetus and newborn due to anti-Dh^a in a Caucasian family, the father having mixed English/Norwegian/French ancestry. Dh^a is a rare antigen first described in a Danish blood donor and later in an English family. Similar to the mechanism of erythroid suppression caused by anti-K, anti-GE3 (high-prevalence GE3 antigen) has been associated with suppression of erythroid progenitor cell growth in the infant as well as with antibody-dependent hemolysis. When fetal anemia in the absence of indication of significant hemolysis is observed in pregnancy, Gerbich or Kell antibodies should be suspected. Although an eluate made from the cord blood was not available for confirmatory testing of anti-Dh^a on the fetal cells, the circumstances are consistent with ANF due to anti-Dh^a.

Plasma Donation

4B-S17-01

KEY FACTORS TO MOVE TO SELF-SUFFICIENCY IN SAFE BLOOD BASED ON VOLUNTARY BLOOD DONATION

M Maghsudlu[1] and AA Pourfathollah[2]

[1]*High Institute for Research and Education in Transfusion Medicine, Tehran, Iran*
[2]*Iranian Blood Transfusion Organization, Tehran, Iran*

One of the oldest resolutions of WHO urges member states to Promote the blood services based on voluntary non-remunerated blood donation (VNRBD). Consequently, achieving Self-sufficiency in six driver products have been focoused by WHO and other international agencies in 2010. However both VNRBD and Self-sufficiency are not a reality in many countries as reported by WHO in 72 countries, more than 50% of the blood supply donated by family and paid blood donors.

In Iran, more than 2.100.000 units of whole blood are being collected annually; all of them from voluntary blood donors. These units are Processed to RBC, Plt and FFP, that cover all the need of hospitals generally. Based on formal agreements between IBTO and the fractionators, redundant FFPs are sent for being processed that cover mostly Albumin and IVIG demand.

Significant measures already made to improve voluntary blood donation and move toward self-sufficiency in Iran in term of quality and quantity are followed.

- Establishment of a well organized national blood transfusion center since 1974 with the most important goal was being to ensure a safe and adequate blood supply.
- Establishment of a central department responsible for all activities related to blood donors including Planning program, Providing SOPs, staff training and supervision over blood centers (2002).
- Adopting some policies to enhance voluntary blood donation in IBTO

No payment is given to donor which could be considered as a substitute for money (1974) Blood drives in places where the prospective blood donors would be under no pressure and not encouraged by awards or pay-off work (2000) Donor motivational messages should be based on altruism and humanitarian driven (2003)

- The media and art Involvement
- Partnership whit NGOs in order to reduce the gap between policy makers and people
- Availability of blood donation facilities as there are 229 ones throughout the country
- Maintain a regular voluntary donor base as more than 75% of blood units are donated by regular and repeated donors.
- Establishment of a donor vigilance system in order to improve donor care
- Establish a national donor database and information management system.
- Establishment of a well-designed blood inventory management system in order to reduce wastage of products as well as reduce the shortage of specific products
- Commencement of the Donor Recruitment Research efforts as the supporter of the Donor Program

Continues efforts have resulted in increasing in blood donation rate (26 per 1,000 population) in the past decade as well as achieving 100% VNRBD in Iran. In order to achieve self-sufficiency Patient Blood Management could play an important role in avoiding unnecessary exposure to blood component and meeting the demand. In spite of some great steps have been taken such as the preparation of the national clinical guidelines and clinical staff training program, more action is required to implement this system in a more efficient way.

4B-S17-02

EXAMINING TRENDS IN FIRST-TIME PLASMAPHERESIS DONORS' RETURN BEHAVIOUR: WHY DO SOME DONORS ONLY DONATE ONCE?

CN Gemelli, A Thijsen, ST Wright and TE Davison

Australian Red Cross Blood Service, Melbourne, Victoria, Australia

Background: The increased demand for plasma and plasma-derived products coupled with the decreased demand for whole blood has shifted marketing and business priorities for the Australian Red Cross Blood Service over recent years. New strategies are required to convert donors from whole blood to plasmapheresis collections and to retain these donors once converted. Previous research has indicated that there are motivational differences between whole blood and plasmapheresis donors that are evident after their first donation; however, there is an absence of information on key factors that enhance or inhibit a new plasmapheresis donor from returning to donate after the first donation.

Aims: The aim of this study was to investigate first time plasmapheresis donors to determine what influences or inhibits their return behaviour over a 2 year period. This information is critical for the development of strategies to optimise retention.

Methods: Donation data were collated for all donors who had given their first plasmapheresis donation between 1 January 2012 to 31 December 2012 (n = 34,605). Donor characteristics examined were age, sex, blood group, previous donation history, and donation behaviour over the subsequent 2 years. Data was also recorded on whether or not a donor experienced an adverse reaction during or after the plasmapheresis donation, and whether or not a temporary deferral was applied to the plasmapheresis donation.

Results: Of 34,605 new plasmapheresis donors, 16,452 donated plasma only once. Fifty-three per cent of these donors were male, with a mean age of 37.7 years, and they had relatively short donor careers (M = 11.9 prior whole blood donations).

Survival analysis revealed that younger age, female sex and having a shorter donation career were associated with increased hazard of not returning to donate plasma. Donors who received a temporary deferral were less likely to return in the 2 year follow-up than non-deferred donors. Furthermore, donors who experienced an adverse reaction during their first plasma donation had a reduced likelihood of returning to donate plasma.

Further analysis on those who did not return to plasmapheresis revealed that thirty per cent of donors lapsed from blood donation altogether. Donors who had received a deferral or experienced an adverse reaction were at an increased risk of lapsing from both plasmapheresis and whole blood donation.

Summary/Conclusions: The process of converting donors from whole blood to plasmapheresis donation focuses largely on procedural and operational differences and largely ignores future donation patterns, including risk factors for non-return. This study highlighted the negative impact of the experience of an adverse reaction or receipt of a deferral for new plasma donors on their likelihood of continuing to donate. There is a need to develop targeted interventions which address these barriers to retention, with particular attention to younger, female and less experienced donors. Recommendations are made for the development of key retention strategies for new plasma donors to help them move from being a new plasmapheresis donor to an established plasmapheresis donor, in order to continually grow this panel and prevent lapse from both plasmapheresis and whole blood donation

4B-S17-03

STUDY OF SERUM FERRITIN LEVELS IN REGULAR PLATELETPHERESIS DONORS OF DUBAI BLOOD DONATION CENTER

R Sharma, MY Raouf, MF Odeh Farajallah and A Nagaramchal

Dubai Health Authority, Dubai, United Arab Emirates

Background: Plateletpheresis donors lose up to 100 ml of blood, which includes lose in the tube samples and residual blood in the tubing sets at each donation leading to concerns that they may become iron deficient. Frequent plateletpheresis donors donating at the maximum donating frequency of every 2 weeks are more at risk of becoming iron deficient as they lose almost about equivalent to 4 units of blood each year.

Aim: To measure 172 regular male plateletpheresis donors for serum ferritin to assess the level of iron stores.

Methods: All plateletpheresis donors in DBDC are regular donors. All male donors who visited DBDC for plateletpheresis in the time period between 15th March 2016 to 30 March 2016 were included in the study. The donors were categorized into two groups-A and B and group I and II. Group A are the frequent donors who donated more than 20 times in 1 year (52 donors) and group B are the donors who donated <20 times (120 donors) in 1 year. Group I donors has been donating platelets since last 3 years (118 donors) and group II donors have been donating platelet more than 3 years (54 donors). All plateletpheresis donors are selected according to AABB guidelines. CBC is performed before each donation and a minimum hemoglobin of 12.5 g% is required for donation. The participants were also asked about their diet, any medical conditions like hemorrhoids and gastritis and if taking any iron supplements. The sample for ferritin was collected in 3 ml plain tube during donation and serum ferritin assessed in batches using ARCHITECT Ferritin assay (CMIA). Plateletpheresis was performed in Trima Accel Automated Blood Collection System V6 and Hemonatics MCS+. P values <0.05 was used for testing significance.

Results: The result shows that 25% (43/172) of the plateletpheresis donors had serum ferritin <12 ng/ml. In the first group, group A, the donors who donated more than 20 times in 1 year (21/52 = 40.4%) had serum ferritin levels <12 ng/ml which was significantly higher than group B, the donors who donated <20 times in 1 year (22/120 = 18.3%) had serum ferritin levels <12 ng/ml. The percentage of donors showing iron depletion is greater (20/54 = 37%) in the group donating for more than 3 years

than in the group who have donated for <3 years (23/118 = 19.5%). Only three of the donors were vegetarian and only one had serum ferritin level below 12 ng/ml.
Summary/Conclusions: The result of the study concludes that frequent plateletpheresis donation and long term platelet donation may lead to iron deficiency. We advise that the accepted Hb level before donation to be 13 g% and minimum donation interval to be increased to 3 weeks and the maximum donation in 1 year to be not more than 15 times to reduce the red cell lose and iron loss.

4B-S17-04

A RANDOMISED TRIAL TO FACILITATE DONOR COMPLIANCE WITH STRATEGIES TO PREVENT VASOVAGAL REACTIONS: COMPARISON OF WEB-BASED AND IN-CENTRE APPROACHES

A Thijsen[1], TE Davison[1], B Bell[1], J Fisher[1], J O'Donovan[1], C Bramble[1], C Del Aguila[1] and BM Masser[2]

[1]Australian Red Cross Blood Service, Alexandria, Australia [2]University of Queensland, Brisbane, Australia

Background: Vasovagal reactions (VVR) have a significant impact on donor health and behaviour. Research has provided preliminary support for applied muscle tension (AMT) and pre-donation water loading (WL) in reducing these reactions. However, Australian blood donors report little knowledge or awareness of these prevention techniques. Written and video approaches have been found to reduce donor concerns and anxiety around potential adverse events but it is unknown whether these approaches increase donor compliance with preventative strategies.
Aims: The aim of this study was to compare alternative methods for communicating VVR prevention strategies which focus on increasing the use of AMT and WL among donors. To meet this aim, two studies were conducted. The objective of the first study was conducted online to assess message appeal, knowledge increase, and donor attitudes towards three communication methods tested. The objective of the second study was to assess changes in donor compliance following implementation of these three communication methods, comparing a web-based to an in-centre approach.
Methods: In study 1, active whole blood donors were randomly assigned to view a webpage (n = 128), a video (n = 110), or an instruction card (n = 100). The online survey included measures of baseline donation behaviours, message appeal, knowledge, attitudes, and preferred communication channels.
In study 2, donors (n = 600) with a whole blood appointment were randomly allocated to receive 1) an email with a link to the webpage, 2) an email with a link to the video, 3) an appointment confirmation email and onsite instructions using the card, or 4) a control group who received only an appointment confirmation email. A post-donation survey assessed use of VVR prevention strategies, self-reported vasovagal symptoms, donation satisfaction, fear, intentions to re-donate, intention to use WL, and intention to use AMT.
Results: At baseline in the first study, a small proportion of participants reported they have previously used AMT 'some of the time' to 'always' (16.2%), with a greater proportion using WL (71.0%) during previous donations. After viewing the study materials, donors indicated greater intention to use AMT (82.0%) and WL (85.0%). Donors assigned to the video had significantly greater intentions to use these strategies compared to those using other materials.
Study 2 revealed low use of the online intervention materials, with <15% of the donors reported having viewed the materials. Donors who had received the onsite instruction card reported the highest use of WL and AMT, with greater intentions to use these techniques at their next donation.
Summary/Conclusions: Despite positive results in study 1 regarding donor attitudes and intention to use VVR prevention after viewing the online video instructions, donors reported low use of the web-based materials in practice. This indicates that efforts to educate donors regarding VVR prevention should occur at the donation site in order to maximise donor compliance.

4B-S17-05

PHYSIOLOGICAL ANALYSIS OF VASOVAGAL REACTION ON PLATELET APHERESIS USING LASER DOPPLER FLOWMETRY

T Yamamoto, H Kikuchi and S Takamoto

Japanese Red Cross Hokkaido Block Blood Center, Sapporo, Japan

Background: Limited number of interventional approaches are reported successful for preventing vasovagal reactions (VVR) in whole blood donation, but not in apheresis. The mechanisms of the reaction are thought to be almost same in each donation. Difficulty to notice the physiological changes prior to VVR may cause the differences.
Aim: To detect the physiological changes, laser doppler flowmeter (LDF) was adopted for measuring hemodynamics on the donors of platelet apheresis. Peripheral blood flow and heart beats were continuously monitored and analyzed focusing on their trends and decreasing rates. In this study, blood flow decreasing rate (BDR) and heart beats decreasing rate (HDR) were calculated to examine the possibility of predicting VVR. VVR related symptoms were also analyzed using Blood Donation Reaction Inventory (BDRI) scores.
Materials and method: 486 high risk donors of platelet apheresis were informed about the study and 354 cases (156 men and 198 women) were tolerable for the analysis. 30 presyncopal VVR (8.5%) and one delayed case were observed in the study. LDF was fitted on donor's ear lobe and the signals were recorded during donation period. BDRI scores were collected at the end of the donation. Following trend analysis, BDR and HDR were calculated using standardized formula. Maximum values of BDR and HDR were obtained in each case. Both values were compared between the cases of VVR and nonVVR. Those values were also compared for BDRI positives and negatives. Based on the preliminary results, physiological condition of the donor were evaluated by stacking the values of BDR and HDR during donation period.
Result: Significant drops in both blood flow and heart beats were observed on the trend chart at the VVR. Average value of maximum BDR and maximum HDR in VVR cases were 64.7 ± 13.7% and 27.6 ± 8.6% respectively. Both values were significantly higher ($P = 4.64 \times 10^{-49}$ and $P = 1.41 \times 10^{-21}$ respectively) than those of 324 nonVVR cases. BDRI positive cases were 90 and the average value of maximum BDR was 34.3 ± 19.6%. The value was also significantly higher (P = 0.0001) than those of 264 BDRI negative cases. By monitoring the physiological conditions, large synchronized progress of BDR and HDR were assessed as decompensation state and attentive sign for VVR. In the case of delayed VVR, decompensation form was observed in physiological analysis at 16 min before leaving donation room. The donor developed VVR in resting room.
Conclusion: Continuous hemodynamics monitoring during donation period is essential for the detection of physiological change of the donor. Real time assessment of the change is necessary for predicting VVR. 45% decrease in blood flow is thought to be most capable alert point for predicting VVR. Prediction of VVR is crucial for preventing VVR.

Emerging Infectious Diseases

4B-S20-01

VIRAL SHEDDING AND ANTIBODY RESPONSE IN MERS

A Memish

Alfaisal University, Riyadh, Saudi Arabia

Background: The Middle East respiratory syndrome coronavirus (MERS-CoV) was first isolated in 2012 in Saudi Arabia. Since 2012, at least 1,731 laboratory-confirmed cases of MERS-CoV infection, mostly with respiratory tract illness, have been reported; 688 of these were fatal. Knowledge of the kinetics of viral shedding from different body regions could help prevent nosocomial transmission and inform clinical management.
Aim: Knowledge of serological features, such as the kinetics of antibody production, could guide decisions regarding diagnostic protocols and provide essential information regarding immunity and virus elimination. Quantitative data, such as viral loads and antibody titers, could enable comparisons with related diseases. In particular, severe acute respiratory syndrome (SARS), for which studies of natural history were conducted in the aftermath of the 2002–2003 epidemic.
Methods and results: In our patients and elsewhere, the Lower Respiratory Tract (LRT) was found to be the main source of MERS-CoV excretion. We have shown, in one of the few available studies, that SARS-CoV was excreted from the LRT at mean concentrations of $1.2–2.8 \times 10^6$ copies/ml, reaching a maximum of 1,010 copies/ml. From this comparison, we can conclude that average and peak LRT viral loads in MERS are equal to those in SARS. Peak URT RNA concentrations in SARS can reach up to 5 × 105 copies/sample between days 7 and 10 after onset. The corresponding number for MERS (peaking at 4.1 × 106 copies/sample), is equivalent or higher. The shedding peak in SARS patients occurred after approximately 10–12 days from symptom onset, which is very similar to the shedding maximum observed in MERS.The detection of MERS-CoV in serum is another similarity with SARS. Up to 79% of serum samples were found to contain SARS-CoV RNA during the first week of illness, and around 50% during the second week. These numbers match our observations for MERS. Viremia despite the presence of neutralizing antibodies indicates a body region that is not accessible to neutralizing antibodies but which releases virus into the blood. Although patients with SARS-CoV showed viral RNA detection rates up to 50% in urine, MERS-CoV RNA was rarely found in urine samples of

MERS patients. A clear difference from SARS was the detection of viral RNA in stool. In SARS, the RNA prevalence in stool samples was high. For MERS, we found stool-associated RNA in only 14.6% of samples, with rather low RNA concentration. The course of MERS antibody development resembles that of SARS. Patients infected with SARS seroconverted during weeks 2 and 3 after onset. Most of the MERS patients had already seroconverted during the first week after diagnosis, which putatively represents the second week after onset.
Conclusions: The timing and intensity of respiratory viral shedding in patients with MERS closely matches that of those with SARS. Blood viral RNA does not seem to be infectious. Extrapulmonary loci of virus replication seem possible. Neutralizing antibodies do not suffice to clear the infection.

4B-S20-02

VIRUS LANDSCAPE OF A BANK OF 600 BLOOD PRODUCTS ELIGIBLE FOR TRANSFUSION

O Preynat-Seauve[1], P Lau[1], D Tirefort[1], S Cordey[1], TJ Petty[1], A Guichebaron[1], E Zdobnov[2], TP Lecompte[1] and S Waldvogel-Abramowski[1]

[1]Geneva University Hospitals, Geneva, Switzerland [2]Swiss Institute of Bioinformatics, Geneva, Switzerland

Background: Although the risk of transmitting pathogenic viruses by transfusion is strongly reduced by highly sensitive laboratory tests and donors selection, many other viruses could still be present at a viraemic stage in the blood of donors and then transferred to the derived manufactured products. A complete picture of the virus lansdcape in blood products eligible for transfusion is the most relevant data to describe exhaustively the risk of virus transmission by transfusion. To date, this virus screen has never been performed.
Aims: This study describe the virus landscape of a collection of 600 manufactured blood products eligible for transfusion including 300 red blood cell units and 300 fresh frozen plasmas.
Methods: Virus screen has been performed through a metagemomics approach combining high throughput sequencing of RNA and DNA purified from blood products and the development of a bioinformatics pipeline for the identification of virus-specific genomes.
Results: Signatures corresponding to viruses known to be frequent at a viraemic stage in the blood donors population were reported and confirmed the robustness of the tool: Torque Teno Viruses, Anelloviruses, GB Virus C. Viruses infecting the normal skin were also found (Human Papillomaviruses, Merkel Cell Polyoma Virus) suggesting skin contaminants in blood products and the high sensitivity of sequencing. Finally, the entire genome of the recently described MLB2 Astrovirus was observed in one plasma unit. It was associated to an asymptomatic and acute viraemia of the corresponding donor.
Summary/Conclusions: The blood products manufacturing process do not completely eliminate the possibility of viral contamination in the final products. One unexpected virus signature was notably found in one plasma unit, and was confirmed by regular techniques to originate from the entire genome of a MLB2 Astrovirus infecting the blood of an asymptomatic donor. MLB2 Astrovirus was recently described to be pathogenic in humans, confirming the presence of unsuited viruses in blood banks despite clinical and biological selection of donors. Moreover, this study demonstrates that high throughput sequencing is a suitable tool for large virus screen in blood products.

4B-S20-03

LOW TRANSFUSION TRANSMISSION OF HEPATITIS E AMONG 25,637 SCREENED BLOOD DONORS

L Harritshøj[1], DK Holm[2], SG Sækmose[3], BA Jensen[4], BM Hogema[5], TK Fischer[6], SE Midgley[6], JS Krog[7], C Erikstrup[8] and H Ullum[1]

[1]Rigshospitalet, Copenhagen, Denmark [2]Department of Clinical Immunology, Odense University Hospital, Odense, Denmark [3]Department of Clinical Immunology, Næstved Hospital, Næstved, Denmark [4]Department of Clinical Immunology, Aalborg University Hospital, Aalborg, Denmark [5]Department of Blood-borne Infections and Virology, Sanquin, Amsterdam, The Netherlands [6]Virus Surveillance and Research Section, Statens Serum Institut, Copenhagen, Denmark [7]Section for Diagnostic and Scientific Advice, National Veterinary Institute, Copenhagen, Denmark [8]Department of Clinical Immunology, Aarhus University Hospital, Aarhus, Denmark

Background: Hepatitis E virus genotype-3 (HEV-GT-3) causes autochthonous infections in Western countries, with a primary reservoir in animals, especially pigs. HEV transfusion transmission has been reported in several countries, and HEV-GT-3 prevalence is high in some european countries and screening of blood donations is discussed in the Western World.
Aim: The prevalence of HEV RNA among Danish blood donors and transfusion transmission among recipients of HEV RNA positive blood components were investigated.
Methods: Samples from 25,637 consenting donors representing all blood centers in Denmark were collected during 1 month in 2015. Samples were screened retrospectively using an individual-donation HEV RNA nucleic acid test (NAT) with a 95% detection probability of 7.9 IU/ml. HEV positive samples were quantified by a real-time polymerase-chain-reaction (PCR) and subsequently genotyped. A donor was considered HEV positive if index sample was repeatedly reactive in either the screening NAT or the PCR test. Transmission was evaluated among recipients of HEV RNA positive blood components. Phylogenetic analyses were performed to compare HEV sequences from blood donors with danish symptomatic HEV-infected patients and HEV-positive porcine samples.
Results: Eleven donations (0.04%) were confirmed HEV RNA positive with a median HEV RNA of 13 IU/ml. Two donations were successfully genotyped as HEV-GT-3. Seven recipients available for look back tested negative for HEV RNA and anti-HEV IGM in follow-up samples drawn 1–4 month after transfusion. In one recipient, IGG anti-HEV was detected, but transfusion transmitted infection (TTI) was considered unlikely based on the fact that no HEV RNA was detectable 46 days post transfusion. phylogenetic analysis showed relatively large sequence differences between HEV-GT-3 from donors and from symptomatic patients and swine.
Conclusions: Despite an HEV RNA prevalence of 0.04% in Danish blood donations; all HEV positive donations carried low viral loads, and no evidence of TTI was found. The findings do not readily justify a general HEV screening of blood donations in Denmark, but in order to evaluate the need of HEV testing in Denmark further studies involving patients at risk are warranted.

4B-S20-04

CYTOMEGALOVIRUS-SERONEGATIVE COMPONENT SUPPLY AND DEMAND: ARE WE ABLE TO MEET OUR FUTURE NEEDS?

H Faddy, T Tran, CR Seed, S Chesneau, S Ismay, C Hogan and RLP Flower

Australian Red Cross Blood Service, Brisbane, Australia

Background: Transfusion transmitted cytomegalovirus (CMV) is well-recognised. The selection of CMV-seronegative donors as well as leucodepletion assist in the prevention of transfusion transmitted CMV. However, in the era of universal leucodepletion, there is debate regarding the necessity for CMV seroselection, and the optimal strategy for managing CMV-safe inventories is currently an area of discussion among the transfusion community. We have previously shown that demand for CMV-seronegative red blood cell (RBC) components increased in Australia and predicted that the future status of CMV-seronegative RBC component inventories may be unable to meet demand (Lancini, *Transfusion*, 2016). In order to evaluate the utility of our approach, we revised our model and refined our estimate of the ability to meet future demand for CMV-seronegative components.
Aims: To extend our previous analysis and model trends in the acquisition and demand for CMV-seronegative blood components and investigate possible discrepancies between the average age of issue of CMV-seronegative and -seropositive RBCs.
Methods: We refined our published model to include two additional time points (financial year (FY) 2013/14 and FY 2014/15). To do this, Blood Service databases were interrogated to provide the number of whole blood donations collected and the number of RBC containing components issued during the 7 years from 2008/09 to 2014/15, inclusive, stratified by CMV serostatus (positive or negative). Linear regression was used to model trends in component acquisition and demand. The age at issue of CMV-seronegative and -seropositive RBCs were also obtained. Statistical analysis was performed using ANOVA.
Results: With the addition of the two extra time points, similar trends in CMV-seronegative component acquisition and demand were modelled, and without actions to influence current trends, it is predicted that supply may be insufficient by FY 2019/20. Nationwide, the average difference in age at issue between CMV-seronegative and -seropositive RBCs ranged from 0.5 days (FY 2014/15) to 1.6 days (FY 2012/13). Nationally, the average age of a CMV-seronegative RBCs at issue was consistently younger than CMV-seropositive RBCs ($P < 0.05$) over the 7 years of the study, with a smaller difference in the most recent year (FY 2014/15).
Summary/Conclusions: This analysis supports and extends our previously reported trend of decreasing CMV-seronegative RBC component excess, however, we revise our predictions to estimate that, without further action to influence trends, supply of CMV-seronegative components may be insufficient to meet demand by FY 2019/20 as opposed to FY 2017/18. We show for the first time that issued CMV-seronegative

RBCs tended to be younger or 'fresher' than CMV-seropositive RBCs; however, in recent years this age difference narrowed. This is supportive of the idea that some of the demand for CMV-seronegative components may be due to a perceived notion of their being 'fresher' at the time of issue. These results will contribute to the current debate regarding optimal strategies for the provision of CMV-safe blood components.

4B-S20-05

VARIANT CREUTZFELDT-JAKOB DISEASE INFECTIVITY AND PRP-TSE IN BLOOD: REFERENCE MATERIALS AND ASSAY DEVELOPMENT

L Gregori, O Yakovleva and DM Asher

Food and Drug Administration, Silver Spring, Maryland, United States of America

Background: Variant Creutzfeldt-Jakob disease (vCJD) and sporadic CJD are rare fatal human neurodegenerative diseases known collectively as transmissible spongiform encephalopathies (TSEs or prion diseases). vCJD is transmissible by blood transfusion and by certain plasma products. Four transfusion-transmitted vCJD infections and one infection attributed to a plasma derivative were reported in the U.K. vCJD risk in the US blood donor population is unknown but expected to be low. However, even a small risk is of concern because vCJD is incurable and individuals can be infected for many years without any overt symptoms. A few blood tests for vCJD are under development, but so far none is available. One major challenge is lack of vCJD human blood reference materials to validate assays. As an alternative to human blood, we proposed to use blood of nonhuman primates experimentally infected with vCJD. Previous studies demonstrated that macaques are susceptible to vCJD infection and that macaque blood contains infectivity.
Aims: One goal was to generate relevant macaque vCJD blood materials in sufficient quantities to support vCJD research and validate candidate vCJD screening tests. The second goal was to develop a prototype vCJD blood test.
Methods: We experimentally infected three cynomolgus macaques with vCJD agent. Blood was collected periodically during pre- and post-clinical phases. At each collection, blood was separated into components and aliquots stored frozen.
vCJD infectivity in buffy coat and plasma from each clinically ill macaque were tested by mouse bioassay; mice were inoculated intracerebrally with 30-µl volumes of blood components and monitored for 2 years for signs of vCJD. At the same time, 100-ml units of whole blood from infected macaques were transfused into four monkeys to assess transmissibility of vCJD by transfusion.
We used an *in vitro* amplification method to detect vCJD-related PrP^{TSE}, the abnormal form of the prion protein associated with TSE infections, throughout the preclinical and clinical phases.
Results: All inoculated macaques were confirmed post-mortem to have died of vCJD. Nineteen blood collections were conducted spanning approximately 2.5 years. After buffy coat aliquots from final bleeds were inoculated into 120 mice, only one mouse developed vCJD. These results indicated that infectivity levels in macaque buffy coat were lower than anticipated. Transfused macaques remain healthy 2 and 4 years after transfusion. *In vitro* protein misfolding cyclic amplification (PMCA) method successfully detected PrP^{TSE} in plasma. PrP^{TSE} concentrations increased during the incubation period and were highest in the final bleeds. The protein was detected in plasma as early as 1 year before first clinical signs of infection. However, multiple repeats of early reactive samples sometimes failed to detect PrP^{TSE}, suggesting that blood of pre-symptomatic animals contains extremely low levels of PrP^{TSE}. This observation has practical implications for development of PMCA as a test to screen blood donors.
Summary/Conclusions: We developed and characterized candidate vCJD blood reference materials. Demonstration of infectivity in blood was challenging; but PrP^{TSE}, the protein target for all vCJD blood screening tests to date, was detectable using an *in vitro* amplification assay.

Transfusion Medicine II

4C-S21-01

TRANSFUSION AND TREATMENT OF SEVERE ANAEMIA IN AFRICAN CHILDREN: A RANDOMISED CONTROLLED TRIAL

K Maitland

Imperial College, London, United Kingdom

Background: In sub-Saharan Africa, where infectious diseases and nutritional deficiencies are common, severe anaemia is a common cause of paediatric hospital admission, yet the evidence to support current treatment recommendations is limited. To avert overuse of blood products, the World Health Organization advocate a conservative transfusion policy and recommend iron, folate and anti-helminthics at discharge. Outcomes are unsatisfactory with high rates of in-hospital mortality (9–10%), 6-month mortality and relapse (6%). A definitive trial to establish best transfusion and treatment strategies to prevent both early and delayed mortality and relapse is warranted.
Methods/Design: The Transfusion and Treatment of severe anaemia in African children: a randomised controlled trial (TRACT: ISRCTN84086586) is a multicentre randomised controlled trial of 3,954 children aged 2 months to 12 years admitted to hospital with severe anaemia (defined as a haemoglobin <6 g/dl). Children will be enrolled over 2 years in four centres in Uganda and Malawi and followed for 6 months. The trial will simultaneously evaluate (in a factorial trial with a $3 \times 2 \times 2$ design) three ways to reduce short and longer-term mortality and morbidity following admission to hospital with severe anaemia in African children.
The trial will compare (i) R1: liberal transfusion (30 ml/kg whole blood) vs conservative transfusion (20 ml/kg) vs no transfusion (control). The control is only for children with uncomplicated severe anaemia (haemoglobin 4–6 g/dl); (ii) R2: post-discharge multi-vitamin multi-mineral supplementation (including folate and iron) vs routine care (folate and iron) for 3 months; (iii) R3: post-discharge cotrimoxazole prophylaxis for 3 months vs no prophylaxis. All randomisations are open. Enrollment to the trial started September 2014 and is currently ongoing. Primary outcome is cumulative mortality to 4 weeks for the transfusion strategy comparisons, and to 6 months for the nutritional support/antibiotic prophylaxis comparisons. Secondary outcomes include mortality, morbidity (haematological correction, nutritional and infectious), safety and cost-effectiveness.
Results: The trial is currently enrolling so the mail results are not available. The presentation will cover areas relating to the blood transfusion component of the trial including type of donor blood being used; haematological quality and operational challenges to delivering GCP-compliant trial in resource-limited health services.
Conclusion: If confirmed by the trial, a cheap and widely available 'bundle' of effective interventions, directed at immediate and downstream consequences of severe anaemia, could lead to substantial reductions in mortality in a substantial number of African children hospitalised with severe anaemia every year if widely implemented.

4C-S21-02

No abstract available.

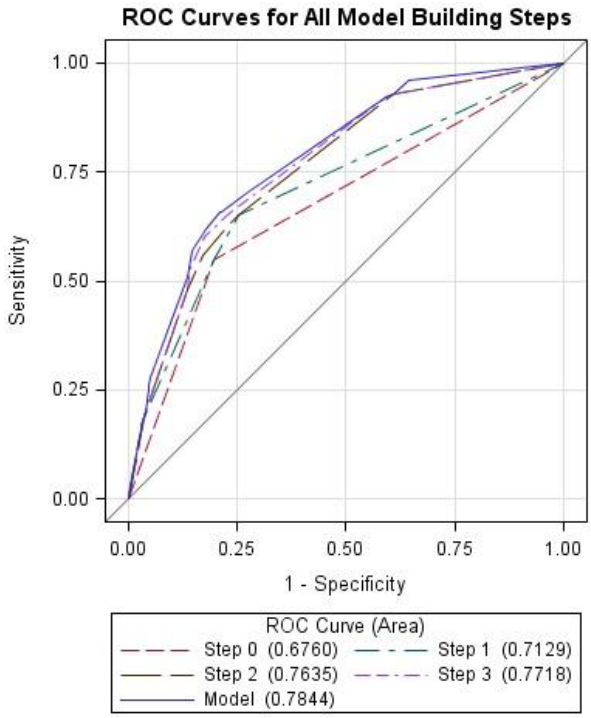
4C-S21-03

PERIOPERATIVE RBC TRANSFUSIONS ARE ASSOCIATED WITH 30 DAY POST-OPERATIVE RISK OF VENOUS THROMBOEMBOLISM: A ROBUST PREDICTION MODEL FROM A LARGE NORTH AMERICAN MULTI-CENTER PROSPECTIVE REGISTRY

R Goel[1], MM Cushing[1], AAR Tobian[2], M Nellis[1], L Vasovic[1], CM Takemoto[2], PM Ness[2] and SM Frank[2]

[1]New York Presbyterian Hospital, Weill Cornell Medical College, New York, New York, United States of America [2]Johns Hopkins Hospital, Baltimore, Maryland, United States of America

Background: Red Blood Cell transfusions (RBCT) have previously been linked with subsequent risk of developing venous thrombosis. This study examined the relationship between perioperative red blood cell transfusions (RBCT) and development of venous-thromboembolism events warranting therapy within 30 days post-operatively.



Caption 1. ROC Curve of the multivariable model for prediction of venous thromboembolism with red cell transfusions using data from the NSQIP database

Study design and methods: The ACS-NSQIP database is a multi-center prospective registry designed to evaluate risk-adjusted outcomes of surgical patients and includes 525 institutions (both teaching and non-teaching hospitals) across North America. Data are submitted electronically to the American College of Surgeons for all processing and analysis.
Using the ACS-NSQIP database, outcomes of patients undergoing elective surgeries were compared. Various preoperative clinical variables significantly associated with the occurrence of postoperative venous thromboembolism (VTE) (i.e. either deep venous thrombosis or pulmonary embolism) warranting therapy on initial univariate logistic regression were included in the multivariable logistic regression model. Stepwise selection was used to select the variables most statistically associated with post-operative VTE after initial clinically informed variable selection.
Results: A total of 750,937 subjects who underwent elective surgery were analyzed from the ACS NSQIP files during 2014. Of these, 7,395 (0.98%) subjects received one or more RBCT's within 72 h of surgery. A total of 4,336 (0.58%) subjects developed a post-operative venous thrombosis which warranted treatment. Subjects receiving RBC transfusions within 72 h before surgery as well as >1 RBCT either intra-op or within 72 h post op had significantly higher unadjusted odds of developing VTE (OR 6.8, 95% CI 5.9–7.9) [DVT (OR 8.2, 95% CI 7.0–9.6) or PE (OR 3.6, 95% CI 2.7–4.9)]. In a risk adjusted multivariable model adjusting for (i) age, (ii) length of hospital stay, (iii) immobilization (including mechanical ventilation), (iv) presence of metastatic malignancy, (v) concurrent sepsis, (vi) concurrent steroid use, (vii) pre-transfusion hematocrit and (viii) pre-transfusion platelet counts, the odds of developing VTE was significantly higher in subjects receiving RBCT's within 72 h before surgery and at least one intra-operative/72 h post op RBCT as compared to those who did not receive any RBCT's (adjOR 2.1, 95% CI (1.7–2.4) [DVT (adjOR 2.3, 95% CI 1.9–2.8); PE (adjOR 1.4; 95% CI 1.0–1.90)]. The model was robust with an excellent discrimination ability.
Conclusions: Perioperative RBC transfusions in elective surgeries are associated with significant risk development of new venous thromboembolism complications warranting treatment within 30 days of surgery, and reinforce peri-operative patient blood management practices.

4C-S21-04

HEMATOLOGICAL MALIGNANCIES, SOLID CANCERS, AND RED CELL ALLOIMMUNIZATION: A CASE-CONTROL STUDY

D Evers[1], JJ Zwaginga[2], J Tijmensen[1], RA Middelburg[1], M de Haas[1], KMK de Vooght[3], D van de Kerkhof[4], O Visser[5], NC Péquériaux[6], F Hudig[7] and JG van der Bom[1]
[1]Sanquin Research, Leiden, The Netherlands [2]Leiden University Medical Center, Leiden, The Netherlands [3]University Medical Center Utrecht, Utrecht, The Netherlands [4]Catharina Hospital, Eindhoven, The Netherlands [5]VU Medical Center, Amsterdam, The Netherlands [6]Jeroen Bosch Hospital, 's Hertogenbosch, The Netherlands [7]Haga Teaching Hospital, The Hague, The Netherlands

Background: Red cell alloimmunization challenges providing compatible donor blood and, most importantly, may induce potentially life-threatening hemolytic transfusion reactions. Identification of risk modifying conditions will support tailoring prophylactic extended matching strategies.
Aim: We set out to quantify the associations of red cell alloimmunization in transfused patients with their hematological malignancy, solid cancer, and treatment regimens.
Methods: We performed a multicenter nested case-control study within a source population of patients who received their first and subsequent red cell transfusion between 2005 and 2013. Cases were patients who developed a first transfusion-induced red cell alloantibody. Each case was randomly matched to two non-alloimmunized control subjects based on the number of (lifetime) red cell units received and the study center. For all cases, the *Nth* transfusion that most likely elicited alloimmunization was defined the implicated transfusion. For their matched controls, the corresponding *Nth* transfusion was similarly marked. Within a 5-week 'alloimmunization risk period' surrounding this *Nth* transfusion, we recorded various potential risk variables for all cases and controls. Using multivariate logistic regression analyses, we then evaluated the association of various types of malignancies and treatment regimens with red cell alloimmunization.
Results: Within a cohort of 24,063 newly transfused patients, 505 cases and 1,010 matched controls received a median of eight (interquartile range 4–16) red cell transfusions. A total of 606 patients (40.0%) had at least one type of malignancy.
The incidence of alloimmunization among patients with acute (myeloid or lymphoid) leukemia, and mature (B or T-cell) lymphoma was significantly reduced [adjusted relative risks (RR) with 95% confidence interval (CI) 0.36 (0.19–0.68) and 0.30 (0.12–0.81)]. These associations were primarily explained by immunosuppressive treatments [RR for (any type of) chemotherapy combined with lymphocyte targeted immunotherapy 0.27, CI 0.09–0.83]. Non-treated patients with these disorders demonstrated risks comparable to the non-oncologic patient population. No association between the other types of malignancies and red cell alloimmunization was observed, including myelodysplastic syndrome and solid cancers.
Enforcement of immunosuppressive treatment effects were similarly observed in recipients of stem cell transplants. Respectively 51, 13, and 10 patients underwent an allogeneic hematopoietic stem cell transplant (HSCT), an autologous HSCT, or both prior to or during the risk period. Irrespective of the type of HSCT i.e. being autologous or allogeneic, alloimmunization incidences were substantially decreased in these transplanted patients (RR 0.34, CI 0.16–0.74), at least during the first 6 months after transplantation.
Conclusion: These findings suggest that alloimmunization may be prevented by treatment-related immunosuppression in patients with hematological diseases. Ultimately, with limited donor resources, prophylactic extensive antigenic donor-recipient matching seems less critical for this patient population.

Red Cell Storage

4C-S22-01

RED CELL STORAGE LESION

A D'Alessandro
University of Colorado Denver, Aurora, Colorado, United States of America

Red blood cell (RBC) storage in the blood bank is a life-saving therapeutic intervention for ~5 million Americans every year. Packed RBCs are routinely stored at ~4°C under sterile blood bank conditions for up to 42 days. However, routine storage is associated with the progressive accumulation of a long series of biochemical alterations to stored erythrocytes, collectively referred to as the 'storage lesion' [1]. Though the clinical relevance of such lesion has not been clearly elucidated, biochemical evidence suggests that RBCs stored longer than 2 weeks are energetically and oxidatively challenged and thus potentially less functional than fresh RBCs [1]. Recently, it has been argued that pre-storage processing strategies, donor-dependent

biological factors and metabolic reprogramming might underlie the onset and progression of the storage lesion [2]. Impairments of energy and redox homeostasis are proposed to trigger alterations to structural lipids and functional proteins (e.g. hemoglobin, peroxiredoxin 2, glyceraldehyde 3-phosphate dehydrogenase), thereby promoting the progressive accumulation of morphological changes and thus reducing RBC deformability and survival upon transfusion. By combining state of the art quantitative and redox proteomics technologies, unsupervised discovery-mode metabolomics and quantitative metabolic flux analysis upon incubation with $^{13}C_{1,2,3}$-glucose, 2,2,4,4-D-citrate, ^{13}C ^{15}N-glutamine, we investigated the RBC storage lesion during routine storage in the blood bank in presence of different additive solutions (SAGM, AS-1, AS-3, AS-5, AS-7). To understand whether a compromised anti-oxidant system may affect RBC storability and post-transfusion behavior, experiments were performed on packed RBCs from glucose 6-phosphate dehydrogenase deficient donors. We thus exploited omics technologies to investigate alternative red cell storage strategies and additive solutions (e.g. cryopreservation, supplementation of antioxidants, rejuvenation, anaerobic storage).

References: D'Alessandro A, Kriebardis AG, Rinalducci S, Antonelou MH, Hansen KC, Papassideri IS, et al. An update on red blood cell storage lesions, as gleaned through biochemistry and omics technologies. Transfusion. 2015;55 (1):205–19.
Nemkov T, Hansen KC, Dumont LJ, D'Alessandro A. Metabolomics in transfusion medicine. Transfusion. 2016; doi: 10.1111/trf.13442.

4C-S22-02

THE EFFECT OF PREFREEZE REJUVENATION ON POST-THAW SHELF LIFE OF RED CELLS

CCM Lelkens, D de Korte and JWM Lagerberg
Sanquin Research, Amsterdam, The Netherlands

Background: Processing thawed, glycerolized red cells in a functionally closed system allows for a 14 day post thaw period, if resuspended in AS-3. In an earlier study (*Lelkens, Vox Sang., 2015*), we have achieved a 28 day post-thaw shelf life, by omitting the centrifugation step to remove the excess glycerol after glycerolization. Further extension of this shelf life would even more simplify the logistics of using frozen red cell concentrates (RCCs) in daily transfusion practice.
Taking into account international guidelines, demanding an *in vivo* 24 h post transfusion survival of at least 75%, we aimed at preserving a total adenylate content of >82% of the original values (*Högman, Vox Sang., 1985*) after 28 days of post-thaw storage.
Aim: To investigate if increasing the energy status of 7 day old RCCs, prior to glycerolization and freezing, could prolong their post-thaw shelf lives beyond 28 days, while at the same time meeting international requirements with regard to 24 h post-transfusion survival.
Methods: Leukoreduced RCCs in SAGM (n = 16) were stored at 2–6°C. On day 8, four RCCs were pooled (n = 4), mixed and split. From each pool, two RCCs were incubated with Rejuvesol® (Citra Labs, Braintree, MA) for 1 h at 37°C. All RCCs were glycerolized using the ACP215 (Haemonetics®, Braintree, MA) to a final concentration of 40% (w/v). The RCCs were subsequently frozen and stored for at least 2 weeks at −80°C. After thawing and deglycerolization using the ACP 215, from each pair (i.e. one rejuvenated and one non-rejuvenated) one unit was resuspended in SAGM (n = 8) and one in AS-3 (n = 8). During storage at 2–6°C stability (hemolysis) and energy status (total adenylate) of the thawed RCCs were determined.
Results: Rejuvenation increased total adenylate content from 6.5 to 8.2 µmol/g Hb and 2,3-DPG from 1.9 to 30 µmol/g Hb. Rejuvenation did not show a negative effect on hemolysis after thawing, which stayed below 0.8% for 7 days (in SAGM) or 35 days (in AS-3). During storage in AS-3 the total adenylate content in non-rejuvenated RCCs went down to 4.9 µmol/g Hb (72% of day1) and to 6.5 µmol/g Hb (96% of day 1) in rejuvenated RCCs.

	Total Adenylate content (µmol/g Hb)			
	SAGM		AS-3	
Day	Control	Rejuvenated	Control	Rejuvenated
0	6.5±0.1	8.5±0.2	6.5±0.1	8.5±0.6
14	6.7±0.3	8.7±0.3	6.6±0.2	8.7±0.3
28	6.3±0.3	8.4±0.4	6.1±0.4	8.1±0.4
35	5.6±0.3	7.5±0.3	5.4±0.4	7.3±0.5
42	n.d.	n.d.	4.9±0.4	6.5±0.5

Caption 1. Total adenylate content (µmol/g Hb)

Conclusion: Based on a maximum allowed hemolysis of 0.8% in Europe and a total adenylate content of >82%, thawed RCCs can thus be stored at 2–6°C for 7 days in SAGM, whereas thawed, prefreeze rejuvenated RCCs can be stored for 35 days, if resuspended in AS-3.

4C-S22-03

COMPARATIVE *IN VITRO* STORAGE ASSESMENT OF PEDIATRIC SAGM RED CELL CONCENTRATES STORED IN DEHP AND DEHP-FREE PVC BAGS

S Begue, S Acquart, F Cognasse and P Tiberghien
EFS Rhone Alpes Auvergne, La Plaine Saint Denis, France

Background: DEHP, a major component of PVC bags used for collection, processing and storage, are known to stabilize the erythrocyte membrane and significantly reduce the aging effects of stored RCC on hemolysis and improve recirculation after transfusion. Nevertheless, by leaching into the blood product, DEHP may, as a suspected endocrine disrupter, be a potential hazard for the new-born, juvenile infants and pregnant women. In order to reduce recipient exposure to DEHP and mitigate the associated risk, the Etablissement Français du Sang (EFS) has initiated a program to assess the quality of RCC stored in DEHP-free containers. Pending the availability of marketed DEHP-free devices for whole blood collection, we focused on the process of pediatric preparation of RCC in DEHP-free PVC-DINCH bags.
Aims: Evaluate *in-vitro* storage parameters of pediatric Rec Cell Concentrates (P-RCC) stored in DEHP-free PVC bags (PVC-DINCH) compared to pediatric P-RCC stored in classic PVC-DEHP bags. We hypothesize that the initial DEHP content in the adult unit prior division into pediatric RCC may ensure the non-inferiority of *in-vitro* quality parameters of pediatric RCC's.
Methods: Thirty adult RCC units suspended in SAGM were split into 2 P-RCC of 100 ml in 500 ml nominal vol. bags, one in PVC-DEHP and one in PVC-DINCH bags (Macopharma). Of these 30 units, 10 were split at day 1 after collection, 10 at day 7 and 10 at day 14. Storage parameters were screened every 7 days up to day 28. *In-vitro* biological parameters were hemoglobin, hematocrit, supernatant free hemoglobin, K^+ and LDH, hemolysis, ATP, 2–3 DPG, annexin V and DEHP concentration.
Results: Determined values are summarized in the Table 1 for 10 units split at Day 1. DEHP concentrations are presented in Table 2.
Paired Student probability show no significant differences ($P > 0.05$) at day 28 for hemolysis, ATP, 2–3DPG, LDH and annexin V. Supernatant K^+ is statistically lower in PVC-DINCH stored RCC, but the relatively low difference with DEHP bags is of weak significance. DEHP concentration increases linearly from 2 mg/l to nearly 40 mg/l at day 28 in PVC-DEHP bags. In comparison, increase of DEHP concentration in adult units is lower than in divided P-RCCs. Results from units split at D7 and D14 show that the initial DEHP content decreases when stored in PVC-DINCH bags. MEHP concentration is bellow quantification level, so we beleive that DEHP is absorbed by the PVC-DINCH plastic surface.

Table 1		Day 1	Day 7	Day 14	Day 21	Day 28	p*
Hemolysis	DEHP	0,04 ± 0,01	0,12 ± 0,04	0,25 ± 0,11	0,37 ± 0,18	0,55 ± 0,25	0,2530
%	DINCH	0,04 ± 0,01	0,11 ± 0,03	0,21 ± 0,06	0,33 ± 0,1	0,48 ± 0,17	
ATP	DEHP	5,58 ± 0,61	5,63 ± 0,57	4,96 ± 0,49	4,43 ± 0,45	3,77 ± 0,41	0,5623
µM/gHb	DINCH	5,58 ± 0,61	5,72 ± 0,52	4,92 ± 0,55	4,29 ± 0,43	3,83 ± 0,51	
2,3DPG	DEHP	5,23 ± 1,79	2,00 ± 1,07	0,44 ± 0,32	0,26 ± 0,16	0,19 ± 0,09	0,6774
µM/gHb	DINCH	5,23 ± 1,79	2,12 ± 1,14	0,52 ± 0,34	0,31 ± 0,15	0,17 ± 0,14	
K+	DEHP	1,3 ± 0,1	16,2 ± 1,4	27,4 ± 2,2	37,3 ± 2,6	44,3 ± 2,6	0,0011
mEq/L	DINCH	1,3 ± 0,1	16,3 ± 1,4	27,2 ± 2,1	36,0 ± 2,4	42,7 ± 2,5	
LDH	DEHP	42 ± 8	92 ± 24	201 ± 119	342 ± 247	509 ± 321	0,1367
UI/L	DINCH	42 ± 8	80 ± 19	161 ± 44	267 ± 129	376 ± 200	
Annexin V	DEHP	0,6 ± 0,6	27,7 ± 2,7	28,2 ± 3,7	nd	18,4 ± 4,6	0,0963
%	DINCH	0,6 ± 0,6	26,1 ± 2,5	25,8 ± 2,7	nd	21,1 ± 2,3	

*Paired Student probability. Significance level is set at 0,05.
nd : not determined

Table 2 : DEHP mg/L	Storage bag	Day 1	Day 7	Day 14	Day 21	Day 28
Units split at D1	PVC-DEHP	2,5 ± 0,2	19,9 ± 1,5	nd	nd	39,3 ± 4,2
	PVC-DINCH		2,0 ± 0,1	nd	nd	1,0 ± 0,1
Units split at D17	PVC-DEHP		10,7 ± 2	26,1 ± 3,3	nd	44,0 ± 6,6
	PVC-DINCH			5,9 ± 0,8	nd	1,9 ± 0,3
Units split at D14	PVC-DEHP			15,6 ± 1,3	32,5 ± 2,9	42,3 ± 4,6
	PVC-DINCH				7,8 ± 0,5	4,3 ± 0,5

nd : not determined

Summary/Conclusions: Dividing adult RCC into small pediatric units dramatically increases (+60%) the concentration of DEHP in product specifically intended for new-born and juvenile infants. By using PVC-DINCH instead of PVC-DEHP bags for splitting, we demonstrate the non-inferiority of *in-vitro* storage parameter's up to 28 days as well as a significant decrease of the initial DEHP content. Approved commercially PVC-DINCH sets for pediatric preparation is a priority.

4C-S22-04

COMPARISON OF *IN VITRO* RED CELL QUALITY PARAMETERS DURING STORAGE IN FIVE DIFFERENT ADDITIVE SOLUTIONS

JWM Lagerberg[1], H Korsten[2], PF van der Meer[2] and D De Korte[1]

[1]Sanquin Research, Amsterdam, The Netherlands [2]Sanquin Blood Bank, Amsterdam, The Netherlands

Background: Red cell concentrates (RCC) are normally stored in SAGM (saline, adenine, glucose, mannitol). During storage, *in vitro* red cell quality declines, like increased cell lysis, decreased deformability and lowered energy status. Recently, several additive solutions, designed to diminish the decline in *in vitro* quality during storage, are developed. These additive solutions allow for prolonged red cell storage.
In a paired study design, *in vitro* RBC quality during storage in SAGM (control), PAGGSM (Fresenius Kabi), PAG3M (Sanquin Research), Erythrosol-4 (E-Sol4, Fenwal) and SOLX (AS-7, Haemonetics) were compared. The new solutions are mainly developed to better maintain 2,3-DPG and ATP levels during storage. High levels of 2,3-DPG allow for better oxygen release while high ATP is necessary for function and survival of RBC *in* vivo.
Aim: To compare the *in vitro* quality of RBC during storage for 56 days at 2–6°C in SAGM, PAGGSM, PAG3M, E-Sol4 and SOLX.
Methods: For each experiment, five overnight stored whole blood units were pooled and split. From four collection systems, SAGM was replaced by PAGGSM, PAG3M, E-Sol4 of SOLX. De whole blood units were processed according to routine procedure, into buffy-coat- depleted RCCs in additive solution, leukoreduced by filtration. RCCs were stored for 8 weeks at 2–6°C and samples for analysis of *in vitro* quality parameters. Deformability was determined with an Automated Rheoscope and Cell Analyzer (ARCA) and expressed as deformation index (DI): ratio of cell length to cell width. With DI >2.5 cells are considered deformable, cells <1.5 are considered undeformable.
Results: Results are summarized in the Table 1.
RCC leukoreduction filtration times were increased with the alternative solutions, especially for SOLX. Haemolysis was significantly lower during storage in PAG3M and E-Sol4. RCCs in PAG3M, E-Sol4 and SOLX showed significantly higher ATP and 2,3-DPG levels compared to SAGM. In PAG3M, 2,3-DPG levels remained high during whole storage period. Storage in PAG3M, and to a lesser content in E-Sol4 and SOLX, resulted in declined RBC deformability compared to SAGM.
Summary/Conclusions: SAGM and PAGGSM allow for storage of RBC up to 35 days. The new additive solutions allow for longer storage, with better maintenance of stability, energy status and 2,3-DPG levels. Deformability might be lesser maintained in the alternative additive solutions.

Parameter	SAGM	PAGGSM	PAG3M	E-SOL	SOL X
Filtration time RCC (min)	25 ± 4	35 ± 4	33 ± 4	41 ± 6	72 ± 26
Haemolysis (%) day 56	0.82 ± 0.11	0.66 ± 0.12	0.36 ± 0.05	0.35 ± 0.11	0.65 ± 0.13
ATP (µmol/g Hb) day 56	2.3 ± 0.1	3.0 ± 0.2	3.2 ± 0.4	3.0 ± 0.2	3.5 ± 0.4
2,3-DPG (µmol/g Hb) day 21	0.51 ± 0.24	0.64 ± 0.26	16.6 ± 6.95	1.97 ± 0.62	2.92 ± 1.57
Deformability, day 56					
DI<1.5 (%)	12	23	67	60	42
DI>2.5 (%)	70	50	16	30	42

Table 1. *In vitro* red cell quality parameters during storage in different additive solutions

4C-S22-05

HEMANEXTTM: DEVICE AND METHOD FOR ESTABLISHING AND MAINTAINING CONTROLLED OXYGEN ENVIRONMENT FOR STORAGE OF RED BLOOD CELLS

A Dunham, T Yoshida, R Cordero, P Keegan and A Blair

New Health Sciences, Inc, Cambridge, Massachusetts, United States of America

Background: Blood transfusions save or sustain thousands of lives every day across the globe. While improvements in pathogen screening, storage solutions, and leukocyte reduction have continued to improve the clinical impact of blood transfusion, sufficient risk from effects of degradation by refrigerated storage remains for some recipients, counterbalancing the clinical benefits. While the direct link between the degrading red cell and transfusion side effects are poorly understood, the biochemical and biomechanical degradation that occurs during blood bank storage is well established. By storing blood under oxygen-depleted conditions Hemanext reduces this degradation by removing one root cause: oxidative damage.
New Health Sciences is developing Hemanext, a novel, proprietary approach to reduce red blood cell (RBC) degradation during cold storage. The Hemanext process is shown in Figure 1.
Aims: An *in vitro* study, closely simulating the process of a final commercial device, was designed to demonstrate the effect on stored red blood cell quality that results from reducing pre-storage O_2 and CO_2 levels and controlling them during refrigerated storage.
Methods: The Hemanext process for reducing O_2 and CO_2 was simulated by using a membrane oxygenator. Eight pools of RBCs were prepared from whole blood collected in CP2D, leukoreduced, and separated RBCs were placed in AS3 additive solution. Prior to storage, oxygen and carbon dioxide levels in the packed RBCs were reduced to 5% SO_2 and <10 mmHg pCO_2, then stored in an anaerobic environment. During the course of refrigerated storage, the stored RBCs were sampled and analyzed for ATP, 2,3-DPG, and hemolysis.
Results: Establishing reduced $\%SO_2$ and pCO_2 of the packed RBCs before refrigeration yielded an increase in 2,3-DPG levels throughout storage, an increase in ATP beyond 2 weeks of storage compared to unprocessed RBCs during 42 days of cold storage. 2,3-DPG levels were maintained at physiologic levels for the first 3 weeks of storage and were higher than the controls across all intervals.
Summary/Conclusions: Using Hemanext to establish and control oxygen and carbon dioxide levels before refrigerated storage improves key quality parameters of RBCs: ATP and 2,3-DPG. The mechanism for achieving higher levels of ATP and 2,3-DPG by reducing O_2 and CO_2 before storage are three-fold: metabolic modulation, initial upward shift in pH, and reduction in oxidative damage. Metabolic modulation occurs when deoxyhemoglobin displaces metabolic enzymes from the Band 3 binding domain accelerating glycolysis during storage, and reduces intracellular concentrations of glycolysis end-products (e.g., ATP and 2,3-DPG, H^+) by binding

Days	ATP µmol / gHb		2,3-DPG µmol / gHb		Hemolysis %	
	Control	Test	Control	Test	Control	Test
1	4.00±0.40	3.78±0.46	8.6±1.03	*14.7±0.9	0.13±0.05	0.17±0.07
14	4.00±0.69	4.00±0.55	1.2±0.5	*18.6±1.0	0.16±0.03	0.20±0.07
28	3.53±0.66	*4.06±0.49	0.8±0.3	*5.9±1.8	0.21±0.07	0.24±0.10
42	2.91±0.63	2.96±0.42	1.0±0.3	*1.3±0.3	0.25±0.11	0.30±0.13

* P < 0.05

Table 1. Data table

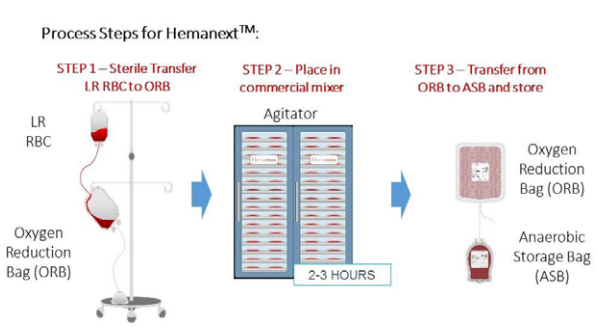


Figure 1. Hemanext process

them, all resulting in acceleration of RBC metabolism. The combination of lower oxygen insult, the maintenance of an anti-oxidant defense coupled with metabolic modulation enhances the quality of RBCs during storage.

Platelet Immunology

4C-S23-01

No abstract available.

4C-S23-02

HUMAN MONOCLONAL ANTIBODY AGAINST HPA-1A INHIBITS ANTI-HPA-1A MEDIATED ENDOTHELIAL DISTURBANCE VIA FC INDEPENDENT

H Wihadmadyatami[1], T Bakchoul[2], B Bayat[3], M Burg-Roderfeld[3], SS Werth[3], C Ruan[4], W Minwang[5], G Bein[3], UJ Sachs[3] and S Santoso[3]

[1]Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia [2]Institute for Immunology and Transfusion Medicine Ernst Moritz Arndt University,, Greifswald, Germany [3]Institut for clinical immunology & transfusion medicine Justus Liebig University, Giessen, Germany [4]Jiangsu Institut of Hematology,TheFirst Affiliated Hospitalof Soochow University, Suzhou, China [5]Dongying Lida Pharmaceutical, Suzhou, China

Background: Foetal/neonatal alloimmune thrombocytopenia (FNAIT) is caused by the destruction of fetal platelets by maternal platelet alloantibodies which crossed through the placenta during pregnancy. Alloantibodies against Human Platelet Antigen-1a (HPA-1a) residing on the b-subunit of the platelet fibrinogen receptor (aIIbb3 integrin) are responsible for intracranial hemorrhage (ICH) in severe FNAIT cases. Recently, we could demonstrate that the deglycosylated mouse mab SZ21 against HPA-1a is able to cross through placenta, and prevents the destruction of platelets in mouse model (Bakchoul et al, Blood 2013). Meanwhile, this mab is humanized as chimeric antibody (mab 813). It is known that the b3 is abundantly expressed as vitronectin receptor (αvβ3 integrin) on endothelial cells.
Aim: In this study, we investigate the effect of deglycosylated mab 813 (deg-813) towards endothelial function and how is the mechanism which is involved
Methods: Deg-813 was prepared and characterized as previously described (Bakchoul et al, Blood 2013). Binding of antibody binding to platelets, endothelial cells and purified αvβ3 integrin was performed by flow cytometry and surface plasmon resonance technology, respectively. Apoptosis and tube formation assays were conducted to analyze the function of antibody binding on endothelial function. To analyse the mechanism from deg-813 toward endothelial cells, removing of Fc part was performed.
Results: Flow cytometry analysis showed similar binding activity of intact 813 and deg-813 with platelets and endothelial cells. This result could be confirmed by surface plasmon resonance technology; native and deg-813 interacted equally with purified αvβ3 immobilized on the sensor chip (Kd 7.4×10^{-11} and 6.7×10^{-11}, respectively). To study the influence of mab 813 on endothelial function, apoptosis and angiogenesis experiment with HUVEC was performed using Caspase-3/7 and tube formation assays. In Caspase-3/7 assay, endothelial apoptosis was not observed with intact 813 and deg-813. In contrast, inhibitory mabs against αvβ3 integrin (clone 23C6) caused significant apoptosis of these cells. Moreover, in contrast to anti-avb3, both intact and deg-813 did not alter angiogenesis as shown by the tube formation assay. Treatment of endothelial cells with deg-813 prevents apoptosis and anti-angiogenesis effect of anti-HPA-1a antibodies and interestingly this protective effects are still presents event we already removed the Fc part from d-813. Furthermore, in comparison to native 813, d-813 was able to inhibit platelet clearance mediated by anti-HPA-1a antibodies in NOD-Scid mouse model.
Conclusions: Taking together, our observations demonstrate that deg-813 can protect not only anti-HPA-1a mediated platelet destruction but also prevent endothelial dysfunction caused by anti-HPA-1a antibodies via Fc independent mechanism. These results suggest that deg-813 may represent a novel drug to prevent ICH in severe FNAIT during pregnancy. This approach may also applicable for the treatment of FNAIT caused by other platelet antibody specificities.

4C-S23-03

FETAL PLATELET GENOTYPING FROM MATERNAL PLASMA USING DROPLET DIGITAL PCR (DDPCR): A NEW TOOL FOR CLINICAL AND BIOLOGICAL FNAIT MANAGEMENT

R Petermann, Y Mammasse, C Chenet and C Martageix

Institut National de la Transfusion Sanguine, Paris, France

Fetal/neonatal alloimmune thrombocytopenia (FNAIT) originates from parental incompatibility in platelet antigen system. FNAIT occurs in 1:1,000 pregnancies and leads to platelet destruction by maternal alloantibodies against fetal platelet antigens inherited from the father and absent in the mother. This may result in serious complications such severe thrombocytopenia leading to bleeding and intracranial hemorrhages. Currently, fetal platelet genotyping is done using invasive procedures such as amniocentesis with a risk of bleeding and miscarriage.
Droplet digital PCR (ddPCR) is a molecular technique which is widely applied in clinical practice. This method allows characterizing tumor-free circulating DNA in patients and achieves high detection sensitivity (0.001%). Here, we carried out a proof-of-concept study for non-invasive prenatal diagnosis (NIPD) of fetal platelet genotyping by ddPCR.
We focused on target amplification and quantification of specific regions carrying polymorphisms of four platelet antigen systems HPA-1, -3, -5 and -15 which are implicated in more than 95% of FNAIT. Fetal platelet genotyping was performed on cell-free DNA extracts from eight maternal plasma of six pregnant women at different weeks of gestation (11–32 WG). To exclude false-negative results caused by the lack of fetal DNA in maternal plasma, an internal control based on the detection by ddPCR of methylation-sensitive restriction enzyme digestion of *RASSF1a* gene promoter sequences was implemented.
Results showed that one pregnant woman was compatible with her fetus, whereas, five of them presented incompatibility in at least one HPA system. Fetal DNA fraction were estimated from all samples by using a reliable marker, it increased with gestational age and represented 0.6–26% of maternal circulating free DNA. Predicted fetal HPA genotypes by ddPCR were confirmed in four FNAIT cases by genotyping studied HPA systems using amniocentesis or after birth. This new NIPD method is currently under a validation process, but these preliminary results suggest that it is of great interest.
Fetal HPA genotyping on maternal plasma based on ddPCR appears as a safe and reliable non-invasive method devoid of risk for the fetus. This technique provides the opportunity to improve early identification of high risk pregnancies, by diagnosing a potential feto-maternal platelet incompatibility and as such should be useful for biological and therapeutic patients management.

4C-S23-04

THE IMMUNIZATION OF CD36$^{-/-}$ MOUSE BY THE USE OF MOUSE CD36 TRANSFECTED CELLS: GENERATION OF NOVEL ANTI-CD36 ANTIBODIES

X Xu[1], D Chen[1] and S Santoso[2]

[1]Institute of Blood Transfusion, Guangzhou, China [2]Institute for Clinical Immunology and Transfusion Medicine, Justus-Liebig Univer, Giessen, Germany

Background: Foetal/neonatal alloimmune thrombocytopenia (FNAIT) as a bleeding disorder in the fetus or neonate caused by maternal antibodies can be caused by CD36 antibodies. And recent data indicate that CD36 antibodies seem to be the most frequent antibodies responsible for FNAIT in asian populations. However, little is known about the pathomechanism of anti-CD36 mediated FNAIT. To examine the effects of anti-CD36 antibodies on platelet clearance and fetal hydrops, we immunized CD36$^{-/-}$ mouse with mouse CD36 transfected cells and generate a panel of murine anti-mouse CD36 MABS.
Study design and methods: Human embryonic kidney cells (HEK293T cells) were stably transfected with mouse CD36 full-length construct and sorted by flow cytometry according to high surface expression. CD36$^{-/-}$ mouse were immunized three times with mouse CD36 transfected cells, and resulted in strong immune responses. Then splenocytes were harvested and fused with mouse myeloma cells (SP2/0-AG14). After selecting in HAT medium, supernatant of hybridomas were screened with mouse CD36 transfected cells by flow cytometry. Positive clones were subcloned twice, and the mouse CD36 antibodies were identified by flow cytometry using WT-mouse platelets.
Results: Full-length CD36 cDNA derived from mouse platelets was cloned into the expression vector pcDNA3.1/V5-histopo and was stably expressed in HEK293T cells (MCD36). For the control experiment, full-length CD36 cDNA from CD36 deficient individual carrying C220T mutation was cloned and transfected into HEK293T cells

(control). Analysis of MCD36 cells by flow cytometry with monoclonal antibody (clones MCA2748) showed mouse CD36 over expression on cell surface. In contrast, MCA2748 did not show any reaction with control cells. This result could also be confirmed by immunoprecipitation analysis; MAB MCA2748 precipitated the expected band with MR of ~95 kDa. Using this mouse CD36 transfected cells $CD36^{-/-}$ mouse were immunized three times, and generated strong immune responses. After screening of antibodies in supernatant of hybridomas, 2/105 wells showed CD36 antibodies positive. By limited dilution assay, two clones were identified that can secrete anti-mouse CD36 monoclonal antibodies by low cytometry using WT-mouse platelets (Figure 1).

Conclusions: By immunization of mouse CD36 transfected cells $CD36^{-/-}$ mouse generated strong immune responses. Splenocytes from these immunized $CD36^{-/-}$ mouse were used to establish new mouse anti-mouse CD36 monoclonal antibodies which were useful for study on the pathomechanism of anti-CD36 mediated fnait.

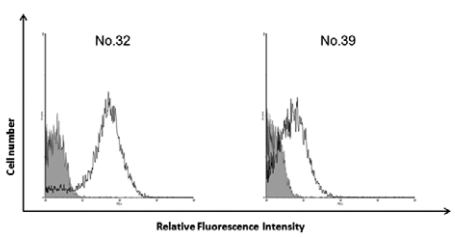


Figure 1: Flow cytometry analysis of anti-CD36 monoclonal antibodies
Cell supernatant of two hybridomas (No.32 and No.39) were incubated with platelets from WT-C57BL/6J mouse (white) or CD36-/- platelets (grey).Bound antibody was analyzed by flow cytometry using fluorescence labelled anti-mouse IgG.

4C-S23-05

INVESTIGATION OF THREE KEL NOVEL ALLELES BY HUMAN ERYTHROCYTE ANTIGENS AND HUMAN PLATELET ANTIGENS PANEL BASED ON NEXT GENERATION SEQUENCING

AJ Halawani, MA Altayar, M Kiernan, TE Madgett and ND Avent
Plymouth University, Plymouth, United Kingdom

Background: Alloimmunisation is a major complication for patients who are multiply transfused. Blood group genotyping (BGG) has helped reduce alloimmunisation by detecting allelic variants, and it may supplant standard serotyping. The current BGG platforms need previous knowledge of the blood group polymorphisms under investigation. Furthermore, they cannot identify any novel mutations, which might be of clinical significance.

Aims: An assay panel has been developed to genotype human erythrocyte antigens (HEA) and human platelet antigens (HPAs) based on the next-generation sequencing (NGS) and is designated as HEA and HPA Panel. We have validated this panel for possible diagnostic use.

Methods: The assay captures the exons of the genes of 11 blood group systems (ABO, MNS, RH, KEL, FY, JK, DI, YT, DO, CO and VEL) and HPAs (1–16). The protocol is based on Ion Ampliseq™ Custom Panel using Ion Torrent™ Personal Genome Machine™ (Ion PGM™). Twenty-four sequencing libraries were generated by amplification of two pools of amplicons using ultra-high multiplex PCR followed by digestion of the primers and ligation to barcoded adaptors. The sequencing libraries were attached to beads to start clonal amplification, which is based on emulsion PCR. The sequencing template was finally loaded onto Ion chip and sequenced on the Ion PGM™. The generated data was produced by Torrent Suite™ Version 4.4 and then analysed using various software for quality control assessment, visualisation and annotation.

Results: The genotyping analysis was carried out using the annotated sequencing data and the phenotypes were predicted. Three novel SNPs, all in the *KEL* gene, were observed in two different samples both serotyped as KEL:-1. The SNP found in the first sample was heterozygous 331G>A (Ala111Thr) in exon 4. The second sample showed two different novel SNPs. The first SNP was heterozygous 1907C>T (Ala636-Val) in exon 17, while the other SNP was found to be heterozygous 2165T>C (Leu722Pro) in exon 19. All three of these mutations have not been described in the literature. Both samples were genotyped as homozygous *KEL*02/02* (encoding the k phenotype), *KEL*02.04/02.04* (encoding the Kp^b phenotype) and *KEL*02.07/02.07* (encoding the Js^b phenotype). We are currently sequencing the entire *KEL* gene of these samples to validate these results, and whether these SNPs have any effect on KEL antigenicity.

Summary: NGS can offer a high-throughput screening for both donors and patients at low cost in comparison to other BGG platforms. Crucially, it provides extensive genotyping for the HEA and HPA and investigates novel allelic variants. We are confident that this will overcome conventional typing of blood groups by serology and the current BGG (array based) methods and aid to reduce the alloimmunisation in multiply transfused patients.

Blood Group Genotyping

4C-S24-01

COST EFFECTIVENESS OF IMPLEMENTING MOLECULAR IMMUNOHEMATOLOGY

A Denomme
Blood Center of Wisconsin, Milwaukee, Wisconsin, United States of America

Molecular immunohematology has been practiced in the clinical laboratory and blood center for some 20 years now. It uses information gained from red cell genotyping transfusion recipients and blood donors. Red cell genotyping is a technology whereby DNA-based techniques are used to evaluate genes for the particular single and multiple nucleotide substitutions, deletions, insertions, and gene conversions that determine the expression of red cell antigens. Its use increases the availability of antigen-negative blood, and is a cost efficient process when introduced through the entire blood supply chain. The practice of molecular immunohematology in the clinical lab uses red cell genotyping as a diagnostic test in pregnancy and has revised antibody investigation algorithms. It is gaining value as a quality assurance tool for reagent red cells and in donor testing.

Fetal red cell genotyping, and in particular *RHD* genotyping, using the discarded cell pellet of amniotic fluid was developed some 20 years ago. It replaced the need for fetal blood sampling and also can determine paternal zygosity with better certainty. Cell free fetal DNA in maternal plasma is now the non-invasive (fetal) alternative to amniotic fluid as the biological material of choice. It is being used in Europe to avoid exposure of Rh immune globulin for the routine Rh-negative pregnancy. At the same time, *RHD* genotyping is used to resolve weak D antigen 'discrepancies' and is a cost efficient alternative to the administration of Rh immune globulin. *RHD* genotyping of transfusion recipients with similar D antigen discrepancies can be done to ensure that Rh-negative blood products go to patients who truly need them.

A short compendium of manuscripts from world-wide investigators proposing the application of mass-scale high throughput red cell genotyping of donors first appeared in a 2005 issue of *Transfusion.* Since then, a plethora of data has accumulated on the value of screening donors for rare blood types. The process is efficient, however if left unabated, the cost of maintaining unused frozen blood in freezers may outweigh the advantages gained by finding such units. Blood centers are presently at a juncture that requires consideration of rarity vs demand for some rare types, and to return liquid units to the general inventory rather than freezing them. It will be prudent to consider limited frozen stock of rare blood types as a back-up in some instances. The use of probability theory will help make cost effective decisions which units to leave as liquid and which to freeze. For routine antigens, red cell genotyping as an historical test-of-record will create efficiencies in the labeling of antigen-negative blood if care is taken in testing appropriate repeat donors. Certainly, it has been shown that exposing antigen-negative types through the entire supply chain efficiently replaces shipping from a central facility. Ultimately, labels will provide antigen attributes and users must accept that an overall incremental cost is inevitable to the benefit of all patients now that genotype dry matching is an alternative to exhaustive antibody investigations in autoimmune hemolytic anemia and possibly multiple myeloma anti-CD38 therapy, and to prevent alloimmunization prior to transplantation.

Molecular immunohematology is not the implementation of cost reductions but the identification of cost efficiencies that show tangible benefits to pregnant women and transfusion recipients alike.

4C-S24-02

SEROLOGICAL AND MOLECULAR CHARACTERIZATION OF RHD VARIANTS IN BLOOD DONORS

B Foglieni[1], L Raffaele[1], I Guarnori[1], M Spreafico[1], F Rossi[2], P Lanzini[3], L La Rosa[4] and D Prati[1]

[1]ASST A. Manzoni Hospital, Lecco, Italy [2]SIMTI, ASST, Monza, Italy [3]ASST Valtellina e Alto Lario, Sondrio, Italy [4]SIMT, ASST, Vimercate, Italy

Background: RhD is the most important, immunogenic and polymorphic Rh antigen, which plays a key role in transfusion medicine. Anti-D antibodies remain the leading cause of the hemolytic disease of the newborn. Screening tests are based on panels of monoclonal antibodies developed to identify the majority of D variants, but Rh D typing is a constant challenge, since in presence of RHD blood group polymorphisms of RH partial D or weak D phenotypes may vary according to reagent and method used. In January 2015, the Lombardy transfusion system has been deeply reorganized, and screening tests performed by 27 transfusion centers have been centralized into eight centers. In our department, we are now testing for ABD about 85,000 units per year.
Aims: The aim of the study was to describe the findings of ABD typing performed with a different system based on agglutination on solid phase technology, different from the ones previously used, based on gel-card technology.
Methods: From March 2015 to March 2016, AB0/Rh typing of blood donations were performed by solid phase technology with a completely automated system (Capture-R Ready-Screen, Immucor). Results were compared with data obtained by gel-card. Samples with negative or weak anti-D reactivity were screened for the presence of RhD variants with different anti-D sera and advanced serological kits such as ID-Partial RhD Typing (Biorad), and, furthermore, they were analyzed for DAT testing. Discrepant or inconclusive samples were selected for further investigation with molecular techniques, based on allele specific PCR for the detection of 14 RhD weak and 48 RhD partial variants (Inno-Train, Essemedical).
Results: A total of 82,000 blood donations, collected from 38,515 donors, were analyzed for AB0 and RhD blood groups. In 130 donors (0.3%) a weak or discrepant RhD typing with different anti-sera were obtained. All of them were analyzed for the presence of RhD variants by molecular approach, which identified: 117 weak D alleles (91 type 1, 7 type 2, 6 type 3, 1 type 4, 1 type 5, 10 type 11, 2 compound heterozygote type 1 + 4 and type 2 + 4) and 12 partial D alleles (nine DFR, 1 DNB, 2 DV). In two subjects none of the RhD variants analyzed were found. All RhD variants were identified by both gel-card and solid phase technologies, except the weak D type 11 (885G>T) variant carried by 10 subjects, which showed a completely negative results with all monoclonal gel-card antisera used, and only the D weak cells analysis on Immucor microplates revealed a weak reactivity. This RHD variant is commonly classified among the Del phenotype, since it can be only identified by adsorption and elution techniques. The RhD typing of blood component of these 10 blood donors were changed from Rh negative to Rh positive. Moreover, all the RhD variants identified showed a strong correlation between serological reactivity obtained with different anti-sera and molecular results. All RHD variants were identified in subjects with Cc or Ee phenotype, and a linkage disequilibrium between RHD variants and RHCE phenotypes were observed.
Summary/Conclusion: Solid phase methods were highly sensitive in detecting very weak RhD expression variants, such as DEL, which is important for the prevention of anti-D post-transfusion or newborn immunizations. Molecular methods help in the differentiation and definition of partial and weak D types, providing additional information for transfusion procedures.

4C-S24-03

IMPACT OF THE MANDATORY DONOR RHD SCREENING IN SWITZERLAND

C Henny, F Still, S Lejon Crottet, H Hustinx, J Aeschlimann and C Niederhauser

Interregional Blood Transfusion SRC, Bern, Switzerland

Background: Serological RhD testing, including indirect antiglobulin test (IAT), is often not sensitive enough to detect very weak RhD variants. However, transfusion of red blood cell concentrates (RBCs) harbouring such RhD variants may cause alloimmunization in RhD-negative recipients as RhD is highly immunogenic. This observation leads to the molecular screening of all serological RhD-negative Swiss donors for the presence of *RHD* DNA sequences. In order to remain cost-efficient mandatory IAT was discontinued. This *RHD* screening was declared mandatory in 2013. Results from transition year 2012 were reported (Lejon Crottet S *et al.*: Transf Apheres Sci (2014) [1]). Herein we summarize the data from the first 3 years of the mandatory screen (2013–2015).
Aim: Detection of *RHD* variants in RhD-negative donors in order to prevent alloimmunization in RhD-negative recipients.
Methods: In our institute EDTA blood samples from about two-thirds of the Swiss RhD-negative blood donations were pooled in pools of up to 23 donors and DNA extracted as previously described [1]. PCR detection of *RHD* exon 3, 5 and 10 was done as previously described [1] or since August 2014 by using the RBC-FluoGene D-Screen kit (Inno-Train, Germany). Pools positive for one or several exons were resolved to the single donation and retested. Samples positive for *RHD* were further characterized using commercially available kits (Inno-Train, Germany and BAGene, Germany) or by exon sequencing and adsorption-elution analysis using polyclonal anti-D pool (in-house) and anti-D clone ESD1 (Bio-Rad, Switzerland).
Results: Between 2013 and 2015 18,537 RhD-negative samples were tested. In total, 154 samples were positive for one or several *RHD* exons. In 65 of 154 samples *RHD* variant alleles classified as serologically positive were detected, with *RHD*01W.01* (n = 13), *RHD*11* (n = 12) and *RHD*01W.31* (n = 7) being the most common identified variants. In one sample no mutation could be determined by sequencing, however by adsorption-elution RhD antigens could be revealed. These 65 donors were reclassified as RhD positive. Further 87 donations harboured a *RHD* variant classified as RhD negative, with the *RHD*Ψ* allele (n = 27) being the most common, followed by diverse *RHD-CE*-hybrid alleles (n = 57). In one sample c.53delT was found. This deletion introduces a frameshift at amino acid 18 and premature stop codon at amino acid 38. To the best of our knowledge this variant, *RHD*53delT,* has not been reported previously. In addition one sample showed, linked to a deletion of *RHD*, a mutation in *RHCE*, revealing variant *RHCE*ceEK*. A second sample is under investigation and RhD status could not be confirmed so far.
Summary/Conclusions: Mandatory screening for the presence of *RHD* in RhD-negative donors revealed 65 donors which must be considered as RhD positive (0.35%). The slightly higher number of RhD positive variants compared to previously (0.15%) [1] is probably due to the discontinuation of IAT as variants such as *RHD*01W.01* and *RHD*11* are often not detected using standard RhD typing. The chosen strategy reduces further the risk of unnecessary anti-D alloimmunization in RhD negative recipients.

4C-S24-04

AFRICAN AND ASIAN RHD BLOOD GROUP GENOTYPES AS A COMPLICATION OF NON-INVASIVE PRENATAL TESTING (NIPT)

CA Hyland, G Millard, EC McGowan, H O'Brien, GH Lopez, EM Schoeman and RL Flower

Australian Red Cross Blood Service, Brisbane, Australia

Pregnancy management for Australian women includes blood group typing, including RhD, and testing for antibodies, including anti-D. RhD negative women without antibodies receive antenatal and postnatal anti-D prophylaxis as standard of care to minimise the risk of antibody formation. Pregnant women with antibodies are at risk of being affected by haemolytic disease of the fetus and newborn when the unborn baby is RhD positive. To guide management for these pregnancies non-invasive prenatal test (NIPT) is performed to assess the fetal RhD type. NIPT is designed on the basis that the *RHD* gene is deleted in the mother however the testing is complicated when the mother is a phenotypic RhD negative but genotypic *RHD* positive due to an *RHD* gene variant.
Aims: To define the rate of blood group variants associated with African and Asian populations in both non-immunised and alloimmunised maternal RhD negative cohorts and review the strategies for application of NIPT to define the fetal blood group where such variants are present.
Methods: Maternal blood samples were collected, total cell-free DNA was extracted from plasma and *RHD* exons 5 and 10 amplified by qPCR. The primers/probes in the NIPT assay will not amplify exon 5 of the African *RHD* Ψ* (*RHD*Pseudogene*). This and other maternal blood group *RHD* variants give high PCR signals for exon 10 at least. An automated algorithm, factoring in gestation age, applies a Z score analysis to detect these variants. Maternal *RHD* variants were defined by SNP array.
Results: Amongst 1,265 non-immunised and 262 immunised cases 18 carried *RHD* variants. These were classified into 13 different *RHD* variants. Three variants were detected amongst the immunised cases: the African *RHD* Ψ*, the *RHD*329–330del* and a *RHD*IVS1 + 1G>A* variant associated with DEL phenotype. Amongst the non-immunised cases the *RHD* Ψ*, the *RHD*1227A* (Asian-associated DEL phenotype), the *RHD*weak partial 15* and the *RHD*D-CE(3–9)-D* recurred. One variant comprised a novel combination of the changes that define the *RHD* gene lacking exon 3 [putative *RHD*D-CE(3)-D*] together with the three single nucleotide variants that define the weak partial D 4.0 phenotype (DAR 3.1).

For cases harbouring either the *RHD* Ψ* or a *RHD*D-CE-D* hybrid a fetal *RHD* prediction was made based on the presence or absence of *RHD* exon 5 signals, as these represented inheritance of a normal paternal *RHD* gene. All neonate RhD phenotypes matched predicted genotype interpretations.

Discussion: The overall rate of *RHD* gene variants was 1.18%.

Amongst the blood group variants, three were detected in mothers with anti-D antibodies, including one with a DEL phenotype. Mothers with variants such as the *RHD*weak partial 15* have been reported to make antibodies. In contrast mothers with the *RHD*1227A* associated DEL phenotype are reported not to make an antibody. Ongoing monitoring of alloimmunisation in mothers with *RHD* variants will contribute to this body of evidence.

We conclude that careful design and performance of NIPT tests overcomes complications arising from the presence of these blood group variants to provide accurate fetal blood group typing in diverse obstetric populations to guide pregnancy management.

4C-S24-05

RAPID RHD ZYGOSITY TESTING USING DROPLET DIGITAL PCR (DDPCR)

KA Sillence, N Avent, T Madgett and A Halawani

Plymouth University, Plymouth, United Kingdom

Background: Determining the precise population frequencies of the different Rh haplotypes is complicated due to the inability to differentiate between CDe/Cde; cDE/cdE; cDe/cde; CDE/CdE genotypes. Haplotype differentiation is not possible since the hemi- or homozygosity of *RHD* in individuals cannot be defined by serology. The presumed genotype, is only based on probability, with the acceptance that this will sometimes be incorrect. However, zygosity determination can be achieved using quantitative DNA typing. Multiplex Ligation-dependent Probe Amplification (MLPA) and real-time PCR approaches are favourable since multiplexing enable many targets for various blood groups to be analysed, but this approach is labour intensive and takes a minimum of 25 h (MLPA) to obtain results.

Aims: To determine *RHD* zygosity of an individual using a rapid (<5 h) ddPCR approach and compare these results to the presumed genotype determined by serological analysis.

Method: DNA was extracted from the plasma and buffy coat of 39 donor human whole blood samples. Two multiplex reactions were carried out on a ddPCR platform to target exon 5 and exon 7 of the *RHD* gene (FAM-labelled) using *EIF2C1* as a reference gene in both reactions. Ratio analysis was carried out using QUANTASOFT™ Software to determine if the *RHD/EIF2C1* was 0, 0.5 or 1, demonstrating a homozygous *RHD*-negative, a hemizygous *RHD*-positive or a homozygous *RHD*-positive individual.

Results: The ddPCR data clearly illustrated *RHD* zygosity, allowing diagnosis to be made. The results revealed discrepancies between the ddPCR determined genotype and the presumed *RH* genotype determined by serological analysis (provided by NHSBT, UK). Serology had predicted these samples 1,777, 729M and 087W to be R_1r, R_1R_2 and R_2R_2, genotype respectively. However, the ddPCR *RHD* zygosity results revealed ratios close to 1 for sample 1,777 indicating that this sample is homozygous for the *RHD* gene and therefore is likely to exhibit the R_1R_0 haplotype. Alternatively, samples 729M and 087W expressed ratios closer to 0.5 indicating *RHD* hemizygosity. These results indicate that samples 729M and 087W are likely to exhibit R_1r''/R_2r' and R_2r'' genotypes, respectively, rather than serologically predicted genotypes (R_1R_2 and R_2R_2, respectively). Sample 729M was typed serologically as weak D with the phenotype R_1R_2. Since sample 729M is classified as weak D and D-variant haplotypes are rare within the population (2.2%) (Wang, Lane and Quillen, 2010), it is unlikely this individual is homozygous for two weak D alleles. However, the ddPCR data illustrated a ratio close to 0.5 for both the exon 5 and exon 7 *RHD* targets, illustrating that the most likely phenotype determined serologically was incorrect since sample 729M was shown to be hemizygous for *RHD* and thus will either express the R_1r'' or R_2r' phenotype. Sequencing data of this sample illustrated multiple intronic SNPs which appear to be associated with the R_2 haplotype, which demonstrates that sample 729M actually possesses the R2r'.

Conclusion: ddPCR can accurately and rapidly be used to determine *RHD* zygosity, and the initial preliminary data reveals that the frequency of r', r'' and R_0 haplotypes may be higher than previously suspected based on serological data. MLPA approaches are more efficient for evaluation of multiple gene regions for a wide variety of blood groups. However, if testing for specific blood groups, ddPCR provides a quicker and more accessible platform for zygosity testing.

Classical Transfusion Transmitted Infections

4C-S25-01

CONCOMITANT PRESENCE OF HBV DNA AND ANTI-HBS AS ONLY MARKERS OF HBV INFECTION IN DONORS WITH OCCULT HEPATITIS B IN DALIAN, CHINA

X Deng[1], D Candotti[2], D Wang[1], X Wang[1], H Chen[1], S Laperche[2] and X Liang[1]

[1]Dalian Blood Centre, Dalian, China [2]Institut National de la Transfusion Sanguine, Paris, France

Background: Anti-HBC antibodies can generally persist for decades in HBV-infected individuals. However, confirmed undetectable levels of anti-HBC antibodies were reported in anti-HBS-only/HBV DNA+ blood donors with occult HBV infection (OBI) from South Africa and South East Asia. The prevalence and the viral and immunological mechanisms of this unusual serological profile in HBV-infected blood donors remain largely unknown.

Aims: To investigate the prevalence and viral features associated with the anti-HBS-only/HBV DNA positive profile in OBI donors from Dalian, China.

Methods: Blood donations were routinely tested for HBV DNA by using cobas-taqscreen MPX (Roche) or procleixultrio ID (Grifols) assays. OBI was further confirmed for research purpose by follow up of HBV DNA+ donors and performing additional anti-HBS antibodies quantification and anti-HBC antibodies testing with three distinct commercial clia assays (anti-HBC Elecsys Roche, architect anti-HBC II Abbott, and HISCL anti-HBC Assay Sysmex). HBV pre-S/S, precore/core and BCP regions were PCR-amplified and sequenced. HBV genotypes were determined by phylogenetic analysis of pre-S/S sequences.

Results: A total of 59,569 repeat and 62,385 first time donors were screened for HBV DNA. Results showed 110 HBSAG non-reactive/DNA+ donors (1:1,438) including 72 confirmed OBI. Among OBI donors, 29 (40%) tested anti-HBC+/ANTI-HBS−, 34 (47%) were anti-HBC+/anti-HBS+, and nine (13%) were anti-HBC−/anti-HBS+. Anti-HBC were consistently undetectable in eight donors overtime (2–6 samples/donor; range: 78–755 days) with all CLIA assays (no follow up for one donor). These nine OBI donors were significantly younger [median age: 21 years (range: 18–38 years)] than those with anti-HBC+/anti-HBS+ [median: 41 years (range: 23–53 years)] and anti-HBC+/anti-HBS− [median: 45 years (range: 21–55 years)] profiles ($P < 0.0001$). HBV vaccination was documented for six of these donors and was reported in one donor but without definitive evidence. No information on vaccination was available for the last two donors. Higher anti-HBS antibodies levels were detected overtime in these donors compared to anti-HBC+/anti-HBS+ OBI donors [median: 61.5 IU/l (range: 24–442 IU/l) vs 32 IU/l (range: 10.4–1,000 IU/l), respectively]. In contrast, extremely low HBV DNA loads (range: <10–155 IU/ml) were transiently detected in eight samples during follow up. Genotypes identified in seven donors were genotype B (n = 2), genotype C (n = 4) and genotype E (n = 1). The preliminary analysis of the core protein sequences obtained for four samples showed no particular genetic feature that could be associated with altered antigenicity. Characterization of BCP sequences is still ongoing in association with *in vitro* functional analysis of core protein production.

Summary/Conclusions: The 13% of OBI carriers in dalian blood donors are anti-HBS-only/HBV DNA positive confirming previous reports from South East Asia. This phenomenon was not restricted to a particular HBV genotype and was not related to core antigenic variations. It was significantly associated with younger age of carriers suggesting a shorter infection history compared with anti-HBC positive OBIS. The over 2 years stability of this serological profile ruled out the hypothesis of acute-phase vaccine breakthrough. Breakthrough in immunized donors may still be suspected. Further studies are needed to evaluate the potential infectivity of anti-HBS-only/HBV DNA+ OBI carriers, and to characterize the viral and immunological mechanisms responsible for this unusual hbvinfection profile.

4C-S25-02

SEQUENCE VARIABILITY OF THE COBAS TAQMAN ASSAY TARGET REGION IMPACTS ACCURATE HBV DNA DETECTION

A Andonov, C Osiowy, J Borlang and K Swidinsky

Public Health Agency of Canada, National Microbiology Laboratory, Winnipeg, Canada

Background: The COBAS TaqMan HBV test and its automated versions are widely used for monitoring response to antiviral treatment and screening of blood donations. These assays target a well conserved pre-Core/Core region of the HBV genome. Natural polymorphisms occurring within the viral genome covered by the specific primer/probe sites may result in erroneous viral load quantification or failure of virus detection.
Aim: To investigate the impact of sequence variability within the primer/probe region of the COBAS TaqMan assay on its performance.
Methods: An in-house multiplex Real-time PCR using the preS/S, Enhancer I (ENHI), and X/Enhancer II (X/ENHII) genomic regions and the Cobas TaqMan HBV test (Roche Diagnostics, Canada) were used in parallel for HBV DNA detection in patients with chronic HBV infection undergoing nucleos(t)ide therapy. The amplified product of the Cobas TaqMan HBV test was cloned to identify the putative pre-Core/Core genomic region targeted by the assay. Clinical samples with discordant HBV DNA results were then amplified by PCR covering that region and characterized by sequencing. Synthetic DNA constructs representative of the wild-type HBV sequence and variants of the primer/probe target region were prepared and used to evaluate the effect of putative mutations on the Cobas TaqMan performance.
Results: We have identified over 20 samples with discrepant viral load results. Two samples were 'Target not detected' by the Cobas TaqMan assay but amplifiable with the in-house multiplex HBV PCR. Alignment of the putative genomic region targeted by the Cobas TaqMan test with the eight HBV genotypes indicates that it corresponds to approximately nt 1827–1970 of reference HBV GenBank accession number KP168424. There were several mismatches within the site of the putative reverse primer used in the Cobas TaqMan assay: (i) T1938C, (ii) G1939C/A, (iii) C1946A, (iv) T1954C, (v) T1961G and (vi) T1961C/C1962A. The latter mutation was always linked to G1939C/A. The C1946A substitution was observed as a single variation in two HBV strains and in two other was combined with T1938C, T1954C and T1961G. The wild-type and variant HBV DNA constructs were diluted to approximately 100 IU/ml and tested with the Cobas TaqMan assay. Most of the observed HBV sequence variations resulted in strong reduction of the viral load (VL) from 77 IU/ml for the wild type to <6 IU/ml for variants A (T1954C + T1961G); B (C1946A); C (T1938C + C1946A + T1954C + T1961G) and D (G1939C/A + T1961C/C1962A).
Conclusion: Sequence variation within the putative reverse primer of the Cobas TaqMan assay had a significant impact on the analytical sensitivity including failure to detect HBV DNA on two occasions. Although the frequency of these HBV variants is not known, the potential for screening errors especially in donors with occult HBV infection and low VL is vital. This study also underlines the importance of publishing the primer/probe sequence data in the public domain by the manufacturers of commercial assays in order to identify the reasons for unusual quantification results or HBV DNA detection failure.

4C-S25-03

THREE YEAR FOLLOW-UP OF HBV-NAT REACTIVE PAKISTANI BLOOD DONORS

SUK Niazi, M Alam and A Mahmood

AFIT, Rawalpindi, Pakistan

Background: Prevalence of transfusion transmissible hepatitis B (HBV) and hepatitis C (HCV) virus is much higher in Pakistani blood donors than their prevalence in the developed countries. A previous study has documented seroprevalence of 2.16% for hepatitis B surface antigen (HBsAg) and 4.16% for anti-HCV in Pakistani blood donors. Nucleic acid amplification (NAT) test was implemented in Armed Forces Institute of Transfusion (AFIT), Rawalpindi, Pakistan in 2012 to enhance safety of blood products. Follow up study with additional investigations is highly important for confirmation of initial NAT test results.
Aims: To conduct follow up study on HBV-NAT reactive blood donors for confirmation and categorization of HBV infection.
Methods: A total 170,519 donors were screened by NAT on cobas s 201 system (Roche) at AFIT from 2012 to 2015. We confirmed 69 HBV (1 in 2,471) and 15 HCV (1 in 11,368) infections during the study period. The infections were confirmed on the basis of results from resolution of the pool (ID-NAT: Individual donation NAT) and by running ID-NAT on retrieved plasma bag (RPB) sample. Centrifugation

Table 1. Results of three years NAT testing for 170,519 donations

Category	HBV		HCV		HIV	
	No. of Reactives	NAT Yield	No. of Reactives	NAT Yield	No. of Reactives	NAT Yield
Initial NAT Reactive - not confirmed	103	1 in 1655	22	1 in 7751	Nil	-
Confirmed NAT reactive donations	69	1 in 2471	15	1 in 11368	Nil	-

Table 2. HBV infection categorization of 42 HBV – NAT reactive donors

HBV Infection Category	No of Donors	Percentage
WP*	4	(9.5)
Acute (Non - WP)	2	(4.8)
OBI#	29	(69)
Chronic HBV	1	(2.4)
Confirmed HBV infection- Seromarker negative	6	(14.3)

* WP = Window period infections
# OBI = Occult HBV infections

(4,000 × *g*) of RPB sample was done to enhance viral detection for initially resolved HBV reactive donations, which were later on found repeatedly non-reactive by ID-NAT. HBV NAT reactive donors were followed up for a period of 3 months. HBV serology (HBsAg, anti HBc total, anti HBc IgM, anti-HBs quantitative) was performed on index samples as well as the samples obtained on subsequent follow up visits.
Results: Of the 69 confirmed – HBV NAT reactive donors, 61 (88.4%) were confirmed by repeating ID-NAT on sample from index donation or RPB, while eight (11.6%) donors were confirmed after ultracentrifugation of samples. During the 3 months follow up, only 42 (60.9%) reactive donors reported for two additional visits for HBV serological testing. On follow up testing, 4 (9.5%) donors were classified as window period (WP), 2 (4.8%) as acute non-WP, 29 (69%) as Occult HBV infection (OBI), 1 (2.4%) as chronic HBV infection and six (14.3%) as Confirmed HBV infection – seromarker negative.
Summary/Conclusions: Follow up study is highly important for confirmation of NAT results, especially for HBV NAT reactive donors. Centrifugation of samples before ID-NAT can be useful while confirming an initially resolved HBV infection, which is afterwards repeatedly ID-NAT non-reactive on index/RPB samples. OBI is the most prevalent type (69%) of hepatitis B infection in Pakistani blood donors.

4C-S25-04

SHOULD ANTI HBC TESTING IN BLOOD DONORS BE PHASED OUT AFTER IMPLEMENTATION OF ROUTINE ID-NAT?

AK Tiwari, RC Dara, D Arora, G Aggarwal and J Sharma

Medanta- The Medicity, Gurgaon, India

Background: In India, mandatory serological tests for donor screening for Hepatitis B is hepatitis B surface antigen (HBsAg). Many studies showed that addition of hepatitis B core antibody (anti-HBc) testing and the HBV NAT to HBsAg testing improves HBV detection. Anti-HBc can be identified in acute, chronic and resolved HBV infection, and persists lifelong in most persons. Furthermore, it can be detected in a high proportion of donors with occult HBV infection, and in the presence of

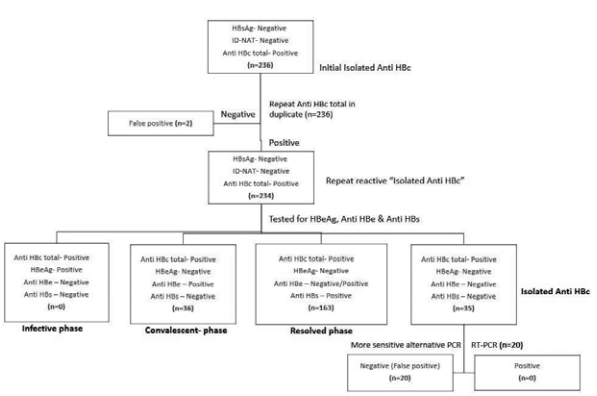


Figure 1. Study algorithm and results

extremely low HBV DNA viral load. Anti-HBc, however, may not identify a subset of donors in the 'window period' of HBV infection, and it is in this situation that HBV NAT plays a vital role. However, continuing anti-HBc testing with routine NAT 'in place' is being questioned. We plan to find answer to this question by testing 10,000 donor samples over a 6 months period.

Aim: In this study, we aimed to assess the role of anti-HBc in HBV screening in presence of NAT and whether 'isolated anti-HBc' reactive donors pose a risk for HBV infection.

Materials and methods: Study was conducted in the Department of Transfusion Medicine at tertiary care hospital. All blood donors underwent mandatory Transfusion Transmissible Diseases (TTD) screening by enhanced chemiluminescence assay (Vitros EciQ, Ortho Clinical Diagnostics, US) for the detection of anti-HIV and anti-HCV antibodies, HBsAg, anti-HBc total and ID-NAT assay using Transcription Mediated Amplification. (TMA; Procleix Ultrio plus Assay, Grifols). Donors negative for HBsAg and ID-NAT but positive for anti-HBc antibody (initial isolated anti-HBc reactive) were retested in duplicate using same platform for anti-HBc and if reactive (repeatedly reactive isolated anti-HBc reactive), tested further for other serological markers (HBeAg, anti-HBe and anti-HBs; Figure 1). Isolated anti-HBc reactive samples stored at −80°C were subsequently tested in batches by alternate PCR (Rotor-gene-Q RT PCR, Qiagen, Germany).

Results: Of 2,652 donor samples tested so far, 8.9% (n = 236) were initial reactive for isolated anti-HBc (HBsAg and ID-NAT- non-reactive; anti-HBc-reactive). 234 were repeatedly reactive for isolated anti-HBc. Out of these 234, none of the donors were in 'infective phase'; 15.4% (n = 36) were in 'convalescent phase'; 69.7% (n = 163) were in 'resolved phase' and only 14.9% (n = 35) donor fell in 'isolated anti-HBc reactive' category. 20 of these 35 donors could also be tested with more sensitive alternate PCR and none exhibited target detection. Less than 1% of the donors had previous history of jaundice (Figure 1).

Conclusion: Our results show that none of the 20 isolated anti-HBc reactive donors were infectious. This study suggests that there is limited value of anti-HBc testing with routine ID-NAT in enhancing blood safety. This would get reaffirmed when all 10,000 samples are analysed.

4C-S25-05

ESTABLISHMENT OF A NEW METHOD FOR PARALLEL DETECTION OF HBV-HCV-HIV BASED ON PCR-CHEMILUMINESCENT ANALYSIS

W Liang[1], Z Li[2], Z Ali[2], R Zhang[1] and J Wang[2]

[1]Jiangsu Province Blood Center, Nanjing, China [2]State Key Laboratory of Bioelectronics, Southeast University, Nanjing, China

Background: Over the past decade, nucleic acid testing (NAT) methods for detection of viral nucleic acids (DNA or RNA) have been developed to reduce this risk around the world. Although PCR-chemiluminescent (CL) analysis has features of ultralow background, high sensitivity, wide calibration range, and simple instrumentation, CL assay is not implemented in blood screening of pathogen infections with multiplex PCR assay, the PCR-fluorescence or transcription mediated amplification technique are usually two methods used in NAT screening.

Aims: To develop a new method for parallel detection of HBV-HCV-HIV based on PCR-chemiluminescent analysis suitable for blood screening on large scale.

Methods: The viral nucleic acid of HBV, HCV and HIV were simultaneously extracted from a single sample with the magnetic particles (MNPs). The isolated viral DNA and RNA were amplified in one-step multiplex RT-PCR. Biotin 11 dUTP was incorporated into in the target segment during amplification. Probes were modified onto the surface of the MNPs. These probes coated MNPs were used to capture viral specific amplified PCR product by hybridization with target segment. The streptavidin-alkaline phosphatase (SA-AP) was added to bind with biotin modified DNA. After washings, signal was generated by incubating the MNPs with AMPDD[3-(2′-spiroadamantane)-4-methoxy-4-(3′-phosphoryloxy) phenyl-1,2-dioxetane].

Results: The detection limits of our present method were 10, 10, and 100 copies/ml for HBV DNA, HCV RNA, and HIV-1 RNA, respectively. This method was used to detect 10,422 samples of blood donors in which 12 were HBV positive and two were HCV positive, these results completely coincided with those of other current diagnostic methods of commercial Kit applied in blood screening.

Summary/Conclusions: A specific, sensitive, simple, combined the magnetic separation and chemiluminiscence technique for simultaneous detection of multiple nucleic acid method has been developed. This method which is based on the use of MNPs, can be modified into a high through put automated set up for simultaneous detection of multiple infections. Simultaneous detection of HBV, HCV and HIV has been practically realized.

4C-S25-06

COMPARISON OF HIV CHANGES AND PATTERNS IN GENERAL AND BLOOD DONOR POPULATIONS IN ZIMBABWE: KEY BLOOD SAFETY CONCERNS

M Tonderai[1], L Marowa[2] and M van Hulst[3]

[1]Groningen University, National Blood Service Zimbabwe, Harare, Zimbabwe [2]National Blood Service Zimbabwe, Harare, Zimbabwe [3]University of Groningen, Martini Hospital, Groningen, The Netherlands

Introduction: Recently a within-gender analysis of HIV prevalence changes between 2005/06 and 2010/11 Zimbabwe Demography and Health Surveys (ZDHS) was conducted. It was noted that whilst Zimbabwe has reported a significant decline among both men and women, there are important differentials across provinces and demographic characteristics. It was concluded that the results tend to suggest that the epidemic in Zimbabwe is heterogeneous and therefore interventions must be targeted in order to achieve epidemic control. In order to assess this heterogeneity effect on blood safety programme, blood donor data analysis over the same study period was conducted and changes and patterns were examined and compared with ZDHS results.

Methods: We analyzed within gender changes in HIV prevalence for 276,720 women and 352,080 men who donated blood nationally for the combined periods of 2002–2006 and 2007–2011 being corresponding periods for ZDHS 2005/6 and 2010/11 respectively. The proportional changes (%) in HIV prevalence were determined and statistical significance using chi-square test was performed over the two periods. Sub-analysis by donation provinces and age categories was done and results compared with corresponding analysed ZDHS data. A blood safety indicator, the Donor Management Factor (DMF) was obtained by dividing the ZDHS's HIV prevalence's by the corresponding blood donor HIV prevalence.

Results: Although there were similar proportional declines in ZDHS HIV prevalence at national level for males (15%, P = 0.011*) and females (16%, P = 0.008*) in the overall blood donors there was a 4% increase in females (P = 0.376) and no change in males (0%; P = 0.929). Sub-analysis for new blood donors showed increases of 10% in females (P = 0.09) and 3% in males (P = 0.573). In repeat blood donors there was no change in females (0%; P = 0.907) and a decline of 3% in males (P = 0.645). There were variations in changes and patterns by provincial setting and age groups. The DMF decreased over the two periods in males from 28 (14.5/0.51) to 24 (12.3/0.51) and woman from 29 (21.1/0.72) to 24 (17.7/0.75). Variations in changes in DMF were also observed across provinces and age strata.

Conclusion: The observed declines in the general population (ZDHS) were not replicated in the donor data, which may signify early trend variation warning to be considered, or it may be a reflection of challenges being faced in further improving blood safety. The DMF indicates that the blood safety programme is increasingly finding it difficult to attract blood donations from HIV negative donors. From the two data sources among both men and women there are important differentials across provinces and age profiles, which jointly supports the view that the epidemic in Zimbabwe is heterogeneous. Therefore, interventions such as donor management need to be strengthened in order to effectively contribute to the HIV epidemic management and control in Zimbabwe.

Cryopreservation of Platelets

5A-S27-01

IN VITRO EVALUATION OF THE HEMOSTATIC CAPACITY OF CRYOPRESERVED PLATELETS AND PLATELET SUBSTITUTES

M Lozano and J Cid

University Clinic Hospital, Barcelona, Spain

The fact that platelets are exquisitely sensitive to a wide variety of stimuli that provokes its activation, essential for its physiologic role, represents a major challenge for the preparation and storage of platelets concentrates for transfusion. Using the most updated techniques for preparing platelet concentrates (PC) from whole blood donations or by apheresis, the maximum time that we can store them currently is 7 days. The short shelf life of PCs presents logistic problems in providing timely PC transfusions for patients highly alloimmunized by HLA/HPA antibodies, and when long or major transportation barriers exist (e.g., rural care settings, severe weather, military operations). Several strategies have been tried to circumvent the limited storage time of PC. Among them the cryiopreservation of human platelets using DMSO, the freeze drying of outdated human platelets and even the manufacture of artificial phospholips that might act as artificial platelets. Several *in vitro* techniques are currently available to characterize the structure and function of those products. Flow

cytometry combined to monoclonal antibodies allows a precise study of the platelet membrane and the glycoproteins that are present there. Platelet aggregation allows studying the functional capacity of the surface receptors and the submembrane pathways of activation response to several agonists. Unfortunately in aggregation studies rheological factors, which play a pivotal role in the regulation of the interaction of platelets with damaged vascular surfaces, are usually disregarded. Studies under flow conditions provide an overall view of the adhesive, aggregatory and procoagulant capacities of platelets mimicking the rheological conditions found at sites of vessel injuries. The method is based on the addition of the platelet product to anticoagulated whole blood from which the platelets have been removed by a leukoreduction filter. Changes in the anticoagulant of the whole blood used for the perfusion allows studying adhesive and aggregatory functions only (in the presence of citrate) or also the procoagulant capacities of the product when low molecular weight heparin is used. In addition the study of the blood that is perfused give a valuable information about the procoagulant effects of platelet products at the level of circulating blood. Another way to evaluate the procoagulant profile of the platelet product is to use thromboelastometry (TEM). The TEM measures in whole blood the viscoelastic properties of the clot and provides information on the speed of coagulation initiation, kinetics of clot growth, clot strength and breakdown. The combined use of those *in vitro* techniques provides valuable information about the structure and functional capacities of those platelet components. Depending on the results the decision can be made if the product can be o not tested on human volunteers and patients.

5A-S27-02

CRYOPRESERVED PLATELETS: PROTECTIVE EFFICIENCY ON VASCULAR PERMEABILITY *IN VITRO* AND IN A MURINE MODEL

G Baimukanova[1], D Potter[1], L Dumont[2] and S Pati[1]

[1]*Blood Systems Research Institute, San Francisco, California United States of America* [2]*Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire, United States of America*

Background: Currently, platelets are held at 22°C for up to 7 days, which results in a storage lesion, compromised platelet function and the potential for bacterial contamination. Platelet supply is severely limited in remote and austere conditions in both military and civilian settings. Cryopreserved platelets (CPP) could increase the availability of a hemostatic agent in locations where platelets are scarce, decrease the risk of iatrogenic sepsis, reduce the current waste of 22°C platelets and furthermore stockpiles can be generated in case of large scale disaster such as a nuclear event. Aside from hemostasis, platelets regulate and safeguard the integrity of the vascular endothelium in both health and disease. This study was designed to assess the vascular stabilizing effects of CPP on the endothelial barrier integrity *in vitro* and *in vivo* in mice. Comparisons were made w liquid apheresis platelets stored at room temperature.
Methods: Leukoreduced apheresis platelets (APU) were collected by standard methods and stored overnight at 20–24°C. CPP were prepared from APU under cGMP conditions with the addition of DMSO to 6%, concentrated, frozen at ≤65°C and shipped overnight on dry ice to the testing laboratory. APU or CPP were evaluated by electric cell-substrate impedance sensing (ECIS) via monitoring transendothelial electrical resistance (TEER) of endothelial cell (EC) monolayers. Platelet effects on preservation of VE-cadherin were also studied *in vitro*. In parallel, platelets were analyzed *in vivo* by using the modified Miles assay of vascular leakage induced by VEGF-A in NSG mice. *In vivo* platelet circulation was measured in blood samples by flow cytometry.
Results: The addition of either CPP or APU platelets to ECs produced almost an identical rapid dose-dependent enhancement in TEER associated with decreased permeability (the normalized resistances of CPP vs APU were 1.12 ± 0.18 and 1.21 ± 0.17 respectively by area under the curve analysis). Both treatment groups exhibited prominent EC protective potency and inhibited VEGF-challenged EC permeability. CPP and APU platelets similarly blocked VEGF-induced alterations in adherens junctions and VE-cadherin disassembly. Consistent with our *in vitro* data, CPP and APU platelets efficiently attenuated the VEGF-induced increase in vascular permeability *in vivo* in NSG mice with absorbances of 0.012 ± 0.002 and 0.011 ± 0.001 accordingly as measured spectrophotometrically. Systemic circulating levels of CPP platelets were diminished compared to APU platelets.
Summary/Conclusions: CPP are capable of preserving endothelial barrier integrity comparable to Day 1 room temperature stored platelets *in vitro* and *in vivo*. This work extends previous findings primarily focused on the hemostatic efficiency of CPP and provides potential novel therapeutic strategies for the treatment of the pathophysiological consequences of vascular permeability.

5A-S27-03

FROZEN PLATELETS IN CLINICAL USE

M Bohonek, L Landova and D Kutac

Central Military Hostpital – Military University Hospital Prague, Prague, Czech Republic

Background: Platelets have a short shelf life. This complicates the logistics of production, distribution and access when required for clinical use. This limitation is especially problematic for emergency and intensive care departments managing massive bleeding. The early and aggressive use of blood products for massive haemorrhage may correct coagulopathy, control bleeding, and improve outcomes. The timely availability of platelets as part of a massive transfusion protocol within the first 'golden hour' after the injury is often problematic. Many hospitals cannot afford to have platelets permanently in stock because of the short shelf life and high price.
Aims: An alternative solution is a stock of frozen platelets. Frozen platelets have successfully been used in military medicine. Since September 2014 we have trialed frozen platelets in routine clinical practice. We present clinical and laboratory data from a comparative study of fresh apheresis platelets (FAP) and deep frozen platelets (DFP).
Methods: In the period, September 2014–March 2016, we transfused a total of 135 units platelets to 41 patients presenting with heavy bleeding. 24 patients were transfused with a total of 75 units of DFP and 17 patients with 60 units of FAP. The International Severity Score (ISS) was calculated for all patients. The diagnoses of transfused patients are shown in Table 1 FAP were apheresis, leucodepleted, platelets, $>280 \times 10^9$ platelets/unit. DFP were apheresis leucodepleted platelets, $>280 \times 10^9$ platelets/unit, frozen in −80°C, with 6% DMSO, and stored in the same temperature for up to 2 years. Before clinical use, DFP (group O) were thawed and reconstituted in thawed group AB plasma. Thawing of DFP and plasma used plasma thawing device Tool (Tool Ltd., CZE). The connection device (CompoDock®, Fresenisus Kabi GmbH, GER) was used for sterile docking of plastics. We evaluated the following criteria: (i) Patient laboratory values (before and after transfusion) – Blood Count, aPTT, PT, Fbg, ROTEM; (ii) Patient vital signs (before and after transfusion) – T, P, BP, GSC; (iii) product testing before transfusion – TEG; (iv) Patient treatment – Number of transfused RBCs, FFP; Dosage of fibrinogen concentrate and tranexamic acid. Calculations were created by statistical software Statistica STATSOFT 11 (Dell, Tulsa, USA).
Results: DFP were, in comparison with FAP, partially activated: the clot strength measured by TEG with citrated kaolin was reduced, and onset of clotting and clot amplification faster. There were no significant differences between the two groups for the parameters PT, aPTT, Fbg, Hb and the mean amount of given blood products, fibrinogen and TXA. The amount of the PLT transfused was significantly higher for the group transfused with FAP ($P < 0.05$). See Table 2 for results. Clinical data in both group did not display any significant differences.

T0 - before transfusion										
Laboratory values	PT (ratio)		aPPT (ratio)		Fbg (g/l)		PLT (× 10^9 /l)		HGB (g/l)	
FAP/DFP										
Median	1,32	1,37	1,26	1,20	1,96	1,69	65,84	61,99	100,68	100,35
p	0,44		0,98		0,36		0,09		0,08	
T1 – after transfusion										
Laboratory values	PT (ratio)		aPPT (ratio)		Fbg (g/l)		PLT (× 10^9 /l)		HGB (g/l)	
FAP/DFP										
Median	1,24	1,32	1,16	1,16	1,97	1,89	**94,75**	**67,62**	95,68	95,05
p	0,21		0,96		0,5		**0,01***		0,09	

* significant difference between FAP and DPF

	PTS transfused with DPF	PTS transfused with FAP
Polytrauma (T068)	14	9
Intracranial bleeding (S065.0)	2	3
Haemoperitoneum (T810)	2	4
Other	6	1
Total	**24**	**17**

Table 2. Comparison and statistical testing of selected values

Conclusion: Frozen platelets are an alternative not only for military blood banks, but also for civilian blood banks which do not have a permanent stock of fresh platelets.. They may be stored in small portable deep freezers until required for clinical use. The thawing and reconstitution is a simple process and takes 30 min at most. Frozen platelet provides a cost effective functional platelets product for the management of bleeding and should be considered for wider use in clinical practice. The additional cost for DPF, to compare with FAP, is at most 10% + plasma for resuspension.

5A-S27-04

GENERATION OF PLATELET MICROPARTICLES AFTER CRYOPRESERVATION OF APHERESIS PLATELET CONCENTRATES CONTRIBUTE TO THE HEMOSTATIC ACTIVITY

I Eker[1], S Yilmaz[1], RA Cetinkaya[2], A Pekel[1], A Unlu[1], O Gursel[1], S Yilmaz[1], F Avcu[3], U Musabak[1], A Pekoglu[1], Z Ertas[1], C Acikel[1], N Zeybek[1], AE Kurekci[1] and IY Avci[1]

[1]Gulhane Military Academy of Medicine, Ankara, Turkey [2]Haydarpasa Training Hospital, Gulhane Military Academy of Medicine, Istanbul, Turkey [3]Memorial Hospital, Ankara, Turkey

Background and aim: In the last decade, substantial evidence has accumulated about the use of cryopreserved platelet concentrates, especially in trauma. However, little reference has been made in these studies about the morphological and functional changes of platelets. Recently, platelets have been shown to be activated by cryopreservation process and to undergo pro-coagulant membrane changes resulted in the generation of platelet derived microparticles (PMPs), platelet degranulation, and release of platelet-derived growth factors (PDGFs). We assessed viabilities, PMP and PDGF levels of cryopreserved platelets and their relation with thrombin generation.
Methods: Apheresis platelet concentrates (APCs) from 20 donors have been stored for 1 day and cryopreserved with 6% dimethyl sulfoxide. Cryopreserved APCs were kept at −80°C for 1 day. Thawed 100 ml of APCs were diluted with 20 ml of autologous plasma and specimens were analyzed for viabilities and PMPs by flow cytometry, for thrombin generation by Calibrated Automated Thrombogram and for PDGFs by ELISA test.
Results: The mean PMP and PDGF levels in freeze thawed APCs were significantly higher (2,763 ± 399.4/µl vs 319.9 ± 80.5/µl; $P < 0.001$ and 550.9 ± 73.6 pg/ml vs 96.5 ± 49 pg/ml; $P < 0.001$ respectively), but the viability ratios were significantly lower (68.2 ± 13.7% vs 94 ± 7.5%; $P < 0.001$) than fresh APCs. The mean endogenous thrombin potential (ETP) of freeze thawed APCs were significantly higher than the fresh APCs (3406.1 ± 430.4 nM min vs 2757.6 ± 485.7 nM min, $P < 0.001$). Moreover, there were a significant positive correlation between ETP levels and PMP levels (r:192, $P = 0.014$).
Conclusions: Our results showed that, after cryopreservation, while levels of PMPs were increasing, significantly higher and earlier thrombin formation was occurring in the samples analysed, despite the significant decrease in the viability. Considering the damage caused by the freezing process and the scarcity of evidence for their *in vivo* superiority, frozen platelets shall be considered for use in austere environments, reserving fresh platelets for prophylactic use in blood banks.

Blood Group Antigens

5A-S30-01

RED CELL ALLOIMMUNISATION RISK IN PATIENTS WITH DIFFERENT TYPES OF INFECTIONS

D Evers[1], JG van der Bom[1], J Tijmensen[1], RA Middelburg[1], M de Haas[1], S Zalpuri[1], KMK de Vooght[2], D van de Kerkhof[3], O Visser[4], NC Péquériaux[5], F Hudig[6] and JJ Zwaginga[7]

[1]Sanquin Research, Leiden, The Netherlands [2]University Medical Center Utrecht, Utrecht, The Netherlands [3]Catharina Hospital, Eindhoven, The Netherlands [4]VU Medical Center, Amsterdam, The Netherlands [5]Jeroen Bosch Hospital, 's Hertogenbosch, The Netherlands [6]Haga Teaching Hospital, The Hague, The Netherlands [7]Leiden University Medical Center, Leiden, The Netherlands

Introduction: Red cell alloantigen exposure can lead to antibody associated morbidity. Identification of clinical factors of influence on alloimmunization might enable allocating extended matched blood principally to high-risk patients, thereby aiming to enhance prevention of alloantibody induction.
Murine models have suggested inflammation as an important modulator of alloimmune responses towards red cell antigens.
Aims: This study sets out to quantify relative alloimmunization risks for patients receiving red cell transfusions during various infectious episodes.
Methods: We performed a multicenter nested case-control study in a source population of patients who received their first and subsequent red cell transfusions between 2005 and 2013. Cases were patients who developed a first transfusion-induced red cell alloantibody. Each case was compared with two randomly sampled non-alloimmunized controls, who received at least the same (lifetime) number of red cell units. For all cases, the *Nth* transfusion that most likely elicited alloimmunization was defined the implicated transfusion. The corresponding *Nth* transfusion was similarly marked for all matched controls. Logistic regression analysis, stratifying for potential confounders, was then used to evaluate the association between red cell alloimmunization and the presence of various infections during a so-called 5-week 'alloimmunization risk period' surrounding this implicated transfusion.
Tissue-invasive bacterial infections were categorized into 'severe' or 'mild' according to an expected degree of associated systemic inflammation. Bacteremia were categorized according to their causative Gram-positive or Gram-negative microorganism. Viremia and disseminated viral zoster infections were defined as 'disseminated viral infections', hereby contrasting local viral infections.
Results: The adjusted relative risk (RR) for red cell alloantibody development during 'severe' bacterial (tissue-invasive) infections was 1.34 [95% confidence interval (CI) 0.97–1.85]. Risks were significantly more pronounced when these infections were accompanied with long-standing fever (RR 3.06, CI 1.57–5.96). Disseminated viral disorders were associated with a RR of 2.41 (CI 0.89–6.53). Gram-negative bacteraemia in contrast coincided with a twofold reduction in alloimmunisation incidences (RR 0.58, CI 0.13–1.14). 'Simple' bacterial infections, Gram-positive bacteraemia, fungal infections, maximum CRP values, and leukocytosis were not associated with red cell alloimmunisation.
Conclusions: These findings are in line with data from murine studies and suggest a possible association between infection-associated inflammation and red cell alloimmunisation in humans. Conformational research is needed before these inflammation-associated risk factors enable selection of high-risk patients who benefit most from extensively matched red cell units.

5A-S30-02

GENETIC POLYMORPHISMS AND SUCEPTIBILITY TO RED BLOOD CELL ALLOIMMUNIZATION IN PATIENTS WITH SICKLE CELL DISEASE

L Castilho, MA Botelho and EA Sippert

University of Campinas, Campinas, Brazil

Background: Red blood cell (RBC) alloimmunization is one of the serious complications associated with transfusion therapy in patients with sickle cell disease (SCD). The presence of the alloantibodies also hinders the provision of compatible blood for these patients and can lead to delayed hemolytic transfusion reactions (DHTR) and autoantibody formation. Most developed antibodies are directed to the Rh antigens and differences in the immune response of transfused patients who develop alloantibodies (responders) and who do not develop antibodies (non-responders) are not completely known. The hypothesis is that susceptibility to RBC alloimmunization is governed by many factors including genetic diversity among individuals.
Aims: The aim of this study was to identify associations of polymorphisms on immunologically relevant genes (*IL1B, IL6, TNFA, IL10, IL4, HLA-DRB1, GZMB, PRKCQ* and *RHAG*) with RBC alloimmunization and specifically Rh alloimmunization.
Methods: We studied one hundred and sixty-one patients with SCD (homozygous for hemoglobin S) on chronic RBC transfusion therapy and 288 unrelated blood donors from the same geographical region of patients. The genetic polymorphisms were analysed by PCR, PCR-RFLP and TaqMan genotyping assay (Applied Biosystems) while, *HLA-DRB1* typing was performed using PCR-SSO (One Lambda). The Hardy-Weinberg equilibrium and the allelic and genotypic frequencies were obtained by Arlequin software. Allele and genotype frequencies were compared using the Fisher's exact test.
Results: Among the 67 patients who were alloimmunized, 35 (52.2%) developed a single alloantibody and 32 (47.8%) developed two or more alloantibodies. The most common specificities identified were anti-E (38.8%), -C (32.8%), -K (20.9) and we also verified that forty-nine (73%) of the patients had at least one antibody with specificity for common Rh antigens (D, C, c, E, e). Our results revealed a statistically significant association among A allele and GA genotype of the *TNFA*-308G/A as well as T allele and TT and CT genotype of the *IL1B*-511C/T polymorphism with susceptibility to RBC alloimmunization (*TNFA*-308A allele: $P = 0.004$; *TNFA*-308GA

genotype: P = 0.0021; *IL1B*-511 T allele: P = 0.0085; *IL1B*-511CT and TT genotypes: P = 0.0071). We also found that *HLA-DRB1*15* (P = 0.044) and *RHAG* 808GA genotype (P = 0.046) could be associated with susceptibility to Rh alloimmunization in Brazilian patients with SCD.

Summary/Conclusions: Our results showed that more than one polymorphism are associated with an increased risk of developing RBC antibodies in Brazilian patients with SCD. Considering these polymorphisms as markers to RBC alloimmunization, 75% of the patients should receive units with extended phenotyping or genotyping (especially to Rh antigens). These findings can contribute, in the future, to improve the transfusion and therapeutic strategies for patients with SCD.

5A-S30-03

ISOHEMAGGLUTININ PRODUCTION AFTER MINOR ABO INCOMPATIBLE PERIPHERAL BLOOD HEMATOPOIETIC STEM CELL AND UMBILICAL CORD BLOOD TRANSPLANTATION

P Solves, A Saus, J Osorio, M Guinot, MJ Pons, J Ibañez, I Gómez-Seguí, N Carpio and MA Sanz

La Fe University Hospital, Valencia, Spain

Background: ABO incompatibility between donor and recipient occurs in 30–40% and more than 50% of patients undergoing peripheral blood transplantation (PBSCT) and cord blood transplantation (UCBT), respectively. A minor ABO mismatch occurs in 15–20% of HLA-matched PBSCT. Minor ABO mismatch is defined when the donor has, or the potential to make isohemagglutinins (IH) directed against recipient red blood cells (RBC) antigens. The fact that cord blood contains predominantly naïve B cells may lead to differences in IH production among patients undergoing cord blood and peripheral blood or bone marrow transplantation. Some authors have published lack of IH production following ABO minor incompatible HLA mismatched UCBT (Snell, Bone marrow Transplant, 2006).

Aims: Our aim was to study the presence of IH in patients after ABO minor incompatible PBSCT or UCBT to assess differences among them.

Methods: We performed IH titers in patients who underwent minor ABO incompatible PBSCT or UCBT. We retrospectively reviewed the patients who underwent a minor ABO incompatible transplant and were alive and determined IH presence and titers. ABO, Rh typing and the Indirect Antiglobulin Test (IAT) were determined in the automated system ORTHO Autovue Innova (Ortho Clinical Diagnostics, England). Presence of IH was identified by reversed grouping and gel titrations in DG Gel Coombs or DG Gel Neutral Cards.

Results: Thirty-two patients were reviewed, 16 who underwent PBSCT and 16 who underwent UCBT. Patients who underwent UCBT had A group (n = 11) and B group (n = 5), while donor group was O in all cases. Median (and range) of days from UCBT until IH determination was 1,393 (132–5,067). All except one patient (3,348 days after UCBT) developed donor derived IH, but not IH against recipient red blood cells (RBC). One A positive patient who had received an O positive UCBT more than 10 years ago, was erroneously transfused with recipient ABO group (A positive), without any adverse effect. However, after transfusion anti-A titer ascended to 2024. This is the only case in which IH against recipient RBC was detected. Ten patients who received a PBSCT developed donor derived IH, but none developed IH against recipient ABO group. Six patients whose ABO group was AB and received O (n = 1), A (n = 2) or B (n = 3) group peripheral blood progenitor cells did not developed any IH. Median (and range) of days from transplantation until IH determination was 597 (88–3,217).

Conclusions: Contrary to previously published, patients who underwent UCBT developed donor derived IH in most cases. According to our data, while hematic ABO group changes to donor ABO group, patients underwent PBSCT or UCBT maintain their seric ABO group. IH against recipient RBC are not produced, probably due to the development of tolerance of B cells or immunosuppressive treatment. However, if the patient is exposed to a high amount of antigen, immune response occurs and IH against recipient are produced.

5A-S30-04

CELL-FREE NUCLEIC ACIDS IN BLOOD PRODUCTS MODULATE THE EXPRESSION OF GENES INVOLVED IN THE INNATE IMMUNE RESPONSE

O Preynat-Seauve, P Lau, D Tirefort, A Guichebaron and S Waldvogel-Abramowski

Geneva University Hospitals, Geneva, Switzerland

Background: Extracellular nucleic acids are present in human blood. They are expected to be present in manufactured blood products eligible for transfusion and little is known about their biological activity on human cells.

Aims: The aim of this study is to investigate if cell-free nucleic acids are present and biologically active in red blood cell units (RBC), fresh frozen plasmas (FFP) and platelet concentrates (PC).

Methods: Cell-free nucleic acids were purified from RBC, FFP and PC. Their nature and structure was analyzed by regular methods of nucleic acid qualification/quantification. Their impact on gene regulation was tested by microarray after co-culture with human peripheral mononuclear cells, macrophages or dendritic cells. The impact of blood product storage was notably tested for RBC.

Results: Extracellular double stranded DNA, RNA were present at very low level (ng/ml) in blood products. A quantitative PCR combining the amplification of a cell-free nucleic acid marker (Alu115) and the amplification of an internal non-human DNA control introduced in all samples (PhiX174) was also developed to study with high sensitivity the impact of RBC storage on cell-free nucleic acids release. Storage did not have any influence on such release in RBC. However, the cell-free nucleic acids concentration highly differed between the different units. Microarray experiments showed that exposition of human mononuclear cells to cell-free nucleic acids purified from RBC and FFP activate numerous genes notably involved in the immune response including chemokines, chemokine receptors and receptors of the innate response. These observations were confirmed by regular PCR.

Summary/Conclusions: Extracellular DNA and RNA are present in blood products and transfused to recipients. Although storage does not have any impact on their release, these cell-free nucleic acids regulate gene expression including activation of pro-inflammatory genes involved in innate immunity. These observations demonstrate immunostimulatory properties of human extracellular DNA and provide new insights in the knowledge of biologically active components of blood products and adverse effects of transfusion.

5A-S30-05

MALDI-TOF MS BASED BCAM GENOTYPING ON 37,234 SWISS PROVES TWO NEW LUTHERAN BLOOD GROUP ALLELES, BOTH POSITIVE FOR AUB SPECIFIC 1,615 G

N Trost[1], S Meyer[1], C Vollmert[2], J Gottschalk[1], J Ries[1], A Markovic[1], L Infanti[3], A Buser[3], SA El Dusouqui[4], D Castelli[5], T Weingand[6], A Sarraj[7], MC Braisch[8], J Thierbach[8], BM Frey[1], T Peyrard[9] and C Gassner[1]

[1]Blood Transfusion Service Zürich, Swiss Red Cross (SRC), Zurich-Schlieren, Switzerland [2]Agena Bioscience, Hamburg, Germany [3]Blood Transfusion Service beider Basel, SRC, Basel, Switzerland [4]Blood Transfusion Service Genève, SRC, Genève, Switzerland [5]Blood Transfusion Service Svizzera Italiana, SRC, Lugano, Switzerland [6]Blood Transfusion Service Zentralschweiz, SRC, Luzern, Switzerland [7]Blood Transfusion Service Neuchâtel-Jura, SRC, Neuchâtel, Switzerland [8]Blood Transfusion Service Ostschweiz, SRC, St. Gallen, Switzerland [9]Département Centre National, Institut National de la Transfusion Sanguine (INTS), Paris, France

Background: The Basal Cell Adhesion Molecule (*BCAM*), also known as CD239 and located on 19q13.2 encodes all Lutheran blood group antigens as exemplified by the antithetic Lu[a] **Lu8/14, Au**antigens and the high-prevalence Lu13 antigen. Genetically, all these antigens are encoded at certain genetic locations of the gene, and by either one of the (usually) two specific nucleotides, defining the respective SNPs. In theory, and looking at the 4 exemplary SNPs, 16 different combinatory haplotypes could be expected. However, only five of them are currently reported as alleles and accepted by the ISBT terminology committee.

Aims: Extended high-throughput *LU* genotyping by MALDI-TOF MS based SNP-detection was originally done to identify rare Lu (a+b−) blood donors. Concomitant findings, however, delivered interesting new evidences with respect to the genetic polymorphism of the Lutheran blood group system in Caucasians.

Methods: Genotyping relied on MALDI-TOF MS based SNP-detection at coding nucleotides 230 (G/A), 611 (T/A), and 1,615 (controversially to the current version of the ISBT table correctly: A/G) for *LU*01/02*, *LU*02/02.14* and *LU*02/02.19*, respectively. Genotyping was performed on 37,234 Swiss blood donors. Of note, the reference *LU*02* allele is defined and considered as the simultaneous carrier of all SNP

current ISBT allelename	simultaneous antigen positivity (exemplary)				new alleles (suggested names)	allele-frequency (Switzerland)
*LU*01*	Lu^a	Au^a	Lu8	Lu13		0.0237
	Lu^a	**Au^b**	Lu8	Lu13	***LU*01.19***	0.0141
*LU*02*	Lu^b	Au^a	Lu8	Lu13		0.6152
*LU*02.14*	Lu^b	Au^a	Lu14	Lu13		0.0092
*LU*02.19*	Lu^b	Au^b	Lu8	Lu13		0.2812
	Lu^b	**Au^b**	**Lu14**	Lu13	***LU*02.19.14***	0.0053
*LU*02.-13*	Lu^b	Au^a	Lu8	**Lu-13**		0.0519
						1.0006

Figure 1.

variations specific for the 'wildtype antigens', e.g. Lu^b, Lu8, Au^a (Lu18) and Lu13. For Lu13, detection of SNP at coding nucleotide 1,340 (C/T) was tested using PCR-SSP on 336 individual DNAs with selected genotypes.

Results: Among the 37,234 Swiss donors investigated, *LU*01*, *LU*02*, *LU*02.14* and *LU*02.19* alleles were observed, in all homozygous and heterozygous combinations as could be expected. However, there were also 6 Lu (a+b−) subjects and 1 Lu (a−b+) donor all typed as Au (a−b+), indicating homozygous presence of two theoretically expectable *LU* haplotypes, proving them as truly new *LU* alleles. Additionally, both new alleles were unambiguously identified in a variety of heterozygous genotypes. Testing 336 genotypically selected DNAs for *LU*02-13*, positive signals were neither encountered in 42 Lu (a+b−), nor among 132 Au (a−b+), but in 16 of 96 Lu (a−b+), Au (a+b−) homozygotes. Applying *LU*02-13* PCR-SSP to all other genotypes of the 336 sample group confirmed this finding. All allele frequencies were calculated (Figure 1).

Summary: The project not only identified 42 Lu (a+b−) and 7 LU: -8,14 homozygous blood donors, but also delivered new data on the genetic polymorphism of the Lutheran blood group system in Caucasians. The existence of two new *LU* alleles, e.g. *LU*01.19* and *LU*02.19* with simultaneous genetic positivity for LU14, could unambiguously be proven in the course of this study. Additionally, linkage of *LU*-13*, to the wildtype allele *LU*02* may be expected and showed an allele-frequency of 0.0519. Consequently, *LU*02-13* homozygosity may be expected at the impressive rate of one among 371 Swiss individuals. However, the detection of one of the new alleles represents a challenge for the current ISBT allele-terminology, since the simultaneous genetic presence of Lu14 and Au^b (Lu19) on a Lu^b allele can be described neither as *LU*02.14* nor as *LU*02.19*. Alternatively, a '2 dot' terminology could be adopted, dubbing the new allele *LU*02.19.14*. This proposal requires further discussion within the ISBT terminology committee.

5A-S30-06

TWO NOVEL HIGH INCIDENCE ANTIGENS IN THE LUTHERAN BLOOD GROUP SYSTEM (LUAC AND LUBI)

V Karamatic Crew[1], R Laundy[2], A Bahashwan[3], P Walser[2], I Skidmore[1], L Wall[4] and N Thornton[2]

[1]NHS Blood and Transplant, Bristol, United Kingdom [2]International Blood Group Reference Laboratory, Bristol, United Kingdom [3]University of Bristol, Bristol, United Kingdom [4]New Zealand Blood Service, Auckland, New Zealand

Background: The Lutheran blood group system (LU) comprises 22 antigens carried on a single type I membrane glycoprotein with five immunoglobulin-like extracellular domains. The glycoprotein is present on red cell surfaces in two isoforms; 85 kD Lu glycoprotein and 75 kD basal cell adhesion molecule antigen B-CAM. Both isoforms are adhesion molecules whose main function is laminin binding and both are products of alternative splicing of a single gene *LU* located on chromosome 19. Lutheran antigens are mostly encoded by single nucleotide mutations, resulting in 10 pairs of antithetical antigens and 12 high incidence antigens.

Aims: We investigated two unrelated cases of individuals with unidentified alloantibodies to high incidence antigens present in their plasma and have shown serological and molecular evidence for two novel high incidence antigens of the Lutheran blood group system.

Case studies and methods: Patient 1 (P1): a 64 year old previously transfused Maori male awaiting surgery. Patient 2 (P2): a 54 year old previously transfused Caucasian female with no known history of pregnancy. For both patients, serological tests were performed by standard IAT (tube and column agglutination) techniques. For P2, a monoclonal antibody-specific immobilization of erythrocyte antigens (MAIEA) assay was carried out using three monoloclonal anti-Lu (BRIC108, BRIC224, BRIC221; IBGRL Research Products, Bristol, UK). Genomic DNA was isolated from whole blood of P1 and P2; all the exons of the *LU* gene were amplified by PCR and analysed by direct Sanger sequencing.

Results: P1′s plasma reacted strongly by IAT and reactivity was marginally weaker with papain treated cells. In (Lu) cells and Lu_{null} cells were compatible with P1′s plasma, confirming a Lu-related specificity. P1′s cells were found to be LU: 1, 2, 4, 5, 6, 7, 8, 12, 13, 16, 17, 19, 20. A novel homozygous mutation c.662C>T was observed in exon 6 of *LU*, encoding p.Thr221Ile in the Lu glycoprotein. P2′s plasma contained a complex mixture of antibodies. Enzyme and chemically modified cells and multiple absorption/elution studies revealed anti-Jr^a present in P2′s plasma, in addition to anti-M, anti-Fy^a and another antibody to a Lutheran high incidence antigen. P2′s cells were found to be Jr (a−) and Lu:2,3,4,5,6,8,13. P2′s plasma gave positive results in the MAIEA assay with three Lutheran monoclonal antibodies, thereby confirming Lutheran specificity. Sequencing of *LU* showed P2 to be homozygous for a novel mutation c.1495C>T in exon 12, encoding p.Arg499Trp. Homology models of the novel Lu glycoproteins of P1 and P2 were subjected to all-atom molecular dynamics calculations to analyse potential conformational changes.

Conclusions: We report serological and genetic evidence for two new antigens of the Lutheran system, which we propose to name LUBI (p.Arg499Trp) and LUAC (p.Thr221Ile) and submit to the ISBT Red Cell Immunogenetics and Blood Group Terminology Working Party for consideration for allocation of antigen numbers. The absence of these high incidence antigens arises from single amino acid changes in the Lu glycoproteins. The presence of anti-LUBI and anti-LUAC in the patients' plasma was presumed to have been made in response to previous transfusions.

Plenary Session IV: Emerging Arboviruses and Transfusion

PL4-01

THE GLOBAL EPIDEMIOLOGY OF ARBOVIRUSES, WITH FOCUS ON ZIKA VIRUS

LR Petersen

Centers for Disease Control and Prevention, Fort Collins, Colorado, United States of America

Background: Arboviruses are increasing in incidence and expanding globally.

Aims: Describe the global spread of arboviruses.

Methods: Review recent data on the epidemiology of arboviruses, with a focus on those of concern for blood safety.

Results: Mosquito-borne arboviral disease transmission can be simplified in two basic patterns: (i) humans serve as the principal host in urban settings and can efficiently infect mosquitoes, and (ii) other animals serve as the principal host and humans are only infected incidentally and do not contribute to transmission of the virus. Both patterns of transmission have contributed to explosive arboviral disease outbreaks in recent years. In the first pattern, dengue, yellow fever, chikungunya, and Zika viruses are spread via Aedes aegypti and to a lesser extent by Aedes albopictus mosquitoes. In the second pattern, West Nile virus is spread among birds by Culex mosquitoes. In this presentation, I will review the global spread of these viruses and the contributing factors leading to this spread.

Summary: Arboviral diseases are a major global health problem. A combination of sociologic, virologic, and environmental factors have contributed to their emergence.

PL4-02

EPIDEMIOLOGY, VIROLOGY AND MOLECULAR ASPECTS OF THE ARBOVIRUSES EPIDEMICS IN BRAZIL

JE Levi

Instituto de Medicina Tropical da Universidade de São Paulo, São Paulo, Brazil

Background: Brazil is the country with the largest number of dengue cases for the last 10 years. Yearly, there is a growing number of reports, with more than 1 million per year since 2014. Vector control has proven unsuccessful while highly effective vaccination for DENV may soon be possible.

Aim: Summarize information on the on-going epidemics of Chikungunya, Dengue and Zika viruses in Brazil.

Method: Review of epidemiological, virological and molecular data from peer-reviewed journals and official governmental information as well as personal and institutional experience.

Results: The risk of transfusion transmitted dengue has never been addressed by Brazilian blood banks, since such TT cases are not commonly verified. However, in centers assisting a significant number of hospitalized transfused recipients and where hemovigilance is implemented, TT dengue has been described with associated morbidity. In 2014 Chikungunya arrived in Brazil from two different routes; a traveler returning from Africa to Bahia and by land from French Guiana, introducing respectively two distinct CHIKV lineages. Thousands of cases are registered but, so far, no TT was reported, not only in Brazil but also globally. In 2015, hundreds of patients in the Northeast states, presenting mild fever, maculopapular rash, conjunctivitis and arthralgia, in whom diagnosis of DENV or CHIKV was ruled out, led to the identification of Zika virus for the first time in the West. The unexpected, and unique among Flaviviruses, association with fetal brain abnormalities, chiefly the dramatic newborns presenting microcephaly, urged WHO to declare ZKV a global health emergency. So far, two TT cases were documented in Brazil, both with no obvious clinical impact to the recipients. From Brazil, ZKV spread incredibly fast through South and Central America countries, where autochthonous cases now occur in more than 20.
Conclusions: Due to the dimension of the problem, with virtually thousands of asymptomatic donors carrying one of the three now endemic arboviruses, laboratorial screening seems the most attainable measure since available pathogen inactivation methods are unaffordable and do not cover red blood cells. Viral nucleic acid detection shall be implemented when licensed and automated systems become available. At the moment, in-house real-time PCR is being adopted by centers with trained personnel and methodology grasp. Universal NAT screening may be difficult and of low cost-effectiveness, but providing ZKV-RNA tested blood to pregnant women and intrauterine transfusions is recommended. The serologic diagnosis of ZKV is hampered by the huge DENV seroprevalence in the general population, and the recognized cross-reaction between DENV and ZKV antibodies. A lack of ZKV specific serological reagents is currently impairing proper risk assessment of pregnant women in addition to epidemiological estimates, that makes the ground for medical interventions. Hopefully, we will have in the short-term better tools to diagnose and prevent vector-borne and blood-borne arboviral diseases. Intense research on basic aspects of the viruses and their ecological interaction with the potential arthropod vectors locally prevailing and on the pathogenesis in humans is of paramount importance.

PL4-03

ZIKA VIRUS (ZIKV) TRANSFUSION TRANSMISSION AND ITS PREVENTION: THE US EXPERIENCE

JS Epstein

US FDA, Silver Spring, Maryland, United States of America

In December 2015, the massive epidemic of ZIKV in the Western Hemisphere was documented to reach US territories with a first reported vectorborne transmission on the Caribbean island of Puerto Rico. Subsequent case reports indicated rapid expansion of the epidemic in Puerto Rico and the US Virgin Islands. Based on those findings and the potential for epidemic spread to the continental US and Hawaii, the federal government took steps to address the public health risks from ZIKV. A need to protect blood safety in areas of vectorborne transmission, and in non-outbreak areas affected secondarily by travelers, was recognized based on: a rapidly spreading epidemic; past experience with flaviviruses including dengue viruses and West Nile virus; high proportion (*c.* 80%) of asymptomatic infections; evidence of ZIKV viremia in 2.8% of asymptomatic blood donors in the 2013–2014 ZIKV outbreak in French Polynesia; and media reports from Brazil of two probable cases of transfusion transmission. On February 1, 2016 AABB issued Association Bulletin #16-03 recommending self-deferral for 28 days of donors potentially exposed to ZIKV in affected and at risk countries of the Western Hemisphere; reporting of suggestive post donation illness (two or more associated symptoms) by donors who failed to self-defer; deferral for 28 days post symptom resolution in affected donors; and recall of in-date components collected within 14 days prior to reported illness. On February 16, 2016, FDA issued recommendations to defer for 4 weeks donors with history of residence or travel to affected areas, diagnosis or suggestive symptoms that developed in an affected area or within 2 weeks of return from an affected area, and sexual exposure to a man potentially exposed to ZIKV in the previous 3 months. Additionally, FDA recommended against collecting blood in affected areas unless tested with a licensed or FDA permitted investigational screening test, or, for platelets and plasma, treated by approved pathogen reduction technology (PRT). Because of the impact in Puerto Rico, the government provided blood from the unaffected continental US from March 5 to April 15, 2016, during which time a commercially developed investigational nucleic acid based screening test for ZIKV RNA was made available. Initial results of screening in Puerto Rico since April 4, 2016 indicated a presumptive viremic rate in donors of *c.* 0.5% consistent with the start of a significant early epidemic. The rate was expected to increase based on experience with prior ZIKV outbreaks and seasonal patterns of dengue and chikungunya virus outbreaks. Efforts are ongoing to establish donor screening for ZIKV in US states at presumed high risk of vectorborne ZIKV due to introduction by travelers and presence of competent mosquito vectors A. aegypti and potentially A. albopictus, and to accelerate development of PRT for red blood cells. Unresolved scientific issues for transfusion transmitted ZIKV include minimal infectious dose, rate of transmission from infected units, viral kinetics and evolution of the immune response, and disease outcomes.

Sickle Cell Disease

5B-S32-01

DEVELOPING NEW PHARMACOTHERAPEUTIC APPROACHES TO TREATING SICKLE CELL DISEASE

MJ Telen

Duke University, Durham, North Carolina, United States of America

Sickle cell disease (SCD) was once nearly uniformly fatal in childhood. Now, even in developing countries with limited healthcare resources, the mean age of death from sickle cell disease may have risen to young adulthood, while in developed countries, the age of death is in middle age. However, prolonged survival for patients with SCD is largely due to improvements in supportive care, including vaccinations, antibiotic prophylaxis, and overall medical management, including transfusion. There is still only one widely recognized and approved drug for sickle cell disease hydroxyurea (hydroxycarbamide). Hydroxyurea Is only partially effective and remains underutilized in both developed and developing countries due to many factors, including reluctance to prescribe 'chemotherapy' and the requirement for repeated monitoring of blood counts. Therefore, we need better ways of treating and preventing both the recurrent painful vaso-occlusive episodes pathognomonic of SCD as well as the widespread end-organ damage that still leads to severely shortened life expectancies.
Over the last several decades, we have learned a great deal about how the abnormal red blood cells of SCD cause both acute painful episodes and end-organ damage in the brain, lungs, kidneys, joints and other tissues. Based on this knowledge, large and small pharmaceutical enterprises as well as individual investigators are now pursuing multiple new avenues for treating SCD, both by using new drugs as well as by repurposing drugs originally developed for other needs. Many classes of compounds are in active development, in preclinical models as well as in phase 1, 2, and 3 clinical trials. Several drugs targeting the selectin class of adhesion molecules are in phase 2 and 3 clinical studies. Other drugs targeting adhesive interactions are in preclinical studies and early clinical trials as well. Pharmacologic agents that address either the overactivity of coagulation pathways or platelet activation are also being vigorously explored. Recent experimental evidence of the importance of inflammatory pathways in sickle cell vaso-occlusion and end-organ damage has also led to interest in a variety of anti-inflammatory compounds for possible use in SCD. And methods to achieve upregulation of hemoglobin F expression, along with compounds that may modulate the chemical and physical behavior of hemoglobin S, remain of interest to clinicians and pharmaceutical firms alike. Finally, recent explorations of the genetic variations that predispose to certain types of SCD-related tissue injury, such as stroke or nephropathy, are expected to lead to identification of drugs targeting the pathways uncovered by such work. Thus, there is reason to be hopeful that the next 5–10 years will bring us new treatments to improve the outlook for patients with SCD worldwide.

5B-S32-02

THE INFLUENCE OF HBF ON THE PATHOPHYSIOLOGY AND PHENOTYPE OF SICKLE CELL DISEASE

AD Adekile

Kuwait University, Safat, Kuwait

HbS results from the substitution of valine for glutamic acid in the 6th amino position in the β-globin chain. Its inheritance as a homozygote or compound heterozygote causes sickle cell disease (SCD). The central paradigm in the pathophysiology of the disease is the reduced solubility of HbS in a deoxygenated medium, which causes rigidity and distortion of the red blood cell (RBC) membrane with increased viscosity

and other rheological abnormalities. There is a chronic hemolytic anemia and occlusion of small blood vessels, which is responsible for the characteristic recurrent pain episodes of SCD. It is, however now recognized that SCD is a chronic inflammatory disease because of extensive vasculopathy, instigated by the free heme from hemolysis, which sets off a cascade of pro-inflammatory signaling mechanisms with upregulation of adhesion molecules, platelet activation, thrombin generation and ischemia/reperfusion injury. Nitric oxide depletion results in vasoconstriction, and eventual endothelial intima proliferation. This endothelial dysfunction drives SCD subphenotypes like stroke, pulmonary hypertension, priapism and chronic leg ulcers. On the other end of the spectrum, the viscosity-driven subphenotypes include recurrent pain episodes, acute chest syndrome and osteonecrosis. Several genetic factors including single-gene polymorphisms within the β-globin gene cluster and in distant loci, act as SCD phenotype modifiers. The most recognized of these is HbF and patients with levels of ≥20% have a mild phenotype. Kuwaiti SCD patients have HbF levels >30% in the first 3 years of life and 15–20% in older patients. They carry the Arab/India β^S-globin haplotype and there is a high prevalence of α-thalassemia trait, which also modulates the phenotype. The patients generally have a mild phenotype in childhood; stroke, priapism and chronic leg ulcers are rare. In addition, silent brain infarcts are uncommon in children and their neurocognitive function is comparable to that in their normal siblings. However, osteonecrosis is common with a prevalence of about 25% in children and >45% in adults. The only predisposing factor is frequent pain episodes; sex, co-existent α-thal trait or bone morphogenic protein polymorphisms have not shown any association with osteonecrosis in these patients. In spite of elevated HbF levels, Kuwaiti patients with HbSD or HbSβthal tend to have a severe phenotype. In the former, it's probably because of the pro-sickling nature of the $\beta^{121Glu>Gln}$ substitution of HbD, while in the latter it may be due to the nature of the β-thal mutation. Even Kuwaiti patients with $HbS\beta^+$-thal do have frequent severe pain crises. Elevated HbF level is therefore not uniformly protective and the SCD phenotype in adult Kuwaiti SCD patients is, quite often, quite severe. More studies are clearly indicated in this group of patients.

5B-S32-03

IMMUNE REGULATION OF SICKLE CELL ALLOIMMUNIZATION

K Yazdanbakhsh

New York Blood Center, New York, New York, United States of America

Red blood cell (RBC) transfusion is the main treatment for acute complications in sickle cell disease (SCD) and the majority of patients receive one or more transfusions by adulthood. However, patients with SCD are at a high risk of alloimmunization, which can cause life-threatening complications for this patient population. The high rate of alloimmunization can in part be explained by chronic inflammatory condition in SCD characterized by significant immune and inflammatory activation. Heightened immune effector cell responses and/or impaired regulatory networks are likely to drive alloantibody production in alloimmunized SCD patients. In support of this, altered T cell immunoregulation, known to control antibody responses, have been reported in alloimmunized SCD patients. In addition, stronger follicular help T cell responses that help antibody production by B cells were described in alloimmunized as compared to non-alloimmunized SCD patients. Furthermore, several innate immune abnormalities have been identified in alloimmunized SCD patients, including a compromised anti-inflammatory response against extracellular cell free heme. The data support a model in which alloimmunized SCD patients are unable to switch off their proinflammatory state in response to the ongoing hemolytic state characteristic of SCD, placing this patient subset at a higher risk to develop a strong immune response against allogeneic determinants on transfused RBCs' thus increasing the risk of further alloimmunization. A detailed mechanistic understanding of the ways in which innate immune abnormalities can contribute to pathogenic T cell responses in alloimmunized SCD patients will lay the foundation for future identification of biomarkers of alloimmunization with the goal that this information will ultimately help guide therapy in these patients.

Management and Organisation

5B-S33-01

No abstract available.

5B-S33-02

STATUS OF BLOOD TRANSFUSION IN WORLD HEALTH ORGANIZATION-EASTERN MEDITERIAN RIGON (WHO-EMRO): SUCCESES AND CHALENGES

A Gharehbaghian, A Darbandi, P Mashati and A Yami

Shahid Beheshti University of Medical Sciences, Tehran, Iran

Background: Blood transfusion has an undeniably crucial role in patients' care and it is a necessary component of health care system. Blood products are used for patients' treatment and survival in the cases of major surgery, cancer therapy, hematological and blooding disorders and organ transplantation. On the other hand main blood components are still not replaceable by artificial products and blood transfusion activity is highly dependent on the health care development. This World Health Organization region comprises 21 member states including Afghanistan, Bahrain, Djibouti, Egypt, Iran, Iraq, Jordan, Kuwait, Lebanon, Libya, Morocco, Pakistan, Oman, Qatar, Saudi Arabia, Somalia, Sudan, Syria, Tunisia, United Arab Emirates (UAE), Yemen and Palestine (West Bank and Gaza Strip). These countries are variable in socio-economic status with an effective impact on the health care system level and subsequently on blood transfusion activities. The fundamental motivation behind this research was to accumulate some data of blood exercises in EMR nations to have a superior and greater picture on the whole region.
Method: The data were collected through published papers or data, the blood transfusion establishment websites and the others official websites such as WHO.
Results: Among EMR countries with a population of roughly 583 million people there are some with a nationally organized blood transfusion establishment such as Egypt, Iran, Iraq, Kuwait, Morocco, Oman, Pakistan and Syria. In a few nations blood transfusion administrations are hospital-based like Saudi Arabia. The others are ruled by Red Crescent involving Bahrain, Tunisia, and UAE or by Red Cross such as Lebanon. Some of them can be grouped in both central and Red Crescent such as Tunisia and some of them are grouped in both hospital-based and central such as Pakistan. Some of them achieved to have 100% voluntary non-remunerated blood donors (VNRBD) such as Iran and UAE. Furthermore some of them are still under the weight of family/replacement blood donors like Afghanistan, Egypt, Iraq, Lebanon, Morocco, Pakistan, Saudi Arabia and Sudan or sometimes even paid donors such as Pakistan and Yemen. The haemovigilance and training program have been implemented in some countries including Bahrain, Egypt, Iran, Jordan, Kuwait, Oman, Qatar, Saudi Arabia, Tunisia and UAE. Shockingly there are rare and inaccessible information about some EMR states like Djibouti, Somalia, and Palestine so that very little data can be independently discovered.
Conclusion: In these countries different measures ought to be additionally designate to ensure blood adequacy and safety including; the development of well-organized and nationally coordinated blood transfusion establishment, the increase in government or policy/makers commitment, the establishment and execution of a practical plan for blood donor recruitment, retention and education based on VNRBD, the establishment of a program to estimate the community blood needs, the establishment of well- equipped laboratories for screening donors' blood samples, producing a feasible program improving appropriate clinical use of blood components, enhancing a training program for the staff working in the blood transfusion chain, to advance territorial joint achievable projects in education, training, research, and impressive correlation of encounters, evaluate regularly the quality and the respectability inside of the blood transfusion chain, bolster the neighboring nations, develop a regional network for the achievement in EMR, and providing key educational materials (i.e. books, pamphlets, brochures, videos or website as hard copy or E-portal resources) in main popular languages in region.

5B-S33-03

IMPACTS OF STRATEGIC LEADERSHIP IN ESTABLISHING SUSTAINABLE BLOOD DONOR SERVICE PROGRAM IN ETHIOPIA, APRIL 2016

A Zewoldie

Ethiopian National Blood Bank Service, Addis Ababa, Ethiopia

Background: The Ethiopian Red Cross Society (ERCS) has been the pioneer organization in developing blood services in the country by establishing the first blood bank center in 1969 and has been organized as a department under the leadership of ERCS with one central and 10 regional blood bank until end of 2012. Increased health coverage, maternal and childhood deaths were unacceptable, increased trauma and car accident related deaths, inadequate blood supply, inequitable access to safe blood and blood products, fragmented blood service, poor government budgeting and reliance on external donor financing was the major national BTS challenges. Ethiopia having a population of more than 85 million was collected 54,693 units in 2012. NBTS strategic leadership outcome analysis will be helpful in ensuring sustainable blood donor program establishment.

Aims: To assess the impacts of strategic leadership in establishing sustainable blood donor service program in Ethiopia and share experience to the world.

Methods: A retrospective analysis and review on newly developed Ethiopian national blood service strategic document and implementation status from 2005 to 2015.

Results: The government developed a new strategy towards sustainable blood service in 2005 and took the commitment towards establishing sustainable blood program. The review on strategy reveals that increasing the community participation on voluntary blood donation and scale up of the blood collection through community mobilization, expansion of Blood Bank centers and mobile blood collection teams, capacity building and strengthening quality system were found the core one. The overall implementation status analysis indicates that Ethiopian NBTS achieved a number of results; some of the success stories achieved within short period of time are below:

The reversion of NBTS administration from Red Cross society to the government health care system made in 2013.

Increased government commitment and support by allocating 4.5 million USD in 2015 and established Nationally coordinated Blood Service as an independent government institution.

Increased the number of standard furnished blood bank centers from 11 to 25 and improved the equitable accessibility towards safe blood nationally by strategically constructing one blood bank center with the principle to supply all transfusing facilities within 200 km of radius.

Increased Mobile blood collection teams from 2 to 31 and mobile collection sites from 27 to >3,200.

Increased blood bank (technical) professionals from 45 to 450.

Year round awareness creation effort and massive mobilization of the public to make voluntary blood donation a nation's culture made.

Increased total blood collection from 25,004 units in 2004 and 54,693 units in 2012 to 142,345 units in 2015.

Increased total voluntary nun remunerated blood donation rate from 28% in 2012 to 95.4% in 2015.

Increased component production from 10% to 40% of the total blood collection.

Conclusions: Strategic leadership with commitment and ownership of the government through developing strategy will have positive impact in establishing sustainable national blood service program and 100% voluntary based blood donation is achievable in developing countries like Ethiopia. Even though the success stories mentioned above are achieved within a short period of time, the BTS remains with challenges like inadequacy of blood supply, inappropriate clinical use of blood, data management system, networking, automation and quality system needs further development.

5B-S33-04

TECHNICAL ASSISTANCE FOR STRENGTHENING THE BLOOD SUPPLY SYSTEM IN TURKEY

N Ertugrul Oruc[1], I Yenicesu[2], PH Conte[3], G Ongun[4], S Eldemir[5], T Cetin[6], MB Saygan[5], IY Avci[6], AE Karakoc[7], E Gul Ibrisim[8], FY Ayhan[9], FR Nascimento[3], M Smid[10], M Farouk[3], N Beckman[3], A Aksoy[5], N Hafizoghu[5], G Ozet[5], T Ilbars[11], U Kodaloglu[11], M Yazici[11], A Kapuagasi[11] and I Sencan[11]

[1]Diskapi Yildirim Bayazit Training and Research Hospital, Ankara, Turkey [2]Gazi University Medical School, Department of Pediatric Hematology, Ankara, Turkey [3]GFA Consulting Group, Hamburg, Germany [4]Hemosoft IT and Training Services, Ankara, Turkey [5]Turkish Red Crescent General Directorate of Blood Services, Ankara, Turkey [6]Gulhane Military Medical Academy, Ankara, Turkey [7]Ankara Training and Research Hospital, Ankara, Turkey [8]Zekai Tahir Burak Women's Health Training and Research Hospital, Ankara, Turkey [9]Behcet Uz Pediatric Diseases and Surgery Training and Research Hospital, Izmir, Turkey [10]Sanquin Consulting Services, Amsterdam, The Netherlands [11]MoH General Directorate of Health Services, Ankara, Turkey

Background: Ensuring the safety and availability of blood and blood products is an essential public health responsibility. Essential functions of a sustainable national blood system include policy formulation, standard setting, strategic and operational planning, provision of sufficient resources, national coordination and management to ensure an adequate supply of blood and blood products as well as safe clinical transfusion. Sustainable national blood system is recognized through a national blood policy, strategic plan and appropriate legal instruments.

Aims: 'Strengthening Blood Supply System' Project (EU-TR080215) conducted in 2012 to contribute to the implementation of the EU acquis communautaire in the area of public health, specifically focusing on strengthening and effective functioning of blood supply system in Turkey and to build a capacity to have an efficient national blood system, regional blood centre based for provision of safest blood from voluntary non-remunerated donors regularly.

Methods: Initially, to observe the best practices of EU Countries, study visits were organised. National survey hanging over 100 questions was conducted to review existing national blood system and collected data were analysed. Based on reults of terms of reference, working groups were created from national and international profesionals to address specific and technical aspects and to bring, to the National Authorities, all the technical elements to adapt those results to the realities of the Country. Hundred professionals were interactive (C3 training) trained as training of trainers (TOT) and 30 of them were additionally trained as inspector trainer with the support of EUBIS Academy.

Results: (i) Analysed survey outcome of existing national blood system was reported. (ii) National Policy and Strategy documents were prepared. (iii) National guides on 'Preparation, Use and Quality Assurance of Blood and Blood Components', 'Standards for Blood Services', 'Total Quality Management in Blood Services', 'Haemovigilance', 'Appropriate Clinical Use of Blood' and 'Inspection for Blood Services' were prepared. All guidelines were sent to scientific societies for public enquiry before finalized. Seventy personnels at administrative level from Turkish Red Crescent (TRC) and Competent Authority of MoH were trained interactive on management and technical level to strengthten the administrative capacity. Interactive technical trainings by TOT's were given to 90 personnel of MoH on Inspection, 250 technical personnel of MoH and TRC on Quality Control, 755 clinicians on Appropriate Clinical Use of Blood. (iv) Total Quality Management and Haemovigilance training were given to 1,200 personnel of MoH and TRC respectively. All the activities of the project were carried by around 15 international and 200 national experts within numerous workshops and meetings during the implementation of the project. (v) Data sets were prepared and 'National Blood Services Information Management System' was established in MoH to provide regular flow of information between the blood services and national authority. Also the users of the system from MoH and blood services were trained on data process, traceability, reporting and notification of serious adverse reactions and events.

Conclusion: Output of the EU Project, supported by European Union, made a significant contribution to the acceleration of the implementation of National Blood System of Turkey and is supposed to be a pioneer to the other developing countries. For the sustainability of national blood system, training activities in each field of the system should be continued and further projects should be implemented such as on 'recruitment of future blood donors', 'haemovigilans' and 'patient blood management' to support it.

5B-S33-05

ADJUSTING THE BLOOD SUPPLY FOR THE FINNISH PATIENT POPULATION: BLOOD GROUP ANTIBODY FINDINGS AND DONOR TYPING

T Jäske, AE Korhonen, I Sareneva and S Natunen

Finnish Red Cross Blood Service, Helsinki, Finland

Background: The Finnish Red Cross Blood Service (FRCBS) is the nationwide blood service provider in Finland, responsible for collection, processing and distribution of blood products to all hospitals and health care providers. The FRCBS also serves as the National Blood Group Reference Laboratory covering the whole country. University hospitals perform antibody identification for their screening positive patients, whereas smaller hospitals leave antibody identification to be done at the FRCBS. Thus the FRCBS is able to monitor RBC antibody frequencies in Finland, which is important to maintain an adequate register of phenotyped donors to meet the needs of the patient population. All donors are phenotyped for ABO, RhD, C, c, E, e and K. Annually about 3,000 donors are phenotyped for Jk^a, Jk^b, Fy^a, Fy^b, M, S and s. Altogether 3,000 donors are genotyped for 37 antigens including DO, CO and LU by using the ID Core XT. The genetic isolation of the Finnish population has been taken in account in the process: the hospitals use an additional C^x, Ul^a and LW^b positive screening cells, and the FRCBS has the reagents to identify antibodies against those antigens. Correspondingly, about 100 donors per year are phenotyped for the Finnish rarities C^x, Ul^a, LW^b, Ls^a, An^a and WES^a.
Aims: The aim of the study was to examine RBC antibody findings in the Finnish patient population in the context of the available blood supply, to evaluate the effectiveness of the donor phenotyping and genotyping strategy at the FRCBS.
Methods: Findings of clinically significant blood group antibodies from patient samples referred to the FRCBS from 2013 to 2015 were retrospectively extracted from our LIMS. The data does not include antibodies identified at university hospitals. However, for the rarer antibodies we expect our data to cover the whole Finnish population, since the FRCBS serves as the national reference laboratory with an access to rare reagents. Phenotype and genotype data of blood donors who donated between 2013 and 2015 was retrospectively collected. A case study of a patient with a challenging antibody combination is presented as a representative example.
Results: Antibody findings of 11,806 patient samples during 2013–2015 are presented. As an example, anti-Jk^a antibody was found in 533 patient samples. During the same interval 5,389 Jk^a negative donors donated blood. An antibody against the typically Finnish rare antigen Ul^a was found in 94 patients, while 5,977 donors typed Ul^a negative. For a patient with a challenging antibody combination: anti-f, anti-C^x, anti-Jk^b, anti-Fy^b and anti-S, there were no fresh units in stock at a given moment, but six frozen units and 22 eligible donors were available. Some rare blood groups have been difficult to find in the Finnish population: for example we have yet to identify an hr^s negative donor, despite over 2,000 donors genotyped for the antigen since 2014.
Summary/Conclusion: The donor phenotyping and genotyping strategy at the FRCBS responds adequately to the needs of the Finnish patient population. Methods for finding certain rare donors still need to be developed.

5B-S33-06

IMPLEMENTATION OF MAXIMUM SURGICAL BLOOD ORDERING SCHEDULE (MSBOS) AT OFF-SITE MEDICAL CENTRES OF A TERTIARY CARE HOSPITAL KARACHI

A Memon, F Karim and B Moiz

Aga Khan University Hospital Karachi Sindh Pakistan, Karachi, Pakistan

Background: Blood ordering is a norm prior to elective surgical procedures. This preoperative request for blood components is usually based on unfavorable clinical speculations, which may exhaust precious resources of the blood banks as it takes time and manpower to cross-match every patient undergoing elective surgery. Several studies conclude that most of the cross matched blood units are not transfused.
Our hospital is a tertiary care center. Four secondary hospitals, namely, Garden, Karimabad, Kharadar and Hyderabad, are linked to our hospitals. The hospital blood bank caters to transfusion needs of all in-patients at the main campus as well as the secondary hospitals. While the transfusion practices at the main campus are controlled by the hospital transfusion committee, we observed that CT ratio was very high for the secondary hospitals. Thus, the hospital transfusion committee identified the need for implementation of MSBOS at secondary hospitals.
Aims: The aim was to establish MSBOS for secondary hospitals to reduce CT ratio.
Methods: CT ratio for elective surgeries performed at the four secondary hospitals was calculated from January 2013 to December 2014. Data was retrieved by using ISD code version 9. Transfusion probability and Transfusion index for each elective surgery was calculated in order to compute MSBOS. Data was entered in Microsoft excel version 2010 and compared in tabulated form.
Results: Total of 3,570 elective surgeries were done at these four centers from 2013 to 2014. Most common surgery was elective C-section (n = 2,843) followed by Dilatation & Evacuation (n = 287), hysterectomy (n = 239), laparotomy (n = 128) and C-section for placenta previa (n = 73). The C/T ratio for C-section was 20 (Garden), 13.4 (Hyderabad), 5.2 (Karimabad) & 3.2 (Kharadar) at the four secondary hospitals. Calculated transfusion index in the above mentioned four centers for C-section was 0.05, 0.07, 0.2 and 0.4, respectively. The transfusion probability for C-section in the four centers was 17.5%, 4.8%, 17.4% and 24.7% respectively. None of the surgeries had a transfusion index of >0.5 and transfusion probability of >30% except for C-section for placenta previa. As per calculated MSBOS, two units of blood needed to be crossmatched prior to C-section for placenta previa. Rest of the surgeries were allotted a 'Group and screen' provision.
MSBOS was implemented in April 2015. Following implementation, CT ratio was again calculated in February 2016. The mean CT ratio for C-section, hysterectomy and laparotomy was found to be 2.3 (Hyderabad), 2.0 (Kharadar), 2.6 (Garden) & 3.5 (karimabad).
Conclusion: Implementation of MSBOS showed a momentous impact in transfusion practices of surgeons with considerable reduction in the C/T ratio. We recommend regular audits in order to improve the quality of services and to ensure high standards.

Red Cell Antibodies

5B-S35-01

COMPLEX RED CELL ANTIBODY CASE ANALYSIS

NM Thornton

IBGRL, Bristol, United Kingdom

Red cell antibody identification is an essential part of pretransfusion and antenatal testing in the immunohaematology laboratory. The requirement to determine the specificity of any antibodies present in a patient's plasma is to enable informed decisions to be made regarding the most suitable blood for transfusion and in pregnant women, to assess the risk of HDFN. Invariably the most complex cases usually involve a situation where finding compatible blood is difficult. The presence of multiple antibodies to 'routine' antigens (D, C, E, c, e, K, M, N, S, s, Fy^a, Fy^b, Jk^a, Jk^b) and/or the presence of an antibody to a high incidence antigen, represent examples of this type of scenario. Antibody identification is a complex analytical process made up of many different elements, which all add an important piece to the puzzle. Serological techniques remain the essential tools required for resolving antibody problems. However there are non-serological elements that are equally as important, especially in the initial stages of an investigation. Information regarding the patient's clinical condition, transfusion and/or pregnancy history, age, sex, medication, ethnicity and previous laboratory results, all has the potential to provide clues to the possible identity of an unknown antibody. Gathering this information can, in some instances, avoid unnecessary testing and can also help to interpret serological results. When analysing complex cases, routine serological techniques may not be enough to reach a resolution. It is then necessary to turn to supplementary manual serological techniques such as adsorption and elution, inhibition tests using soluble recombinant blood group proteins and naturally found inhibitory substances and the use of enzyme treated and chemically modified cells. In addition, molecular based tests now play an important role in helping to resolve the most complex of cases. Knowledge of blood groups and antibody characteristics is key to being able to recognise clues and ensure that decisions in the antibody identification process are made in a logical and informed way, because although there are many tools available for investigating complex red cell antibody cases, knowing when to employ those tools is a key factor to ensuring complex cases are solved with minimal delay.

5B-S35-02

THE S ANTIGEN ENCODED BY GYPB*MIT IS A PARTIAL ANTIGEN: FIRST REPORT OF ALLOANTI-S IN A PERSON WITH S+MIT+ RBCS

C Lomas-Francis[1], M Louzon[2], S Vege[1], S Harris[3], J Hess[2], ZG Hu[1], MB Pagano[2], G Teramura[3], RW Velliquette[1], M Delaney[3] and CM Westhoff[1]

[1]New York Blood Center, Long Island City, New York, United States of America [2]University of Washington Medical Center, Seattle, Washington, United States of America [3]Bloodworks NW, Seattle, Washington, United States of America

Background: An 81 year old White male presented with a history of anti-c, -E and an anti-S. At the time of the initial antibody identification, the anti-S was suspected to be autoantibody because the patient's red blood cell (RBC) phenotype was S+s+. On a subsequent admission, an unequivocal alloanti-S and RBCs confirmed to be S+s+ prompted further serologic and molecular investigation.
Methods: Genomic DNA was extracted from peripheral blood leukocytes and analyzed by PreciseType HEA (Immucor), RBC-FluoGene vERYfy, (inno-train Diagnostik), and *GYPB* sequencing of exons 1 to 6. Standard hemagglutination methods were used for antigen typing and antibody testing. Multiple anti-S, licensed and unlicensed, and in-house reagents were used to investigate the possibility that the patient's RBCs expressed a variant S antigen. Eluates were made using Gamma Elu-Kit II.
Results: The patient's RBCs were confirmed s+ and S+ (polyclonal anti-S: Immucor, Ortho, unlicensed in-house; monoclonal: Bio-Rad), reacting to the same strength as S+s+ controls. The anti-S in the patient's plasma reacted with S+s- and S+s+ RBCs by solid phase and in tubes by PEG IAT. The plasma did not react with S- RBCs or with the autologous control. DNA testing by HEA PreciseType predicted a S- s+ phenotype; RBC-FluoGene vERYfy predicted S+s+. Gene sequencing of *GYPB* exon 4 showed c.143T/C (p.48Met/Thr), consistent with *GYPB*S/*s (GYPB*03/*04)* but also identified an additional change c.161G>A (p.54Arg/His) associated with a Mit+ phenotype. No other changes were found. The RBCs typed as Mit+ (2 examples of anti-Mit), He-, Mi (a−), TSEN- and M+N+. The plasma did not react with two examples of S+ Mit+ RBCs. An acid eluate prepared from Mit+ RBCs sensitized with the patient's anti-S was non-reactive by PEG IAT suggesting that the Mit+ RBCs express S antigen compatible with the patient's anti-S, although the plasma sample was insufficient to perform control eluates.
Summary/Conclusion: Several glycophorin alleles encode for altered S (or s) antigen expression and associated expression of low prevalence antigens; e.g., S antigen expression is altered not only in the presence of Mit antigen but also if M^V or TSEN antigens are expressed. It is reported that some anti-S reagents do not react with S+Mit+ RBCs; this is consistent with the theory that, in addition to Met48 amino acid associated with S antigen expression, some anti-S also require Thr44 and/or the GalNAc attached to this residue, Glu47, His53, and Arg54 for full expression. The patient's sample has nucleotide change c.161G>A encoding p.Arg54His and expression of the Mit antigen (prevalence of 0.1% in Western Europeans). This change is primarily found on alleles encoding S, and only rarely on alleles encoding s antigen. The c.161G>A change was associated with failure to detect the S antigen specific c.143T by HEA PreciseType, and along with the plasma antibody reactivity supports Mit antigen expression from the S allele in here. This case is the first evidence that the *GYPB*Mit* (*GYPB*24)* allele encodes a 'partial S' antigen with risk for production of allo-anti-S.

5B-S35-03

QUANTIFICATION OF AUTOANTIBODIES IN ELUATE OF IMMUNOGLOBULIN G SENSITIZED RED CELLS IN AUTOIMMUNE HEMOLYTIC ANEMIA

SS Das

Apollo Gleneagles Hospitals, Kolkata, India

Background: A reactive direct antiglobulin test (DAT) with clinical and laboratory evidences of *in vivo* hemolysis establish the diagnosis of autoimmune hemolytic anemia (AIHA). Concentrated autoantibodies obtained by elution can be subjected to total serological characterization including thermal amplitude, specificity and autoantibody quantitation which help in proper diagnosis and management of the patients.
Aims: The present study was performed to correlate the quantity of immunoglobulin (Ig) G (IgG) autoantibodies in eluate with DAT reactivity and markers of *in vivo* hemolysis in warm AIHA (WAIHA).
Methods: Antibody class, IgG subclass, DAT dilution and quantification study on eluate was performed on 41 samples. Hemolysis in a patient was documented as per the previous guidelines. Cold acid elution was performed on DAT positive red cells and quantitation of IgG in eluates was done using ELISA. From the concentration of IgG in eluates, number of Ig molecules eluted per RBC was calculated using Avogadro's number.
A calibration curve was generated using corrected OD values of known different concentrations (dilutions) of standard. IgG antibody in eluate was quantified from the calibration curve obtained with the known concentration values of standard. The sensitivity of the assay was 7.8 ng/ml.
Statistical analysis: Pearson correlation between the amounts of IgG in eluates with markers of hemolysis was calculated by multiple linear regression analysis and calculating '*r*' value for all the markers. A $P < 0.05$ was considered statistically significant.
Results: We observed a significant correlation between concentration of IgG in eluate and DAT strength ($r = 0.49$, $P = 0.011$), hemoglobin (Hb; $r = -0.44$, $P = 0.03$), serum bilirubin ($r = 0.57$, $P = 0.000$) and serum lactate dehydrogenase (LDH) ($r = 0.52$, $P = 0.03$). The number of IgG molecules/RBC also correlated significantly with the DAT strength ($r = 0.63$, $P = 0.001$) as well as DAT dilutions ($r = 0.57$, $P = 0.006$). With DAT strength ranging from 1+ to 4+, the number of IgG molecules eluted per RBC varied from 343 to 1,291 IgG/RBC.
Conclusions: Quantification of autoantibodies in eluate and measuring the number of IgG molecules per red cell are sensitive methods for clinical and serological characterization of AIHA. Such methods are more useful in the evaluation of DAT-negative AIHA or DAT positive patients without AIHA where suboptimal autoantibodies on red cells affect the sensitivity of the conventional DAT methods. Immunohematological laboratories with no facilities of high sensitive methods such as flow cytometry may quantify autoantibodies in eluate that may help in labelling the severity of AIHA and plan appropriate management.

5B-S35-04

INDIVIDUALS HARBORING THE P.GLN47ARG SUBSTITUTION IN THE APQ1 GENE SHOW A WEAK COA EXPRESSION AND SHOULD NOT BE CONSIDERED AS CARRYING A RARE CO (A−) PHENOTYPE

V Thonier[1], A Willemetz[1], I Morenne[1], D Gien[1], G Alluin[2], O Fontaine[2], J Babinet[1] and T Peyrard[1]

[1]Institut National de la Transfusion Sanguine (INTS), Paris cedex 11, France [2]EFS Nord de France, Lille, France

Background: The Colton blood group system currently comprises three high-prevalence antigens, Co^a, Co3 and Co4, and one polymorphic antigen Co^b, carried by the water channel aquaporin-1 coded by the *AQP1* gene. Co:-3 (Co_{null}) individuals do not express any Colton antigens. The rare Co:-4 individuals were described to harbor the p.Gln47Arg substitution, coded by the *CO*01-04* allele at the homozygous state. This mutation was previously claimed to abolish the expression of Co^a and all Co:-4 people were shown to appear Co (a−b−) 'Arnaud et al., *Transfusion* 2010'.
Aims: We report here the case of two donors previously typed as Co (a−b+), found to be incompatible with a potent anti-Co^a developed by a Co (a−b+) patient.
Methods: Standard haemagglutination techniques and molecular investigations (BeadChips HEA v1.2 – BioArray Solutions/Immucor, *APQ1* sequencing on genomic DNA with an 'in house' technique) were performed.
Results: The serum of a pregnant woman was referred to our IRL in order to confirm an anti-Co^a. Her medical history reported an anti-Jk^a. Anti-Co^a was confirmed with a titer 32 in IAT-IgG-gel test. Autologous controls and DAT were negative. Anti-Jk^a was undetectable and allogeneic adsorptions ruled out other common alloantibodies. The patient was Co (a−b+); this was confirmed by *AQP1* sequencing. Surprisingly the RBCs of two rare Co (a−b+) sibling donors were unambiguously found to be positive ($1+^S$) by crossmatch. The presence of an antibody to a low-prevalence antigen was ruled out on two alloadsorbates. Both donors were genotyped as *CO*1/CO*2*, suggesting a variant *CO*1* allele. *AQP1* sequencing was consistent with a wild-type *CO*2* allele and a rare *CO*1* allele with a c.140A>G heterozygous change, known as *CO*01-04*. Adsorption/elution test of the proband's serum on one of the two 'incompatible' donors' RBCs was performed. The eluate (checked not to contain anti-Jk^a) was positive with one Co:-4 sample (2+), positive ($1+^S$) with the other sibling's RBCs, positive (3+) with one Co (a+b−) sample, and negative with 3 Co (a−b+) samples. Thirty donors from our cryobank known to carry a rare Co (a−b+) phenotype were tested nonreactive with the proband's serum.
Conclusions: We report here the first evidence that the *CO*01-04* allele, encoding the p.Gln47Arg substitution, does not fully abolish the expression of the Co^a antigen

as was previously assumed, but is responsible for its weakened expression. Only a few individuals were described with this mutation, all of them being homozygous and mistyped as Co (a−b−). The strength of the anti-Co^a reagent used very likely explains the Co^a typing discrepancies. Of note, a weak expression of Co^a could be suspected by flow cytometry in K-562 p.Gln47Arg transfectants (Arnaud & al, *Transfusion* 2010). It was also suggested that the full Co^a antigen expression could be dependent on the two close amino acids Ala45 and Gln47. If Gln47 is missing, the expression of Co^a could be dramatically weakened. Despite being seemingly infrequent, we advise blood bankers involved in a rare blood program to screen all their Co (a−) donors for the p.Gln47Arg substitution, either by molecular techniques (*CO*1/CO*2* study with genotyping platforms or RFLP-PCR p.Gln47Arg analysis), or by phenotyping with a sufficiently potent anti-Co^a reagent.

5B-S35-05

A UK NEQAS PILOT EXERCISE DEMONSTRATES THE IMPORTANCE OF INCLUDING AN ANTIBODY SCREEN WHEN UNDERTAKING ABO TITRATION TO SUPPORT ABO INCOMPATIBLE RENAL TRANSPLANTATION

CE Milkins, J White, A Mavurayi and M Rowley

UK NEQAS (BTLP), Watford, United Kingdom

Background: Eligibility for the ABO incompatible renal transplantation (ABOiRTx) programme is partly determined by the initial titre of ABO antibodies in the recipient. Although there is no universally accepted cut-off, potential transplant recipients with high ABO antibodies may be rejected. Patients receive immunosuppression, plus sessions of plasma exchange or immunoadsorption to reduce their ABO antibody levels to a point deemed safe for transplant; a titre of eight is used in many UK centres.

The UK NEQAS (BTLP) ABO titration pilot scheme has been running since 2010. A plasma sample distributed in December 2014 contained anti-D, which did not affect the results because cde/cde red cells were provided for titration. However, it raised awareness that in a clinical situation, a spuriously high result could be reported if an antigen positive cell was selected for ABO titration in a patient with an unexpected red cell alloantibody.

Aim: To demonstrate that ABO titres can be affected by the presence of an unexpected alloantibody, and assess the potential impact on clinical decision making in patients receiving ABOiRTx.

Method: A total of 97 laboratories participated in an exercise distributed in December 2015, 45 of which undertake titration to support ABOiRTx. Two 'Patient' samples were prepared from a single pool of 800 ml of group B FFP. 50 ml of group AB IgG anti-K plasma was added to one half ('Patient 1') but not the other ('Patient' 2). Participants were provided with group A K+ red cells to titrate each 'Patient' sample against, by direct agglutination at room temperature (DRT) and/or by indirect antiglobulin test (IAT), reflecting their clinical practice. An accompanying questionnaire asked whether antibody screening (AS) is routinely undertaken on patient samples submitted for ABO titration in clinical testing.

Results: Results were submitted by 90/97 (92.8%) participants. There were minimal levels of IgG anti-A present in this plasma pool, and the higher median IAT titres in 'Patient' 1 were due to IgG anti-K (Table 1). Only 54 (60.7%) of 89 laboratories responding to the question, stated that they routinely undertake AS on ABO titration samples; of the 35 that do not, 13 titrate ABO antibodies to support ABOiRTx. All 13 reported an IAT titre of ≤8 for 'Patient' 2, and all but two reported an IAT titre of ≥64 for 'Patient 1' (highest 256).

Conclusions: In a clinical situation, a falsely high anti-A IAT titre could have been reported for Patient 1 in almost 40% of laboratories because those not undertaking an AS would have been unaware of the need to select a K- cell. In 11 centres this would have resulted in renal transplant patients receiving additional unnecessary antibody reduction procedures or potentially being ineligible for the ABOiRTx programme. Any contaminating antibody, whether IgG or IgM, could affect the ABO titration result and an antibody screen (and antibody identification where the screen is positive) should be routinely undertaken before an appropriate cell is selected for titration of ABO antibodies.

Technique	Range of median titre (different by technology)	
	Patient 1 (anti-K)	Patient 2 (no anti-K)
DRT	0 to 2	1 to 2
IAT	64 to 128	0.5 to 3

Table 1. Median reported titres

Transfusion Technologies

5B-S36-01

IN-VITRO QUALITY ASSESMENT OF THAWED WHOLE BLOOD FP24 STORED FOR UP TO FIVE DAYS AT +4°C.

S Begue[1], M Colombat[2], A Assal[2], R Djoudi[2] and P Tiberghien[1]

[1]*EFS Rhone Alpes Auvergne, La Plaine Saint Denis, France* [2]*EFS Aquitaine Limousin, Bordeaux, France*

Background: In France, Frozen Plasma (FFP and FP24) is collected, prepared and delivered by the Etablissement Français du Sang (EFS). For this product, 6 h after thawing is the maximum time limit for transfusion defined by the national regulation. This regulatory constraint forbids the distribution centers to stock thawed plasma at +4°C that would be readily available for massive transfusion protocols and life-threatening bleeding. In order to change this regulation, EFS has initiated a study to assess the quality of thawed plasma stored for up to 5 days at +4°C ± 2°C.

Aims: To assess hemostatic factors and major quality markers in quarantine FP24 during 5 days after thawing and storage at +4°C ± 2°C in relation with regulatory conformity requirements for thawed FFP in order to propose a time limit change for transfusion.

Methods: 30 filtered whole blood plasma frozen within 24 h post-collection were thawed at +37°C and stored for 5 days at +4°C ± 2°C. Mean frozen storage time before thawing was 142 days. Of the 30 units, 10 were of group O and 20 of non-O. Sampling occurred at 6, 24, 48, 72 and 120 h after thawing. Immediately after sampling, samples were split and stored at −80°C prior to analysis. *In-vitro* biological parameters are listed in Table 1

Results: Determined values are summarized in the Table 1.

Fibrinogen, Factor IX, XI, protein C, AT III, plasminogen, C1-inh, α2-antiplasmin, C5a and Von Willebrand (Ag and activity) do not evolve by more than 5% than their initial activity as determined at thawing.

Factor II, V, X, protein S, TAT and ADAMTS13 do not evolve by more than 10% of their initial content. The most impaired factors during storage at 120 h are FVII (−33%), FVIII (−32%), C3a (+23%) and protein S (−21%). However, despite this decrease, mean FVIII activity at 24 h (0.72 UI/ml) remain l in accordance with regulatory requirement (>0.7 UI/ml) while reaching 0.62 UI/ml at 120 h.

For these last markers, activities at the end of the 5 days storage are still within the normal ranges. Thrombin generation test do not show signs of any impaired hemostasis function.

Table 1

		Activity at x hours after thawing (% of initial activity or content)					
	Activity at Thawing	6h	24h	48h	72h	120h	evolution ± 5% at 120hrs
TQ (%)	**101,1**	99%	94%	89%	86%	82%	Yes
TCA (ratio)	**1,12**	101%	104%	106%	106%	107%	Yes
Fibrinogen (g/L)	**2,48**	98%	99%	98%	98%	99%	No
Factor II (UI/ml)	**1,07**	95%	98%	95%	97%	92%	Yes
Factor V (UI/ml)	**1,01**	100%	95%	92%	92%	91%	Yes
Factor VII (UI/ml)	**1,15**	95%	89%	77%	74%	67%	Yes
Factor VIIIc(UI/ml)	**0,91**	93%	79%	71%	68%	68%	Yes
Factor IX (UI/ml)	**1,00**	nd	94%	96%	nd	97%	No
Factor X (UI/ml)	**1,04**	nd	96%	96%	nd	93%	Yes
Factor XI (UI/ml)	**0,97**	97%	94%	98%	98%	96%	No
Protein S (% activity)	**88,70**	95%	94%	90%	88%	79%	Yes
Protein C (% activity)	**98,00**	nd	100%	100%	nd	99%	No
AT III (% activity)	**98,93**	nd	102%	102%	nd	100%	No
Plasminogen (% activity)	**91,07**	nd	101%	101%	nd	100%	No
C1inh (g/L)	**0,22**	nd	100%	100%	nd	100%	No
TAT Complex (µg/L)	**3,26**	nd	98%	nd	nd	94%	Yes
α2 antiplasmin (%)	**95**	nd	99%	99%	nd	98%	No
C3a (µg/l)	**202,72**	nd	107%	nd	nd	123%	Yes
C5a (µg/l)	**9,50**	nd	99%	nd	nd	103%	No
VW Factor (Ag) (%)	**90,13**	nd	101%	99%	nd	101%	No
VW Factor (UI/ml)	**85,93**	nd	100%	103%	nd	100%	No
ADAMTS 13 (% activity)	**81,43**	nd	103%	103%	nd	109%	Yes

Summary/Conclusions: Balance between the observed factors is preserved throughout the 5 days of storage after thawing. At 24 h and at 5 days, *in-vitro* parameters are all within regulatory requirements with the exception of factor VIII that nevertheless remains at day 5 within the normal range. We believe that these results support prolonging storage of plasma after thawing up to 5 days and thus allowing transfusion centers in France to significantly improve plasma availability for immediate use in massively bleeding patients.

5B-S36-02

DO DONOR AND/OR PROCESSING-RELATED DIFFERENCES IMPACT ON BLOOD PRODUCT QUALITY?

MM Dean[1], KM Rooks[1], FN Chong[1], HM Faddy[2], JP Tung[1] and RL Flower[1]

[1]Australian Red Cross Blood Service, Kelvin Grove, Australia [2]University of QLD, Brisbane, Australia

Background: During routine storage packed red blood cells (PRBC) undergo numerous biochemical and biophysical changes collectively referred to as the 'RBC storage lesion'. Variability in red blood cell (RBC) characteristics and onset of the storage lesion has been reported. We investigated changes in levels of potential biological response modifies (BRMs) in PRBC relevant to storage, donor demographics and parameters associated with processing of PRBC units.

Aims: To characterise potential biological response modifiers in PRBC units and determine whether donor-and/or processing- related parameters could contributed to variation in blood product quality.

Methods: PRBC units (n = 200) prepared in accordance with standard operating procedures were stored at 2–6°C with sterile sampling of 10 ml at Day (D) 2, D14, D28 and D42). Supernatant (SN) was prepared at each time point. Concentrations of ICAM-1, VCAM-1, E-selectin, P-selectin, RANTES, sCD40L, Angiogenin, Eotaxin, IP-10, IL-1α, IL-8 and IL-9 were assessed in the PRBC-SNs using cytometric bead array (CBA). For each unit the following information was assessed: blood group, donor gender, donor age, donor BMI, blood collection volume (pre-separation), PRBC volume (final) and time of collection until processing. Multiple-regression analyses was used to determine whether variation in concentration of BRMs in PRBC was associated with donor- and/or processing- related parameters (95% CI).

Results: PRBC storage had no impact on levels of ICAM-1, VCAM-1, E-selectin, P-selectin, RANTES, sCD40L, IP-10, IL-1α or IL-8. Angiogenin, Eotaxin and IL-9 levels significantly declined during routine storage. In terms of donor-related parameters we found that age, BMI, nor blood group contributed to variation in levels of BRMs. We found longer processing time was associated with significantly increased levels of E-Selectin, IL-1α and IL-9. Collection volume and final PRBC unit volume were associated with increased levels of ICAM-1, VCAM-1 and P-selectin, while final PRBC volume was associated with increased levels of RANTES and Angiogenin. We identified a subset of donors who had 10–100 fold higher levels of multiple BRMS. Males were significantly overrepresented in this high BRM sub group, and these units were predominantly from the top quartile in terms of final PRBC volume.

Summary/Conclusions: There was minimal change in the BRMs studied relevant to storage duration of the PRBC units. However, significant variation in levels of BRMS was identified with a clear sub population of 'high BRM' units identified. The most notable parameters contributing to levels of biological mediators were processing time, PRBC volume and donor gender. We hypothesise that high levels of BRMs may contribute to immune modulation in transfusion recipients. Further investigation of how donor gender and blood processing characteristics can contribute to variation in blood product quality is warranted.

5B-S36-03

A PAIRED COMPARISON OF THAWED AND LIQUID PLASMA

S Huish[1], L Backholer[2], L Green[3], S Platton[3], M Wiltshire[2], H Doughty[2], E Curnow[2] and R Cardigan[2]

[1]NHSBT, Cambridge, United Kingdom [2]NHS Blood and Transplant, Cambridge, United Kingdom [3]Barts Health NHS Trust, London, United Kingdom

Background and objectives: Early resuscitation of trauma patients with blood components is becoming increasingly widespread in both civilian and military practice. In order to make plasma readily available to treat major haemorrhage, internationally some centres are using either pre-thawed plasma (TP) or 'never frozen' liquid plasma (LP). Despite the routine use of both, there is limited data comparing the two. The haemostatic properties of LP were evaluated and compared to TP (stored at 2–6°C for up to 7 days) in a paired study, with LP further assessed until day 28 of storage.

Materials and methods: Two ABO matched plasma units were pooled and split to produce one unit for LP and one unit for TP, this was repeated 17 times in total (4 × group A, 5 × group O, 5 × group B and 3 × group AB). TP was analysed up to 7 days of storage at 2–6°C and LP up to 28 days of storage at 2–6°C. Samples of TP and LP were tested for APTT, PT, FII, FV, FVII, FVIIa, FVIII, FXI, FXII, protein C, protein S, fibrinogen, C1 inhibitor (C1INH), C1INH-FXIIa complex, thrombin generation (using 1 & 5 pmol of tissue factor) and rotational thromboelastometry (ROTEM). An additional 119 units of LP were collected and analysed for markers of contact activation (S-2302 cleavage) and cellular content.

Results: LP and TP were compared, up to 7 days of storage, with results showing APTT, FVII, FXI, fibrinogen and protein C to be higher in TP compared to LP (but by <5%). In addition, Lag time (1 & 5 pmol), ttPeak (5 pmol) and clot time were also higher in TP compared to LP. There was no difference in the rate of change over time (the interaction between type of plasma and time) for any parameter measured up to 7 days storage.

LP was compared on day 7, 11 and 14 of storage to that at day 5. At day 7 we found no difference for any factors measured, however at day 11 FII, FV, FVII, and protein S (activity) were lower compared to day 5. A decrease in peak thrombin and ETP (1 & 5 pmol) and an increase in lag time and time to peak (5 pmol) were observed at day 11.

Analysis of 119 LP units showed 26/119 (22%) exhibited cold induced contact activation by day 28, with one as early as day 8, but most after day 14. All units met current FFP specification in terms of residual cellular content.

Conclusions: Although we observed differences in the coagulation factor content of TP and LP up to 7 days storage, the magnitude of this difference was small and there did not appear to be a benefit of not freeze-thawing plasma in terms of coagulation factor content. In LP there was a further reduction in some clotting factors between day 7 and 14 and cold induced contact activation from day 14 in most units. These factors need to be considered and balanced against availabilty to supply and clinical need in determining a shelf life for these components.

5B-S36-04

VALIDATION OF A NOVEL DOUBLE DOSE BUFFY COAT PLATELET POOLING SET IN COMBINATION WITH PHOTOCHEMICAL TREATMENT

P Sandgren

Karolinska University Hospital, Stockholm, Sweden

Background: The INTERCEPT™ Blood System (Cerus Europe BV) uses a photochemical treatment (PCT) with amotosalen and UVA light to prevent pathogens and leukocytes from replicating and functioning. A novel process allows for the production of two pathogen-inactivated therapeutic platelet units from one double dose (DD) platelet concentrate (PC). Buffy coat (BC) platelets are generally obtained from pools of up to six BC. A new I-Platelet Pooling Set (Kansuk) allows producing DD PLT from pools of up to eight BC.

Aims: The objective of this study was to evaluate the preparation of DD platelets from eight BC pools followed by the INTERCEPT treatment with Dual Storage Set to obtain two inactivated PC. Functional, phenotypic and mitochondrial properties of platelets stored for up to 7 days were analyzed.

Methods: Platelets were collected from 16 BC′s individually stored overnight (mean volume 42.5 ± 2.2 ml, hematocrit 32.8 ± 8.3% (QC data, n = 10). BC′s were then pooled together with 2 × 280 ml of SSP+ (Macopharma) and split in a double bag set (DonoPack, LMB). One sub-pool was sterile docked (Terumo BCT) to an I-Platelet Pooling set. This set includes an Octopus with nine tubing leads (not used in this study), a 700 ml pooling bag, a PLX-5 leukodepletion filter (Asahi Kasei) and a temporary platelet storage container. The other sub-pool was connected to a conventional pooling set (Fenwal) also including a PLX-5 filter and served as control. Subpools were submitted to soft spin in Macospin centrifuge (Macopharma) to obtain two DD leukodepleted BC PC.

BC PC (Test and Control) were photochemically treated (150 μM amotosalen, 3 J/cm^2 UVA) at day 1, transferred to a Compound Adsorption Device, stored 15 h under agitation, split into two equal single dose units and stored for 7 days. Eight replicates were produced (n = 8).

Functional, phenotypic and mitochondrial properties of platelets were analyzed through storage.

Results: A repeated measures ANOVA '*Post Hoc*, Bonferroni's adjustment' was performed to compare Test (Kansuk) and Control (Fenwal). No statistically differences were observed between groups at any time point. All Test and Control Platelets were

	INTERCEPT DD BC platelet units		
Variables	DAY 2	DAY 5	DAY 7
Platelet yield (10^9/unit)	283 (14)	283 (14)	277 (8)
MPV (fL)	9.3 (0.4)	9.3 (0.5)	9.4 (0.3)
LDH (%)	4.9 (1.4)	4.3 (0.4)	4.8 (0.8)
pH (37°C)	6.756 (0.110)	6.953 (0.160)	6.956 (0.107)
Bicarbonate (mmol/L)	5.8 (1.1)	4.4 (1.4)	4.1 (1.2)
Glucose (mmol/L)	5.0 (1.2)	3.0 (1.5)	1.5 (1.0)
Lactate (mmol/L)	12.8 (2.2)	15.6 (2.9)	18.5 (2.1)
ATP (µmol/1011 plts)	7.02 (0.57)	7.09 (0.54)	7.05 (0.44)
Mitochondrial Membrane Potential (JC-1 / %)	95.76 (1.68)	92.60 (4.95)	89.80 (6.74)
CD62P (%)	22.50 (5.64)	36.24 (6.91)	38.08 (7.43)
CD42b (%)	99.14 (0.66)	98.76 (0.90)	98.56 (0.66)
Pecam-1 (%)	99.66 (0.12)	99.56 (0.21)	99.53 (0.17)
PAC-1 (%) unstimulated	0.39 (0.18)	0.62 (0.38)	0.33 (0.12)
PAC-1 (%) ADP	47.98 (3.74)	35.81 (12.36)	25.94 (7.29)
PAC-1 (%) Collagen	38.93 (4.74)	42.82 (13.77)	28.48 (7.50)
PAC-1 (%) Thrombin	29.44 (10.68)	30.61 (13.79)	14.03 (3.55)
sCD40L (pg/mL)	5529 (2739)	9469 (1810)	9662 (1398)

Table 1. *In vitro* analysis of DD BC PLTs (n = 8) stored for 7 days in SSP+ with INTERCEPT treatment. Mean ± standard deviation (SD).

in conformity with EDQM guidelines for platelet, residual WBC count and pH at the end of shelf life. *In vitro* measurements are shown in Table 1 for the Test series only (Test and Control being statistically similar at all time points). Platelet volume was 205 ± 13 ml and platelet concentration 1,384 ± 78 × 10^9/l.

There was no indication of platelet lysis with lactate dehydrogenase activity (LDH) levels below 5% on average. Swirling was maintained and mean platelet volume (MPV) was stable. Glucose was consumed and lactate produced without pH decrease. Mitochondrial Membrane Potential (JC-1) was preserved. There was no decline in adenosine triphosphate (ATP) values.

Platelet activation (CD62P) was observed with storage in platelet containers. Nevertheless platelets retained their ability to respond to strong and weak agonists (PAC-1). The expression of phenotypic markers CD42b and Pecam-1 remained stable. A progressive accumulation of biological response modifier sCD40L was observed.

Summary/Conclusions: Photochemical pathogen inactivation with INTERCEPT Blood System of Double Dose Buffy-Coat Platelet Concentrates produced with a novel eight BC pooling set maintain adequate platelet *in vitro* quality over the 7-day period of storage.

5B-S36-05

IN VITRO ASSESSMENT OF UNTREATED, UVC-TREATED AND GAMMA-IRRADIATED PLASMA REDUCED PLATELET CONCENTRATES PREPARED FROM POOLS OF 5 BUFFY COATS UNDER ROUTINE CONDITIONS

V Brixner[1], A Seltsam[2], S Dombos[1], I Weber[1], M Schmidt[1], F Tolksdorf[3], P Pohler[2], U Gravemann[2] and T Tonn[4]

[1]German Red Cross Blood Donor Service Baden-Württemberg-Hessen, Frankfurt, Germany [2]German Red Cross Blood Service NSTOB, Springe, Germany [3]MacoPharma International GmbH, Langen, Germany [4]German Red Cross Blood Donation Service North/East, Dresden, Germany

Background: Pathogen inactivation technology may enhance microbial safety of platelet transfusion by reducing bacterial and viral contamination. We established the manufacturing of UVC-treated platelet concentrates (PCs) under routine conditions.

Aims: The objective of this study was to evaluate potential *in vitro* effects of the THERFLEX UV-Platelets treatment on platelets produced from pools of five buffy coats (BCs) in comparison to untreated and gamma-irradiated platelets.

Methods: For this study, leukoreduced and plasma-reduced PCs were prepared from five BCs using 280 ml of SSP+ additive solution (Macopharma). We established the preparation of three different platelet products: PCs treated with the THERAFLEX UV-Platelets system (Macopharma) within 6 h after PC preparation, untreated PCs and gamma-irradiated (30 Gy) PCs. Platelet products were all stored for 7 days in the storage bag of the THERAFLEX UV-Platelets kit.

To evaluate the quality of these three products, we analyzed 100 PCs for volume, residual erythrocytes, residual leukocytes, platelet content, protein concentration and sterility. For a smaller number of PCs, CD62P (P-selectin) surface expression, a marker of platelet activation was analyzed by flow cytometry with and without activation by thrombin-receptor activating peptide (TRAP) after production and at the end of shelf life. *In vitro* parameters were compared by an unpaired Welch's t-test due to different sample size. A $P < 0.05$ was considered statistically significant.

Results: UVC-treated PCs showed no significant differences compared to untreated or gamma-irradiated PCs for residual leukocytes, residual erythrocytes, protein concentration and pH at the end of shelf life. The volume of the UVC-treated PCs was significantly lower than the volume of the untreated or gamma-irradiated PCs due to volume loss (5–12 ml) by the additional transfer into the illumination bag. Differences in CD62P expression between the PC groups varied during storage, depending on the day of storage and the use of TRAP for activation. Tests for bacterial contamination were negative for all tested PCs (Table 1).

Summary/Conclusions: This data indicates that the plasma-reduced, UVC-treated PCs meet the quality standards for PCs products of the German Guidelines. Studies in patients are necessary for the evaluation of safety, tolerance and efficacy of PCs manufactured by the THERAFLEX-UV Platelets system.

	Untreated PC	Welch's test comparing untreated and THERAFLEX UVC-treated PC	THERAFLEX UVC-treated PC	Welch's test comparing Gamma irradiated and THERAFLEX UVC-treated PC	Gamma-irradiated PC
Volume (ml)	N=30 362 ± 11	**p=0.0007**	N=47 352 ± 12	**p=0.01**	N=23 360 ± 12
Leukocytes per Unit (10^6)	N= 30 0.09 ± 0.2	p=0.18	N=47 0.17 ± 0.31	p=0.67	N=23 0.14 ± 0.19
Erythrocytes per Unit (10^9)	N=30 0.9 ± 0.6	p=0.80	N= 47 0.9 ±0.6	p=0.44	N=23 0.8 ± 0.3
Platelet content (10^8)	N=30 3.14 ± 0.38	p=0.95	N=47 3.06 ± 0.43	p=0.18	N=23 3.2 ± 0.42
Protein concentration (g/l)	N=30 22.6 ±1.3	p=0.77	N=47 22.5 ± 1.7	p=0.26	N=23 22.9 ± 1.2
pH	N=30 7.4 ± 0.2	P=0.78	N= 47 7.4 ± 0.1	p=0.15	N=23 7.4 ± 0,1
Day 2 %CD62P positive	N=8 21.5 ± 6.6	**p=0.0027**	N=26 31.6 ± 5.3	p=0.45	N=12 29.9 ± 6.6
Day 2 %CD62P positive /TRAP	N=8 90.9 ± 2.4	**p=0.0241**	N=26 88.5 ± 1.5	**p=0.01**	N=12 89.7 ± 1.0
Day 6 %CD62P positive	N=8 41.6 ± 10.1	p=0.70	N=26 43.1 ± 7.4	**p<0.0001**	N=12 31.6 ± 5.1
Day 6 %CD62P positive /TRAP	N=8 87.6 ± 4.1	p=0.64	N=26 86.9 ± 2.2	**p< 0.0001**	N=12 88.3 ± 1.6
Bacterial contamination (BacT/ALERT® 3D)	N=30 100% negative	not tested	N=26 100% negative	not tested	N=23 100% negative

Table 1: *In vitro* assessment of untreated, UVC-treated and gamma-irradiated PCs.

5B-S36-06

INACTIVATION OF CHIKUNGUNYA VIRUS WITH AMUSTALINE/GSH IN RED BLOOD CELLS

A Laughhunn[1], YJS Huang[2], D Vanlandingham[2] and A Stassinopoulos[3]

[1]Cerus Corporation, Microbiology Department, Concord, California, United States of America [2]Biosecurity Research Institute, Kansas State University, Manhattan, Kansas, United States of America [3]Cerus Corporation, Global Scientific Affairs, Concord, California, United States of America

Background: Chikungunya virus (CHIKV) is an RNA virus of the genus *Alphavirus*, a group of arboviruses in the family *Togaviridae*. CHIKV has been detected in human blood products from asymptomatic infected donors. Transfusion-associated transmission of CHIKV has become a significant health concern in endemic areas but a TTI has not been demonstrated yet. The objective of this study was to evaluate the inactivation of CHIKV with the INTERCEPT Blood System for RBC prepared in Optisol (AS-5). The INTERCEPT Blood System for RBC uses the small molecule amustaline to form covalent crosslinks within nucleic acids of leukocytes and contaminating pathogens to prevent replication. The process includes addition of 0.2 mM amustaline and 20 mM glutathione (GSH) and an 18–24 h incubation at RT. Inactivation is complete after 3 h, and the additional incubation ensures complete decomposition of the active ingredient. A final centrifugation and exchange of the supernatant with the additive solution SAG-M provides pathogen-reduced RBC for transfusion.
Methods: For each experiment, a single RBC unit was spiked with CHIKV to a final concentration of ~$10^{7.0}$ pfu/ml and treated with amustaline. The control sample was removed prior to the addition of amustaline and serially diluted up to 10^{-7}. The appropriate dilutions were inoculated onto Vero76 cell monolayers to determine pre-amustaline titer. Each unit was then dosed with amustaline and a test sample was removed after 3 h to determine the levels of inactivation. Test samples were diluted 10^{-1} through 10^{-3} and inoculated onto Vero76 cells. The plates were incubated for 5 days at 37°C, stained with crystal violet and the plaques enumerated. Log reduction was calculated as the difference between the mean titer in pre-amustaline samples and the mean titer in the 3 h post-amustaline samples.
Results: Robust inhibition of CHIKV infectivity was achieved (Table 1).
Conclusions: Chikungunya virus was inactivated to the limit of detection in RBC after treatment with amustaline and GSH. Inactivation of >7.1 log or >7.1 log/ml of chikungunya virus was achieved in the Vero76 cell infectivity model. The INTERCEPT Blood System for RBC is not approved for use.

Chikungunya virus Inactivation in Red Blood Cells

Replicate	Log Titers (pfu/mL)		Log Reduction per mL
	Control T=0	Test T=3	
1	7.7	<0.5	>7.2
2	7.3	<0.5	>6.8
3	7.7	<0.5	>7.2
4	7.7	<0.5	>7.2
Mean ±SD	7.6 ± 0.2	<0.5 ± 0.0	>7.1 ± 0.2

Table 1.

Transfusion Practitioner Session: Managing blood use appropriately

5B-S37-01

IDENTIFICATION OF FACTORS CAUSING THE EVIDENCE-PRACTICE GAP IN TRANSFUSION PRACTICE AMONG NURSES IN MEDICAL EMERGENCY UNIT AT A GENERAL HOSPITAL

J Svanholt Seeberg

Capital Region Blood Bank, Copenhagen, Denmark

Introduction: Implementation of new evidence in clinical practice is known to be a challenge. In Copenhagen a gap between evidence-based transfusion medicine/transfusion guidelines and bedside transfusion practice of allogeneic red blood cells was documented prior to initiating patient blood management. It has previously been shown that nurses in Copenhagen have a pivotal influence on the decision to transfuse, and lack knowledge of guidelines/evidence. In spite of knowledge about transfusion evidence and guidelines, doctors have a weaker impact on transfusion decisions. At the medical emergency unit at a general acute 700 bed hospital in Copenhagen, a high percentage of red blood cell transfusions were initiated at haemoglobin trigger above the upper guideline limits and two units was the standard dose. Medical emergency unit is a 23-bed unit receiving acutely admitted adult patients with a variety of medical conditions. We hypothesized that targeting the evidence-practice gap by identifying the barriers among nurses could improve transfusion practice. The aim of the study was to identify potential reasons for the evidence-practice gaps among the clinical nursing staff of the medical emergency unit.
Materials and methods: Data were collected by questionnaires and semi-structured focus group interviews to the nurses. Doctors were not involved. In the questionnaires, the nurses were asked about their knowledge of transfusion medicine including guidelines, transfusion triggers and the effect of transfusion red blood cells. The questionnaires were followed up by focus group interviews in order to collect more detailed data among nurses on consensus, attitudes/beliefs of the value of evidence-based medicine, and whether their decision to transfuse are based on evidence, guidelines or other factors. Data was fed back to the head nurse, the nursing unit manager and the clinical head nurse, and they were involved in planning educational intervention in nurses with data feedback and demonstrating of the evidence-knowledge-performance gap.
Results: Questionnaires demonstrated that 91% knew the hospital guidelines, but only 35% reported consensus in the nursing team about when to transfuse a patient relative to guidelines. The focus group interviews showed that the nurses had little knowledge of the principle of evidence-based transfusion medicine and its important role in defining transfusion triggers and guidelines and nurses generally preferred using other factors than guideline triggers to decide when to transfuse. Focus groups also indicated the lack of consensus on transfusion trigger among nurses.
Conclusion: Targeting the evidence-practice gap by identifying the barriers among nurses' with questionnaires and semi-structured focus group interviews demonstrated factors, which were important causes of the evidence-practice gap.

5B-S37-02

DIFFERENT STRATEGIES USED TO IMPLEMENT PREOPERATIVE ANAEMIA ASSESSMENT IN AUSTRALIA

LJ Bielby[1], L Campbell[2], M Burgess[3], F Nasra[4] and C Hogan[5]

[1]Blood Matters, West Melbourne, Australia [2]Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia [3]The Canberra Hospital, Canberra, Australian Capital Territory,, Australia [4]Austin Health, Heidelberg, Victoria, Australia [5]Australian Red Cross Blood Service, Melbourne, Australia

Background: Undiagnosed anaemia is common in the surgical setting and is independently associated with increased perioperative morbidity and mortality. If uncorrected, there is an increased likelihood of blood transfusion, which is independently associated with increased morbidity, mortality and hospital length of stay (Thomson A, 2009).
The Australian National patient blood management (PBM) guidelines 'Module 2: Perioperative' contains recommendations/practice points related to assessment and management of reversible anaemia, and optimisation of iron stores prior to surgery

where significant blood loss is anticipated. This includes a template to guide practice which can be modified for local use.
Key steps to implementing a preoperative anaemia assessment pathway include:

- communication
- identifying of roles and responsibilities
- timely assessment with appropriate screening
- management options considering urgency of surgery – types of investigations/ treatments

Across Australia many different strategies have been implemented. Three examples are described.
A hospital funded service, with a dedicated PBM Clinical Nurse Consultant (CNC):
The PBM CNC ensures that patients undergo PBM assessment, treatment and post treatment evaluation according to National guidelines prior to surgery. A 6 month audit of this model showed in the first 2 years of the program that 99% and 100% (n = 208 and 196) of all primary elective joint replacement patients were screened for anaemia preoperatively with over 30% requiring treatment. The success of this program is due to collaboration between, and across many departments, with the overall responsibility of preoperative anaemia management resting with the PBM CNC.
Hospitals (2) without a dedicated PBM Nurse:
The assessment of patients 'fit for surgery' status is conducted in this hospital, at the preadmission clinic (PAC). This often results in a small window of opportunity to identify and manage IDA preoperatively. Patients have often been on the elective surgery waitlist for months prior to PAC assessment. This model focuses on engaging general practitioners (GPs); supporting their skills and knowledge in the context of screening and management of IDA in the primary care setting. A dedicated GP IDA symposium was held to support this model, and has highlighted GPs' willingness, and improved confidence in managing IDA in the primary care.
Another hospital modified existing workflows for elective surgery patients to identify patients with IDA. Here the focus is on patients undergoing major elective procedures, such as cardiac, joint replacement, or colorectal surgery. Patients are reviewed in anaesthetic PAC and screened for anaemia, triaged and then treated according to surgical urgency. Anaemia has been identified in 14% of patients, with 21% of these anaemic patients with IDA. The success to implementation and sustainability has been due to collaboration between and across departments, and inclusion in existing workflows.
Conclusions: The strategies used to implement preoperative anaemia assessment should take into consideration the local level of engagement from executive and clinicians. Consider if the process could be moulded into an existing service, or is there an option for additional funding? Evaluation is important, and with that the consideration of how the strategy could be made sustainable.

5B-S37-03

BLOOD USE IN MAJOR HAEMORRHAGE

S Allard and On behalf of the BCSH Guideline Writing Group

Consultant Haematologist, Barts Health NHS Trust & NHS Blood and Transplant, London, United Kingdom

Management of major haemorrhage in any setting requires a multidisciplinary approach. While there are arbitrary definitions of massive blood loss, e.g., loss of one blood volume within a 24-h period, 50% blood volume loss within 3 h, loss of 150 ml/min, these may be difficult to apply in the acute situation. Physiological parameters may be used in the definition of major haemorrhage such as bleeding which leads to a heart rate >110 beats/min and/or systolic blood pressure <90 mm Hg. However such triggers may not be appropriate in the post-partum setting. Early recognition of significant blood loss is essential to allow prompt action and locally agreed triggers are needed as part of major haemorrhage protocols.
The provision of emergency blood requires the use of specifically designed protocols with robust and clearly understood communication channels between clinical staff and the blood transfusion laboratory. Blood components should be released promptly and without delay. The Hospital Transfusion Committee (HTC) has a pivotal role in overseeing protocol development and effective implementation with oversight of key activities including training and audit.
Following trigger of the major haemorrhage protocol, there must be a clear mechanism for contacting all relevant team members and a designated Team Leader should then co-ordinate further management. The Team Leader should nominate a specific clinical team member to co-ordinate communication with Transfusion Laboratory staff and support services for the duration of the incident.
The optimum target haemoglobin concentration (Hb) in the management of bleeding is not established. Red cell transfusion is usually required when 30–40% of blood volume is lost (1,500 ml in a 70-kg male) and more than 40% blood volume loss (1,500–2,000 ml) is life threatening and requires immediate transfusion. Hospitals must have a strategy to ensure that red cells are readily available for life-threatening bleeding. Patients should have correctly labeled samples taken before administration of emergency Group O blood with a switch to group specific blood as soon as possible.
FFP should be given in the initial resuscitation process in at least a 1:2 unit ratio with red cells: in traumatic bleeding, plasma: red blood cells transfusion is often given initially in a 1:1 ratio. Further transfusion administration should be guided by results of conventional laboratory-based (PT, APTT, fibrinogen) or near-patient tests (e.g. TEG/ROTEM), if part of a clinical trial, to evaluate their utility. Fibrinogen replacement should be given if fibrinogen levels are <1.5 g/l. Platelet transfusion should be given when the platelet count falls below 50×10^9/l. Many hospitals do not keep a stock of platelets and therefore platelets need to be ordered from the Blood Transfusion Centre early (e.g. when the platelet count has fallen below 100×10^9/l).
Whilst massive blood loss is less common in children specific protocols should be considered to guide management including appropriate transfusion volumes based on weight in very young children.
Audit of the management of traumatic major haemorrhage is essential to assess adverse events, timeliness of blood component support, patient outcome and component wastage

Arbo Viruses

5C-S38-01

ZIKA VIRUS AND BLOOD TRANSFUSION, EXPERIENCES FROM FRENCH POLYNESIA

D Musso[1], J Broult[2] and M Aubry[1]

[1]Institut Louis Malardé, Papeete Tahiti, French Polynesia [2]Blood Bank Center, Papeete Tahiti, French Polynesia

Background: French Polynesia is a high endemic area for arboviruses with recurrent outbreaks of dengue virus (DENV), and recent outbreaks of Zika virus (ZIKV) in 2013–2014 and chikungunya virus (CHIKV) in 2014–2015 in a context of co-circulation with DENV and probable circulation of Ross River virus (RRV). The epidemiological context is similar in several American Countries with possible co-circulation of ZIKV with DENV, CHIK, Yellow fever, Mayaro fever, Oropouche fever . . . Transfusion transmitted arboviruses has been reported for West Nile virus, DENV and recently RRV.
Aims: We hypothesized that ZIKV can be transmitted by blood transfusion. In order to prevent this transmission, we implemented preventive measures during the ZIKV French Polynesian outbreak.
Methods: Implemented measures were: pre-donation information, deferral of blood donors with clinical symptoms of ZIKV infection, implementation of an in-house Nucleic Acid Testing (NAT) for screening of blood donations, quarantine of blood components, pathogen inactivation (PI) of aphaeresis platelets, importation of plasmas, and donor self-reporting of ZIKV symptoms post-donation. We also investigated the efficacy of amotosalen combined with UVA light to inactivate ZIKV in fresh frozen plasma.
Results: During the outbreak, 42 of the 1,505 (2.8%) asymptomatic blood donors were found positive using the in house ZIKV-specific reverse transcription PCR (viral loads ranged from 2.5×10^3 to 8.1×10^6 copies/ml). Some donors developed ZIKV symptoms up to day 10 post-donation. Moreover, we confirmed the efficacy of amotosalen combined with UVA light to inactivate ZIKV in fresh frozen plasma (>6.57 $\log_{10}$ reduction).
Summary/Conclusions: In French Polynesia, prevention of ZIKV transmission by blood transfusion was challenging for several reasons: most of the infections were asymptomatic; donors deferral and post-donation information reporting were limited; the whole population was at risk of infection because ZIKV spread in all French Polynesian islands; there was no commercially available licensed NAT assay for ZIKV; ZIKV was co-circulating with DENV and we had to test for both pathogens (multiplex assay was not available); PI is currently only available for plasma and platelets but not for red blood cells; it was not possible to supply French Polynesia (remote area in the South Pacific) with fresh blood imported from non-endemic areas. The context is the same in other endemic areas for ZIKV in the Americas (remotes areas of Latin America and Caribbeans).
As ZIKV usually co-circulates with other arboviruses, there is an urgent need to develop multiplex assay that can detect the main co-circulating arboviruses (DENV, CHIKV, and ZIKV) and to develop PI system that can inactivate these pathogens in

whole blood and red cells. Countries with limited capacities should focus on safety procedure for transfusion of at-risk recipients (pregnant women and intra-uterine transfusions).
The prevention measures that were implemented in French Polynesia are now recommended by the Food and Drug Administration and the World Health Organization. The potential for ZIKV transmission by blood transfusion in French Polynesia was recently confirmed in Brazil with two possible cases of transfusion-transmitted ZIKV infections.

5C-S38-02

ZIKA VIRUS OUTBREAK IN THE AMERICAS: DEVELOPMENT OF TOOLS TO ASSIST RESPONSES

M Rios, C Chancey, RCG Fares, A Grinev and E Volkova
FDA, Silver Spring, Maryland, United States of America

Background: Zika virus (ZIKV) is a Flavivirus transmitted to humans mainly by *Aedes aegypti* mosquitoes, first isolated from a rhesus monkey in the Zika Forest, Uganda, in 1947 and from a human sample in 1968 in Nigeria. Epidemiological studies suggest that the virus circulated in humans between 1951 and 1981 in Africa and Asia. The first ZIKV outbreak outside Africa/Asia occurred on Yap Island, Micronesia in 2007. ZIKV was next reported in French Polynesia in October 2013/February 2014, followed by an extensive outbreak that started in Brazil in 2015, and spread through the Caribbean and the Americas.
About 80% of human infections are asymptomatic, and symptomatic infections include low-grade fever, arthralgia/myalgia, headache, non-purulent conjunctivitis, rash, and asthenia. ZIKV infection has been associated with severe neurological manifestations such as Guillain-Barré syndrome and with congenital abnormalities including microcephaly in the regions most affected by the ZIKV epidemic in French Polynesia and the Americas.
There are no vaccines or specific treatments for ZIKV infection. There were two probable transfusion-transmitted ZIKV infections in Brazil, and because 80% of infections are asymptomatic, transmission via this route is very likely during epidemics in the absence of blood screening assays. Viremic blood donations were reported at about 3% during the French Polynesia outbreak. Although the outcome of transfusion-transmitted ZIKV is unknown, neurological manifestations and congenital anomalies are potential undesirable outcomes that need to be prevented. Laboratory diagnosis of ZIKV is made by serology, viral isolation and nucleic acid testing (NAT), the latter being considered the most sensitive viral detection method. There are various NAT assay protocols including one FDA-cleared NAT for diagnostic use in clinical samples, but there are no FDA-approved ZIKV blood screening assays. The lack of a reference reagent for ZIKV-RNA is a barrier to evaluation of existing NAT assays and development of novel ZIKV assays. Our aim was to produce ZIKV-RNA Reference Reagents for use in the development and validation of ZIKV-NAT assays.
Methods: The ZIKV-RNA reference reagents were prepared using cell-culture-grown virus stock from two strains (PRVABC59, Puerto Rico-2015, GenBank #KU501215, from CDC; and FSS13025, Cambodia-2010, GenBank #JN860885, from UTMB.), heat-inactivated and diluted in human plasma. Heat-inactivation was confirmed by back-titration and the material was further characterized by six well-established laboratories according to a recommended plan as follows: participants were asked to test the RNA from reagents using their NAT assay(s) in serial dilution to determine the end-point, followed by testing of half-log dilutions around that end-point to confirm titer. Estimated NAT-detectable units/ml was calculated using Probit analysis after adjustment for the volume of reagent used for testing.
Results: The ZIKV Reference Reagent had an estimated overall mean of 4.4 $\log_{10}$ detectable units/ml, ranging from 4.2 $\log_{10}$ to 4.5 $\log_{10}$ units, for PRVABC59 and 4.8 $\log_{10}$ detectable units/ml, ranging from 4.7 $\log_{10}$ to 5.0 $\log_{10}$ units, for FSS13025.
Summary: The Center for Biologics for Evaluation and Research/FDA ZIKV RNA Reference Reagent for NAT was established with a concentration of 4.4 $\log_{10}$ NAT detectable units/ml for PRVABC59 and of 4.8 $\log_{10}$ NAT detectable units/ml for FSS13025.

5C-S38-03

LESSON LEARNED FROM THE 2015 DENGUE OUTBREAK IN TAIWAN

MH Lin, JW Chen and CC Yeh
Taiwan Blood Service Fundation, Taipei, Taiwan

Background: Most (53–87%) dengue infections are asymptomatic or mildly symptomatic (Mohammed H, et al., Transfusion, 2008). In 2015, a total of 42,513 dengue cases were found in southern Taiwan, indicating an incidence of 910/100,000 in population. To uncover the potential transmission of dengue virus through blood transfusion, blood donations were tested for dengue virus (DENV) RNA and NS1.
Aims: This study was conducted to assess the threat that dengue imposed on blood safety during the 2015 endemic.
Methods: The frozen stored donor samples were selected from the two epidemic locations (Tainan and Kaohsiung) during the 2015 epidemic. The serum samples (n = 6,515) were subjected to reverse transcription (RT) PCR (LightMix Modular Dengue Virus, TIB MOLBIOL) and NS1 antigen (PLATELIATM DENGUE NS1 AG Assay, Biorad) detection. Samples with repeat reactive (RR) result were interpreted as positive.
Results: Of the 6,515 donor samples, 16 were detected as dengue RNA positive and 2 were NS1 Ag positive, as showed in Table. Of the DENV viremic donors, NS1 Ag assay detected 1 out of 16 DENV RNA positive cases.
Conclusion: (i) Our data show a low sensitivity of dengue NS1 Ag assay in detecting DENV viremia in asymptomatic blood donors. (ii) During the 2015 endemic peak, characterized by an incidence of 9.1/1,000 in population, donor viremia were detected to be 2.4/1000 (16/6,515). Recent study in Brazil (Sabino et al., J Infect Dis, 2015) indicated approximately one third of the DENV RNA positive units resulted in transfusion transmitted (TT) infection. This suggest that one TT case per thousand may occur during the epidemic. (iii) Dengue surveillance data for the outbreak in the past provide an essential information about the potential treat and severity of the future endemic. Blood collector may rely on a real-time comprehensive surveillance program to trigger endemic preparedness plan, which may include blood collection from non-endemic areas and laboratory screening of blood donors for dengue viremia.

NS1	DENV RNA	
antigen	Positive	Negative
Positive	1	1
Negative	15	6498

Table 1. Detection of DENV RNA and NS1 in donors during endemic

5C-S38-04

DENGUEPLEX ASSAY: THE DEVELOPMENT OF A NEW PROTEIN-BEAD ARRAY FOR DENGUE

RCG Fares[1], A Teixeira-Carvalho[2], EG Kroon[3], SL Stramer[4] and M Rios[1]
[1]FDA, Silver Spring, Maryland, United States of America [2]Fundação Oswaldo Cruz, Centro de Pesquisa Rene Rachou, Belo Horizonte, Brazil [3]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil [4]American Red Cross, Gaithersburg, Maryland, United States of America

Background: Dengue disease is caused by any of four dengue virus serotypes (DENV-1 to -4) and is transmitted by Aedes mosquitoes. DENV infection may be asymptomatic or lead to symptoms ranging from flu-like illness (dengue fever, DF), to a life-threatening condition (severe dengue, SD), which may be fatal in the absence of early supportive therapy. Transfusion-transmitted DENV (TT-DENV) has been reported in dengue endemic regions including the U.S. territory of Puerto Rico. Laboratory diagnosis of dengue is made by serology, viral isolation and nucleic acid testing; however, there are no FDA-approved ZIKV blood screening assays and current antibody assays take days to be completed, sometimes jeopardizing patient care.
Aims: This project aims to develop a rapid and specific test for early antibody detection of the four DENV serotypes. The development of the proposed assay is expected to: (a) obviate the limitations imposed by cell culture and conventional

serology; (b) have capability for detection of concurrent infections by multiple serotypes in a single testing sample reducing volume required of either serum or plasma; (c) be used for routine diagnosis, prognosis and therapeutic monitoring of DENV patients.

Methods: The test is based on an antigen-coupled bead array (DenguePlex) for the identification of antibodies to DENV in human plasma and serum. Essentially, 7.5 mm color-coded polystyrene beads (Becton-Dickinson, BD) and 4 mm Ni-coated polystyrene beads (Kisker Biotech) were coupled to E. coli-derived His-Tag DENV recombinant E-proteins from UFMG, Prospec and Fitzgerald Industries. The 7.5 mm beads coupled to Fitzgerald E-proteins were selected after evaluation under different conditions.

Results: Envelope proteins from the four dengue serotypes were successful coupled to beads of different sizes. The prototype assay was first standardized for DENV-1 and -4 based on sample availability. Mean Fluorescence Intensity data were acquired in a BD FacsCanto-II instrument, and analyzed using FlowJo software. Assay sensitivity was 87%, and comparison of serum and plasma samples from the same collection showed 100% correlation of results. Thus, we have successfully developed the DenguePlex assay for DENV-1 and -4, and demonstrated assay feasibility by testing 84 archival specimens, including 74 known DENV-positive and 10 DENV-negative for antibodies and RNA. We are now testing DENV-2 and -3 known positive samples, which will be followed by the use of DenguePlex for serotype identification in a multiplex format; optimization of overall sensitivity; determination of analytical sensitivity, specificity, likelihood ratio, and cross-reactivity with other flaviviruses including JEV, SLE, WNV and ZIKV.

Conclusion: We have successfully developed and optimized the prototype DenguePlex assay for all serotypes envelope proteins and standardized a preliminary test for evaluation of the assay using serum and plasma samples. This assay may be helpful for diagnosis and confirmation of dengue infection in transfusion settings.

5C-S38-05

ROSS RIVER VIRUS IN 'AT-RISK' AUSTRALIAN BLOOD DONORS: IMPLICATIONS FOR BLOOD SUPPLY SAFETY

H Faddy[1], T Tran[1], E Hewlett[1], CR Seed[1], V Hoad[1], HT Chan[1], R Harley[1], A Keller[1], RA Hall[2], N Prow[3], H Bielefeldt-Ohmann[2] and RLP Flower[1]

[1]Australian Red Cross Blood Service, Brisbane, Australia [2]The University of Queensland, Brisbane, Australia [3]Queensland Institute of Medical Research, Brisbane, Australia

Background: Emerging transfusion-transmissible pathogens, including arboviruses such as West Nile and dengue viruses, are a threat to transfusion safety. The most prevalent arbovirus in humans in Australia is Ross River virus (RRV); however, prevalence varies substantially around the country. Outbreaks of RRV have also occurred across The Pacific region, in the Solomon Islands, Papua New Guinea, and Fiji. This virus can cause debilitating, rheumatic symptoms in humans. Viraemia prior to symptoms presentation can occur, so transmission through blood transfusion is possible. Until recently, the risk of transfusion-transmission was theoretical; with risk during the peak of an outbreak estimated from population data as one in 4,917 (range 1 in 1,730 to 1 in 12,269). However, in 2015 a probable case of transfusion-transmitted RRV was reported and subsequent modelling estimated a yearly risk of 8–11 potentially RRV viraemic fresh blood components. In order to help maintain blood safety in Australia, individuals diagnosed with RRV are ineligible to donate blood for 2 weeks after symptom recovery. This is followed by a 12 month period when only plasma for fractionation can be utilised. An additional safety precaution is the quarantine or recall of a blood component for donors reporting any illness within 7 days of donation.

Aims: To measure the rate of RRV RNA carriage among donors in regions of Australia 'at-risk' for RRV transmission during peak seasons.

Methods: Plasma samples were collected from blood donors (n = 7,500) residing in areas considered 'at-risk' for RRV transmission during the peak season (based on historical notifications). Viral RNA was extracted from individual samples and RT-PCR performed in duplicate using in-house primers and probes targeting NS3. MS2 phage was used as an extraction and amplification control for each sample, and RRV strain T48 RNA as a positive control for each RT-PCR plate. A corrected estimate for the assay limit of detection, based on a dilution series of synthetic oligonucleotides containing the target RRV sequence (T48 strain), was used.

Results: The 'highest-risk' regions for RRV transmission were not areas where blood donor centres were located. The limit of detection of our in-house RT-PCR was estimated to between 8 and 12 copies per reaction. None of the 7,500 samples were positive for RRV RNA, resulting in a zero risk estimate with a one-sided 95% confidence interval (95% CI: 0–1 in 2,019).

Summary/Conclusions: We did not detect RRV RNA among 7,500 donations collected from blood donors residing in regions 'at-risk' for RRV transmission during peak periods of transmission. Our results suggest the yearly risk of collecting a RRV infected donation in Australia was low, and at the lower range of previous risk modelling. This was expected due to the observation that the majority of donor centres were not in areas known to be at the highest risk for RRV transmission, the stringent donation restrictions utilized in Australia, and the fact these previous models were based on notification data. We therefore believe the risk of RRV transfusion-transmission in Australia is adequately managed through existing donation restrictions and recall policies.

TRALI Mechanisms

5C-S39-01

NON-ANTIBODY MEDIATED TRALI – CURRENT UNDERSTANDING

APJ Vlaar

Academic Medical Center, Amsterdam, The Netherlands

Transfusion related acute lung injury (TRALI) is suggested to follow a 'two hit' mechanism. The 'first hit' is the underlying condition of the patient (e.g. sepsis) resulting in priming of neutrophils and/or endothelium. The 'second hit' is any transfusion resulting in activation of the primed neutrophils and/or endothelium. The transfusion factors can be divided in antibody mediated and non-antibody mediated TRALI. Antibody mediated TRALI is caused by passive infusion of donor antibodies reacting with the cognate antigen of the recipient. Non-antibody mediated TRALI is thought to be caused by the transfusion of stored cellular blood products. Although the mechanisms involved in antibody mediated have been well outlined and confirmed in pre-clinical and clinical studies this is not the case for non-antibody mediated TRALI. Pre-clinical studies show a strong association between storage time of cellular blood products and the onset of lung injury. However, recent clinical studies show contradictory results. In this presentation the latest insights on the pathogenesis of non-antibody mediated TRALI will be discussed as well as the areas future research should be focused on.

5C-S39-02

INTERACTION OF NEUTROPHILS ANTIBODIES WITH ENDOTHELIAL CELLS IN TRALI

B Bayat

Clinical Immunology and Transfusion Medicine Justus-Liebig University Giessen, Giessen, Germany

Transfusion related acute lung injury (TRALI) is severe pulmonary reaction induced by transfusion. This reaction is characterized by pulmonary edema, breathing distress and consequently hypoxia temporally associated with transfusion. In immune TRALI, alloantibodies reactive with recipient's neutrophils are mainly considered as responsible antibodies that activate neutrophils and involve in TRALI reaction. These antibodies targeting either human leukocytes antigen (HLAs) class I or II or human neutrophils antigens (HNAs). Antibodies interaction with cognate antigens is believed to induce neutrophils activation and sequestration that consequently leads to endothelial damage and lung injury. Analyzing the effects of these antibodies on neutrophils depleted animals, however has doubted efficiency of this dogma that consider neutrophils as a sole inducer of lung injury during TRALI. For antigens which are not exclusively expressed on neutrophils or leukocytes (such as anti-HNA-3 and HLA-class I and class II), the 'neutrophils only' theory has overlooked the effects of antibodies binding on other antigen positive cells. However our knowledge about participation of direct antibody binding on endothelial in mechanism of TRALI is still limited.

Previous studies have documented that anti-HNA-3a are implicated in sever and fatal TRALI. Anti-HNA-3 epitope resides on choline transporter like protein (CTL) two with broad expression patterns on different cells including neutrophils and endothelial cells.

Recent *in vitro* as well as *in vivo* research of our laboratory has identified the binding of anti-HNA-3a to CTL2 protein on endothelial cells, as initiator of lung injury in anti-HNA-3a mediated TRALI. Binding of anti-HNA-3a antibodies to endothelial cells triggers production of endothelial reactive oxygen species (ROS) and damage

therefore endothelial junctions that consequently leads to endothelial dysfunction. Although *in vivo* neutrophils depletion alleviated severity of lung injury however failed to protect animals from anti-HNA-3a induced TRALI.
This presentation will summarized findings on effects of antibody binding on endothelial cells and highlight the contribution of endothelial reactive antibodies in mechanism of immune TRALI.

5C-S39-03

SEVERE ADVERSE TRANSFUSION-RELATED REACTIONS REPORTED TO THE NATIONAL HEALTHCARE SAFETY NETWORK HEMOVIGILANCE MODULE, UNITED STATES, 2013 TO 2015

SV Basavaraju, KA Haass, MRP Sapiano and MJ Kuehnert

Centers for Disease Control and Prevention, Atlanta, Georgia, United States of America

Background: Transfusion-related reactions are estimated to occur in 1–2% of all transfusions and are often preventable. The National Healthcare Safety Network (NHSN) Hemovigilance Module (HM) is a U.S. national surveillance system that tracks transfusion-related adverse reactions. In 2010, U.S. healthcare facilities began voluntary enrollment in the system. Facilities monitor transfusion recipients and report the occurrence of 12 transfusion-related adverse reactions as defined by the HM case definition criteria. We present a summary of transfusion-related adverse reactions reported to NHSN from 2013 to 2015.
Aim: The purpose of this study was to determine overall and reaction-specific rates of 12 adverse transfusion reactions, including serious reactions, occurring among transfusion recipients as reported by participating healthcare facilities to the HM during 2013–2015.
Methods: Participating facilities report the total number of blood components transfused monthly and transfusion-related adverse reactions. These analyses include all reports from participating facilities between January 1, 2013 and December 31, 2015. Frequencies of transfusion-related adverse reactions associated with red blood cells (RBC), platelets (PLT), plasma, or cryoprecipitate that met definite, probable, or possible case definition criteria were calculated. These reactions were classified by severity (categorized as severe, life threatening, and fatal), and imputability (i.e., likelihood that the transfusion caused the reaction). Rates were calculated for each of 12 reaction types occurring per 100,000 total transfused units, and further stratified by component type (RBC, PLT, plasma, and cryoprecipitate).
Results: A total of 165 facilities were included and reported 3,849,955 transfused components during the study period. These included 2,171,816 RBC, 693,331 PLT, 636,206 plasma, and 348,602 cryoprecipitate units. A total of 8,911 reactions meeting case definition criteria were reported. The overall adverse reaction rate was 231.5 per 100,000 blood components. Reaction rates per 100,000 RBCs and PLTs components were 219.2 and 430.4, respectively. Plasma (131.7 per 100,000 components) and cryoprecipitate (22 per 100,000 components) units were implicated in fewer reported reactions. Of 8,911 total reactions reported, 813/8,911 (9.1%) were serious. Of these, 701/813 (86%) were severe; 105/813 (13%) were life-threatening; and 7/813 (1%) were fatal. The most frequently reported reactions were allergic (n: 3,833/8,911, 99.6 per 100,000 total components), febrile non-hemolytic (n: 3,257/8,911, 84.6 per 100,000 total components) and delayed serologic (n: 656/8,911, 17.0 per 100,000 total components). Allergic reactions were the most commonly reported serious reactions (n: 369/813, 9.6 per 100,000 total components) followed by transfusion associated circulatory overload (n: 200/813, 5.2 per 100,000 total components). Acute hemolytic reactions (n: 50/8,911, 1.3 per 100,000 total components), transfusion-associated lung injury (TRALI) (n: 36/8,911, 0.9 per 100,000 total components), and transfusion-transmitted infections (TTI) (n: 20/8,911, 0.5 per 100,000 total components) were uncommon. TRALI (36 total; 15 severe; eight life-threatening; one fatal) and TTI (20 total; 11 severe; 2 life-threatening; 2 fatal) had more life-threatening and fatal outcomes.
Summary/Conclusion: Serious transfusion reactions, though rare, continue to result in significant morbidity and mortality in the United States. These findings suggest that interventions to prevent serious transfusion reactions are important for recipient safety.

5C-S39-04

YIELD OF HLA AND HNA ANTIBODY SCREENING IN DONORS ASSOCIATED WITH CLINICAL TRALI: THE DUTCH EXPERIENCE (2007–2015)

CM Weller[1], L Porcelijn[1], E Huiskes[1], M de Haas[2], AG Bokhorst[1], MGJ van Kraaij[1], RMMW Koopman[1] and C So-Osman[1]

[1]*Sanquin, Leiden, The Netherlands* [2]*Sanquin, Leiden University Medical Center, Leiden, The Netherlands*

Background: Transfusion-related acute lung injury (TRALI) is a severe transfusion reaction with a mortality rate up to 20%. It is characterized by diffuse pulmonary edema, hypoxemia and hypotension within 6 h of transfusion of blood products. Recognition of passive transfusion of HLA and HNA antibodies as the main cause of TRALI has led to a male-only quarantaine plasma policy by the national Dutch Blood Bank Sanquin since 2007. As of 2009, also plasma of pooled platelet concentrated (PPCs) is from males only. From 2014 onwards, single donor quarantaine plasma has been replaced by pooled solvent detergent (SD) plasma from males and females, which contains less antibodies due to the manufacturing process. Despite these precautions TRALI still occurs, although nowadays most cases are thought to be mediated by other factors.
Aims: The aim of the study is to describe the frequency with which TRALI was reported to Sanquin after introduction of male-only plasma and to evaluate the yield of HLA and HNA antibodies screening in donors associated with TRALI in recipients of blood products (red blood cells (RBCs), plasma (Q and SD plasma) and PPCs).
Methods: Data on all suspected TRALI cases reported to Sanquin between January 2007 and May 2015 were extracted from the QA registry and the laboratory information system.
Results: After the introduction of male-only plasma, the number of suspected TRALI cases decreased from 29 in 2007 to 8–13 per year since 2010. Over these years the number of transfusions in the Netherlands declined with 15% (data not shown). After evaluation of all clinical data TRALI remained the most probable clinical diagnosis in 27 of the 134 reports (20%). After reviewing all available data TRALI was indicated as a possible diagnosis in 58 cases (43%) or TRALI was excluded or highly unlikely in 49 cases (37%). More than 600 donors were involved and were considered for antibody screening.
HLA or HNA antibodies were detected in nine of 27 cases (33%) with a probable TRALI diagnosis. All these cases date from the years 2007 and 2008. One case concerned a reverse TRALI caused by HLA antibodies reactive with epitopes on donor cells and eight patients received passively transfused antibodies. Not in all cases the source of antibodies was a plasma-containing blood product (Q plasma or PPC), as three patients received only RBCs. Screening identified antibodies in ten donors involved in these eight TRALI cases and they were subsequently excluded from donating whole blood or Q plasma.
Summary/Conclusions: From our preliminary analyses we conclude that the number of reported TRALI cases decreased more than expected based on the decline in the number of transfusions. We have not seen any cases with a causative role for passively transfused HLA or HNA antibodies since the introduction of the male-only plasma policy, suggesting an impressive protective effect of this measure. However, in three of the cases with HLA or HNA antibodies a RBC was the source. Currently, we are processing the results from the evaluation of the laboratory work-up of the donor screening.

Impact of Drugs on Transfusion

5C-S42-01

EXTENDING THE REACH OF THERAPEUTIC AGENTS TO TRANSFUSION

P Bartolucci[1], A Habibi[1] and F Noizat Pirenne[2]

[1]*CHU Henri Mondor. UPEC. APHP. INSERM, Créteil, France* [2]*EFS. UPEC, Créteil, France*

Delayed hemolytic transfusion reaction (DHTR), a life-threatening complication, particularly in sickle-cell disease (SCD), occurs about 5–20 days post-transfusion. It is diagnosed based on the conjunction of clinical and biological parameters: decreased HbA concentration (transfused red blood cells), appearance of hemoglobinuria, increased biological hemolytic parameters and, generally, a vaso-occlusive crisis. Its

evolution can lead to an acute chest syndrome and/or multiorgan failure, highlighting the need for rapid diagnosis and onset of management. The only consensual guideline is to avoid a new transfusion, except in an imminent life-threatening situation.

Many treatments have been tried in this context with poor results, e.g. intravenous immunoglobulins and steroids. Herein, we review new therapeutic strategies for DHTR. Rituximab, a monoclonal antibody targeting CD20 expressed on B lymphocytes, has been used during the acute phase of DHTR and to prevent its recurrence before transfusing a patient with a history of DHTR. Indeed, although rituximab use is restricted to SCD patients with known alloantibodies, 30% of DHTRs occur without alloantibodies. This absence of alloantibodies highlights the need to search for new pathophysiological pathways and therapies. Complement activation in DHTR seems to be a very promising avenue of investigation. Complement might be activated via the classical pathway in the presence of mono- or polyclonal alloantibodies but, more intriguingly, could represent an amplification system via the alternative pathway, either in the presence or absence of alloantibodies. For those reasons, eculizumab, which inactivates the C5-convertase complex, might be a good option to treat severe DHTRs. It has been used in some patients with good results.

DHTR is an increasing cause of SCD patients' death. New therapeutic strategies that modulate the immune system are emerging and should be investigated in prospective studies.

5C-S42-02

INCIDENCE OF PLASMA TRANSFUSION REACTIONS AND COMPARISON OF PLASMA PRODUCTS – AN ANALYSIS OF 7 YEARS OF ISTARE DATA

NH Saadah[1], JG van der Bom[2], RA Middelburg[3], JC Wiersum-Osselton[4], P Renaudier[5], C Richardson[5], P Robillard[5], C Politis[5] and MR Schipperus[6]

[1]Leiden University Medical Center, Leiden, The Netherlands [2]Sanquin CCTR, Leiden, The Netherlands [3]Department of Clinical Epidemiology; LUMC, Leiden, The Netherlands [4]Sanquin Donor Services Unit, Leiden, The Netherlands [5]International Haemovigilance Network Steering Committee, Manchester, United Kingdom [6]Haga Teaching Hospital, The Hague, The Netherlands

Background: The International Surveillance of Transfusion-Associated Reactions and Events (ISTARE) database contains annual aggregate nationally-reported data from 24 countries on plasma transfusion reactions in the period 2006–2012.

Study aim: We compared plasma transfusion reaction incidences of various plasma types within this database. We further compared the variance in these nationally-reported incidence data to that of published (peer-reviewed) data.

Study methods: Country-anonymized data was provided by the ISTARE steering committee. Random effects pooled average incidences and inter-quartile ranges of national annual rates were calculated for commonly reported plasma transfusion reactions. Binomial-normal regression was used to compare plasma types (apheresis-derived vs whole blood derived, un-treated single donor vs pathogen inactivated single donor vs solvent/detergent pooled) with regard to common transfusion reaction incidences. Levene's test (significance of difference in variances) was used to compare variance in nationally-reported incidences to that of peer-reviewed data from our recent meta-analysis of plasma transfusion reaction incidences.

Results: 111 annual national datasets representing transfusion of over 15 million units of plasma were analyzed. Annual datasets reported incident count data on whole blood derived untreated (57 datasets; 10.3 million units transfused), whole blood derived pathogen inactivated (5 datasets; 77,000 units), apheresis derived untreated (22 datasets; 1.5 million units), apheresis derived pathogen inactivated (4 datasets; 856,000 units), solvent/detergent pooled (nine datasets; 588,000 units), and unspecified (14 datasets; 2.2 million units) plasmas. The five most commonly reported transfusion reactions are allergic reactions {pooled average incidence 45.3 reactions per 10^5 plasma units [Interquartile Range (IQR) 16.8–96.2]}, Febrile Non-Hemolytic Transfusion Reactions [FNHTR; $6.2/10^5$ units (IQR 2.1–17.6)], Transfusion Associated Circulatory Overload [TACO; $2.2/10^5$ units (IQR 1.1–6.8)], Transfusion Related Acute Lung Injury (TRALI; $1.4/10^5$ units (IQR 0.8–3.4)], and Hypotensive reactions [$1.3/10^5$ units (IQR 0.7–3.6)]. For allergic reactions, transfusion with apheresis-derived plasma was associated with 2.00 times as many events as whole-blood derived plasma (95% CI 1.75–2.27), while transfusion with pathogen inactivated single donor and solvent/detergent plasma was associated with 0.47 (95% CI 0.40–0.54) and 0.24 (95% CI 0.18–0.31) times as many events as untreated plasma, respectively. For FNHTR, transfusion with apheresis-derived plasma was associated with 1.51 times as many events as whole-blood derived plasma (95% CI 1.01–2.25), while transfusion with pathogen inactivated single donor plasma was associated with 0.61 (95% CI 0.37–0.99) times as many events as untreated plasma. No statistically significant differences between products were observed in incidences of TRALI, TACO, or hypotensive reactions. Variances in reported incidences for nationally-reported transfusion adverse event data were statistically significantly smaller than variances in peer-reviewed transfusion adverse event data for allergic reactions [W (Levene's test statistic) = 7.69, P = 0.006] and TACO (W = 10.92, P = 0.002) while the differences were not significantly different for TRALI (W = 2.27, P = 0.135) or FNHTR (W = 0.002, P = 0.965).

Conclusion: Apheresis derived plasma is associated with more allergic reactions and FNHTRs than whole blood derived plasma. Pathogen-inactivated single donor and solvent/detergent pooled plasmas are both associated with fewer allergic reactions and pathogen inactivated plasmas are associated with fewer FNHTRs than untreated, single donor plasmas. Variance in reported transfusion reaction incidences is significantly smaller in nationally-reported data than in published data for allergic reactions and TACO.

5C-S42-03

TRANSFUSION ADVERSE EVENTS IN PATIENTS WITH HAEMOGLOBINOPATHY – REPORTS TO THE SERIOUS HAZARDS OF TRANSFUSION UK HAEMOVIGILANCE SCHEME 2010–2015

S Allard[1], P Bolton-Maggs[2], D Poles[2] and On behalf of the SHOT Steering Group

[1]NHS Blood & Transplant, London, United Kingdom [2]SHOT, Manchester, United Kingdom

Introduction: Blood transfusion is essential in the management of many patients with haemoglobinopathy, namely thalassaemia major and intermedia and is increasingly indicated in sickle cell disease (SCD). These patients are particularly vulnerable to transfusion complications.

Aims and methods: The Serious Hazards of Transfusion (SHOT) haemovigilance scheme collates data submitted by hospitals in the UK on adverse clinical incidents in patients with haemoglobinopathy. Data available on key adverse events over a period of 6 years (2010–2015) were analysed and categorised by the type of complication.

Results: Cumulative data available for 2010–2015 are summarised in Table 1 with some key outcomes shown in parenthesis.

Summary and Conclusions: Patients with SCD are known to be at particular risk of red cell alloimmunisation with SHOT data highlighting potential serious consequences including death and major morbidity following haemolytic transfusion reactions. It is vital that clinical staff inform the laboratory of the diagnosis of haemoglobinopathy to permit selection of red cells of suitable phenotype and minimise risk of inadvertent allo-immunisation.

Hyperhaemolysis remains a challenging complication that can be potentially fatal. More efforts are needed to better understand the mechanisms and pathophysiology behind this complex phenomenon to guide appropriate therapy.

Specialist clinical input is also essential to expedite appropriate transfusion therapy for patients with haemoglobinopathy; a child with SCD died following very delayed transfusion.

The advice not to transfuse at night issued by SHOT in previous years was sometimes misinterpreted and has been revised as it is in the interests of patients

Table 1:

Type of adverse reaction	Sickle Cell Disease	Thalassaemia
Haemolytic transfusion reaction (HTR)	53 (2 deaths, 24 MM)	3 (1 MM)
Specific requirements not met	39 (1 alloimmunisation)	7
Acute transfusion reaction	16 (minor morbidity)	18 (minor morbidity)
Near miss	13	2
Incorrect blood component transfused	3 (2 ABO-incompatible, 1 D pos to D neg female)	4 (3 ABO incompatible)
Avoidable, delayed or under transfusion	8 (1 death from delay)	1
Transfusion-associated circulatory overload	2 (1 MM)	1
Transfusion associated dyspnoea	1	0
Transfusion-transmitted infection	1 (parvovirus)	0
Total	136	36

MM–major morbidity e.g. renal failure, ICU admission

Table 1. MM = major morbidity, e.g. renal failure, ICU admission

receiving regular transfusion to reduce time lost to education or employment. However it is essential that the standard of care during transfusion is the same at whatever time a transfusion takes place including stringent attention to patient identification with prompt detection and management of reactions.

5C-S42-04

RED BLOOD CELLS TRANSFUSED IN THE LAST 7 DAYS OF THEIR 42-DAY STORAGE LIMIT ARE ASSOCIATED WITH ADVERSE CLINICAL OUTCOMES IN HIGH RISK PATIENTS: EVIDENCE FROM A LARGE ACADEMIC TERTIARY CARE CENTER

R Goel[1], D Johnson[2], A Scott[2], AAR Tobian[2], PM Ness[2], E Nagababu[2] and SM Frank[2]

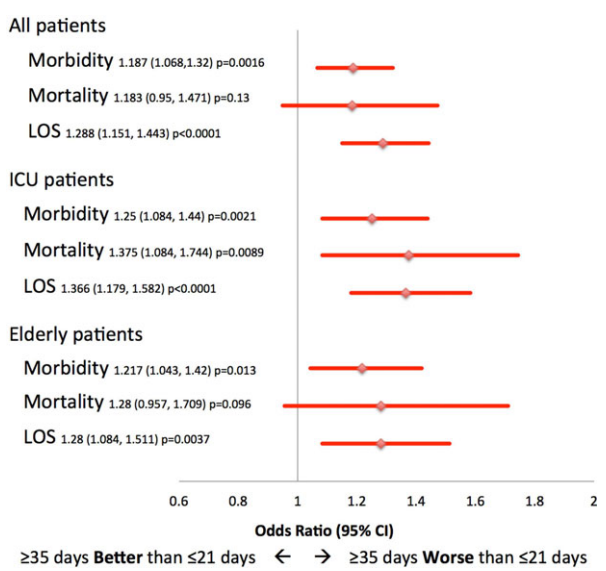
[1]New York Presbyterian Hospital, Weill Cornell Medical College, New York, New York, United States of America [2]Johns Hopkins Hospital, Baltimore, Maryland, United States of America

Background: Clinical trials have shown that longer red blood cell (RBC) storage duration does not worsen outcomes, however these studies included very few RBCs transfused near the end of the 42-day storage limit. We tested the hypothesis that these 'oldest' RBCs i.e.those stored ≥35 days and near the 42 day storage limits are associated with adverse outcomes.

Study design and methods: We acquired electronic medical record data from a web-based intelligence portal and our hospital's database for inpatients discharged between January 2009 and June 2015. For the purposes of risk-adjustment, collected data included pre-hospital comorbidities, and the weighted Medicare severity diagnosis related group (MSDRGWt), a comprehensive surrogate marker of overall disease severity and complexity of hospital procedures, otherwise known as the casemix index. Morbidity, mortality, and length of stay (LOS) were compared in patients transfused exclusively with RBCs stored ≤21 days vs patients transfused exclusively with RBCs stored ≥28 days and patients transfused exclusively with RBCs stored ≥35 days.

Results: A total of 28,247 transfused patients given 129,483 RBC units were assessed. After risk adjustment, ≥35 day RBCs were associated with increased morbidity [adjusted OR (adjOR) 1.19 95% CI (1.07,1.32), P = 0.002], but ≥28 day RBCs were not [adjOR 1.06 95% CI (0.97,1.15), P = 0.2]. Neither ≥35 day nor ≥28 day RBCs were associated with increased mortality. In critically-ill patients, ≥35 day RBCs were associated with increased morbidity [adjOR 1.25 95% CI (1.08,1.44), P = 0.002] and mortality [adjOR 1.38 95% CI (1.08,1.74), P = 0.009], but ≥28 day RBCs were associated with neither. In older patients, ≥35 day RBCs were associated with increased morbidity [adjOR 1.22, 95% CI (1.04,1.42), P = 0.01], but not mortality [adjOR 1.28, 95% CI (0.96,1.71), P = 0.1], and ≥28 day RBCs were associated with neither. LOS was increased for the both ≥28 and ≥35 day RBCs for all patients, and the critically-ill and older subgroups.

Conclusions: The findings in this retrospective outcome study suggest that RBC units stored ≥35 days, but not those stored ≥28 days are associated with increased morbidity, and these oldest RBC units were associated with with adverse clinical outcomes including increased mortality in the highest risk patients especially the critically ill and the elderly patients. These findings may help to explain why the randomized trials on age of blood were negative, since the majority of these trials did not evaluate the oldest RBC units. The clinical implications of our findings are that consideration should be given to whether blood units should be 'triaged' to avoid giving oldest blood to the sickest patients, or to those anticipated to receive large numbers of transfused units.



Caption 1. Risk adjusted odds ratios for morbidity, mortality and prolonged length of stay for >35 day old RBC units vs <21 day old units.

5C-S42-05

THE ASSOCIATION OF STORAGE TIME OF PLATELET CONCENTRATES WITH INCIDENCE OF BACTERAEMIA IN HAEMATOLOGICAL PATIENTS

AL Kreuger[1], R Middelburg[1], JL Kerkhoffs[1], CMC Bank[2], EAM Beckers[3], AJ van Gammeren[4], A Leyte[5], JMM Rondeel[6], KMK Vooght[7], F Weerkamp[8] and JG van der Bom[1]

[1]Sanquin, Leiden, The Netherlands [2]ADRZ, Goes, The Netherlands [3]Maastricht University Medical Hospital, Maastricht, The Netherlands [4]Amphia Hospital, Breda, The Netherlands [5]OLVG Hospital, Amsterdam, The Netherlands [6]Isala Klinieken, Zwolle, The Netherlands [7]University Medical Center, Utrecht, The Netherlands [8]Maasstad Hospital, Rotterdam, The Netherlands

Background: Blood banks are seeking to extend maximum shelf life of platelet concentrates for logistic reasons. As a consequence, the risk of bacterial infections could increase, directly via transfusion of contaminated products or indirectly via transfusion related immunomodulation.

Aim: The aim of this study was to investigate the association of storage time of platelet concentrates with the incidence of bacteraemia in patients with haematological malignancies.

Methods: We performed a case-cohort study in eight hospitals in the Netherlands. All patients with a haematological malignancy or aplastic anaemia who received at least one platelet transfusion between 2006 and 2015 were included. Platelet concentrates were prepared from buffy coats of five donors and resuspended in plasma of one of those donors, or in platelet additive solution. Cases were defined as patients who had a bacteraemia, i.e. a positive blood culture, the day after transfusion. Cases were matched to as many controls as possible, based on hospital, day of the week, number of transfusions, ABO blood group and storage medium. Storage time was calculated from day of donation and categorized in three categories: ≤2, 3–4, ≥5 days. A conditional logistic regression was performed using the youngest storage time category as reference, adjusted for matching factors and stratified on storage medium. As sensitivity analyses the effect of storage medium and amount of plasma was investigated, and a follow-up period of 2 and 3 days was taken.

Results: The cohort consisted of 4,545 patients, who received 42,066 transfusions on 38,235 days. Among 3,054 patients receiving 30,258 plasma stored platelet concentrates, 533 cases of bacteraemia were found. The day after transfusion of a plasma stored platelet concentrate, the adjusted relative risk of bacteraemia was 0.70 (95% CI 0.50 to 0.99) for concentrates stored 3–4 days and 0.59 (95% CI 0.43–0.82) for concentrates stored ≥5 days, compared to concentrates stored ≤2 days.

Among 1,527 patients receiving 11,808 PAS stored platelet concentrates, 182 cases of bacteraemia were found. The adjusted relative risk of bacteraemia was 1.13 (95% CI 0.71 to 1.80) for 3–4 days and 1.18 (95% CI 0.72 to 1.94) for concentrates stored ≥5 days. Storage medium was not associated with the risk of bacteraemia (RR 0.94 95% CI 0.74 to 1.18). After transfusion of hyperconcentrated products the risk of bacteraemia was 1.43 (95% CI 0.96 to 2.14) compared to normal volume plasma platelet concentrates. Storage time was not associated with the risk of bacteraemia 2 or 3 days after the transfusion.

Conclusion: With increasing storage time of platelets stored in plasma, the risk of bacteraemia 1 day after transfusion decreased, whereas storage time was not associated with bacteraemia after transfusion of PAS stored products. Therefore, it seems safe to store platelets for a maximum of 7 days with regard to the risk of bacteraemia.

Post Partum and other Major Haemorrhage

6A-S44-01

THE INDONESIAN APPROACH TO REDUCE MATERNAL MORTALITY

Y Soedarmono

Indonesian Association of Transfusion Medicine, Jakarta, Indonesia

Background: Blood service is an important part of health services for Indonesia with a high maternal mortality. There are three main reasons of maternal mortality in Indonesia: haemorrhage (31%), hypertension (27%) and infections (6%); and 78% of the maternal death occurs within 48 h post partum. The unavailability of blood for transfusion has contributed to a 9% of mortality death.
Aims: To describe government strategy in ensuring availability of safe blood supply in an effort to reduce maternal mortality due to haemorrhage.
Method: Improving maternal health is one of the 2015 MDGs and post MDGs goals with maternal mortality as one of its indicator. The Ministry of Health Decree on Collaboration work between the Primary Health Centres (PHCs), Blood Centres (BC) and Hospitals on Blood Services has been issued in 2015 to increase people's awareness and willingness in donating blood voluntarily for every pregnant woman in the village. The trained PHC staff will do donor recruitment and early donor selection according to blood donor requirement, blood group and haemoglobin test, and simple physical check of the donor. Four eligible donors for every pregnant woman will be requested to donate blood at the BCs on 7–10 days before the delivery time. The excess donated blood that is not used by intended pregnant woman will increase the availability of blood at the BC that can be later used by other patients.
Results: There are 1,786 PHCs located in 16 provinces, 73 districts with high maternal mortality that made collaboration work with the BCs and hospitals and some of them have been trained for donor recruitment and selection. The impact of this program on blood supply will be evaluated in 2017.
Conclusion: Indonesia has high maternal mortality that is mostly due to haemorrhage. The unavailability of blood contributed into 9% of the maternal mortality. Increasing people's awareness and willingness on donating blood voluntarily at the village level is believed to increase blood supply in the BCs and to prepare for the post partum haemorrhage.

6A-S44-02

No abstract available.

6A-S44-03

USING MAJOR HAEMORRHAGE PRESCRIPTIONS TO IMPROVE DELIVERY – A 3 YEAR AUDIT OF UTILISATION OF A MAJOR HAEMORRHAGE PROTOCOL IN A UK MAJOR TRAUMA CENTRE

RS Hawes, J Orr, S Avery, H Watson, Y Scott and J Wallis

Royal Victoria Infirmary, Newcastle upon Tyne, United Kingdom

Background: Major haemorrhage remains the leading cause of death in trauma, which has a large economic burden in the UK. Recent British military experiences in Afghanistan have highlighted the importance of early aggressive management of major haemorrhage with the aim of preventing the development of acute coagulopathy of trauma, which results in significantly morbidity and mortality. Recent civilian studies have provided further evidence showing improvement in outcomes using optimised transfusion ratios which has led to the development and introduction of Major Haemorrahge Protocols (MHP) in most UK Major Trauma Centres (MTC). A review of major haemorrhage management in our MTC in 2012–2013 highlighted good mortality outcomes but suggested that transfusion ratios could be optimized and identified that what was delivered to the clinician in the MHP was not always administered to the patient. Several reasons were highlighted, including the lack of clarity for recording administered products in an emergency situation involving large transfusions (>10 RBC).
Aims: This study aims to review the introduction of simple 'Human Factors' interventions including pre-thawed FFP, and a 'major haemorrhage prescription' template and their effect on accurate administration of the MHP, blood product transfusion ratios and patient outcomes over a 3 year period.
Methods: We reviewed the management of all 174 adult trauma patients admitted via the Emergency Department who were managed using the MHP or who received group O emergency blood in the Emergency Department in the 3 year period from April 2012 to March 2015. Patients <18 years old and concomitant medical event were excluded. Patient demographics, mechanism of injury, prehospital fluid volumes, blood products administered, pre and post resuscitation lab FBC, Coag, injury severity score (ISS), predicted survival (PS) and 30 day mortality were analysed and correlated with expected outcomes. Following the introduction of a major haemorrhage prescription, which was delivered to the clinician in the MHP cool box along with the products (and supportive 'human factors' teaching) the delivery of blood product ratios and patient outcome data was re-audited.
Results: Transfusion ratios of red cells (RBCs): fresh frozen plasma (FFP) deteriorated from 1.3:1 in April 2012 to March 2013 to 1.7:1 in April 2013 to March 2014 but then improved to 1.1:1 in April 2014 to March 2015 following the introduction of Major Haemorrhage Prescriptions. 30 day mortality reflected this pattern at 33%, 59% and 33% respectively. Correction of coagulation laboratory parameters particularly fibrinogen, PT and Haemoglobin also reflected this pattern but platelet count correction was poor in all three time periods. Admission thrombocytopenia, hypofibrinogenaemia and anaemia were associated with increased mortality. There was no clear relationship between use of pre-hospital fluids and coagulopathy. Use of the MHP increased and use of emergency group O blood reduced over the 3 year time period.
Conclusion: Optimised transfusion ratios were associated with improved mortality and improved post resuscitation PT and Fibrinogen within our cohort of trauma patients with major haemorrhage. Transfusion ratio of RBCs: FFP and mortality have improved in April 2014–March 2015 compared to the previous year. This appears to have been related to use of a major haemorrhage prescription template associated with educational intervention.

6A-S44-04

IN-HOSPITAL MORTALITY OF CRITICALLY BLEEDING PATIENTS REQUIRING MASSIVE TRANSFUSION IN AUSTRALIA AND NEW ZEALAND

R Ruseckaite[1], ZK McQuilten[2], JC Oldroyd[2], KM Venardos[2], TH Richter[2] and EM Wood[2]

[1]Transfusion Research Unit, Mebourne, Australia [2]Transfusion Research Unit, Monash University, Melbourne, Australia on behalf of the Australian and New Zealand Massive Transfusion Registry Steering Committee

Background: Massive transfusion (MT) given in response to critical bleeding, following trauma, surgery and childbirth, is potentially lifesaving. However, evidence to guide MT remains limited and few data exist on patient characteristics and outcomes following MT. The Australian and New Zealand Massive Transfusion Registry (ANZ-MTR) generates observational data on MT management and outcomes in patients receiving MT in any clinical setting.
Aims: To describe patient characteristics, transfusion support, and short-term clinical outcomes from MT.
Methods: All adult patients receiving a MT [≥5 units red blood cells (RBC) in 4 h (h)] were identified at 20 participating ANZ-MTR hospitals between April 2011 and March 2015. Patient data, including hospital admission data, transfusion history and laboratory results were extracted. Post-hospital discharge data were collected from the Australian National Death Index and New Zealand Ministry of Health to assess in-hospital and extended mortality status. Associations between patient characteristics and in-hospital mortality were modelled using multiple logistic regression. Patients were classified into three groups according to survival status: (i) Group 1 – patients who died in hospital, (ii) Group 2 – patients who died after hospital discharge, and (iii) Group 3 – patients who were alive until October 2015.
Results: A total of 3,560 MT patients were identified. Patients were predominantly male (62%). The vast majority (77.7%) of all MT cases were admitted to an intensive care unit. The majority of patients (69%) had at least one comorbidity, with a median (IQR) Charlson Comorbidity Score (CCS) of 2 [1–4]. Median (IQR) age of the study cohort was 63 (48–74) years. The same proportion of patients died in-hospital (n = 690, 19.4%) and after discharge (n = 687, 19.3%). Overall mortality was 38.7%. Patients who died in hospital were significantly older than those who survived [69 (87–78) vs 59 (42–72) years, $P < 0.05$]. For in-hospital deaths, cardiothoracic surgery was the most frequent bleeding cause for MT (24.5%) followed by trauma (18.3%).

Other non-specified type of bleeding (30.4%) and gastrointestinal haemorrhage (22.1%) was the most frequent cause of MT in those who died after discharge. Factors independently associated with in-hospital mortality were age (OR = 1.03; 95% CI 1.02–1.04); CCS (OR = 1.17; 95% CI 1.10–1.24); larger volume of RBC in the first 4 h post-MT onset (OR = 1.07; 95% CI 1.04–1.08), higher platelet to RBC ratio at 4 h post-MT onset (OR = 1.82; 95% CI 1.02–3.24) and highest International Normalized Ratio (OR = 1.27; 95% CI 1.17–0.137).

Conclusions: This study describes characteristics of critically bleeding patients from a large group of diverse hospitals and clinical contexts across Australia and New Zealand. This study found that patients in the ANZ-MTR who die in hospital are generally older and with more comorbidities, with cardiothoracic surgery and trauma accounting for most deaths. Volume of transfused blood products was strongly associated with in-hospital mortality. The ANZ-MTR provides a unique opportunity to explore differences in MT patient profile, clinical management and blood utilisation and short and long-term outcomes. Future studies will be conducted to better understand hospital preparedness for MT. Additional ANZ-MTR data linkages will allow better understanding of how bleeding is managed in specific patient populations.

Bacterial Detection

6A-S46-01

RBC ADHESION MOLECULES AND VESICULATION ARE ESSENTIAL FOR EFFICIENT IMMUNE ADHERENCE CLEARANCE

R van Bruggen[1], DZ de Back[1], EB Kostova[1], TRL Klei[1], BM Beuger[1], R van Zwieten[1], TW Kuijpers[2], TK van den Berg[1], D de Korte[1] and M van Kraaij[1]

[1]Sanquin, Amsterdam, The Netherlands [2]Academic Medical Center, Amsterdam, The Netherlands

Background: Red blood cells (RBC) are capable of binding complement-opsonized pathogens via complement receptor 1 (CR1, CD35, Knops blood group antigen). It has been known since the 1950s that this immobilization of bacteria on RBC facilitates pathogen phagocytosis, a process that has been termed immune adherence clearance (IAC). Thus, the ability of RBC to not only bind complement-opsonized pathogens and immune complexes but also deliver them to macrophages in the spleen and liver where phagocytosis takes place, can serve as an important host defense mechanism against pathogens. Even though IAC was discovered decades ago, there is little information on the exact molecular mechanisms involved in the process. To understand the underlying mechanisms of IAC, we developed an assay to monitor the transfer of opsonized pathogens from RBC to human monocytes and macrophages under flow conditions.

Aims: In this study we aimed to unravel the interactions that take place between RBC and phagocytes during transfer of pathogens bound by RBC.

Methods: The transfer of pathogens bound by RBC to peripheral blood monocytes and macrophages isolated from human spleen was studied under flow by confocal microscopy. Various pathogens were used in this study including S. aureus, E. coli, S. typhimurium and C. albicans. To assess the transfer of membrane of the RBC during phagocytosis of the pathogen by the monocytes/macrophages the RBC membrane was labeled with the fluorescent dye PKH26. To investigate the role of different membrane proteins on the RBC or the phagocytes, blocking antibodies were used. On one occasion, monocytes, derived from an individual that lacks β2 integrins, were used to establish the role of β2 integrins in this process.

Results: We have gained evidence that RBC are actively involved in IAC. RBC vesiculation coincides with pathogen transfer and pathogen transfer can be inhibited by fixation of the RBC membrane. Furthermore, we identify β2 integrins on the phagocyte to be essential for the binding of RBC-pathogen complexes and subsequent transfer of the pathogen to the phagocyte. Moreover, we found that blocking antibodies directed against ICAM-4 (Landsteiner Wiener (LW) blood group) and CD147 (Basigin, Ok blood group) on the RBC inhibit efficient transfer of pathogens bound by human RBC to phagocytes under flow.

Conclusion: Transfer of pathogens bound by RBC to phagocytes is dependent on β2 integrins expressed by the phagocyte. In addition, we demonstrate that RBC vesiculation is essential for transfer of pathogens from RBC to monocytes and macrophages. Finally, we propose that CD147 and ICAM-4 play an essential role in the efficient transfer of pathogens by mediating the interactions between RBC carrying pathogens and phagocytes under flow.

6A-S46-02

RAPID BACTERIAL DETECTION IN BUFFY COAT DERIVED POOL-PLATELETS AND APHERESIS PLATELETS BY BACTIFLOW AND NAT, FIVE YEAR EXPERIENCE

K Hourfar, K Gubbe, B Rüster, K Frank, A Karl, T Tonn, U MayrWohlfart, H Schrezenmeier, E Seifried and M Schmidt

German Red Cross, Frankfurt, Germany

Background: The residual risk of bacterial contamination of blood components is approximately 1 to 2 log periods higher than that for virus infections. Especially platelets which are stored at room temperature represent good growth conditions for a broad range of bacteria strains. In order to improve blood safety the shelf life of platelets was limited to 4 days in 2008 in Germany. Next to the introduction of pathogen inactivation systems the German regulatory authority (Paul-Ehrlich Institute) allowed testing by rapid bacterial detection systems like NAT or Bactiflow to improve blood safety and to re-extend the shelf life to a maximum of 5 days.

Aim: The aim of this study was to show the routine experience of the last 5 years by using an inhouse 16s DNA NAT system and to evaluate the Bactiflow method in individual donation testing, in mini-pools of 5 or 10. Validation of Bactifow method was done using seven transfusion relevant bacterial strains in buffy coat derived pool-platelets (BC-platelets) as well as in apheresis platelets (A-platelets).

Methods: For routine testing (2012–2016) samples from BC-platelets or A-platelets were collected 49 h after blood donation and pooled into mini-pools with a maximum pool size of 10 samples per pool. Mini-pools were screened by using a NAT system targeting the the 16s DNA region.

For the Bactiflow validation study BC-platelets as well as A-platelets were screened for bacterial contamination at day 0 by BacT/ALERT and by Bactiflow. Negative platelet bags were spiked in independent experiments with a low bacterial concentration of 0.03 CFU/ml with *Bacillus cereus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia marcescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes*. Test samples were collected after 48 h, 72 h and 96 h. All experiments were done for BC-platelets in replicates of five and for A-platelets in duplicate. The minipool bacterial detection method Bactiflow was validated at three screening sites (Frankfurt, Plauen and Ulm) of the German Red Cross blood donor service Baden-Wuerttemberg – Hesse and North-East.

Results: Bacterial detection was 100% on individual donation, mini-pools of five and mini-pools 10 for BC-platelets and A-platelets without any exception. Data were comparable at all three test sites. Robustness tests demonstrate that test samples can be shipped for 24 h at 4°C as well as at 21°C without impacting detection of bacterial contamination. Over the last 5 years in total 60,000 platelets were tested by NAT (Table 1). Five platelet concentrates were confirmed positive for bacterial contamination, which represents a yield rate of 1 in 12,000.

Conclusions: The rapid bacterial detection method Bactiflow was able to detect transfusion medicine relevant bacterial strain in mini-pools up to a maximum pool size of 10 samples per pool either in BC-platelets as well as in A-platelets and is comparable to the 16s DNA NAT system. The introduction of rapid bacterial detection systems enables blood donor services to detect bacteria in platelets before products are release. After introduction of the rapid bacteria screening no TTBI fatalities were observed. Rapid bacterial tests convinced with a high efficiency and are cost-effective.

	Frankfurt	Plauen	Ulm	Total
NAT tested	23040	19771	9284	
POS	2	2	1	5
Incidence	0,004340278	0,005057913	0,010771219	
Bacterial strain	*S. epidermidis + S. warneri*	E.coli, S. aureus	*S. aureus*	
	S. aureus			

Table 1.

6A-S46-03

COMPARISON OF BACTERIAL CONTAMINATION RATES OF PLATELET CONCENTRATES PREPARED FROM BLOOD DONATIONS ON THE DAY OF COLLECTION AND AFTER HOLDING OVERNIGHT

NK Chan, CK Wong, RWM Lo, VKY Lee, KK Lai, CMY Chan and WC Tsoi

Hong Kong Red Cross Blood Transfusion Service, Hong Kong, China

Background: The Hong Kong Red Cross Blood Transfusion Service has adopted the practice of blood component preparation from blood donations after holding overnight at 20–24°C since 1996. While such practice of blood component preparation

Table 1: Bacterial Contamination Rates of Platelet Concentrates (April 2009 to April 2016)				
(a) Platelet Concentrates Prepared from 450-mL Blood Donations				
	No. of units tested	No. of units confirmed bacterial culture positive	Bacterial contamination rate (%)	p-value
Day 0 PC	72,562	3	0.0041	0.70
Day 1 PC	769,056	40	0.0052	
(b) Platelet Concentrates Prepared from 350-mL Blood Donations				
	No. of units tested	No. of units confirmed bacterial culture positive	Bacterial contamination rate (%)	p-value
Day 0 PC	37,024	1	0.0027	0.85
Day 1 PC	302,537	10	0.0033	
(c) Platelet Concentrates Prepared from Both 450-mL and 350-mL Donations				
	No. of units tested	No. of units found bacterial culture positive	Bacterial contamination rate (%)	p-value
Day 0 PC	109,586	4	0.0037	0.64
Day 1 PC	1,071,593	50	0.0047	

offers logistic convenience to the operation of blood centres, it may give rise to concerns about possible increase in bacterial contamination of blood components, in particular platelet concentrates, prepared from blood donations after overnight hold.

Aims: To conduct a retrospective analysis to compare the bacterial contamination rates of platelet concentrates prepared from blood donations on the day of collection and after overnight hold.

Methods: Routine pre-release bacterial surveillance of platelet concentrates by BacT/ALERT 3D automated blood culture system (bioMérieux, Durham, NC, USA) has been implemented since 1998 (Liu HW, et al. Vox Sang 1999; 77:1–5). Initial positivity in the pooled sample was subject to resolution and confirmation by repeat culture of samples taken from individual platelet concentrates and/or other blood components of the implicated initial positive pool/donations. Bacteria isolated from confirmed positive samples were subject to bacterial identification. For the purpose of this study, bacterial culture results of platelet concentrates during the period from 19 April 2009 to 11 April 2016 were retrieved from database. The bacterial contamination rates of two groups of platelet concentrates prepared from blood donations (i) on the day of collection and (ii) after holding overnight were computed and analysed by the z-test. A P-value < 0.05 was considered as statistically significant.

Results: The bacterial contamination rates and P-values of platelet concentrates prepared from 450-, 350-ml and both 450 + 350 ml blood donations on the day of collection (Day 0 PC) and after holding overnight (Day 1 PC) were shown in Table 1. The bacterial contamination rates of platelet concentrates prepared from blood donations (450 + 350 ml together) on the day of collection and after overnight hold were 0.0037% and 0.0047% respectively. The P-value was 0.64.

Conclusions: As all the P-values were >0.05 at 95% significance level, there is no signicant difference between the bacterial contamination rates of platelet concentrates prepared from blood donations regardless of blood volume on the day of collection and after overnight hold (not more than 24 h) at 20–24°C. Our retrospective study on bacterial contamination rates of more than 1 million platelet concentrates provided strong evidence that there was no increase in risk of bacterial contamination of platelet concentrates prepared from an overnight storage of blood donations at 20–24°C. This was consistent with the findings of similar studies reported in the literatures that overnight hold of whole blood would potentially reduce risk of bacterial contamination due to ingestion of contaminating bacteria by leucocytes in the whole blood during ambient storage. Therefore, increase in risk of bacterial contamination in platelet concentrates is not a factor to consider when planning to implement overnight hold of blood donations for preparation of blood components.

6A-S46-04

COMPARISSON OF TPHA RESULTS 6 MONTHS PRE AND POST REAGENT CHANGE FOLLOWING THE EVALUATION OF FOUR TPHA REAGENTS

WA Sykes, C Coleman, C Mmenu, L Ford, J Mhlanga, N Nyuswa and M Vermeulen
SA National Blood Service, Durban, South Africa

Background: The South African National Blood Service (SANBS) tests all donations for Treponema Pallidum (TP) antibodies using a TP Haemagglutination (TPHA) reagent on the Beckman Coulter PK7300 instrument. Prior to the evaluation Axis Shield TPHA supplied by Bioweb in South Africa had been in use since 2000.

Supplier	Period	Donations Tested	TPHA Rep Pos	% RR	TPHA Conf Pos	% Conf Pos	Specificity	VD Pos	% VD Pos	% RR = Conf	% Conf = VD Pos	% RR = VD Pos
Bioweb	April – Sept 15	429449	695	0.162	692	0.16	99.999	195	0.05	99.57	28.18	28.05
BioRad	Oct 15 - March 16	415588	1587	0.382	1408	0.34	99.957	284	0.07	88.72	20.17	17.90
Difference		-13861	892	0.22	716	0.18	0.04	89	0.02	-10.9	-8.01	-10.15

Table 1. Results for the 6 months pre and post reagent change

As part of a tender process four TPHA Reagents were evaluated in 2015; Axis Shield TPHA (Bioweb), Spin React TPHA Automatic (Sitetech), BioRad OC2000, and Micro-Trak Syphilis (Scientific Group).

Sensitivity was calculated using panels supplied by the four suppliers and tested on each of the reagents as 88.89%, 94.44%, 94.44% and 94.44% respectively. Sensitivity to IgG antibodies was 100% for all reagents and to IgM antibodies 33.33%, 66.67%, 66.67% and 66.67% respectively. 12 FTA positive donor samples gave a comparative sensitivity of 16.67%, 75%, 75% and 100% respectively. IgG reactivity indicates past infection and IgM represents active infection.

Specificity was calculated from 3,588 negative donor samples as 100%, 99.89%, 100% and 99.80% respectively.

The Biorad OC2000 assay was recommended and implemented in October 2015.

Aims: The aims of this study were to compare data from the 6 months before and 6 months after changing to the Biorad reagent to establish if what was seen in the evaluation carried through to laboratory testing of donor samples.

Methods: Results from two periods April to September 2015 (Bioweb) and October 2015 to March 2016 (Biorad-OC2000) were compared. Sensitivity, specificity and proportion of active infections were calculated. Chi Square statistics were used to assess significance.

Results: During the respective 6 month periods under review 429,449 and 415,588 donor samples were tested on Bioweb and Biorad. The specificity was 99.99% and 99.96% respectively. There were 695 (0.162%) and 1,587 (0.38%) repeat reactives (RR), of which 692 (0.161%) and 1,408 (0.34%) confirmed positive and195 (0.05%) and 284 (0.07%) tested VDRL Positives (Table 1).

Using Axis Shield, 28% of TPHA RR were also VDRL reactive compared to 17.9% using Biorad.

Summary/Conclusion: In the evaluation Bioweb and Biorad reagents showed equal IgG sensitivity (100%). IgM sensitivity using Bioweb was significantly less at 33.3% than Biorad at 66.7%. This difference is particularly relevant in a transfusion environment as IgM antibodies indicate a recent/active infection. Specificity was 100% for both reagents.

In the 6 months following implementation of Biorad OC2000 TPHA, confirmed TPHA reactives increased from 0.161% to 0.339% ($P < 0.05$). Active infections (VDRL positive) increased from 195 (0.05%) to 284 (0.07%) indicating an increased sensitivity for both IgG and IgM with Biorad.

The proportion of active infections detected by Biorad was significantly less in relation to TPHA repeat reactives ($P < 0.05$) due to the increased sensitivity of IgG positives.

Specificity decreased by 0.04%. This will result in an additional 332 false positives per annum. Although the increase in RR and IgG positives will lead to an additional 0.22% of units being discarded an additional 0.02% VDRL positive units will have been detected and have prevented the issue of 89 potentially infectious units during the 6 months under review.

6A-S46-05

EXPERIENCE FROM THE IMPLEMENTATION OF THE REVERSE SYPHILIS SCREENING IN GREEK BLOOD DONORS

M Pape, A Konstantinidou, V Bakaloudi, E Ntinopoulou, F Girtovitis, M Chatzikyrkou, D Pantelidou, P Chalkia, V Papageorgiou, D Gountias and E Hasapopoulou-Matami

Ahepa University Hospital, Thessalon Iki, Greece

Background: Current Greek guidelines for serological screening for syphilis in blood donors recommend starting with a non-treponemal test [rapid plasma reagin (RPR)] followed by confirmatory treponemal tests [fluorescent treponemal antibody absorption test (FTA-ABS) and T.Pallidum haemagglutination assay (TPHA)].
However, the ease of performing all five obligatory tests (HBsAg, anti-HCV, anti-HIV1/2, anti-HTLV I/II, syphilis) on an automatic analyzer, prompted us to install reverse screening for syphilis (treponemal screening test) last year. Additional advantages would be to avoid false negative RPR results that occur in early primary syphilis, latent phase and treated secondary cases (or due to technical errors and the prozone phenomenon), as well as false positive results due to cross reactivity.
Aims: The aim of our study was to evaluate the consequences of performing reverse screening for syphilis.
Methods: We analyzed data on 22,369 voluntary blood donors, including first-time and repeat donors, all apparently healthy, who gave blood in 2015. Blood samples were tested on the Architect platform for treponemal syphilis antibodies by a highly sensitive chemiluminescent microparticle immunoassay (CMIA) that qualitatively detects IgG/IgM. Commercial negative and positive control reagents were routinely used as recommended by the manufacturer. Samples with S/CO values ≥1.00 were considered reactive (*R*). In order to maximize the safety of blood supply, samples with S/CO ratios between 0.70 and 0.99 were scored as grey zone (GZ) and subjected to the same protocol as *R*, that includes a new centrifugation and repeat testing in duplicate by CMIA. If repeat testing was positive or in the grey zone, the non-treponemal RPR test was performed in serial dilutions (to avoid false negative results due to pre-zone phenomenon) as well as confirmatory tests (FTA-ABS and TPHA) to assess the stage of the disease and take therapeutic decisions. A positive confirmatory test with negative RPR, would indicate past, successfully treated or latent syphilis.
Results and conclusions: Before the introduction of the reverse syphilis screening (2012–2014) *0.016–0.089% donors tested positive by RPR. This figure rose to* 0.17% (39/22,369) when reverse screening was performed (during 2015).
Among positive screening results, 0.085% (19/39) tested positive by at least one confirmatory test.
Reverse screening apparently results in higher seroprevalence compared with the traditional screening (RPR), due to its capacity to detect different stages of the syphilis infection, including cases unlikely to be diagnosed by nonspecific tests.
The relevance of a confirmed positive syphilis test result in an apparently healthy blood donor, is an issue. By reverse screening cases of past successfully treated infections are expected to be positive at all subsequent donations, with either positive or negative confirmatory testing. Therefore, for the more accurate determination of the stage of the infection, it might be necessary for blood bank laboratories to additionally perform IgM antibody testing.

Plenary Session V: Future Direction in Blood Transfusion

PL5-01

CULTURED RED BLOOD CELLS FOR TRANSFUSION, HOW FAR ARE WE?

E van den Akker

Sanquin, Amsterdam, The Netherlands

Erythrocyte transfusion is the most common form of cellular therapy. It rapidly restores the oxygen supply to the tissues in patients suffering from sudden blood loss, reduced erythrocyte production following for instance cytotoxic cancer treatment, or in patients suffering from chronic anaemia due to congenital diseases such as sickle cell anaemia. Generally, matching for ABO and Rh blood group antigens is common practice and sufficient for most transfusions. However, over 200 additional blood group antigens are known, belonging to in total 34 blood group antigen systems and unmatched transfusions can result in alloimmunisation. In fact, chronic exchange transfusion in sickle cell patients results in >17% allo-immunization. Once allo-immunized, it becomes difficult to find appropriate donor erythrocytes, especially when multiple antibodies or rare combinations of antibodies are present. This problem becomes increasingly urgent in an aging population where the transfusion need increases and the donor population decreases, and in a multicultural society in which the population at risk for chronic anaemia, for instance sickle cell disease, does not match with the general Caucasian donor population. Besides allo-immunization due to blood group mismatches, also exposure to unknown or ill-screened blood borne diseases and a minimal availability of specific blood group donors are all important reasons to start research into the production of *in vitro* cultured customizable red blood cells (cRBC). Importantly, even though cultured erythrocytes may initially only be required for a small percentage of transfusions, the number of yearly transfusions is so large that the availability of erythrocytes with rare blood groups for allo-immunized patients constitute may solve a major health problem. Particularly sickle cell disease patients would benefit immediately from better matched or autologous cRBC because of the increased risk of stroke in SCD patients with low hemoglobin. Several research institutes, mostly associated with Blood supply centres, are developing protocols to culture RBC for transfusion purposes. Primary human erythroblast cultures can be expanded from hematopoietic stem cells to large numbers, and differentiated to functional hemoglobinized enucleated red blood cells (RBC) that express the appropriate blood group antigens. However, many challenges remain to be solved: (i) from which source, e.g. immortalized erythroblasts, induced pluripotent stem cells or hematopoietic stem cells, should we expand erythroblasts, (ii) how can we render the expansion phase efficient taking into account the unprecedented upscaling required, (iii) can we regulate the expression of foetal vs adult hemoglobin, (iv) can we obtain a functional transfusion product comparable to erythrocytes, and (v) should we aim to transfuse autologous or allogeneic. Here we will address and discuss these issues, indicate the state of the art of *ex vivo* produced transfusion-ready red cells and address the different strategies that are currently taken.

PL5-02

ETHICAL ASPECTS OF MEDICAL PRODUCTS OF HUMAN ORIGIN

DE Martin

Deakin University, Geelong, Australia

Medical products of human origin (MPHOs) are 'substances derived wholly or in part from human biological materials and intended for clinical application'. In 2014, the World Health Organization (WHO) launched an Initiative for MPHOs. One of the primary goals of this Initiative is to establish global consensus on core principles that should guide policies and practices involving MPHO, particularly with regards to the procurement, distribution and use of these products. Like any medical products with therapeutic value for humans, the sufficiency of their supply, risks and benefits for recipients, and equity in their allocation present ethical concerns for health policy makers, professionals and patients. Unlike other medical products, however, MPHO present additional ethical concerns because their source materials are procured from living or deceased human beings.
The clinical and psychosocial risks and benefits of donating specific materials for use in MPHOs vary according to characteristics of the donor, the material procured, and the procurement process used. Risks and benefits, for example, continue to evolve as new procurement technologies, standards of donor care, and new donor populations emerge. Nevertheless, several core ethical concerns remain constant, distinguishing MPHO as 'exceptional' health products. They include concerns about donor autonomy, duties of care towards donors, commercialization of 'donation', and inequities in donor populations. I review these issues in this paper using illustrative examples from the history of medical products derived from the various components of human blood.
I also outline the rationales for seeking global consensus in the light of the WHO Initiative for MPHOs and discuss some of the challenges inherent in achieving consensus. Finally, I review principles that might justifiably be included in a global ethical framework for governance of MPHO and briefly discuss their potential implications for policy and practice.

PL5-03

PATIENT BLOOD MANAGEMENT – IMPROVES RED BLOOD CELL USAGE AND PATIENT SAFETY

K Zacharowski[1], P Meybohm[1], E Herrmann[2], AU Steinbicker[3], M Wittmann[4], M Gruenewald[5], D Fischer[1], G Baumgarten[4], J Renner[5], HK van Aken[3], CF Weber[1], M Mueller[6], C Geisen[6], J Rey[2], D Bon[2], G Hintereder[1], S Choorapoikayil[1], J Oldenburg[4], C Brockmann[5], R Geissler[3] and E Seifried[6]

[1]University Hospital Frankfurt, Frankfurt, Germany [2]Goethe University Frankfurt, Frankfurt, Germany [3]University of Muenster, Muenster, Germany [4]University Hospital Bonn, Bonn, Germany [5]University Hospital Schleswig-Holstein, Kiel, Germany [6]German Red Cross Blood Transfusion Service Baden-Wuerttemberg – Hessen, Frankfurt, Germany

Patient Blood Management (PBM) is a concept that emerged from the need to improve patient safety and is promoted by the World Health Organization encouraging all member states to implement PBM programs. These include for example to increase and preserve autologous erythrocyte volume that in turn minimize the demand for red blood cell (RBC) transfusion. To date, statistically powered data regarding safety issues are not sufficiently available. We performed a prospective, multicenter, controlled, non-inferiority cohort study including more than 120,000 patients. Preoperative optimization of hemoglobin levels, implementation of blood sparing techniques and standardization of transfusion practice amongst others reduced not only the number of transfused RBC units but also the incidence of acute renal failure.

The profound and sustainable implementation of new standards of care, however, is an immense task. Risk perception, clinical knowledge and perioperative practice need to be assessed and modulated by a multidisciplinary team. Based on our experience we suggest following key strategies: (i) Identify subject matter experts and local opinion leaders within your organization to provide guidance. (ii) Identify elective procedures where patients will benefit from early anemia screening and therapy. (iii) Appoint local guideline implementation teams to teach and maintain the program's components. (iv) Design and provide educational material for regular training programs on PBM for medical school, continuing professional education in the residency curriculum. (v) Create check-lists on PBM measures for the walk-in-clinic and operation rooms. (vi) For the new standards to be practical and feasible, assess and adapt infrastructure as necessary, including the preoperative flow of patients. (vii) Foster team spirit and corporate identity by distributing marketing gadgets (such as PBM-buttons etc.). (viii) Standardize performance metrics and data collection to allow valid benchmarking within your hospital as part of ongoing professional practice evaluation. Used as feedback, these data will keep the motivation high and might induce healthy competition.

In conclusion: Our study show that implementation of PBM with a more conscious handling of transfusion practice can be achieved even in large hospitals without impairment of patient's safety. To ensure successful implementation of PBM a concept should be developed that fits into your hospital.

Posters
Management and Organisation: Organisational issues

P-001

DETERMINATION OF THE RATE AND CAUSES OF WASTAGE OF BLOOD IN A DEVELOPING COUNTRY LIKE BANGLADESH, A THREE YEAR STUDY

SS Miah

Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh

Background: A developing country like Bangladesh is always facing challenges in the world of blood transfusion. The growing health issues are more concerned to furnish the demands in applying blood as a remedy. 40–60% of whole blood and components are regularly used up by the patients and therefore also return back owing to rescheduling of operation or not utilized in time. But when this blood remains for a longer period of time in normal temperature that is unfortunately sometimes unreliable to be used up for the next time. Occasionally, the patients party collect unscreened blood from the remote area that could not be used up in time and has to be discarded promptly. Component wastage is more because of the ignorance of the traditional physicians for using only the fresh whole blood. These all issues need to be focused more to get back from the entire loss.

Aims: The aim of this study is to notify the way of wastage of blood components and the foremost reasons stay behind for that .

Materials and methods: This is a retrospective, analytical study based on the statistical data available from hospitals blood banks. In this study total bloodletting from RD was18,858 to 22,167 in the last 3 year. Total components prepared 18,179 to 21,329. Among them RCC 27.56–35.65%, FFP 26.99–35.41%, PC 27.59–35.34% and PRP 0–0.68% were prepared.

Results: The leading reasons included time expiry, wasted imports, blood medically or surgically ordered but not used. Data indicated that total wastage were 5.31–7.28% in these years. Amid this 9.47–26.81% were platelet concentrate; most of prepared from RD and wasted mainly due to time expiry. Packed cell wastage were 0.41–3.03% due to collection in pediatrics bags, expired dates, formation of micro clots, failure of transfusion for bacterial contamination and haemolysis. Whole blood wastage were 0.2–2.2% as formation of bulky clot owing to inappropriate mixing with anticoagulant solution during collection. Plasma wastage were 0.26–1.52%.Therefore, 1.20% were cracking in blood bags due to mishandling by unskilled passing and 0.32% were blocking of the flow during transfusion.

Conclusion: Modern blood transfusion requires proper storage, rational use as well as wise distribution. To reduce this wastage, the entire transfusion team should recognize the problems in a comprehensive and coordinated way. Blood component wastage is a major concern as we don't have adequate supply of blood and a reputable donor pool. The traditional physicians should also change their perception to use the conventional remedy which is only based on practicing whole blood. This is a common barrier that should be changed as quickly as possible. Consequently, if suitable actions are taken for the above distinguishing reasons then it will be possible to save more blood and the limited capital of this poor state.

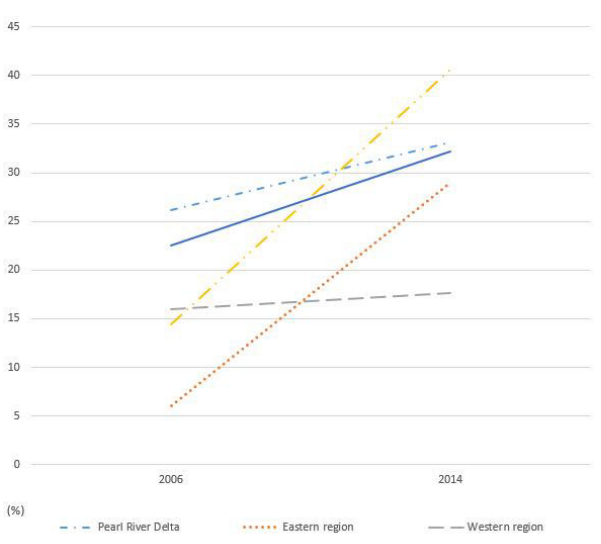
P-002

BLOOD DONATION IN GUANGDONG PROVINCE, CHINA, FROM 2006 TO 2014

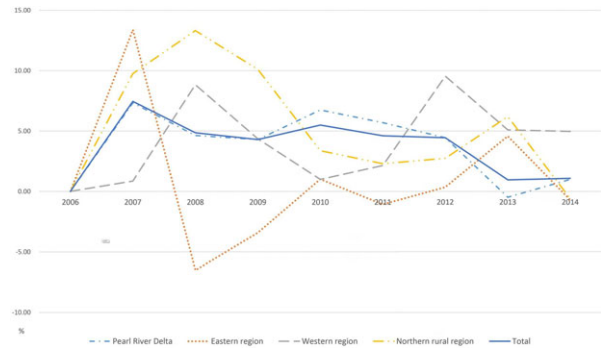
J Ouyang and C Bei

Guangzhou Blood Center, Guangzhou, China

Background: Although the Blood Donation Law of the People's Republic of China was implemented in 1998, voluntary non-remunerated blood donation (VNRBD) has been promoted fully for only a decade. The provincial and local governments of Guangdong, one of the most well-developed provinces in China, have promoted blood donation by various means.



Caption 1. Rates of regular blood donation in 2006–2014 by region



Caption 2. Annual rates of increase in all types of blood donation in 2006–2014 by region

Aims: To explore the trends in blood collection from 2006 to 2014 in Guangdong, China.
Methods: Official data on blood donation from 2006 to 2014, including the number of blood donations and the family replacement/mutual blood donation rate, were collected from all blood collection and supply institutions in Guangdong. These data were analyzed to explore trends in blood donation in Guangdong Province, and to detect differences among the province's four regions.
Results: The number of blood donations in Guangdong increased 38.23% from 2006 to 2014; overall, the rate increased annually, although it fluctuated in the eastern region. Family replacement/mutual whole blood and platelet donation rates decreased dramatically from 2006 to 2014 (from 39.99% to 20.16% and from 64.15% to 26.51% respectively), but remained high. Marked disparities in blood donation development were detected among the four regions.
Summary: With nearly a decade of efforts, blood donation in Guangdong has developed rapidly and sustainably. All blood collection and supply institutions must strengthen efforts to improve awareness of blood donation among the population, retain repeat and regular donors, and reduce the rate of family replacement/mutual blood donation in favor of the development of VNRBD.

P-003

This abstract has been withdrawn.

P-004

LABORATORY PERFORMANCE IMPROVEMENT, PREDICTING THE FUTURE VIA SIMULATION THE POWER OF CERTAINTY, ADDRESSING LEAN PITFALLS BY PROVIDING CERTAINTY AND CONFIDENCE IN STRATEGIC DECISION MAKING

FA Kloren[1] and M Vermeulen[2]
[1]*LTS Consulting, Bellville, South Africa* [2]*SANBS, Johannesburg, South Africa*

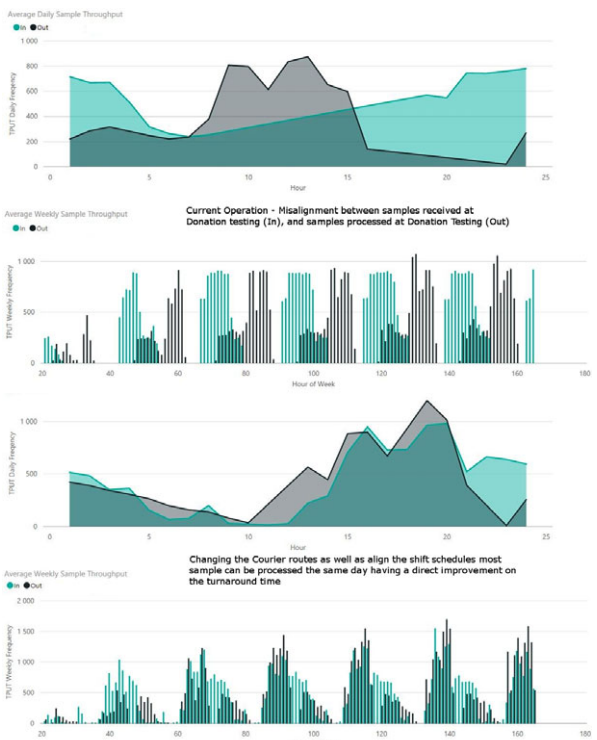
Background: The South African National Blood Service (SANBS) provides human blood for transfusion for most of South Africa. SANBS has 84 fixed donor centers, 3,000 mobile blood drives, seven processing zones and two donation testing sites. SANBS wants to improve their TAT for specimens from the zones to the testing centers to meet the platelet production pathogen inactivation requirements.
Improvement will have a positive effect on platelet shortages and potential wastage. This is a complex process influenced by a lot of different variables like the different zones, transport, laboratory testing capabilities, availability of samples and shift-schedules. Usually lean would be the methodology of choice to reduce waste. However, it is impossible with Lean to quantify the effect that individual changes in the different areas will have on the overall TAT.
Aims:

- Look at the entire operational process to provide end to end transparency;
- Identify bottlenecks and their impact on the specimen turnaround time (TAT);
- Provide solutions to improve TAT and select the best possible solutions;
- Ensure that the solutions provided are sustainable;
- Project time 2 months to provide results.

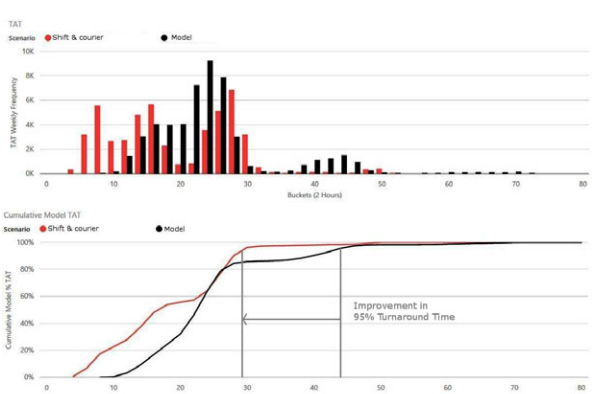
Methods: The PinpointBPS methodology was used for the SANBS project and is based on simulation of the process to identify bottle necks. Proposed improvement scenarios can be tested with the simulation model prior to implementation. The simulation provides answers on how changes in demand, workflow, operating hours, staffing, equipment, automation, etc. will impact on KPI's.
Advantages:

- Quantifies the impact of change with a high degree of certainty;
- Reduces risk by first assessing the impact of change in the model;
- Evaluates the complexity, variability and dynamic nature of real world problems;
- Simplifies and shortens implementation process;
- Ability to compare different solutions and technologies;
- Facilitates benchmarking.

Results: The PinpointBPS methodology identified multiple improvements in the complex operational process. After analyzing more than 1.5 million rows of data the simulation highlighted the following main process bottlenecks:



Caption 1. Scenario and Model



Caption 2. Overall turn-around time improvement

- Misalignment between sample arrival patterns and shift schedules;
- Unnecessary movement of specimens between different locations;
- The power of simulation enables us to look at the individual impact of the identified bottlenecks on the TAT as well as the combined impact. The results indicated that only changing the one bottleneck do not have the same impact as making multiple changes. It's essential to align the courier arrivals with the shift schedules to have an ideal enhancement in TAT. The simulation indicated that this improvement will reduce the overall TAT from 43.5 to 29.8 h.

Summary/Conclusions: CEOs and CFOs of healthcare organizations want to know if continuous improvement is going to give them a strong return on investment. Once they see the financial benefits (in addition to improved work processes), they will be more open not only to implementing continuous improvement but also sustaining it. Using Lean one would look at changing arrival patterns, implement changes, review and change again till satisfied, the same will apply for the shift schedules. Simulation eliminates trial & error to make decisions swiftly, with understanding and certainty. Testing potential changes in a safe environment eliminating the process of trial and error in real world simplifies implementation. It provides transparency into the strengths, and weaknesses as well as the connections between every process, person and piece of technology, close enough is no longer excusable.

P-005

APPLICATION OF SERVICE BLUEPRINT TECHNOLOGY IN OPTIMIZATION OF VOLUNTARY BLOOD DONATION SERVICE

JY Chen, SJ Li, RR Ke, FF Feng, YY Xu and YS Fu

Guangzhou Blood Center, Guangzhou, China

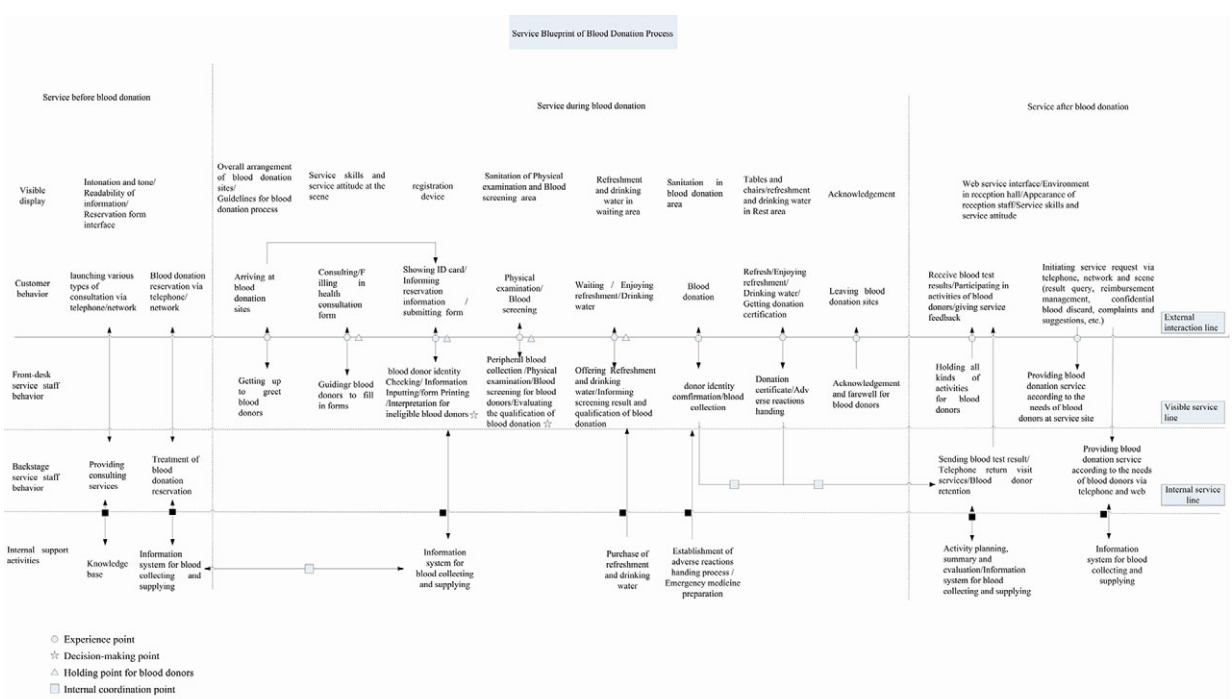
Background: High-quality service covering the whole process blood donation is important work of blood service organizations. At present, blood service organizations usually understand their own service quality through satisfaction of blood donors; however, this approach is hard to acquire the problems existing in service support links and internal employee collaboration as donors do not participate in these processes. The optimization of the whole process of voluntary blood donation service needs to be carried out with the aid of other tools. In the 1980s, American scholars Shostack et al. applied related technologies in industrial design, decision science, military logistics and computer graphics to service design and service management, and proposed the concept of 'service blueprint', which highlights the 'customer centered' idea, describes the picture or map of service system in detail and the service process using visualized chart from the view point of customers, providing a powerful tool for the optimization of service process in service industry.

Aim: Using the principles of service blueprint technology to draw a blueprint for voluntary blood donation service, searching and determining important points in order to optimize the service process and improve the quality of service.

Methods: The activities of blood donors and staff during the blood donation process were classified according to customer behaviors, service staff behaviors and support activities .The above parts were connected by the flow lines which to connect behavior,dividing lines–including interaction line between customers and service organizations, visible dividing line and internal interaction line– and visible display parts to draw a service blueprint for voluntary blood donation process.

Results: The drawn service blueprint for voluntary blood donation process revealed that above and below external interaction line was a direct contact between blood donors and staff and 10 experience points for blood donors were found there. As visible display was an important factor affecting blood donors' perception of service quality, we strengthened the overall layout of environment and ensured the clarity of process guidelines in related work. Four holding points for blood donors were also found in the external interaction line, which very easily led to dissatisfaction of blood donors, so we optimized work processes and established the dynamic adjustment mechanism of human resources, to ensure enough human resources at the scene in most of the time and to reduce the waiting time of donors as much as possible. Two decision-making points were determined, on which we strengthened training and improved the decision-making level of employees. There were three internal coordination points which needed strengthened cooperation among service departments. We optimized the work flow between departments with the help of information system. Additionally, we strengthened the input of human resource, realizing the seamless link of service work. There were seven support activity points, on which we communicated regularly with logistic and support departments to strengthen the guaranteeing role of supporting work in service work. After the process optimization, the total degree of satisfaction of blood donors increased by 5.3%, demonstrating a good effect.

Conclusion: Application of service blueprint technology to the management of voluntary blood donation service is a powerful tool for searching key points in blood donation service process for the optimization.



Caption 1. Service Blueprint of Blood Donation Process

P-006

THE CONTRIBUTION OF AVIS IN THE ITALIAN BLOOD SYSTEM

V Saturni

AVIS Nazionale, Milano, Italy

Background: Avis (Italian Voluntary Blood Donors Association) is probably one of the first blood volunteering associations in the world and the biggest one in Italy.
Our association has always played an essential role in the Italian transfusion system in order to grant access to whole blood, labile blood components and plasma-derived medicinal products coming from regular, voluntary, non-remunerated, responsible, associated donors.
Aims: Such a system represents a unique model of cooperation between different players: public institutions, hospitals and not for profit organizations. The Italian law recognizes the role of these associations as essential players for the national blood self-sufficiency.
Methods: Therefore, AVIS gives a strong qualitative contribution to this system through its involvement in the health and social planning of public institutions such as CNS, the National Blood Centre, the Welfare and Health Ministries. AVIS also gives an important contribution to blood collecting thanks to its self owned blood units and by directly contacting the blood donors, in order to plan their periodical donations. AVIS also promotes healthy lifestyles, the values of volunteering and solidarity and encourages the adoption of deferred criteria for the first blood donation.
Results: According to the most recent data published by CNS (National Blood Centre), in 2014 in Italy there were 1.712.456 blood donors (28.1 donor every 1,000 inhabitants)
Men: 69.3%
Women: 30.7%
Periodical donors: 1.426.187 (83.3%)
Donors at first donation: 286.269 (16.7%)
Total number of donations: 3.081.777 (50.7 every 1,000 inhabitants)
Whole Blood (84.0%): 2.587.869
/1.000 pop: 42,6 ‰
Apheresis (16.0%): 493.908
/1.000 pop: 8.1‰
(Plasmapheresis: 395.102)
Whole blood donations/whole blood donors/year: 1,6
Apheresis donations/apheresis donors/year: 2,1
Avis blood donors and donations
Donors: 1.288.322 (75.23% of the total number)
Donations: 2.022.763 (65.63% of the total number)
Whole Blood: 1.687.985
Plasmapheresis: 289.632
Other donations: 45.146
Conclusions: As we can see, AVIS plays a very important role in the Italian trasfusion system in order to grant self sufficiency of blood and hemo derivatives. However, one of the main challenges that Italy is currently facing is the growing number of elderly people. For this reason, AVIS must intensify its presence all over the Country and must strengthen its promotional campaigns towards the youngest generations, in order to spread the culture of solidarity among those who will be tomorrow's blood donors.

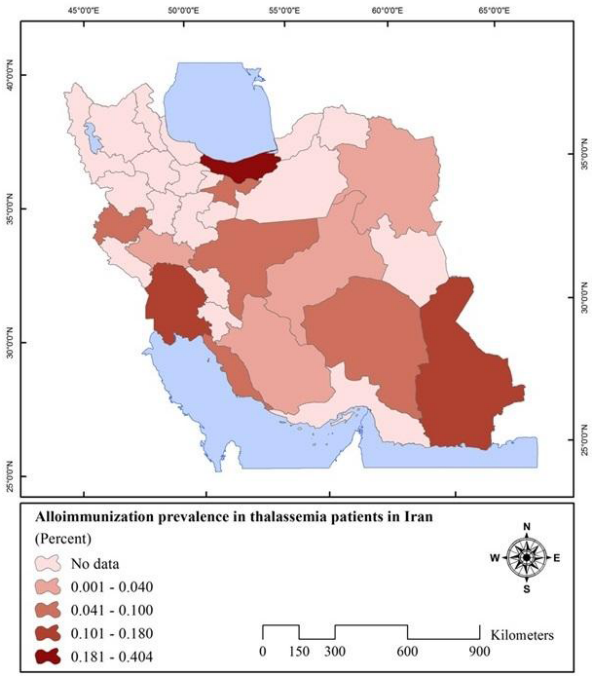
P-007

RED BLOOD CELL ALLOIMMUNIZATION IN IRANIAN BETA-THALASSEMIA PATIENTS: A SYSTEMATIC REVIEW AND META-ANALYSIS

P Darvishi[1], AA Pourfathollah[2], K Sayehmiri[3] and A Azarkeivan[4]

[1]Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran [2]Iranian Blood Transfusion Organization (IBTO), Tehran, Iran [3]Psychosocial Injuries Research Center, Ilam University of Medical Sciences, Ilam, Iran [4]Pediatric Hematology Oncology. Associated Professor. Transfusion Research Center, Tehran, Iran

Background: Red blood cell alloimmunization may be associated with hemolytic reactions and severe clinical consequences. The rate of alloimmunization in multi-transfused patients such as Thalassemia is high, that is a major challenge.
Aim: The present meta-analysis study was conducted to determine the prevalence of alloimmunization in Iranian Thalassemia patients.
Methods: This study was constructed based on the computerized literature database. English and non-English articles were searched in PubMed database, ISI Web of Science, Iranmedex, Magiran, Google Scholar, SID, Scientific Journal of Iran Blood Transfusion Organization (SJIBTO) and Directory of Open Access Journals (DOAJ) from 1994 to 2013. Alloimmunization rates and 95% CI calculated by random effect model. Statistical analyses were performed using STATA 11.2 and ArcGIS 10.3 was used for map construction.



Caption 1. Alloimmunization prevalence in Iranian thalassemia

Results: Eighteen papers from 297 studies involving 4,677 patients met our inclusion criteria. The prevalence of alloimmunization in Iranian thalassemia was 10% (CI 95%: 0.07–0.13%). The most prevalent alloantibody was anti-K (37%), followed by anti-D (29%) and anti-E (20%) in country.
Conclusions: Prevalence of alloimmunization in Iranian Thalassemia from 1994 to 2013 is not decreased. In spite of detecting D antigen in pre transfusion test, the rate of anti-D is high in our population. It shows the importance of more investigation on data on D variants. In spite of low alloimmunization rate in Iran compared to many other countries, some strategies should be taken to reduce it.

Information Technology

P-008

This abstract has been withdrawn.

P-009

BLOO DONOR IT SYSTEM IMPLEMENTATION

T Makarovska Bojadzjieva, E Velkova, L Mitevska, M Jakimovska and M Blagoevska

Institute of Transfusion Medicine, Skopje, Macedonia

Background: The attitude to quality has dramatically changed over the past decade in our Institution. Nowadays the existence of a blood bank software is an essential tool for the quality and safety of blood products.
Well known for developing blood bank software, Hemasoft has desined e-Delphyn Blood Bank which offers a complete data traceability from the Donor Recruitment and Collection until the definitive Patient Transfusion Confirmation ('Donor-to-Recipient' tracking system).
Aim: The aim of this work is to present the implementation process of e-Delphyn software at the Institute of Transfusion Medicine-Skopje.

Method: In the period from April 2015 to April 2016 the following activities toward the implementation of the software were conducted: technical equipment procurement, training of the master users; translation of the software messages in Macedonian language; configuration of the general, promotion, donor, laboratory, processing, inventory and distribution parameters; testing of the system functionality, training of the staff and finally start-up with the system *ex vivo* for 2 months.
Results: As an outcome of the training period we can present the following data: 28 blood components were defined, 22 user profiles were created for 84 trained users. In the trial period about 700 blood donors were registered and completely processed including labeling and issuing of the products to reveal any anomaly or malfunction of the system. The revealed nonconformities were mainly due to human errors concerning validation of bags and samples, or validation criteria concerning the test results. After additional training of the staff we started with the system *in vivo* on April, 13, 2016. The statistics for 2 weeks period revealed the following results: 1,202 registered blood donors (69% male, 30% female), 1,105 realized donations, 1,023 accepted donations, 69 (7%) deferred donors (35% male, 34% female) and 3,079 blood components processed (1,057, RBC, 1,056 FFP, 872 PLT, 75 CRYO).
Conclusion: According to the preliminary results, we believe that the IT system is going to improve every segment of the vein-to vein process as a reliable link between the blood donors, blood products and the recipients thus enabling an effective haemovigilance and high level blood safety.

P-010

THE EFFECTS OF CHANGES IN MOBILE BLOOD DRIVES AFTER THE INTRODUCTION OF MOBILE DEVICES

S Lee
Korean Redcross Blood Services, Gangwon-do, South-Korea

Background: Staff at mobile blood drives had limited access to information technology (IT) system that manages every process of blood services from collection to supply. In order to access to the system to know the real time status of mobile drives, staff members had to contact workers at the office or work overtime. With the advanced IT and proliferation of mobile devices, Korean Red Cross (KRC) introduced tablet PCs (mobile devices) to staff working at mobile blood drives in 2015 in order to ensure work efficiency.
Aims: This research is to analyze the effects of implementing mobile devices at Korean Red Cross Blood Services.
Methods: Compare and analyze various indicators such as changes in the work environment, working hours, cost, and productivity before and after the introduction of mobile devices.
Results: Korean Red Cross introduced tablet PCs for the blood donor recruiters to better manage organizations taking part in mobile drives in 2015. As a result, staff members at mobile blood drives were able to share the real-time information and communicate with other workers much more effectively, resulting in the efficient management. Therefore, the amount of data related to mobile blood drives such as the estimated number of blood donors, schedule, and organization information has increased by 17% (10 am–4 pm) while working outside and by 52% (6 pm–11 pm) after working hours in 2015 compared to 2014. The total amount of information has shown the 1.26% of increase with 112 more cases in 2014 compared to 2013, and 10.13% of increase with 915 more cases in 2015 compared with 2014.
Also, tablet PCs have replaced paper-based donor registration cards for donor interview. The implementation of tablet PCs has reduced the workload of scanning cards and economized space of card storage. Also, Korean Red Cross has saved the printing cost (27,650,580 Korean Won with 921,686 copies) and reduced manpower (512 h). Additionally, the introduction of donor registration on tablet PCs has strengthened blood safety by preventing human errors such as omitting questions and selecting deferred donors as blood donors. In present, 99.8% of donor registration at mobiles drives are carried out by this system.
Summary/Conclusions: Employees at mobile blood drives had limited access to effective work environment as they work outside. This research shows that the introduction of tablet PCs has improved work productivity and efficiency at mobile blood drives.

P-011

ADVANTAGES OF BLOOD BANK MOBILE APPLICATION

R Abdullah, A Ahmed, L Borhan, M Labib, D Shokry and H Abady
National Blood Bank Transfusion (NBTC), Giza, Egypt

Background: Finding appropriate donors at the right time poses a challenge, especially in the event of an accident or disaster .Many patients encounter problems regarding the unavailability of their blood type due to shortage of it. This leads to a continuous and arduous search by the family of the patient in all blood banks and hospitals this search wastes a great deal of time and may end up fruitless due to insufficient units. This search is thus expedited through the sharing of emergency notices via social media like Facebook and others. These social media channels contribute to helping find the appropriate blood type and a matched donor,
To solve this problem some countries have started to use a Blood Bank Mobile Application For example; (F2S In India, EXPO2020 in Dubi and 'DSC Lebanon' In Lebanon) all which have shown a positive effect, and have saved many lives since their implementation.
Aim: The aim is to endeavor to save more lives through a speedy response using the various social media, and ultimately through the building of a huge network of healthy voluntary and ready donors.
Methods: Blood donors download the application from Google Play or iTunes.
Donors then must fill the form with their personal information (ID, Name, Region, Phone Numbers and Blood Group).
Data saved on a database, which is a centralized web portal database available 24/7 (24 h a day, 7 days a week).
All data is then made accessible to the blood banks, to hospitals and doctors; however only registered donors can see the results, this is to avoid issues that may arise concerning privacy of donor information as blood groups and addresses.
The system is smart enough to retain donor information for at least 3 months.
Communication with the nearest donor can be achieved through the mobile phone.
Results: Increase in the number of the Donors:

- When Donors download the application, they will receive reminders to donate every 3 months, which will ultimately lead to more blood collection.
- Once a blood drive is in a certain area, the application will send an alert to notify registered donors in that area, these donors may then possibly recruit friends and relatives in the same area to share the experience, leading to a greater number of donors gained for every alert sent.

More lives are saved through:

- Facilitating the finding of a matched donor in the nearest area; this is achieved through the GPS function that will locate the nearest registered donor and send a notification to him.
- Solving the problem of blood shortage; through the mobile alert which is sent to donors.

Conclusion: Using Blood donor Mobile Application with a cloud database, will ease the search for matched donors, will save valuable time that is otherwise wasted in finding a suitable healthy matched donor for a patient in need. It ultimately also solves the problem of blood shortage and will recruit more donors indirectly. This application given all these advantages can safely claim to save lives.

P-012

THE IMPACT OF INFORMATION TECHNOLOGY IN TRANSFUSION SERVICES – EXPERIENCE FROM A DEVELOPING COUNTRY

A Sadagopan
Sri Ramachandra University & Hospital, Chennai, India

Background: Information Technology plays a vital role in transfusion medicine services, which includes donor center activities like registration, selection, rejection of blood donors, blood collection, processing, storage and transfusion services activities like issue of blood components and compatibility testing, blood inventory and traceability, tracking the transfusion service data with interfacing
Information technology helps in accessing the data easier and faster to process. It also helps in generating blood supply chain including manufacturing and labeling, facilitating and improving compliance with good manufacturing practice.
Aim: Development and implementation of an in-house software and assessing its utility in transfusion medicine services.
Method: The study was done in the Department of Transfusion Medicine at a tertiary care hospital, South India April 2014 to March 2016.
Only Manual method of documentation was in practice in the Transfusion Medicine Service for the past two decades. Though there were many Blood Bank Management

Software (BBMS) commercially available, it was decided to upgrade the department functionality by developing and implementing an in-house software to reduce cost, errors, time and the software developed was custom made as per the requirements of the department. The in-house software was designed to meet all the requirements of daily activities and step-by-step process and it was validated. Training was provided for a period 3 weeks by the IT team in the hospital to the entire medical, paramedical, and nursing and other ancillary staff in the department. The training included handling of computers, releasing of critical results and facing trouble shooting issues. The software helps to monitor 'vein to vein' process, which includes registration of donors till the final issue of blood and blood products. The in-house software was compared and validated in concurrence with the manual records. Whenever a deviation was observed Route Cause Analysis (RCA) was carried out and Corrective and Preventive Action (CAPA) was implemented. Action Taken Report (ATR) was documented for future follow-up.

Result: The in-house software was developed and implemented after validation over a period of 2 years. The software helped in reducing errors, maintaining confidentiality, tracking the data and retrieval of data, interfacing with the semi-automated machines, releasing the critical results, and look-back policy. It helps to monitor the process flow and the deviations thereof.

Conclusion: Implementation of in-house blood bank management software is a step forward for ensuring safe and smooth functioning of the department activities. The software also helps in developing a data base of blood donors.

P-013

ENHANCING THE SECURITY OF THE EGYPTIAN NATIONAL BLOOD TRANSFUSION SERVICES' (ENBTS), BLOOD MANAGEMENT SYSTEM (BMS) BY TRACKING GENERATED LOG FILES

R Abdullah[1], L Bohran[2], M Labib[2], M Abbas[2], D Shokri[2], A Gohry[1], A Ahmed[1] and M Hashem[1]

[1]NBTC, Giza, Egypt [2]National Blood Transfusion Center, Giza, Egypt

Background: The BMS is a project which will automate all manual operations in the ENBTS. Guaranteeing security is a critical point in BMS execution that can be achieved through monitoring all BMS log files. These logs record all events that occur in the BMS operating system or software, and are used to monitor the vulnerability of the security system.

Aim: To illustrate how analyzing and tracking generated log filescan insure that optimum security is achieved and the integrity of the system is preserved.

Methods:

i). Log files were analyzed and categorized during the continual process of Non Functional Testing using a qualitative value:According to the following levels of security

a. Firewall: An application to prevent unauthorized internet users from accessing private networks. Manually reviewing firewall log reports can check attacks and calculate how many times your system has been attacked.

b. Antivirus: An Application used to monitor warning log files, that can detect threats and inform the user regarding what action has be taken against the malware, whether it was successfully removed or not types of log files

c. Windows Server 2012: Throughthe Event Viewer command line tool in Window servers, logs of warning messages will be seen and abnormal behavior of the users can be monitored.

d. E-Delphyn Application (User Module):This module has the capability to track users who access the system module, and to record the entire process and all actions in log files.

ii). At the Pre Go live log files were monitored and tracked.

Results: Number of analyzed security test cases was 55 cases, 10 out of these were exclusively covering log file security issues,as the project is still at the Pre-Go Live testing phase, it is anticipated that other test cases can added throughout the project execution.

Monitoring warning errors in logs files allowed the administrator to reduce traffic by uninstalling unwanted applications, closing unused ports, and detecting vulnerable ports/zones that were prone to hacking.

Tracking users in the system was useful to track user activity on the system and apply training when needed, or identify those who exhibited continuous abnormal behavior.

Conclusion: A system is never 100% secure; however sharing the responsibility of maintaining security with all users in an organization and educating them regarding the importance of updating antivirus, and not downloading any harmful files, will help create a virus free environment.

Log file analysis and securing the log files themselves, in addition to log file tracking to monitor behavior of BMS users,isvital to enhancing the quality of the BMS' security systemand can allow neutralization of risky ports that allow hackers access. Additionally protecting a system requires a certain level of ingenuity and continuous quality control. Applying this analysis is an easy, resourceful and efficient way that will provide insight into the existing operational level of security and help create a healthier safer cyber environment.

P-014

ENHANCED QUEUE MANAGEMENT SYSTEM TO IMPROVE BLOOD DONATION EXPERIENCE

AC See[1,2]

[1]Health Sciences Authority, Singapore, Singapore [2]Blood Services Group, Health Sciences Authority, Singapore

Background: The decision to donate blood and return for further donations depends not just on altruistic reason such as helping someone in need of transfusion, but also on social responsibility, personal credit, social pressure, satisfaction, family motivation, and most importantly, **positive donation experience**. This poster shares how Singapore's Health Sciences Authority (HSA) uses the enhanced Queue Management System (QMS) to improve blood donation experience by:

Aiding staff to address donors by name

Replace manual computation of turnaround time

Call doctor for assistance in reaction cases in a swift and hassle-free manner

The QMS manages the queue at the three service stations; the medical interview room, blood test station and donation room. A queue number will be issued to blood donor at the registration counter. The QMS will display the queue number when donor is being called at respective service stations. The enhanced QMS is able to display name of donor at the respective stations when donor is called. This allows staff to know the donor's name beforehand aiding staff to greet donor. Dale Carnegie once said, 'A person's name is to that person the sweetest, most important sound in any language'. When the blood bank addresses donors by their name, it shows the interest and respect for the individual. The enhanced QMS also replaced the manual computation of turnaround time. This has saved staff about half an hour daily for manual data entry to compile the turnaround time report. Staff can now make use of the time saved to focus more on donors attending to their needs. In addition, notifying doctor of a reaction case is just a click away. There is no need for a nurse to go to the doctor asking for help. This improves the efficiency and in return building up donor's confident level of the service provided.

Aim: To ascertain if the enhanced QMS system improve blood donation experience.

Methods: Two blood banks with similar facilities (i.e. located near train station and shopping malls, refreshment provided etc.) were selected for this project; Bloodbank@Dhoby Ghaut (BB@DG) which is without the enhanced QMS, and Bloodbank@Westgate Tower (BB@WT) which is installed with the enhanced QMS. Compliments from the two blood banks were collected over 6 months from June 2015 to February 2016 (excluding August to Oct 2016 where BB@DG was closed for renovation) to compare the effectiveness of the enhanced QMS.

Results: A total of 621 compliments were received; 176 compliments from BB@DG, and 445 compliments from BB@WT. We can see the blood bank installed with enhanced QMS receiving 153% more compliments.

Summary/Conclusions: The result shows blood banks installed with the enhanced QMS received more than double the compliments as compared to blood bank without the enhanced QMS which is a positive sign of a better blood donation experience.

P-015

IMPROVING BLOOD MANAGEMENT SYSTEM (BMS) EFFICIENCY AT THE ENBTS (EGYPTIAN NATIONAL BLOOD TRANSFUSION SERVICES) THROUGH IMPLEMENTING RAS (RELIABILITY, AVAILABILITY AND SERVICEABILITY) TESTING

MHS Elsayed[1], A Elgohary[2], M Abbas[1], H Abady[1], E Hussain[1], L Samir[1], D Shokri[2] and M Labib[1]

[1]NBTC, Giza, Egypt [2]National Blood Transfusion Center, Cairo, Egypt

Background: Every organization must plan to perform appropriate testing covering all elements of the system. (RAS) is an essential step in the application of the (BMS) that was initially implemented as a pilot between Tanta (RBTC) (Regional Blood

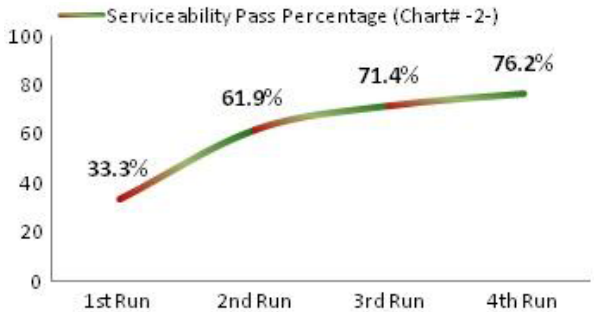


Table 1. Increased efficiency for serviceability

Transfusion Centre) and Cairo (NBTC) at Egyptian blood banks. RAS testing is a type of non-functional testing performed using Test Cases (TC). A TC is a method to verify compliance against specific requirements; A TC acts as the starting point for any test execution.

1. Reliability: It is measured by MTBSI- Mean Time between System incidents, which reflects the ability of a system to perform its required functions under stated conditions for a specified period of time, it is divided into:
   1. Avoidance:Avoiding failure of the system through strategic planning.
   2. Detection:Detecting if services are stopped, and what specific decision must be taken.
   3. Repair:Repairs events and problems and provides solutions to help admin intervention.
2. Availability: It is measured by MTBF-Mean Time between Failures; to reduce and eliminate downtime; The BMS divided availability into two levels:
   1. High availability:Characterized by redundancy (duplicity) of most hardware components.
   2. Continuous Availability:Non-stop service, with planned or unplanned outages (single point of failure).
3. Serviceability: It is measured by MTTR-Mean Time To Repair (downtime);early detection of faults that can decrease or avoid system downtime.

Aims: To show how RAS testing can ensure the efficiency of the BMS system in order to classify it as an ideal system.

Methods:

A Using dynamic testing (white box technique) TCs were generated by qualitative values to comply with IEEE 829 specifications, by utilizing (Check lists, scenarios and diagrams); to ensure effective testing by generating the least number of test cases that will produce the largest number of the defects.

B All the providing company's documents were implemented eg. Bid Proposal; in order to determine the scope of RAS testing which includes (coverage rate of TCs, item description, site, reference, severity, results, impact)

Results: The following are results for TCs executed for the pilot project at Pre-GoLive stage.

A Reliability: The IT Team Generated 109 TCs, that are ready to run;at the next cycle of project.

B Availability: The executed TC illustrated how the efficiency of availability increased from 45.2% to 74.2%, by an average of 29% (Table 1; Chart 1).

C Serviceability: The executed TC illustrated how the efficiency of serviceability increased from 33.3% to 76.2%, by an average of 42.9% (Table 2; Chart 2).

Table 2. Increased efficiency for availability

Conclusions: Any efficient system cannot operate without RAS testing, This was clearly illustrated by our study that showed how testing Serviceability and Availability had a direct impact on raising the efficiency of the BMS system from 40.4% to 75.0%,on average 34.6%. This highlights how it is fundamental in converting the BMS system from a traditional to a highly efficient Ideal system.

Such an increase in efficiency is advantageous as can avoid downtime, can cause auto detection and auto repair of errors and greatly reduces effort and cost for admin and system support respectively. It also constitutes a building block when considering future scalability and KPI (Key Performance Indicators) preparation.

P-016

EVALUATION OF ACCEPTANCE TESTING METRICS (AT) TO ASSESS THE EGYPTIAN BLOOD MANAGEMENT SYSTEM (BMS) AT THE PILOT PROJECT PHASE

AG Elgohary, M Hashim and S Lamia

NBTS-Egypt, Giza, Egypt

Background: Acceptance testing (AT) is a vital prerequisite for the implementation of BMS in all its phases is needed to fulfill the business rules of any blood transfusion centre.

AT is a means to measure quality by challenging the efficiency of the utilized software. This can be achieved by choosing reflective in-process metrics which validate and quantify the ability of the system in place.

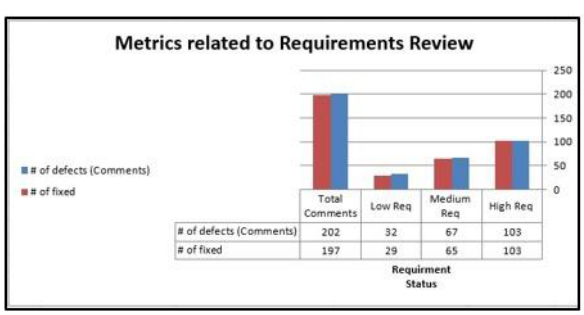
At the NBTS two sites were chosen to conduct the BMS pilot project in Cairo (NBTC) and Tanta (RBTC). This phase is a critical milestone in implementation that is necessary to detect any errors in the system prior to the Go live phase of actual real time implementation and rollout to the remaining 23 sites all over Egypt.

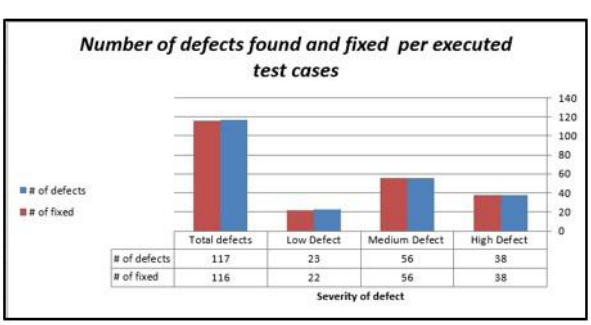
Aims: To quantify the accuracy of (AT) performed on the BMS software at the critical pre go live phase using in-process metrics as useful indicators that highlight problems,ensure the quality of all BMS application components and their successful module integration from donation to patient care.

Methods: Test data was collected from Dec to March 2016.

A review of 'Software requirement specifications'(SRS) was done to enable the detection of missing requirements, detect the need for more information, and identify mistakes in SRS as 'comments' which are analyzed in terms of severity.

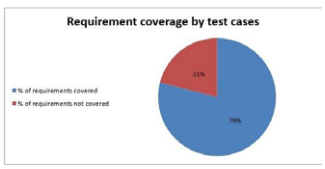
The ability of the system to meet these comments was evaluate defects were recorded and assigned the metric 'requirement review'.





Caption 1. Metric related to Number of defects found and fixed per executed test cases

Requirement Coverage		
Basic Metrics		
Item	**Value**	**Unit**
Total # of requirements	285	Req.
Total # of identified (planned) test cases	421	TCs
Total # of requirements supported by TCs	225	Req./UC
Derived Metrics		
Item	**Value**	**Unit**
% of requirements covered	79%	%
% of requirements not covered	21%	%
Requirement Coverage=Total # of requirements supported by TCs /Total # of requirements		



Caption 2. Metric related to Requirement coverage by test cases

Test cases (TCs) were developed based on the SRS,subsequently the degree of requirements coverage by (TCs) was calculated using an equation and named the metric 'requirement coverage'Requirement Coverage = Total no. of requirements supported by TCs/Total no. of requirements.

The third important metric is number of defects vs fixed defects. These defects are weighted in terms of severity and this evaluation process must be reflected in the acceptance criteria.

Finally TCs were executed, recorded and analyzed,subsequently execution results were categorized as: pass/fail. The 'fail' status generated a defect record and indicated that the targeted function hadn't been achieved. This metric was named '*Number of defects/executed TCs*.

Result: Regarding the metric 'Requirements Review',our study found 202 missing requirements, in the SRS which otherwise wouldn't be included in the final applied software.

The percentage of requirement coverage found by (TCs) was 79%. However the remaining 21% lacking coverage need to be addressed by increasing number of TCs (effectiveness) or enhancing the efficiency of the existing TCs.

The percentage of fixed defects in relation to total defects was calculated at 99% (116 fixed/117 Total defects), it is noted that 99% defects fixed, is a positive indication of software integrity and functioning.

Conclusion: These metrics are vital to evaluate (AT), through test case analysis,The continued utilization of (AT) metrics throughout the testing procedure is necessary to isolate missing requirements,evaluate requirement coverage, detect application defects of varying severity and ensure the correction of these defects Quality assurance can be achieved by assessing the number of fixed defects.

Our study illustrated how the development of these metrics was achieved through the customization of already existing data. Such metrics can be similarly utilized by other blood banks,in the process of (AT) provided that they adapt these metrics to conform to their own business rules.

Once these defects have been tackled rollout implementation can be performed with greater confidence and less risk.

P-017

ENHANCING THE NATIONAL BLOOD TRANSFUSION SERVICES IN EGYPT, THROUGH THE INTRODUCTION OF SPEECH RECOGNITION TECHNOLOGY

A Lotfy[1] and M Sewefy[2]

[1]NBTS, OCTOBER, Egypt [2]Ahmed Maher Hospital, October, Egypt

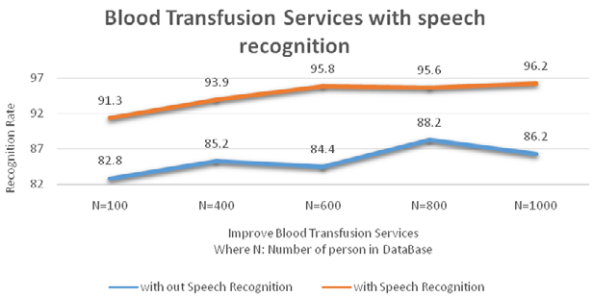
Background: The Egyptian national blood transfusion services (ENBTS),is currently engaged in an ground breaking process that aims to automate all of it manual work by using the blood management system (BMS).

The need for the technology of automatic speech to text conversion is continuously increasing, especially for donors with special needs. Given the importance of Blood Transfusion Services (BTS) in saving people's lives worldwide efforts have been undertaken to utilize speech recognition and smartphone applications to make the blood donation process easier,

The blood donation process usually consumes a great deal of time and effort from both donors and medical staff, this can be overcome by introducing an information system that allows donors and blood donation centers to use speech recognition, to achieve a accelerate the donors' registration process on the BMS.

Aims: To describe how developing BMS based on speech recognition system can improve the system performance by saving effort taking in donors' registration process and typing time of donors questionnaires, which permits text dictation and voice commands interchangeably.

Methods: The BMS based on automatic speech recognition consists of five layers, namely, input speech, preprocessing, speech feature extraction, and classification scheme.



Caption 1. Improve performanc blood transfusion service with speech recogntion

The input layer represents a dataset of short length voice that is focused on the voice signal. Those voices are fed to preprocessing layer, in which voice localization algorithms are applied. And then voice detection algorithm is applied to extract and isolate the noise from the background. Finally, the voice localization is applied based on specific threshold to extract the signal.

The feature extraction stage seeks to provide a compact representation of the speech waveform. This form should minimize the loss of information that discriminates between words, and provides a good match with the distributional assumptions made by the input speech. Feature vectors are typically computed every 10 ms using an over-lapping analysis window of around 25 ms.

ASR systems can be classified according to some parameters that are related to the task. Some of the parameters are:Vocabulary size, Speaking style, Speakermode, Channel type, Transducer type. We use a version of open-source Sphinx4 speech recognizer which is faster than any other speech recognition system.

In the blood bank Medical transcriptionists listen to voice recordings that physicians and other healthcare professionals make and convert them into written reports. They may also review and edit medical documents created using speech recognition technology. Transcriptionists interpret medical terminology and abbreviations in preparing patients' medical histories, discharge summaries, and other documents.

Results: In this section, the evaluation of NBTS BMS with speech recognition, and a comparison of their performance are given. The system model is tested using cross validation and the evaluation is based on: (i) Accuracy, which is the correct classified records, over all records, (ii) true positive rate (TPR) where improve performance from 86.2% to 96.2%.

Conclusions: Developing a BDS through a speech recognition system, is a revolutionary idea that if utilized can be of great value in the blood bank in saving time and effort spent on documentation, transcription and donor identification. It will also include donors with special needs in the donation pool, and have great community impact.

P-018

AUTOMATIC TESTING FOR ABO ISOAGGLUTININ TITERS USING IMMUNOHEMATOLOGY ANALYZER, QWALYS-3

JH Hwang, SJ Kang, HS Seo, HL Lee, TK An, SY Shin and SY Kong

National Cancer Center, Goyang-si, South-Korea

Background: ABO isoagglutinin titer is important follow-up marker for patients with ABO incompatible bone marrow or solid organ transplantation. It has been known the column agglutination technique (CAT) is more sensitive than manual tube methods. However, both CAT or tube methods are labor intensive and time consuming.

Aim: We developed the automated ABO isoagglutinin titration methods using QWALYS-3 (DAIAGAST, Loos Cedex, France) which use erythrocyte magnetized technology then compared the results from automated method, tube method and and CAT with or without DTT.

Methods: A total 120 (40 with each blood group A, B and O) healthy adults (20–70 years old), who visited health checkup in National Cancer Center between April and October 2015 were included. The three different methods were compared.

Results: The median (range) IgG titers of anti-A in group O by IS, CAT and QWALYS-3 methods were 16 (2–256), 512 (128–8,192) and 2,048 (8–8,192), respectively. The median (range) IgG titers of anti-B in group O by IS, CAT and QWALYS-3 methods were 4 (2–128), 128 (64–8,192) and 256 (16–8,192), respectively. The

median (range) IgG titers of anti-B in group A by IS, CAT and QWALYS-3 methods were 2 (2–8), 32 (8–128) and 2 (1–32), respectively.
The median (range) IgG titers of anti-A in group B by IS, CAT and QWALYS-3 methods were 2 (2–4), 64 (8–256) and 12 (1–1,024), respectively.
The median (range) IgM titers of anti-A in group O by IS, CAT and QWALYS-3 methods were 16 (4–32), 32 (8–512) and 32 (2–256), respectively. The median (range) IgM titers of anti-B in group O by IS, CAT and QWALYS-3 methods were 8 (2–32) and 16 (4–512) and 12 (2–128), respectively. The median (range) IgM titers of anti-B in group A by IS, CAT and QWALYS-3 methods were 8 (4–32), 8 (2–32) and 16 (2–64), respectively. The median (range) IgM titers of anti-A in group B by IS, CAT and QWALYS-3 methods were 8 (2–32), 8 (4–64) and 16 (4–256), respectively.
The concordance of anti-A and anti-B titers between IS and CAT was 0% and 0% (IgG), 78% and 84% (IgM). The concordance of anti-A and anti-B titers between IS and QWALYS-3 was 19% and 41% (IgG), 65% and 84% (IgM). The concordance of anti-A and anti-B titers between CAT and QWALYS-3 was 40% and 30% (IgG), 75% and 66% (IgM).
Conclusion: Though there was difference between IS and sensitive methods CAT, the ABO isoagglutinin titers by QWALYS-3 were comparable with CAT. Therefore QWALYS-3 might be useful automatic tools for ABO isoagglutinin titers in clinical laboratory.

P-019

APPLYING ISBT GUIDELINES FOR VALIDATION OF AUTOMATED SYSTEMS IN THE ENBTS (EGYPTIAN NATIONAL BLOOD TRANSFUSION SERVICES)

M Abd El Hafez
NBTS, Giza, Egypt

Background: ISBT guidelines for validation of an automated system was developed by a validation task force formed of the international Society of Blood Transfusion working party on information Technology (ISBT WPIT) in 2010 then updated and published in 2010 to provide guidance on the validation of automated systems in blood establishments.
The blood establishment bears the responsibility for the regulatory compliance of the automated/computerized systems used. Full validation of the computerized system is required for all systems that are critical to preserving product (blood) quality (information management, storage, tools for operational decision-making, and control)
Aims: To illustrate how following the ISBT Guidelines and creating a responsibilities matrix between the customer and supplier in Blood Management system, in ENBTS this can have a major effect on the safety and quality of blood components.
Methods: Before formally embarking on ISBT validation, The Egyptian NBTS had already taken steps in applying any of the validation process outlined in the guidelines, through automated system life cycle of E-Delphyn software, these steps included:
Well defining URS (User Requirements Specification)
Defining good selection criteria for System Selection
Inviting potential suppliers.
Conducting system evaluation against international regulations, WHO standards (World Health Organization),requirements for installation,configuration and training.
Taking financial considerations as a cost for life cycle like software licensing, establish a data center,added an extra Hardware or interfacing
Conducting assessment classified into (High–medium–low)
Validation Plan for testing and other verification or validation process consists of cycles and iterations in central and peripheral sites by a team (IT plus physicians as owner)
Following problem solving techniques in problem resolution and issuing a report with full documentations.
Ensuring a Validation State maintenance.
Promoting a Business continuity plan
Results: Ready made BMS Software had to be customized/A manner of customization was done for the software to be suitable for use in Egypt
This added features on standard system
Data Center established to host BMS for all sites
26 Persons are already trained on software
19 persons are already trained on SQL Server
11 persons are already trained on Windows Server 2008.
Establish a WAN connection between NBTC and RBTCs with Leased lines.
Developed and execute test cases for Testing Non Functional was passed by 89% in first run.
Conclusion: Any BMS system requires that it be customized to the needs of the establishment, however following international guidelines for validation insures that steps aren't missed and that a clear outline is followed. This will standardize validation and serve to enhance quality of work, saving time.
Validation is doubly important when the system to be customized and validated exists in a health facility, as it ultimately, ensures the safety of an establishment as critical as a blood bank and the blood supply of a country.

P-020

AUTOMATION OF DONATION DEPARTMENT USING BLOOD MANAGEMENT IT SYSTEM – THE EGYPTIAN EXPERIENCE

HA Abady[1], M Labib[1], LB Ragheb[1], MA Ibrahim[1], A ElGohary[1], D Shokry[1], M Hashem[2], R Abdallah[1] and KB Mokbel[1]
[1]National Blood Transfusion Center, Giza, Egypt [2]Sohag Regional Blood Transfusion Center, Sohag, Egypt

Background: The Blood Management Information Technology System (BMS), which started implementation in 2014, is an automated system that aims to improve the efficiency and productivity of routine blood transfusion process: Donor-to-Patient workflow. The project aims to connect 17 out of 24 Blood Transfusion Centers (BTC) including the National Blood Transfusion Center (NBTC), the head quarters of the Egyptian National Blood Transfusion Services (ENBTS), through a secure Wide Area Network. The system will connect all technical departments of each blood bank together. The donation department plays a vital role in the blood transfusion process, without it the rest of the departments can't function. The donation module is also the vital starting point for the BMS to function well.
Aims: This is a quantitative research that aims to describe steps in automating the donation department through designing our own test cases (TC). TCs are a means to test compliance of the software against the Software Requirement Specifications (SRS) documents and the ENBTS Standards.
Methods: The IT, Quality Department and Head of donation department at NBTC carried out a number of meetings with the software house to explain their work flow, business rules, the SOP's and forms. This was to guarantee that the BMS complies with the ENBTS standards. This resulted in the issuing of Donation SRS annex, which describes how the software will be able to serve the departments functional needs.
Using the SRS document, the same team was able to create functional test cases repository for the validation of the donation module.
Results: We had two testing cycles, with the following results, please refer to Table 1.
Results of the first cycle reflect a big number of failures because of the following:
1. Some TCs were built on wrong understanding of how screen functions, due to lack of training.
2. Some errors needed configuration by the user such as Hemoglobin, Age
3. Part of the failures were related to Egypt-specific local problems for example
   a. We are still one of the countries manufacturing Frozen Plasma, the problem was that the system wasn't taking into consideration that any donation exceeding 10 min can't be processed into Fresh Frozen Plasma
   b. The company presumed the continual use of the shaker balances in the donation department, and that all weights are correct, when actually the balances are used intermittently and subsequently we have four weight categories
   i. Ideal weight -all components are separated

Basic Metrics		Iteration 1	
Item	Unit	Cycle 1	Cycle 2
Total # of test cases executed	TC	241	252
Total # of test cases passed	TC	198	232
Total # of test cases failed	TC	43	16
Total # of critical defects found	defect	30	14
Total # of medium severity defects found	defect	7	2
Total # of low severity defects found	defect	6	0

Table 1

Table 1. Donation module testing results

ii. Overweight- units are discarded
iii. Underweight -units are discarded
iv. Accepted weight- we produce RBCs only

Concerning this Hemasoft held a number of sessions with us, to tackle these issues, and major problems were solved by cycle 2.

Conclusion: The implementation of the BMS is a positive step for the ENBTS, as it has great advantages in terms of traceability, accountability and reporting. The thorough testing of the donation module specifically is vital as it gives users more trust in the software capabilities, and any module modifications will be reflected in the quality of the all subsequent components separated from each blood unit. This leads to insuring greater blood safety in all other departments.

P-021

WORKFLOW ANALYSIS STUDY AT SHARJAH BLOOD TRANSFUSION AND RESEARCH CENTER, UAE – FIRST WORLDWIDE FULL AUTOMATION SOLUTION IN BLOOD BANK LABORATORY

MZ Rabeh Zouaoui, S Ahmad, Z Ahmad, KJ Almazem and FS Al Souedi

Sharjah Blood Transfusion and Research Centre, Sharjah, United Arab Emirates

Background: Blood Banking is an evolving field where quality must be a primary goal. Efficiency and Effectiveness in blood banks is of vital importance to ensure the availability and safety of blood and its components. Evidence demonstrates that pre and post-analytical steps of the total testing process (TTP) are more error-prone than the analytical phase hence Automation helps in improving the laboratory processes and safety by minimize the handling errors and reduce manual steps.

Aims: Aim of this case study (Workflow Analysis) is to compare workflows into the blood screening laboratory in a 'Before' and 'After' consolidation of platforms setting of total automation with metrics such as Workflow Efficiency, Higher staff utilization and Less Manual interactions and to evaluate the potential challenges in the new automation process.

Methods: 'Before and after' comparison study have been performed. International company consultant in cooperation with local consultant collected workflow data by observation and measurement. The assessment takes 2 days on-site in comparable routine setting, same tube amount and same system parameters. Follow up reviewing and evaluating of workflow study data and results, supported by pictures, movies, and layout and measurement analysis. Study setting 'Before': Manual sample preparation including manual sample sorting into centrifugation devices and sample re-capping for storage, semi-automated platform for NAT from, cobas s 201 Roche and automated platform for Serology from Abbott, Architect *i2000*. Study setting for 'After': Full Automated Workflow for both NAT & Serology Testing disciplines with fully automated sample preparation including built-in centrifugation with cobas p 612 and cobas p 471 as well as automated re-sealing of the tested tubes for mid-term storage, full automated NAT with cobas 6,800 and full automated Serology with cobas 6,000.

Results: The overall operation time could be reduced significantly, in addition a decrease in hands-on-time of more than 50% in total was observed in a combined NAT and Serology workflow. In comparisons of the Operational time and of the TAT as described The comparison was between cobas s 201 vs cobas 6,800 as well as for Architect I 2,000 vs cobas 6,000 reduction in both is being observed too. The 'After' full automated layout shows a reduced demand of space and compact user workflow, leading into significant less 'ways-to-go' of more than 70% for the users and increases of workflow efficiency, The overall manual steps are being mainly minimized to prepare the instruments, loading tubes in a single point of entry and minor general interactions with minimized error-proneness in the pre-analytical process.

Conclusions: An awareness of the potential benefits and challenges can assist in the planning of the implementation of the automated platform in blood bank. A fully automated blood screening laboratory design, where NAT and Serology is physically connected to pre- and post-analytics, along with the integrated software system (LIS) offers new workflow opportunities that could provide reduced need of space, a compact user workflow for high workload with minimized ways-to-go, significant reduced manual steps and hands-on-time of more than 50%. Further evaluation for the challenges need to be conducted.

P-022

DIGITAL QUESTIONNAIRE IN THE DANISH BLOOD DONOR STUDY

K Burgdorf

Copenhagen University Hospital, Copenhagen, Denmark

Background and aim: The Danish Blood Donor Study is a prospective, population-based study and biobank. Since 2010, 107,000 Danish blood donors have been included in the study. Prior to July 2015 all participating donors had to complete a paper-based questionnaire. Here we describe the establishment of a digital tablet-based questionnaire platform implemented in blood bank sites across Denmark.

Methods: The digital questionnaire was developed using the open source survey software tool LimeSurvey. The participants accesses the questionnaire online with a standard SSL encrypted HTTP connection using their personal Civil Registration Numbers. The questionnaire is placed at a front-end web server and a collection server retrieves the completed questionnaires. Data from blood samples, register data, genetic data and verification of signed informed consent are then transferred to and merged with the questionnaire data in the DBDS database.

Results: The digital platform enables personalized questionnaires, presenting only questions relevant to the specific donor by hiding unneeded follow-up questions on screening question results. New versions of questionnaires are immediately available at all blood collection facilities when new projects are initiated.

Summary/Conclusion: The digital platform is a faster, cost-effective and more flexible solution to collect valid data from participating donors compared to paper-based questionnaires. The overall system can be used around the world by the use of an internet connection, but the level of security depends on the sensitivity of the data to be collected.

Cost/Effectiveness

P-023

COST-BENEFIT ANALYSIS AND VALIDATION OF THE RFID-BASED TRACEABILITY SYSTEM DEPLOYED IN SEVILLE-HUELVA'S BLOOD ESTABLISHMENT

JM Aznar[1], A Vega[2], MC Rodriguez[1], MT Martinez[1], B Cumbrera[2], J Diez-Yanguas[2], A Parreño[2], S Oyonarte[1] and JP Benitez[2]

[1]*CTS Seville-Huelva, Seville, Spain* [2]*AT-Biotech, Malaga, Spain*

Background: The collection, production, storage and distribution of human blood products (HBP) often use manual registering in Blood Establishment Computer Software (BECS) and traffic light rating systems to trace process steps of working lots and therefore to confirm task completion of individual units.

The effectiveness of decisions and the improvement of processes depend then on the grade of adherence to protocols and on the accuracy of assumptions made when information is not available in semiautomatic databases, and GMP compliance and other quality requirements are difficult to prove as well.

Aims: To present the results of the cost-benefit analysis and the validation of the system deployed in Seville-Huelva's BE that tackles the outlined challenges.

Methods: The system allows tracing all units individually at any point in the process and automatically registering a big amount of data for later analysis, by using diverse devices powered with RFID (RadioFrequency IDentification) technology adapted to the specific needs of each operation.

The validation was performed by BE's staff following the ISO.2859-1 norm.

The analysis of benefits that can be offered by the system was carried out using operation time measurement methodologies for calculating the productivity and real data collection for calculating the rest.

For quantifying the benefits, the new system mode of operation and the operations -or their equivalent- that were being undertaken before its deployment were compared, and typical economic conversion factors -labour costs, price of HBP, etc.- were applied.

Cost-benefit was calculated as the difference between the economical quantification of all benefits and a selling price of the system -cost-.

Results: The system was progressively deployed hence, the analysis used data registered of 35.000 whole blood donations and the processing, storage and distribution of more than 190.000 HBP during 18 months of operation.

29 test cases were run while processing 7.255 HBP in 10 different operations of the production cycle to validate the system, resulting in a 100% conformance for all tests.

The theoretical cost-benefit (net saving) calculated for 12 months of operation with the system in the BE – 80,000 donations processed yearly- was 245.000€/year, broken down as:
* Direct net savings of 69.000€/year (28% of the total): reduction of operational expenditures and investments and 31% productivity improvement (16.000€/year), and the theoretical reduction of detectable unit losses (53.000€/year).
* Indirect net savings of 176.000€/year (72%): estimation of operations' optimization achieved when using automatic controls enabled and data registered by the system. 99.000€/year would come from a theoretical increase of 20 IU/l of FVIII in plasma delivered to the fractionation industry.
Summary/Conclusions: The RFID-based traceability system deployed in Seville-Huelva's BE was validated −100% conformance- using ISO.2859-1 norm and it has been used in the production of more than 190.000 HBP.
The system could deliver a calculated cost-benefit -net saving- of 245.000€/year and some additional intangible benefits such a better production process control to facilitate compliance with GMP.

P-024

REDUCTION IN TRANSCRIPTION ERRORS IN LABORATORY REPORTS USING LEAN SIX SIGMA

ST To, J Tang, AG Alcantara, MN Abella, V Voon, CY Phang and M Chan
Health Sciences Authority, Singapore, Singapore

Background: Due to the complexity of the human leucocyte antigen (HLA) and limitations of the current blood-bank computer system, majority of the fields in the tissue typing laboratory (TTL) reports have to be entered manually, and errors occur during transcription. These errors ranged from minor ones such as wrong patients particulars to more serious ones such as wrong results which might affect clinician's diagnostic decision making, patient management and transplant outcomes. Therefore, it is crucial to reduce errors in clinical laboratory reports.
Aims: To measure the rate of transcription errors in TTL reports and identify factors contributing to the errors using the lean six sigma model in order to reduce error rate.
Methods: In this retrospective study, TTL reports with errors detected during internal review by laboratory managers were consolidated and the factors which caused the errors were analyzed and tabulated using lean six sigma tools.
Results: From July to September 2015, 21 (1.84%) transcription errors were detected in 1,144 TTL reports. The transcription errors comprised of patients' particular errors (14%), wrong hospitals registered (9%), and errors in results reporting (76%). The fishbone model highlighted several factors causing the errors: the time of the day when the reports were generated, the specific technicians making the errors, urgency of the test request, and report information overloading. Transcription errors were significantly noted to occur in the afternoons ($P = 0.04$). Measures were then introduced to reduce transcription error rates, including the introduction of a surcharge to urgent test requests and an improvement in the computerisation of result entry process. These measures reduced the error rates from 1.84% (21 out of 1,144) to 0.68% (7 out of 1,035).
Conclusions: Manual transcription of laboratory data is prone to error but fortunately most of the errors were detected before results were released, hence there were minimal impact on patients' safety. It is important for TTL to continue to monitor and control the error rate in laboratory reports in order to produce reliable test results for clinical diagnosis.

P-025

COMPARISON OF MANUAL AND AUTOMATED BLOOD GROUPING METHODS AT NAKASERO REGIONAL BLOOD BANK – KAMPALA UGANDA

A Elaborot, R Sunday, W Wambi and O Grace
Blood Transfusion Service, Kampala, Uganda

Background: Uganda blood transfusion service is a semi-autonomous institution made up of seven regional blood banks and eight collection centers. As the rest of the world is embracing new immunohematology technologies the transfusion service is still embroiled in manual blood grouping methods for routine work. In the last 8 months, 23 units were returned to the blood bank due to changing blood groups and many other cases may not be reported. From the route cause analysis of the errors, the quality team found that errors were mainly attributed to data entry and clerical errors. However in March 2016 the laboratory at Nakasero regional blood bank received two new Immucor Neo machines to move towards embracing modern technology in Uganda and away of mitigating these errors. This study focused on comparing results and processes of manual and automated techniques of blood grouping as a validation process.
Methods: A total of 560 EDTA blood samples were subjected to routine conventional micro titer blood grouping and automated technique on immucor gamma machine. It took about 5 h to produce results for 200 units that is grouping manually 200 samples on twenty micro plates for forward and reverse by two people as opposed to 1 h 30 min on seventeen plates by the Galileo Neo automated machine. Results were analyzed manually.
Results: The automated and manual techniques of blood grouping showed 100% concordance, three inadequate samples were identified by both methods. From the samples analyzed 31 were heamolysed (5.5%),6 samples were mixed field reactions, 1.1% were three samples, Invalid 1.4%, 0.5% was rhesus positive grouped as negative. From these results it showed the machines are reliable and could be adopted by ubts.
Conclusion: The manual method took automated meth a long time to produce results it had less determined error due to haemolysis. It is cheaper compared to the automated method. The errors in manual are fatal but can be minimized by automation. Though Uganda is a low developing country its worth investing in automation when comparing the number of lives that could be saved by getting the right blood group and the advantages that come with it outweigh the manual methods. This automation has opened the gates of research in immunohaematology in Uganda blood transfusion service which could not be done manually. It has also simplified the accreditation process by providing a platform for antibody and high titer typing. However; the discrepancies that were identified were mainly due to sample integrity which is to be addressed in the near future.

P-026

EFFECT OF RED BLOOD CELL TRANSFUSION FOR TOTAL HIP REPLACEMENT ON THE UNIT COSTS OF INPATIENT DAYS

HH Hsieh[1], KM Lu[2], JH Lin[2] and TH Lin[2]
[1]*Taichung Veterans General Hospital, Taichung, Taiwan* [2]*Section of Transfusion Medicine, Taichung Veterans General Hospital, Taichung, Taiwan*

Background: Total hip replacement (THR) is the common clinical surgery in Taiwan. Bleeding is a risk factor for adverse outcomes after THR operation. Red cell blood (RBC) transfusion therapy may be useful and necessary in the bleeding patients. There is still not enough data to confirm RBC transfusion will extend the period of hospital days and cost in Taiwan.
Aims: Our aim is to identify the effect of RBC transfusion in patients having THR surgery on the total hospital cost and hospital days.
Methods: This retrospective cohort study was used the inpatient sample database from the Clinical Informatics Research & Development Center of Taichung Veteran General Hospital. We were searching THR patients by the ICD-9 code 81.51 from 2005 to 2014. THR patients were separated into treatment group (RBC transfusion within 72 h) and control group. RBC transfusion during nonsurgical period will meet the exclusion criteria. Propensity score method was used to balance confounding variables such as gender, age, diabetes mellitus, hypertension, autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus), and coronary artery disease. The possible relationships among the proportions of transfused THR patients, the total hospital cost and hospital days will be analysis.
Results: We studied 1,555 primary THR cases after exclude 44 RBC transfusions during nonsurgical period. The proportions of transfused THR patients was 39.87% (620/1,555). THR patients who received RBC transfusions with longer mean hospital stay (7.04 days vs 6.38 days; $P < 0.017$). The mean expense for transfused THR patients was higher than nontransfused THR patients (TWD 110,520 vs TWD 104,008; $P < 0.001$).
Summary/Conclusions: Although RBC transfusion is associated with prolonged hospital duration after THR surgery, the proportion of transfused THR patients in our hospital was much higher than other country. RBC transfusion is widely used in THR surgery as it is relatively inexpensive and reduced surgical risk. The criteria for reimbursement on transfusion services are relatively unrestricted. Antibiotic prophylaxis during THR surgery and transfusion may increase the hospital cost. To improve the safety and effective use of transfusions, a formal educational and training program should be held in the future.

P-027

COST CALCULATION AND PAYMENT MODELS IN BLOOD TRANSFUSION SERVICE, PAKISTAN

HA Zaheer

Safe Blood Transfusion Programme, Ministry of National Health Services, Islamabad, Pakistan

Background: Availability, safety, quality and accessibility of blood and blood products for transfusion are a global concern. In resource limited countries blood transfusion services often struggle against competing demands for increasingly scarce resources. As a result blood transfusion often receives low priority. It is therefore essential to be able to have accurate information on the cost of provision of blood transfusion service. In Pakistan, blood transfusion system reforms are being implemented since 2010 and a new infrastructure is being developed. Blood costs largely rely on the number of activities performed to deliver the final product and generally cover blood donor recruitment, Blood collection, processing, storage and distribution. However indirect costs are often not incorporated.
Aims: To review the comprehensive costing system in the blood transfusion sector of Pakistan.
Material and methods: Information was gathered from the major service providers during field visits and practices observed. In a diverse system architecture of Blood Transfusion Services in Pakistan there are different systems of costing and payment.
Results: Three categories of blood service providers exist in Pakistan; public, private and the NGO sector. The costs are usually calculated only for the production process. The public sector typically does not charge the patients directly while the private and NGO sector follow the Activity Based Costing (ABC). The capital costs (buildings, equipment, vehicles, equipment, training, furniture) of the NGO and private sector are contributed by external sponsors and mostly not considered in their accounting. The cost recovery approaches usually are focusing on operational or recurrent costs only. Certain variables are also present including donor mobilization, blood processing, immunohaematology, TTI screening test methods and production capacity. Regarding the cost recovery, in public sector, it is reimbursed by the government. In the private sector, patients are charged for every blood bag and the charges include the cost of blood bag, screening, blood grouping and pre-transfusion testing (Patient's blood group, cross-match, antibody screening, etc.). In NGO sector, cost is recovered from charity and donations. Similarly, there are five payment systems namely Out-of-pocket, Health Insurance, Corporate Panel, Govt. Panel and through Charity/Donations.
Conclusion: The outlined model and approach enables the determination of the actual cost of blood transfusion system in Pakistan.

P-028

ECONOMIC MODELLING FOR TRANSFUSION MEDICINE IMPROVEMENT INITIATIVES IN NSW, AUSTRALIA

S Francis and C Choi

NSW Clinical Excellence Commission, Sydney, Australia

Background: Since 2006, Blood Watch has had a focus on the appropriate use of red cells, specifically in the elective surgical setting, as well as other key transfusion quality improvement strategies. In 2014, the program expanded to include:
All blood product utilisation where evidence for use exists and/or data is available
Blood product inventory wastage reduction strategies.
The Blood Watch Program objectives are:
To promote, support and improve the provision of world-class transfusion medicine practice in NSW, based on a blood management framework and relevant best practice guidelines.
To provide leadership, strategic direction, advice, and support to ensure NSW is able to identify and improve systems at local and jurisdictional level as outlined in the National Blood Agreement and by the NBA including:
Supply Security
Clinical Safety and Quality
Sector Management
In 2013, as part of the NSW Health Program Management Office Road mapping program, two financial metrics were identified: (i) cost savings related to reducing inappropriate use of blood porducts, and (ii) savings related to the reduction of fresh blood product wastage.
Aim: To develop a model that could be used to predict the financial impact of two system wide improvement initiatives: The NSW red cell wastage reduction working party and action plan, and the progressive implementation of patient blood management principles in NSW hospitals.
Method: The program has access to quality data on the issue and discard of blood products at the state, district and hospital level, as well predictive supply planning excercies. The data was reviewed and two models were developed.
Results:
Model 1: Indirect benefits derived from appropriate use of blood products
Estimated Supply reduction (in units) = [|Planned supply (roadmap year) $\pm$ Planned supply (financial year)|]
Estimated savings (avoidable cost) per year = Supply units reduction $\times$ forecast unit prices (roadmap year) $\times$ 37%
Model 2: Indirect benefits derived from blood products wastage reduction
Keeping the wastage rate (DAPI) below the benchmark wastage (WAPI) target
Reducing the wastage rate (DAPI) to meet the benchmark wastage (WAPI) target
Total Cost of Issues = Number of Issues (units) $\times$ price per unit
DAPI (%) = Discard (units)/Number of issues (units) $\times$ 100%
Estimated wastage (%) = Discard (units) $\div$ Number of issues (units)
Estimated potential savings ($) = Total Invoice Cost of Issues for NSW $\times$ (|DAPI $\pm$ WAPI|)
Summary/Conclusion: The models were accepted for the road map project and approved by NSW Treasury. Following validation at 6 and 12 months, including realisation of actual savings, the models were identified as approapriate for baseline prediction, and re-modelling is now in progress in collaboration with Treasury for the development of monitoring the ongoing improvemetn work in a sustained project.

P-029

EVALUATION OF ECONOMIC AND SAFETY BENEFITS OF THE INTERCEPT BLOOD SYSTEM FOR BUFFY COAT DERIVED PLATELETS BY COMPARISON OF PRE- AND POST-IMPLEMENTATION AND THE RELATION TO PRODUCT QUALITY

LM Larsson, HS Ahlzén, M Kaarlenkaski and B Diedrich

Karolinska University Hospital, Stockholm, Sweden

Background: Karolinska University Hospital produces about 9,000 units of buffy coat derived platelets (BCPs) per year for patient use. Until 2014, they were produced using the OrbiSac System (OrbiSac) together with enhanced Bacterial Detection System (eBDS) as safety measure. To further improve blood safety, pathogen inactivation was considered, and to avoid increased costs, Intercept Blood System (Intercept) was combined with manual production of double dose buffy coat derived platelets (DD-BCPs).
Aim: The aim of this study was to perform an extensive retrospective analysis to evaluate implementation of Intercept with regard to economy, blood safety and blood component quality.
Method: After 1 year of Intercept treated BCPs, production cost (labor and material cost), number of produced BCPs and number of transfused platelet units (PLTs), percentage of BCPs subjected to safety measures, as well as the quality of BCPs, plasma and RBCs were analyzed and compared to Orbisac eBDS BCPs.
Results: Production cost per BCP unit decreased by 16% after Intercept implementation. This was mainly driven by 21% per unit decreased material costs, whereas labor cost increased by about 10% per unit. Increases in both BCP production (7%) and PLT transfusions (5%) were observed.
After OrbiSac production, all BCPs were released immediately. Due to patient demand, transfusion of 22% of BCPs was done without eBDS, 23% after eBDS was started (but not completed) and 55% after completion of eBDS. All BCPs were gamma-irradiated. After manual production, 98% of BCPs were Intercept treated before release and transfusion. Only 2% of BCPs (the not Intercept treated) were gamma-irradiated.
In the 2 years preceding Intercept implementation, two severe bacterial transfusion reactions occurred, whereas none was observed during the first 12 months of Intercept.
Due to a volume limit on the DD-PLTs for Intercept treatment, BCP volume was reduced while PLT count per liter was concomitantly increased. Quality data showed that BCP volume decreased by 47%. However, the PLT count per unit only decreased by 30% (average PLT count of 239 $\pm$ 40 $\times$ 10^9/unit), which is still well above our quality limit (>200 $\times$ 10^9/unit). Quality of plasma and RBCs remained unaffected. Due to the new production procedure, a large number (representing about 8% of all BCPs produced) of extra quality controls were performed during the first year of Intercept usage.
Summary: Intercept implementation has improved the safety of BCPs with reduced production cost per unit, while blood component quality is still in agreement with

national guidelines. However, extra quality controls during the first year have cancelled about 0.6% of the reduced cost per unit.
The increase in PLT transfusions is comparable to previous years, implying that the reduced platelet count per unit has not affected clinical transfusion effectiveness.
The potential cost saving related to gamma-irradiation has not been possible to quantify.
We have identified areas in the production that can be streamlined, and predict that revising the production procedure will further decrease labor cost and eliminate extra quality controls, saving money and effort. However, this remains to be confirmed.

Training and Education

P-030

APHERESIS EDUCATION EXPERIENCE

H Vrielink[1] and MM Neyrinck[2]
[1]*Sanquin Blood Supply, Amsterdam, The Netherlands* [2]*AZ Delta, Roeselare, Belgium*

Background: Workers need to be qualified and competent for their job. Based on this idea, learning objectives for various areas within the field of apheresis for a course for apheresis nurses were described by a group of experienced apheresis nurses and physicians (Joint Task Force for Apheresis Education and Certification; JTFAEC).
Materials and methods: We composed a course with 13 lessons and tests with >100 different and independent questions based on these learning objectives. Tests are made prior and after the course. In co-operation with colleagues, ASFAs 5th edition of 'Principles of Apheresis Technology' was written to cover the majority of the learning objectives, and can therefore applied as manual.
Results: Indonesia (December 2013, March 2015, nurses and physicians from Cambodia, Indonesia and Singapore).
Belgium and the Netherlands (March, September, October 2014; January, May 2015; January 2016, nurses and physicians).
Austria (February 2015, nurses from Austria, Italy, Spain, Sweden, Switzerland, The Netherlands, UK and USA).
South Africa [April 2015, train-the-trainer course for trainers from SANBS (South African National Blood Service)]. The new trainers gave a successful course (nurses and physicians).
All 152 nurses and 115 physicians showed a significant increase of knowledge (post- vs pre-course test).
All nurses could be certified according to the JTFAEC requirements.
Conclusions: This program provides an approach to educate and certificate apheresis nurses.
This course can be of value for physicians increasing their knowledge regarding apheresis.
The course can be given in local languages in case English is not the native language.
Training of apheresis nurses (and physicians) according to the concept of this course is discussed in various countries.

P-031

BEDSIDE BLOOD TRANSFUSION PRACTICES AND SAFETY: A SURVEY OF THE NURSING STAFF OF BONE MARROW TRANSPLANTATION UNIT OF A TERTIARY CARE HOSPITAL

N Jain and J Chakrabartty
AMRI, Hospitals ltd, Kolkata, India

To establish the evidence based institutional guidelines for safe and secured blood transfusion practices.
Background: Blood is a living tissue and its transfusion, from one individual to another, is not without risk. Significant among these risks is the potential for human error and the subsequent transfusion of the incorrect blood component. Alarmingly, the number of errors would appear to be increasing, with the second annual report of the Serious Hazards of Transfusion (SHOT) Steering Group reports a 36% increase in these cases. Reversing this trend can only be achieved by adopting all inclusive and rigorous blood transfusion procedures. The registered nurse is central to such processes and therefore plays a crucial role in the safe administration of blood and blood products. This article considers the nurse's responsibilities and offers evidence-based guidelines for practice.
Materials and methods: We have around 2–3 haematopoitic stem cell transplantation in a month and the average requirement of blood products per patient throughout their hospital stay is 14 (SDP-8 and PRBC-6). A survey questionnaire was prepared and distributed amongst the nurses posted in the BMT unit, which they had to fill in front of the surveyor. Also surveyor has observed the process of blood transfusion from the receiving of blood from the blood bank till 15 min post transfusion and at the end of the transfusion. Also, the nurses have been asked certain questions including the management of a blood transfusion reaction.
Results: Of the 50 nurses posted in BMT unit on rotation basis, 43 (86%) have answered correctly when questioned by the surveyer, but only 33 (66%) nurses correctly demonstrated the sequence of transfusion from receiving of blood till the onset of transfusion. 34 nurses experienced the blood transfusion reaction but five (14%) of them could not answered correctly the steps of management of a blood transfusion reaction. A significant delay was noted in turned around time (TAT) from sending the requition to get calls for issue slip and then from sending the issue slip to receiving the blood at nursing station, then from nursing counter to starting the transfusion.
Discussion: In last 18 months a total of 648 blood units (415 SDP and 233 PRBC units) have been transfused to the patients during stem cell transplantation. 13 transfusion reactions were noted during this period, but none of them caused serious hazard to the patient. Also one near miss error took place, detected just prior to the onset of transfusion. In conclusion, nurses play a very crucial role in the safe blood transfusion practices. Repeated training can definitely help in reducing the human errors, hence there has to be an Institutional blood policy which must ensure the basic blood transfusion training for nurses.

P-032

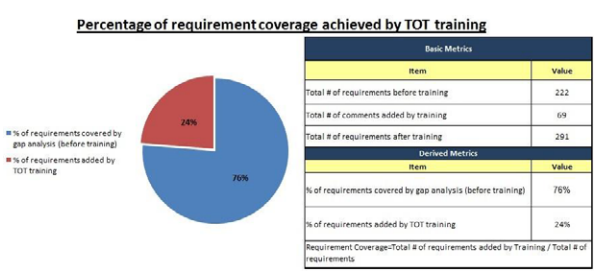
This abstract has been withdrawn.

P-033

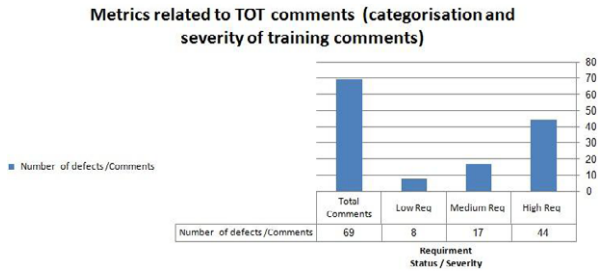
IMPACT OF TOT (TRAINING OF THE TRAINERS) METRICS, ON IMPLEMENTATION OF BLOOD MANAGEMENT SYSTEM (BMS) AT THE EGYPTIAN NATIONAL BLOOD TRANSFUSION SERVICES (ENBTS): THROUGH ADJUSTMENT OF WORKING RULES

L Borhan Ragheb[1], A Al Gohary[1], H Abady[1] and M Hashem[2]
[1]*National Blood Transfusion Centre, Cairo, Egypt* [2]*National Blood Transfusion Services, Sohag, Egypt*

Background: TOT for BMS super-users at the NBTS is vital to achieve maximum familiarity and competence regarding all implementation aspects of the E-Delphyn application.
Training of super-users can deliver constructive feedback, provided by blood bank staff who are the ultimate software users. Comments are then utilised to enhance application quality and minimise implementation errors.
Aim: To illustrate how TOT is not only training of the users,to further propagate training, but feedback can be channelled to produce data and improve software accuracy and reliability and subsequently adjust working-rules.
Methods: Training of the super-users was initiated 4/4/2016 for 16 working days, with 24 trainees composed of blood bank staff from all departments, divided into two groups (12), with 40 training hours in total. These 40 h constituted 8 days (5 h/day).The first 4 days (theoretical), whilst the remaining 4 days (practical).



Caption 1. Percentage of Requirement Coverage Achieved by TOT training



Caption 2. Metrics Related to Training Comments

Qualitative assessment of comments during gap analysis (before TOT training),and comments from TOT was done. Comments were recorded/categorised according to module and severity (High/Medium/Low)

Result: When training is propagated it will exponentially increase the number of trainers, but there was an added-value in that metrics could be produced from these comments:Total software requirements before training were **222 (76%),** after TOT **69 (24%)** requirements were added.

This meant that resulting comments were so pertinent that they led to an **increase in number of software requirements by 24%,** and once these metrics were incorporated into the software they caused an enhancement of accuracy, and markedly decreased cost and time before implementation and risk of fatal errors after implementation.

Examples of high priority errors:

Donation: In case of an underweight unit the software allowed the donor to donate again on the same day. Donors should be allowed to give only one donation whether it is:acceptable/underweight/overweight, and only be accepted again after 3–4 months.

Laboraotory: Antibody results are not saved or retrieved at donation by the result screen.

Processing: Centrifugation date should be fixed, and unchangeable .The old version allowed the user to enter a centrifugation date in the past (before the donation took place).

Platelets should not be allowed to be separated if plasma is entered as Frozen Plasma (devoid of coagulation Factors).

The underweight unit weighing 400–449 g should only be separated to 'RBCs only' (to avoid citrate toxicity).

Patient: When searching for a patient, who was previously a donor, all data appears, but when trying to enter the civil ID the field is blocked.

Conclusion: TOT is a necessary step in propagating training, but when trainees are chosen intelligently based on experience and pro-activeness,gleaned feedback can detect hidden glitches that only blood bank staff can discover through medical perspective. This is seen when training increased software requirements by 24%.These metrics are invaluable to the IT staff, causing an alteration of working rules,and enhancement of application accuracy,safety and reliability,through shedding light on points that staff outside the regular work flow could overlook

Propagation of this customised collaborative training is an irreplaceable,step towards NBTS,BMS implementation as identifying and communicating such errors will help other blood banks in the country and elsewhere, considering BMS installation.

P-034

QUALIFIED APHERESIS NURSE' CERTIFICATION BY THE JAPAN SOCIETY OF TRANSFUSION MEDICINE AND CELL THERAPY; A 5-YEAR EXPEIENCE OF CERTIFICATION AND RENEWAL PROCESS

K Ikeda[1], T Iseki[2], K Yamamoto[3], Y Okuyama[4], H Kanamori[5], K Matsuzaki[6], E Sueoka[7], K Muroi[8] and H Ohto[9]

[1]Okayama Red Cross Blood Center, Okayama, Japan [2]Chiba University Hospital, Chiba, Japan [3]Saitama Medical Center, Kawagoe, Japan [4]Komagome Hospital, Tokyo, Japan [5]Kanagawa Cancer Center, Yokohama, Japan [6]Fukuoka Blood Center, Tsukushino, Japan [7]Saga University Hospital, Saga, Japan [8]Jichi Medical University Hospital, Shimotsuke, Japan [9]Fukushima Medical University, Fukushima, Japan

Background: In 2014, 1,791 autologous and 963 allogeneic (907 from related and 56 from unrelated donors) peripheral blood stem cell (PBSC) transplants were carried out in Japan. These PBSCs were harvested at transplant centers throughout Japan. Platelets and plasma for transfusion therapy and plasma fractionation products were harvested from 864,898 and 529,508 donors at blood donation rooms operated by the Japanese Red Cross Society (JRCS). The Japan Society of Transfusion Medicine and Cell Therapy (JSTMCT) started certifying experienced nurses through lecture and examination as JSTMCT-Qualified Apheresis Nurse to encourage them to be more knowledgeable and to contribute to safe apheresis through adequate nursing.

Aims: In order to review the certification and renewal process and to improve the certification system.

Methods: To be eligible, nurses were required to (i) work at hospitals or blood donation sites operated by the JRCS, (ii) have more than 3 years of clinical experience at hospitals or blood donation sites, (iii) have experience of more than 1 year and 10 cases of apheresis, (iv) get recommended by director of nursing and by physician in charge of transfusion at hospitals or director of blood donation sites. The curriculum included basics for transfusion and cellular therapy, colleting blood components at hospitals and blood donation sites, therapeutic apheresis, hematopoietic stem cell mobilization and transplantation, nursing for patients and donors in apheresis and Japanese guidelines for transfusion and in-house cell processing. The certification was supposed to be renewed every 5 years by acquiring defined credit points. A questionnaire survey was conducted on the renewal process 5 years after the certification.

Results: Between 2010 and 2015, a total of 254 nurses, of whom 93 belonged to hospitals and 161 belonged to blood centers, respectively, applied, 246 (86 and 160) were considered eligible for the examination and 239 (81 and 158) passed the examination. As of April 1, 2016, 214 nurses were actively certified. During the last 5 years, one passed away and nine withdrew from JSTMCT. During the fiscal 2015, 27 nurses, all of whom belonged to hospitals and passed the examination in 2010, were supposed to renew their certification. Of the 27, 12 successfully renewed the certification. Of the 15 who did not renew, six declined to renew, two retired from work, five postponed the renewal due to inadequate credit points and two lost contacts. The questionnaire survey showed the certification was stimulatory for most of the nurses who participated, and they desire continued education even after certification. The survey also showed their relocation among departments and financial burden to attend meetings to gain credits for renewal tended to hamper their renewal.

Summary/Conclusions: We established certifying 'Qualified Apheresis Nurse' system in 2010 and certified 239 nurses. Our review on the certification and renewal process suggest the need to enrich sessions for post-certification education at JSTMCT Annual Meetings and to care for their financial burden for renewal.

P-035

EVALUATION OF DONORS' AND POTENTIAL DONORS' EDUCATION REGARDING HBV, HCV AND HIV INFECTION IN REGIONAL BLOOD CENTRE IN POZNAN IN 2012–2013 AND 2014–2015

A Zawadzka and K Olbromski

Blood Center, Poznan, Poland

Background: Haemovigilance is the set of procedure regarding the proper conduct in reference to the occurrence of serious adverse reactions after transfusions in blood donors and serious reactions in blood recipients, including the donors that were tested positively for HBV, HCV and HIV.

Aim: Evaluation and comparison of the effectiveness of educational activities in the years 2012–2013 and 2014–2015 in the group of blood donors and potential blood donors in the area of activity of the Regional Blood Center in Poznań and the analysis if providing intensive education activities for blood donors can minimize in the future the risk of viral complications after blood transfusion in blood recipients.

Methods: The education of donors and potential donors was started in years 2012–2013 and continued in years 2014–2015. Various educational materials were used: leaflets, brochures, press articles, internet websites, resources and tools on Facebook as well as individual tutorings for donors registering for blood donation. For high school students dedicated lectures were held, including visiting the premises of blood center. Within the regular events such as Open Door exhibitions and presentations giving insight into details of processing blood took place. The effectiveness of activities was evaluated randomly using survey questionnaire (100 surveys per quarter of the year). The knowledge regarding the possibilities of HBV, HCV and HIV infection was the focus of the survey. The effectiveness proved to be very high – approx. 96%. The educational activities were continued after the analysis of the epidemiological data from 2010 to 2011 (prior to the education) and 2012 to 2013 (after introduction of educational activities) with the assumption of effectiveness at least on the same level if not higher. Eventually, data from three periods (2010–2011, prior to the educational activities), 2012 to 2013 (introduction of the activities) and

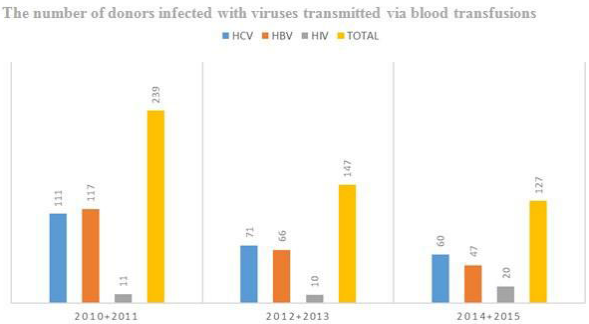


Chart 1.

Table 1

Years	Total number of donors	Number of infected donors			
		HCV	HBV	HIV	TOTAL
2010+2011	97979	111	117	11	239
2012+2013	97406	71	66	10	147
2014+2015	105756	60	47	20	127

2014 to 2015 (continuation of the activities) were summarized and compared. The table below (No. 1) presents total number of donors for years 2010–2011, 2012–2013 and 2014–2015 and the number of donors tested positively for the presence of markers of the blood-borne viruses.

Results: The analysis proved definite declining trend of total number of virus infections (Chart 1). Considerable statistical significance was found in favor of the events in years 2012–2013, compared to 2010–2011. The comparison of data from years 204 to 2015 with 2012 to 2013 shows persistent positive results on verge of statistical significance as the total number of donors increased by 8,350 in years 2014–2015 compared to years 2012–2013 and the number of infected donors decreased by 20.

Conclusions: The undertaken educational activities proved successful and effective and should be continued in the future.

Risk Models, Standards and Regulation

P-036

HEMATOLOGICAL REFERENCE INTERVALS IN IRANIAN BLOOD DONORS

M Pourmokhtar[1], S Sarkardeh[2], M Rasouli[3], F Abbasi[4] and M Eftekhari[4]

[1]Blood Transfusion Research Center,High Institute for Research & Education, Tehran, Iran [2]Faculty of Pharmacy, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran [3]Department of Biostatics, School of Public Health, Iran University of Medical Sciences, Tehran, Iran [4]Tehran Blood Transfusion Center, Tehran, Iran

Background: Hematological reference intervals are essential for the interpretation of laboratory test results in clinical practice, research studies and for patient management. Since there is considerable variation in reference values due to ethnic, social and environmental variables, therefore determination of locally-derived hematological reference intervals of Iranian population is a necessity.

Aims: The aim of this study was to determine the hematological reference intervals for healthy adults in Iran.

Methods: A total of 394 healthy adults, donating blood at Tehran Blood Transfusion Center, and negative for HIV, HBV, HCV and RPR were randomly selected. Complete Blood Count indices were measured for all blood samples using Sysmex automated hematology analyzer. Data were analyzed by SPSS software (version 22) and median and 95% reference intervals were determined for hematological values.

Results: The median and 95% reference intervals for hematologic parameters were as follows: WBC ($\times 10^3/\mu l$) = 7.00 (4.87–10.42), RBC ($10^6/\mu l$) = 5.54 (4.60–6.27), Hgb (g/dl) = 15.85 (13.7–17.9), HCT (%) = 47.90 (41.07–54.2), MCV (fl) = 86.15 (80.40–95.6), MCH (pg) = 28.70 (25.70–31.2), MCHC (g/dl) = 33.30 (30.50–35.3), PLT ($\times 10^3/\mu l$) = 233.00 (144.70–332.0).

Summary/Conclusions: The results of present study showed that the upper limits of the platelet reference intervals for the Iranian population were lower than the corresponding international values. While some differences were also observed in other hematological intervals. Therefore given the importance of hematological reference intervals, conducting more studies at the country level are recommended to determine those intervals for Iranian population.

P-037

GLOBAL RISK MANAGEMENT SYSTEM: A PART OF MANAGEMENT AND DECISION MAKING

W Sghaier, E Hergon, S Jullien and X Ortmans

Etablissement Francais du Sang, La Plaine Saint Denis, France

Background: Daily the company takes risks. It is source of itself risks because of its activities, organization, staff and the top management decisions. On the other hand, company's environment is a source of hazard that has to be reduced by minimizing the economic impacts.

The risk management is an activity as important for the companies as the stake in optimal safety of products and services is big. This is the case particularly for healthcare organization such as the 'Etablissement Français du Sang' (EFS).

Aims: The objective of our work is to present tools and methods used for the implementation of a global risks management system within the EFS.

Methods: The initial approach of risk management is based on a process approach, more technical than strategic. It is very useful for the process pilots and based on two sub-systems:

-Proactive and systemic approaches: The method of risk management used is the Preliminary Hazard Analysis (PHA) which led to define and describe the system to be analyzed, to design of the hazard checklist, to define severity classes, frequency, effort and risk matrix, then build the hazardous situation mapping and the risk mapping for each event and the elaboration of the follow up actions with the catalogue of safety parameters.

-Reactive risk management which is based on the implementation of a system of non-conformity management. It is continuously realized from the data stemming from inspections, from vigilances, from audits, from voluntary statements, from complaints. . .

These two sub-systems are put in coherence by a common nomenclature (hazard checklist) and a common severity scale. So the PHA will be put in the day during the processes review from the synthesis of the declared non-conformity.

This approach based on process is completed by a governance risks management system. This system allows, thanks to its macroscopic dimension, to visualize better the major risks to which the EFS is exposed. It is intended to establish the risk dashboard that can be exploited by the top management for decision-making. For this aim the strategic risks of the EFS are identified on the basis of semi-directive interviews with the top management.

Results: The PHA results are represented in the form of a process and hazard risks mapping. These mappings allow to prioritize and to decide on plans of risks reduction to implement at the analyzed processes.

The interviews with the top management allowed to identify 25 major risks among which 10 were prioritized and modeled within a major risk mapping. This mapping as well as the risks reduction plans are the object of a quarterly reporting on a risks dashboard.

Conclusion: The implementation of a global risks management is strategic and contributes to the sustainability of the organization. However, changing structures and constraints on the availability of human and financial resources can make it challenging to apply industry-leading risk management practices.

P-038

LOW HAEMOGLOBIN IS NOT ASSOCIATED WITH INCREASED RISK OF INFECTION

SR Kotzé[1], OB Pedersen[2], MS Petersen[1], E Sorensen[3], KM Dinh[1], KA Kaspersen[1], AS Rigas[3], H Hjalgrim[4], K Rostgaard[4], H Ullum[3], G Edgren[5] and C Erikstrup[1]

[1]Aarhus University Hospital, Aarhus V, Denmark [2]Naestved Hospital, Naestved, Denmark [3]Copenhagen University Hospital, Copenhagen, Denmark [4]Statens Serum Institut, Copenhagen, Denmark [5]Karolinska Institutet, Stockholm, Sweden

Background: Low haemoglobin (Hb) is associated with poor general health and adverse outcomes in a wide range of diseases, and patterns of declining Hb are seen in patients with cancer and auto-immune disease. However, a link between Hb and risk of infection among healthy individuals has yet to be investigated.

Table 7. Hemoglobin and risk of hospital or outpatient contact for infection, 3 months of follow-up

	Pre-menopausal women				Post-menopausal women				Men			
	Events	Person years at risk	HR	95%CI	Events	Person years at risk	HR	95%CI	Events	Person years at risk	HR	95%CI
Deferral	558	76,114	1.04	0.95-1.14	30	10,876	0.77	0.54-1.11	128	25,598	0.97	0.81-1.16
No deferral (reference)	2,622	387,238	1.00	Ref	396	116,675	1.00	Ref	4,007	722,861	1.00	Ref
Hb decline	70	9,726	1.01	0.83-1.24	9	1,825	0.86	0.31-2.39	71	12,001	0.86	0.52-1.41
No Hb decline (reference)	2,303	361,293	1.00	Ref	370	112,875	1.00	Ref	3,457	651,571	1.00	Ref
Very low Hb	4	460	1.07	0.95-1.21	0	98	n/a	n/a	4	681	0.92	0.72-1.18
No very low Hb (reference)	3,176	462,892	1.00	Ref	426	127,453	1.00	Ref	4,131	747,778	1.00	Ref

Note: numbers in table may not add up due to rounding. Hazard ratios (HR) were computed using an Andersen-Gill model for Cox regression.

Aim: The aim of the study was to investigate the association between low Hb levels and risk of infection.
Materials and methods: Using data from the Scandinavian Donations and Transfusions (SCANDAT) database, 497,390 donors were followed-up in health registers after 5,458,499 donations. With 1,339,362 person years of follow-up, we performed Andersen-Gill Cox regression to study the association of Hb below deferral guidelines, very low Hb (lowest 0.1 percentile) and patterns of Hb decline with risk of hospital or outpatient contact for infection and risk of filling a prescription for anti-microbials within 3 months of donation. Analyses were stratified by sex, menopausal status and frequency of donation.
Results: Hb below deferral guidelines was not associated with risk of hospital contact due to infection for premenopausal women [Hazard Ratio (HR): 1.04, 95% Confidence Interval (CI): 0.95–1.14], post-menopausal women (HR: 0.77, 95% CI: 0.54–1.11), or men (HR: 0.97, 95% CI: 0.81–1.16). Nor was there any association for very low Hb or patterns of declining Hb. Hb below deferral guidelines was associated with a reduced risk of filling a prescription for anti-microbials for pre-menopausal women (HR: 0.92, 95% CI: 0.91–0.93), post-menopausal women (HR: 0.93, 95% CI: 0.89–0.97) and men (HR: 0.91, 95% CI: 0.88–0.94).
Conclusions: Neither Hb below deferral guidelines, very low Hb or declining Hb was associated with increased risk of infection in active whole blood donors. This is reassuring, as blood donation can lead to lower Hb.

Table 8. Hemoglobin and risk of filling a prescription for anti-microbials, 3 months of follow-up

	Pre-menopausal women				Post-menopausal women				Men			
	Events	Person years at risk	HR	95%CI	Events	Person years at risk	HR	95%CI	Events	Person years at risk	HR	95%CI
Deferral	**29,539**	**74,158**	**0.92**	**0.91-0.93**	**8,741**	**11,858**	**0.93**	**0.89-0.97**	**5,994**	**28,903**	**0.91**	**0.88-0.94**
No deferral (reference)	159,025	375,022	1.00	Ref	41,685	122,975	1.00	Ref	174,135	746,528	1.00	Ref
Hb decline	3,930	9,531	1.01	0.98-1.04	685	1,983	1.08	1.00-1.16	2,944	12,958	1.01	0.98-1.05
No Hb decline (reference)	140,870	350,059	1.00	Ref	38,931	117,318	1.00	Ref	153,531	668,649	1.00	Ref
Very low Hb	167	517	0.80	0.67-0.93	46	232	0.67	0.45-1.00	329	1,502	1.06	0.89-1.25
No very low Hb (reference)	188,397	448,663	1.00	Ref	45,380	134,601	1.00	Ref	179,800	773,928	1.00	Ref

Note: numbers in table may not add up due to rounding. Hazard ratios (HR) were computed using an Andersen-Gill model for Cox regression.

Blood Supply Management and Utilisation

P-039

BLOOD TRANSFUSION QUALITY INDICATORS AS REFERENCE TOOL TO EXAMINE AND IMPROVE OWN CLINICAL PRACTICE

V Polanda Lasica[1] and S Oslakovic[2]

[1]General hospital Dubrovnik, Dubrovnik, Croatia [2]County hospital Cakovec, Cakovec, Croatia

Background: Large differences are observed between hospitals and clinical teams in the use of red cell transfusions for the same surgical procedures without clear explanation. That cause unnecessary requests, reservation, high cost and wastage of blood. County Hospital Cakovec and General Hospital Dubrovnik are equal in capacity and medical procedures, but in different regions of Croatia.
Aim of study: To evaluate the routine blood requests and consumption of blood products in surgery and its wards (abdominal, trauma, vascular, orthopedics and urology) in both hospitals for 1 year.
Methods: Data has been processed according to the urgency of surgery, urgency of transfusion treatment, clinical indicators for transfusion therapy and hemoglobin values. The plans have been used for ordering blood products for elective surgical procedures in order to optimise the practise of clinical transfusion. Clinical quality indicators C/T ratio, % T – the likelihood of transfusion and TI – transfusion index are used to screen and evaluate these plans and transfusion of clinical practice.
Results: Total number of transfused patients, who were in the surgical departments, are 24.26% in Cakovec and 28.63% in Dubrovnik. However, the number of surgical requests for blood components make 35.83% in County Hospital Cakovec and 32.32% in General Hospital Dubrovnik out of the total number. That results significantly affect the organization of transfusion services. These two Hospitals from different regions of Croatia have differences in percentage of realised surgical requests, nevertheless both of them have the acceptable usage rate which can not be <30%.
Conclusion: The role of clinical quality indicators is important in assessment of blood use. Comparison analysis should help to examine and improve our own clinical transfusion practice and ensure continuous quality improvement. It should also reduce consupmtion of blood products for elective surgical patients. Maximum Surgical Blood Ordering Schedule for routine elective procedures will prevent unnecessary blood reservations and diagnostic screenings.

P-040

BURDEN OF HEMOGLOBINOPATHIES ON THE BLOOD BANK OF A TERTIARY CARE SETTING

R Akbar, L Zafar, A Khalid and SS Shafaat

Foundation University Medical College Rawalpindi, Rawalpindi Cantt, Pakistan

Background: There is dearth of blood banking facilities in Pakistan. Only a few tertiary care hospitals are offering blood banking facilities. Amongst these, many blood banking services cannot cater to special needs of antibodies identification and provision of washed, leucodepleted or irradiated blood products etc. Besides this hemoglobinopathies impose additional burden to the blood transfusion services because of lack of availability of rare blood groups and blood components and lack of replacement donors. These patients are also at high risk of transfusion transmissible diseases. The aim of this study was to identify the burdon imposed by various hemoglobinopathies on the blood bank of a tertiary care hospital.
Materials and methods: In our study, the transfusion requirements of all the patients having various hemoglobinopathies were studied from January 2015 to January 2016. The data of all the patients who were issued red cell concentrates during this period was recorded. Particulars of those patients who needed washed red cells were also recorded in the proforma. Forward and reverse ABO grouping and Rh grouping was done on all samples along with cross match and all the findings were recorded in the proforma.
Results: A total of 4,688 red cell concentrates were issued during the study period. Out of these, 1,284 (27.41%) units were issued to patients admitted to medical wards, 840 (17.93%) red cell units were issued to gynaecology and obstetrics department,768 (16.39%) units were issued to patients admitted in oncology ward,624 (13.32%) units were issued to patients admitted in surgical department,480 (10.24%) to patients in intensive care unit,156 (3.33%) units were issued to paediatrics patients other than those having various hemoglobinopathies, while 84 (1.79%) red cell concentrates were issued to the patients in whom the ward could not be identified because of incomplete

filling of blood requisition forms .452 (9.64%) red cell concentrates were issued to the patients having various hemoglobinopathies. A total of 429 (95%) of these red cell concentrates were issued without replacement donations. The frequencies of A^+, B^+, AB^+ and O^+ blood groups were 22.0%, 35.65%, 6.40%, and 33.65%, respectively. Twenty four washed red cell concentrates were issued during this period.
Conclusion: It is necessary to design effective ways and means to counter haemoglobinopathies with adequate preventive methodologies and treatment strategies. Moreover awareness among general population about voluntary blood donations is key to keep pace with increasing load of haemoglobinopathies on blood banks.

P-041

BETTER BLOOD TRANSFUSION PRACTICES

B Raghuwanshi
AIIMS, Bhubaneswar, India

Background: Blood ordering is a common practice however over ordering leads to loss of health care resources and health burden.
Aims: To audit blood ordering and blood utilization practices and evaluate factors predictive of transfusion requirements.
Methods: A hospital based cross sectional study was conducted in those patients who underwent elective and emergency surgeries in AIIMS Bhubaneswar hospital over a period of 19 months. Blood requisition and transfusion of surgical, obstetrics and gynaecology, medicine and oncology cases were compiled and reviewed. Patient's age and sex, number of units prepared, cross matched, and transfused number of patients cross matched and transfused, source of blood donation or collection, were collected from discharged patient medical records and blood bank registries. Data were coded, entered, and analysed using SPSS Version 20. Blood utilization indices were computed with the equation.
Result: During the study period a total of 11,412 patients underwent major elective and emergency procedures. Among these, 2,574 patients were requested to prepare 2,558 units of blood. Majority of the patients were females 1,507 (59.2%), who underwent surgery in elective schedule, and blood for transfusion was arranged by replacement
Donation. Patients prepared blood ranging from 1 to 6 units; averagely it was 1.06. From 2,558 units prepared, 1,473 (57.6%) units were transfused and 1,085 (42.4%) were prepared but not transfused. These showed that 57.6% of total blood cross matched was utilized, leaving 42.4% of the units cross matched but not transfused to the patient for whom it was prepared, that is, wasted. Surgery department was the department with the highest number of both patients cross matched 988 (38.7%) as well as with the highest no of units reserved but not transfused 504 (46.5%). On the other hand, obstetric and gynaecology was the department with the second highest number of units cross matched and reserved for transfusion 761 (29.8%) but not transfused 447 (41.3%).
Summary: Preoperative over ordering of blood has been documented since 1976, when Friedman et al. published their findings. Subsequently, a number of studies have also showed over ordering of blood in different parts of the countries. Data from developing countries have shown gross over ordering of blood in 40% to 70% of patients transfused.

DEPARTMENT	C/T RATIO	%T	TI	TIX1.5
SURGERY	2.04	48.9 %	0.48	0.73
OBSTETRIC AND GYNECOLOGY	1.8	53.0 %	0.53	0.79
ONCOLOGY	1.17	85.4%	0.85	1.28
MEDICINE	1.15	87.1%	0.87	1.3
TOTAL	1.59	63	0.63	0.94

Picture 1.

DEPARTMENT	C/T RATIO	%T	TI	TIX1.5
SURGERY	2.04	48.9 %	0.48	0.73
OBSTETRIC AND GYNECOLOGY	1.8	53.0 %	0.53	0.79
ONCOLOGY	1.17	85.4%	0.85	1.28
MEDICINE	1.15	87.1%	0.87	1.3
TOTAL	1.59	63	0.63	0.94

Picture 2.

Since the introduction of blood transfusion into clinical practice, its appropriate use has been the subject of debate. It has been reported that only 30% of cross matched blood is used in elective surgery. Generally the percentage of cross matched patients receiving transfusion for general surgical procedures ranged from 5% to 40%. Therefore, it is essential that the usage of blood and blood products should be rationalized and saved for crisis situations.
The current study revealed 42.4% of the cross matched blood was unutilized. Blood ordering pattern needs to be revised and over ordering of blood minimized by the estimation of MSBOS for each procedure. The hospital blood transfusion committee should formulate blood order schedules for selected surgical procedures, conduct regular auditing about effectiveness of the blood requesting policy and offer periodic feedbacks to improve blood ordering, distribution, and utilization practices of this scarce resource.

P-042

REVIEW OF BLOOD CROSS-MATCH ORDERING AND TRANSFUSION PRACTICE FOR ELECTIVE HYSTERECTOMIES AT SAINT PAUL'S HOSPITAL, ADDIS ABABA, ETHIOPIA: FIRST STEP TOWARDS MAXIMUM SURGICAL BLOOD ORDER SCHEDULE

K Lemma, D Bekele and T Mena
St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

Background: Preoperative requesting of blood is a common practice. Studies show that majority of blood requested by surgeons are not utilized and this over ordering of blood will create a burden on transfusion services and will divert blood from the pool making it unavailable for other needy patients. Such practices have greater implications in resource constrained settings like ethiopia where the blood bank struggles to avail blood and blood products. Maximum surgical blood order schedule (MSBOS) is an evidence based system which recommends the maximum number of units of blood to be cross-matched pre-operatively reducing the cost associated with inappropriate cross-match ordering. At saint paul's hospital, one of the largest tertiary referral centers in the country which provides specialized gynecology services, two units of blood are cross-matched routinely for all elective hysterectomies.
Aims: The objective of this study was to assess the blood requesting and utilization pattern in relation to elective hysterectomies performed at Saint Paul's Hospital, as a first step to introduce maximum surgical blood ordering schedule in the hospital.
Methods: A hospital based retrospective study was conducted at St. Paul's Hospital. All elective hysterectomies performed in the time period between September 2011 and August 2013 were included for the study and the associated blood cross-match ordering and transfusion practice was audited. The various transfusion indices were calculated separately for the different indications and modalities of hysterectomies and the msbos for each indications were determined.
Result: A total of 532 units of blood were cross-matched for the 265 patients who underwent hysterectomy. From these only 74 units of blood was transfused to 39 patients, making the blood utilization 13.9%. With regard to the operative procedure and the transfusion induces; the maximum cross match to transfusion ratio of 96 was found for vaginal hysterectomy and the minimum was for abdominal hysterectomy done for

gestational trophoblastic disease which was 1.5. The transfusion probability was least for vaginal hysterectomy (1.4%) indicating that <2% of blood is being utilized.the application of msbos showed that for vaginal hysterectomy there is no need to prepare blood.
Conclusion: The results of the study showed that in most of the elective hysterectomies performed at St. Paul's Hospital the level of blood utilization was minimal and there is significant over ordering of blood and associated wastage of resources. The process whereby fixed units of blood are requested for each patient irrespective of the diagnosis and type of surgical procedure should be revised.

P-043

ESTABLISHMENT OF PAKBLOOD: AN ASSOCIATION OF BLOOD TRANSFUSION STAKEHOLDERS IN PAKISTAN

HA Zaheer

Safe Blood Transfusion Programme, Ministry of National Health Services, Islamabad, Pakistan

Background: The Blood Donor Organizations (BDOs) are an integral component of the blood transfusion system in Pakistan working all over the country, though isolated. The potential importance of BDOs in the BT system reform process, however, lies in the fact that they are collecting enormous quantities of (voluntary) blood donations through their donor motivation and mobilization programmes. The Safe blood Transfusion Programme's national strategy to promote VNRBD aims to encourage the conversion of the vast population of replacement donors into regular blood donors as many of these replacement donors are suitable to become VNRBDs. The second strategy to increase reliance on VNRBDs is to strengthen the capacity of the BDOs which exist all over the country. To achieve the WHO recommended goal of 100% voluntary blood donations till 2020, these BDOs can be the strongest partners because they can do outreach work and already have a huge network of voluntary donors.
Aims: To establish a network of Blood Donor Organizations of Pakistan.
Material and methods: The SBTP identified 60 small and large BDOs and formulated an Inventory of BDOs. The representatives of these BDOs were regularly invited to the training sessions organized by the Programme to develop capacity of these BDOs to unlock their true potential. Need was felt to bring these BDOs on one platform and develop possible strategies for cooperation between the newly constructed Regional Blood Centres and BDOs. This will have the advantage of combining strengths of centralized model with decentralized approaches.
Results: The SBTP in December 2015, established a network of BDOs called PakBLOOD following a national consultation of key stakeholders. The objective is to bring them together on one platform with the aim of interchanging experience and best practices, while on the other hand being open to policy advice from the SBTP and joint strategies and actions for the promotion of blood donation and the advancement of blood safety. Reliable statistics related to every aspect of blood transfusion services are being collected directly from the blood centres and BDOs resulting in credible data from the source directly for onwards reporting to WHO Global Database on Blood Safety. The earlier practice of collecting the data from the Provincial Programme Managers affected the quality of data and lacked consistency. To develop the capacity and future strategy of PakBLOOD, the SBTP is regularly organizing national level consultations to strengthen the process of BDOs' networking and enabled interaction of these organizations in the reformed blood transfusion system. The consultations also served to develop a mechanism to make public private partnership in the VNRBD sector by the provincial blood transfusion programmes. During the course of development process, the Association is expanding its role and now all the blood centres, technical experts involved in BT sector are becoming members.
Conclusion: The PakBLOOD Association is developing close liaison with WHO, IFBDO and other international partners. Credible data will be generated and reported to WHO for the year 2015.

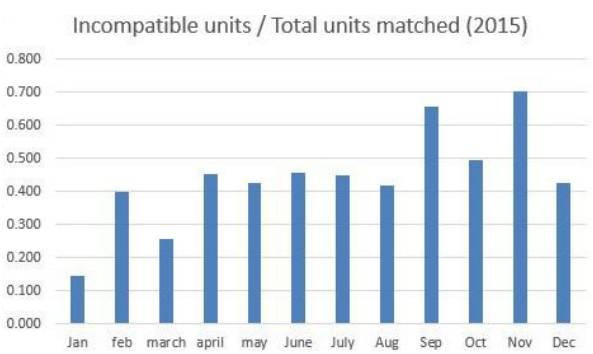
P-044

EFFECT OF AUTOMATION ON THE KEY PERFORMANCE INDICATORS OF ISSUING DEPARTMENT IN EGYPTIAN NATIONAL BLOOD TRANSFUSION SERVICES

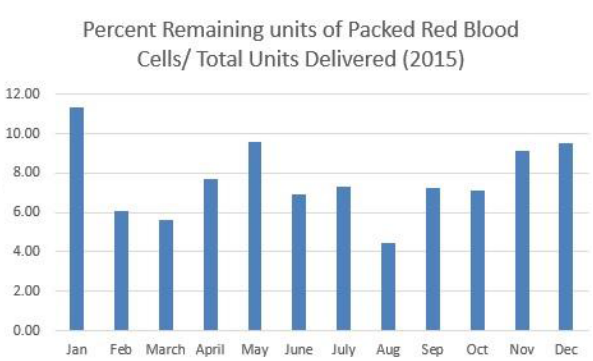
D Shokri, MM Nicola, G Salem, R Omer, M Labib, A Ahmed and R Abdallah

Egyptian National Blood Transfusion Center, Giza, Egypt

Background: Egyptian National Blood Transfusion Services are going through an automation process, aiming to connect 15 regional blood transfusion centers to the Headquarter in Agouza, Giza. The governmental hospitals' blood banks will be simultaneously. Indicators will be set to quantitatively assess how the automation process will make a positive shift in performance.
Aim: Having a look on some already-used-indicators in Issuing Department of Abbassya Regional Blood Transfusion Center, which are now tedious to calculate but will be more simple and more easily tracked after automation.
Methods: The main key indicators are now being calculated manually. The main focus of the study is on the time and effort that will be saved, and how some defects can be avoided upon the implementation of E-Delphin Blood Management System.
Some of these indicators that will be studied:
Indicators that Are Manually Calculated:
Ratio between Incompatible Units and Total Units Matched (Graph attached)
This indicator will help determining:
Causes of technical errors (human errors or pipetting errors)
The kits' quality.
If a patient has developed antibodies.
The Actual Monthly Stock Issued to Contracted Hospitals: which is currently calculated for financial departments, but is not easily accessible by technical lab staff to assess the blood stock needed by each hospital.
Percentage of Remaining Units of Each Blood Component/Total Units Received by Issuing Department: Aiming to have a random figure about the relation between the stock available and actual need (Graph attached).
Indicators That Can't Be Calculated Now but Automation Will Make Them Possible:
Rate of Positive Antibody Screening Cases among Total Number of Samples Tested: Patients with previous positive screening tests will be identified by the system and will be automatically directed for antibody identification. This is expected to increase cost effectiveness.
Ratio between Units in Unfulfilled Requests (due to the lack of inventory stock) and Total Requested Units: the relation between the stock available and actual need will be more accurately calculated.
Percentage of Each Blood Group of Units Issued will be a good indicator of the blood groups distribution among the Egyptian population and can be considered in donor recruitment system.



Graph 1.



Graph 2.

Results: An Example of key indicators manually calculated of the period from January to December 2015:
Incompatible units / Total units matched (Compatible units).
Remaining units of blood component/Total Units Delivered.
Other indicators discussed in this study are set to be calculated with the first implementation of E-Delphin Blood Management System.
Conclusion: Automation of Egyptian blood banks is expected to result in more accurately calculated key performance indicators, in an easier and less tedious, which will eventually lead to more productivity and improvement in performance.
A comparative study will be done to investigate the differences between key performance indicators before and after implementation of E-Delphin Blood Management System.

P-045

A REVIEW OF RED BLOOD CELL AND FRESH FROZEN PLASMA UTILIZATION BASED ON HAEMOGLOBIN LEVELS AND COAGULATION PROFILES

N Muhammad[1], NA Hamzah[2], NL Ramly[1], NN Hamdan[1], NA Hamzah[1], NA Rosli[1], SA Mohd Rosli[1], NA Berhan[1], R Ismail[3], AN Talib[1] and S Shahid[3]

[1]International Islamic University Malaysia, Kuantan, Pahang, Malaysia [2]Hospital Melaka, Bandar Melaka, Melaka, Malaysia [3]Hospital Tengku Ampuan Afzan, Kuantan, Pahang, Malaysia

Background: Red blood cells (RBC) and fresh frozen plasma (FFP) are two blood component types that are frequently transfused. Although the indications for transfusion are specific and limited, they are often inappropriately transfused leading to shortage of supply as well as development of both immunological and non-immunological adverse effects.
Aims: We performed a retrospective review of RBC and FFP utilization based on the haemoglobin levels and coagulation profile results respectively.
Methods: Records of patients from transfusion unit who received RBC and FFP transfusions from July to December 2014 in Hospital Tengku Ampuan Afzan, Kuantan, Pahang, Malaysia were reviewed. The haemoglobin (Hb) levels, prothrombin time (PT) and activated partial thromboplastin time (aPTT) results of the patients at the time of request were also noted.
Results: In all there were in all 3,224 RBC transfusion episodes over the 6-month period of study. The total units of RBC transfused was 6,307, ranging from 1 to 8 units per transfusion. The medical-based departments utilized the highest number (36.8%). The Hb levels of patients ranged from 1.6 to 16 g/dl. Approximately 20% of RBC units were administered to patients with Hb levels of ≥10 g/dl and they involved mainly patients of surgical-based units. In 76% the indications of RBC transfusions were stated in the request forms as 'for supportive management'. As for administration of FFP, there were 729 transfusion episodes involving 2,562 units. Approximately 60% of transfusions were administered to patients with prolonged PT and aPTT (>1.5 times of reference range). In about 40% of patients who received the FFP, the coagulation profile results were either mildly prolonged or normal with two third of them being from surgical-based units. The indications for the transfusions were also stated as 'for supportive management' in about 70% of patients.
Conclusions: This review highlights that the practice of RBC and FFP transfusions could be improved. Since in most patients the indications of transfusion were not specifically stated, both the RBC transfusions in patients with haemoglobin ≥10 g/dl and FFP in patients with normal or mildly prolonged coagulation profile results could not be justified. Continuous medical education with regular audit exercise could contribute to the improvement in practice.

P-046

COORDINATION BETWEEN BLOOD COLLECTION, USE AND SUPPLIES OF CONCENTRATED ERYTHROCYTES IN NORTHEASTERN SLOVENIA

E Kavaš[1] and I Muršic[2]

[1]Centre for Transfusion Medicine, University Medical Centre Maribor, Maribor, Slovenia [2]University Medical Centre Maribor, Maribor, Slovenia

Background: Rational usage of blood and uninterrupted treatment of patients with blood is based upon safe and sufficient blood supply, which can be achieved with coordinated policies of blood collection on both regional and national levels.
In Northeastern Slovenia (NE), the Center for Transfusion medicine (CTM), University Medical Centre (UMC) Maribor is responsible for uninterrupted supply of blood for UMC Maribor, Murska Sobota General Hospital (GH) and Ptuj GH. CTM Maribor cooperates with the Institute for Transfusion Medicine Ljubljana (ITM) and Transfusion center Celje in the events of blood deficit.
Collection of blood is organized through pre-planned blood drives in collaboration with main organizer of blood drives in Slovenia – the Slovenian Red Cross (SRC).
Aims: In 2009, after the adjunction of Unit for transfusion activity (UTA) Ptuj and UTA Murska Sobota, we have issued a proposition in northeastern (NE) Slovenia to organize the plan of blood collection in centers for transfusion medicine based on average weekly demand for concentrated erythrocytes (CE) in an individual region. We proposed that it is necessary to determine the optimal, minimal and maximal supply of CE of particular blood group per individual hospital. We proposed an estimate of 500 accepted blood donors weekly in NE Slovenia. We were interested in the outcomes of our predictions.
Methods: We analyzed statistical data about collection and use of CE from 2010 to 2014. We reviewed the average weekly, monthly and annual use of CE in individual hospitals.
Results: Results of average CE use show that we are justified in planning 500 accepted blood donations weekly (Table 1). We also correctly estimated the proportion of declined potential blood donors, which is approximately 10%.
Optimal supply of CE at the CTM is influenced by the number of donations, use in the previous time interval as well as minimal and maximal use in the CTM and in particular UTA.
Optimal blood supply in the individual transfusion establishment as well as on a national level can be achieved only with cooperation between ITM Ljubljana, CTM Maribor and Transfusion center Celje.
Safe supply of CE to the transfusion departments of general hospitals are also influenced by: average and maximal use of CE in the previous time interval, the date of collection of blood donation and the time necessary for transport of CE from another institution, as well as by all other aspects of work organization.
Conclusion: To improve the supply of CE, it is necessary to define the exact terms of cooperation between centers for transfusion medicine on a national level and to determine the additional activities of the SRC. Only with these measures it will be possible to take appropriate action in the event of surplus or shortage of CE of particular blood group.

		2010	2011	2012	2013	2014
MIN.	CTM	270	306	287	274	248
	Ptuj	24	37	22	28	32
	MS	61	65	35	54	66
	combined	355	408	344	356	346
MAX.	CTM	378	356	346	333	334
	Ptuj	53	48	47	58	58
	MS	92	110	81	89	123
	combined	523	514	474	480	515
AVERAGE	CTM	315	333	312	297	303
	Ptuj	37	45	39	47	45
	MS	75	84	72	81	83
	combined	427	462	423	425	431

Table 1. Minimal, maximal and average weekly demand for CE in NE Slovenia

P-047

MANAGEMENT OF FFP INVENTORY IN PRIVATE HOSPITALS – A SINGAPORE PERSPECTIVE

L Wong and M Chan

Health Sciences Authority, Singapore, Singapore

Background: The inventory management and distribution of Fresh Frozen Plasma (FFP) for private hospitals in Singapore is handled by our national bloodbank laboratory. Blood request is routed to the laboratory and ABO-compatible FFP units are assigned to specific patients after blood grouping is confirmed. Untransfused units are returned for redistribution. However, additional measures to maintain cold chain are necessary to minimize impact on product integrity and quality and could potentially put them at risk of exceeding the expiration date.
Aim: To minimise the risk of compromising the cold chain as a result of redistribution, an initiative was undertaken to recycle the untransfused FFP units within the hospitals. This could also possibly reduce the turnaround time (TAT) for the patients to receive their required FFP units.

Methods: A retroactive multi-centre study was done to analyse the usage and discard rates of FFP by the major private hospitals. Hospitals with higher usage rates of FFP were encouraged to keep FFP as an inventory item and to reassign the untransfused units.

Results: Data from April 2015 to March 2016 was tabulated and of the 5,526 units of FFP distributed to the nine private hospitals, 4,234 units were transfused, 1,009 units were discarded and 283 were channelled to their respective inventories. The discard rate was almost 20% and only 5% of the FFP units were reassigned.

From Table 1, we can see this initiative appears more successful in smaller hospitals like facility 5, 6 and 7 as the inventory is more manageable due to the low volumes of FFP units and for larger hospitals like facility 2 and 3, only <3% of distributed FFP was 'recycled'. These hospitals may choose to discard the untransfused units resulting in a higher discard rate.

Table 2 revealed a savings of an average 119 mins in transport time which in turn means a reduction of TAT for patients to receive their required FPP units.

Summary: The recycling initiative resulted in savings in transport time which leads to a reduction in TAT for patients. It also ensured that FFP is not subjected to multiple transport trips thus maintaining product integrity and quality of the plasma factors as cold chain is not compromised. However, this will require the hospitals to adopt the 'first in first out' strategy and closely monitor the expiration dates to optimise usage of the FFP inventory. Unfortunately, the initiative also led to a higher discard rate possibly due to the risk-adverse approach that hospitals adopt to prevent possible transfusion errors. For a holistic approach, a training program can be implemented to educate clinicians on Patient Blood Management to minimize excessive orders which lead to higher wastage rates or product unavailability. This will also help manage plasma as a limited resource if we were to adopt the male-only plasma initiative in future to mitigate the threat of TRALI.

Facility	Total no of FFP transfused		Total no of FFP discarded by hospitals		Total no of FFP channelled to hospital inventory	
1	50	(83.33%)	8	(13.33%)	2	(3.33%)
2	1151	(77.14%)	323	(21.65%)	18	(1.21%)
3	1136	(70.69%)	434	(27.01%)	37	(2.30%)
4	769	(80.61%)	165	(17.30%)	20	(2.10%)
5	321	(77.54%)	20	(4.83%)	73	(17.63%)
6	73	(82.02%)	3	(3.37%)	13	(14.61%)
7	335	(76.14%)	1	(0.23%)	104	(23.64%)
8	7	(100.00%)	0	(0.00%)	0	(0.00%)
9	392	(84.67%)	55	(11.88%)	16	(3.46%)
TOTAL	4234	76.62%	1009	18.26%	283	5.12%

Table 1. Transfusion and discard rates of FFP in private hospitals (April 2015–March 2016)

	Average time saved with on-site FFP inventory (min)
Facility 1	158
Facility 2	137
Facility 3	136
Facility 4	141
Facility 5	92
Facility 6	102
Facility 7	88
Facility 8	102
Facility 9	114
Average	119

Table 2. Time saved for patients receiving FFP with on-site inventory

P-048

A SURVEY OF RED CELL AND PLASMA USE IN 25 HOSPITALS IN CHINA

L Li[1], S Hong[2], R Zhang[1], W Li[1], Y Wu[3] and Z Liu[1]

[1]Institute of Blood Transfusion. CAMS & PUMC, Chengdu, China [2]Academy of Public Health, Anhui Medical University, Hefei, China [3]Bloodworks (Formerly Puget Sound Blood Center), Seattle, Washington, United States of America

Background: Establishing audit system of rational blood transfusion for clinical purpose will contribute to its improvement. The analysis of the relationship of the clinical condition of transfusion in hospitals and the number of the inpatients, the operations and the usage of red blood cell, plasma will provide supportive data for the establishment of audit system of reasonable blood transfusion in clinic.

Aims: The purpose of this study was to establish baseline information on red cell and plasma use in relation to the number of inpatients and operation to identify patterns of blood use that may be used for blood program planning and transfusion audits.

Method: The total number of inpatients, operations and the usage amount of red blood cells, plasma were collected from 25 grade 3A hospitals of 25 provinces in China, during January 1, 2015 to December 31, 2015, including the number of inpatients in internal medicine, surgery, obstetrics and gynecology, pediatrics, ICU. SPSS software were performed to investigate the correlation of total annual number of inpatients, operation and the usage of red blood cells, plasma as well as their average amount. In addition, we analyzed the usage proportion of red cells and plasma in internal medicine, surgery, obstetrics and gynecology, pediatrics, ICU .

Results: The correlation coefficient of the annual number of inpatients, operations with red blood cells, plasma usage are 0.917, 0.712, 0.809 and 0.677, respectively. Average red blood cells and plasma usage of hospitalization were 0.2589 ± 0.08552, 21.8160 ± 16.53207, those in every surge were 0.6568 ± 0.28008, 54.0126 46.66553. The usage ratio of red cells and plasma in different apartments of surgery,

Table 1 The correlation coefficient, mean and standard deviation of the annual number of inpatients, operations with red blood cells, plasma

	$\bar{x}\pm sd$	The correlation coefficient(R)
Average red blood cells usage of annual number of inpatients（U）	0.2589±0.08552	0.917
Average red blood cells usage of annual number of operations （U）	0.6568±0.28008	0.809
Average plasma usage of annual number of inpatients （ml）	21.8160±16.53207	0.712
Average plasma usage of annual number of operations （ml）	54.0126±46.66553	0.677

Table 2 The proportion of the usage amount of red blood cells and plasma of different department in that of all departments (data from 25 hospitals)

	red cell (U)		plasma (ml)	
group	dosage	percent(%)	dosage	percent(%)
Internal medicine	172625.2	24	16965979.3	27
surgery	282981.5	39	26620354.6	42
obstetrics and gynecology	45454.3	6	1792824.1	3
pediatrics	21884.7	3	1664748.8	2
ICU	67129.8	9	9475729.7	15
others	133343.9	19	7005833.7	11

obstetrics and gynecology, pediatrics, ICU were 24%, 39%, 6%, 3%, 9% and 27%, 42%, 3%, 2%, 15% (Tables 1 and 2).
Summary/Conclusions: There are good correlations of the annual number of inpatients, operations with red blood cells and plasma usage respectively (R = 0.917 and 0.809).The information of average red blood cells and plasma usage of hospitalization and surgery may can be as a reference for the establishment of evaluation system of rational blood transfusion for clinical purposes.

P-049

STANDARD BLOOD TRANSFUSION INDICES AND THE EFFICIENCY OF BLOOD UTILIZATION

HA Alsulami and S Almarshad
IAFH, Riyadh, Saudi Arabia

Introduction: A number of indices are used to determine the efficiency of blood ordering and utilization system. Boral Henry was the first that suggested the use of crossmatch to transfusion ratio (C/T ratio) in 1975. Ideally, this ratio should be 1.0, but a ratio of 2.5 and below was suggested to be indicative of efficient blood usage. The probability of a transfusion for a given procedure is denoted by %T and was suggested by Mead et al. in 1980. A value of 30% and above has been suggested as appropriate. The average number of units used per patient crossmatch is indicated by the transfusion index (TI) and signifies the appropriateness of number of units crossmatched. A value of 0.5 or more is indicative of efficient blood usage. The aim of this study is to assess the efficacy of blood transfusion indices and the efficiency of blood utilization.
Methods: In this cross-sectional study, 349 patients were studied over 9 months in 2015 for whom blood supply was requested in different wards of IAFH. Patients' demographic data, hospitalization ward, blood group, hemoglobin, the number of requested and crossmatched blood units, the number of transfused blood units were collected. Standard indices of blood transfusion including C/T, TI and T% were calculated.
Result: In this study, of 623 blood units requested, 48% of units were transfused. 61% of the transfused patients have Hemoglobin (Hb) level over 10 g/dl. Blood transfusion indices C/T, T% and TI, were 2.02, 39.8% and 0.85, respectively, all within accepted range. However, more than half of the transfused patients have Hb level over 10 g/dl and only two of those patients have cardiac or pulmonary disease.
Conclusion: Given the obtained blood transfusion indices together with fact that 61% of the transfused patients have Hb level above 10 g/dl, means that normal transfusion indices values do not indicate appropriate use of blood and other factor should be looked at to assess quality of blood utilization.

P-050

This abstract has been withdrawn.

P-051

UTILISATION OF BLOOD COMPONENTS IN A TERTIARY CARE HOSPITAL

H Qadir and F Mahar
Aga Khan University Hospital, Karachi, Pakistan

Background: Human blood is a life-saving therapeutic option in many conditions and an important part of patient management. Monitoring of blood utilization helps in effective management of blood bank inventory and to meet present and future demands in a hospital. It also helps to prevent blood products misuse or overuse, which may lead to shortage of available blood to a life-threatening situation. Blood utilization data help us in developing strategies locally to improve usage of blood products.
Aim: The aim of the study was to gather information on blood component utilization by various specialties in a tertiary care hospital.
Material and methods: This was a retrospective cross sectional descriptive study covering a period from 1st January 2015 to 31st December 2015. The study was conducted at the Blood Bank of The Aga Khan University Hospital. Data was extracted from blood bank information system. Statistical package for social sciences 21 was used for data entry and analysis. The frequency of whole blood, packed red blood cells (RBCs), fresh frozen plasma (FFPs) and platelets (pooled and random) utilization was calculated. Blood product utilization was stratified into five groups according to age as 0–1 year, 1–16 years, 17–40 years, 41–64 years and ≥65 years. Pattern of distribution of blood products to various specialties in the hospital was determined.
Results: The total number of blood components transfused during the study period was 60,252. Packed red blood cells were the most consumed product (37.8%) followed by platelets concentrates (36.6%) and fresh frozen plasma (20.3%). Recipients of 41–64 years received most 21,406 (35.5%) blood components followed by 17–40 years age group [n = 19,524 (32.4%)]. Maximum number of blood products were utilized by hematology and oncology patients [n = 17,249 (28.6%)] followed by emergency [n = 9,097 (15%)], internal medicine (7.9%) and cardiac surgery (7.1%).
Conclusion: This study demonstrates that packed red blood cells are the most utilized blood component in our patient population. The highest demand for blood products is of hematology and oncology patients. Regular review of utilization of blood products is important to see the trend and manage our inventory.

P-052

ANALYSIS OF THE REASONS FOR DISCARDING BLOOD AND BLOOD PRODUCTS IN DUBAI BLOOD DONATION CENTRE

N Hamad, M Akmal, M Ayoob, A Al Ali and MY Raouf
Dubai Health Authority, Dubai, United Arab Emirates

Background: Implementation of quality indicators and continuous monitoring and evaluation will lead to the reduction in the discard rate and proper blood and blood components utilization.
Aim: The main purpose of this study was to determine the rate and causes of discard of blood and blood products (packed red cells, plasma, platelets, and cryoprecipitate) in Dubai Blood Donation Centre, in order to introduce an appropriate improvement interventions.
Methods: This is the retrospective study of the collection of 2014 and 2015 in Dubai Blood Donation Centre. The study data on the number of discard of whole blood units and its components obtained using the daily blood components discard specific form and available elicited data from the Immunohematology laboratory at Dubai Blood Donation Centre. The collected data were then analyzed using descriptive statistic method. Reasons of discarding blood and blood components are generally related to either donor screening result (TTI), inappropriate blood collection or components processing.
Results: The total number of whole blood collected in this study were 92,646 whole blood units, from which units of 124,644 of components were prepared.
The amount of discard of blood products in the study, revealed that packed red cell has the highest percentage rate of discard 9.1% (7,521 units), followed by fresh frozen plasma with percentage rate of discard of 8.8% (2,263 units).The major cause of discarding blood components was seroreactivity for transfusion transmitted infections (TTI) results 4,723 units (59%), mainly Hepatitis B core total (HBcT), followed by underweight or low volume 779 units (10%), CBC results in total of 667 units (8%), clotted units counted as 533 units (7%), RBC expired units 568 units (7%), lipemic units 174 (2%) and incomplete filtration units 173 units (2%), ABO/Rh discrepancy, antibody screen or DCT positive 148 units (1.8%), manufacturer defect 87 units (1%), punctured and leakage fresh frozen plasma 90 units (1%), punctured units of packed red cells 40 units (0.5%), hemolysis 13 units (0.2%). Overall blood and blood product wastage was 9,884 units (7.9%) of the total of 124,644 units of blood components.
Summary/Conclusions: A properly conducted donor screening will help in discarding less number of blood bags which are positive for (TTI). Advancements in areas such as donor management, good donor selection, proper donor interview, proper unit collection, training and evaluation of the staff will reduce the discard of underweight and clotted units. Moreover, effective communication with Marketing department and the session organizing staff, will support in reducing the collection of surplus specific blood group in order to manage the expiration. Also the introduction of the monthly quality indicators evaluation form with the set target of each indicator and the implementation of blood inventory management policies will assist in reducing the discard blood products and to improve the output.

P-053

AN ANALYSIS OF BLOOD ORDERING AND UTILISATION AT A PRIVATE HEALTH INSTITUTION IN HARARE ZIMBABWE

JJ Parirewa, G Mavunganidze and T Mapako

National Blood Service Zimbabwe, Harare, Zimbabwe

Background: National Blood Service Zimbabwe (NBSZ) launched a document on Standards for Blood Donation, Processing and Clinical Transfusion in 2010 with the aim of promoting blood safety through appropriate use of blood components. Trainings on the standards were conducted through out the country with emphasis on setting up of Hospital Transfusion Committees (HTCs). Standard documents for providing feedback to NBSZ were developed and distributed to hospitals for monitoring and evaluation purposes. A few health institutions from both public and private have established HTCs and are submitting data on blood ordering and usage to NBSZ regularly. In this study, we identified and selected one private health institution in the main city of Harare that has been consistently providing feedback on blood utilisation. We share the analysis of findings on blood ordering and utilisation from this private health institution as this might help to highlight blood utilisation practices in the other private health institutions.

Aim: To establish trends from data submitted by the hospital on ordering and utilisation of blood at this health institution.

Methods: A retrospective desktop review of data on blood ordering and utilisation submitted for the period January–June 2015 was analysed. The corresponding NBSZ blood request forms received from the hospital for patients requiring transfusion during this period was checked for completeness. Data on blood units requested and shipped to the hospital was extracted from the electronic blood bank software 'e-Delphyn' system for this hospital for comparison purposes. The actual number of units transfused and blood units returned to NBSZ was analysed.

Results: Data submitted by the hospital indicated that 795 units of blood were ordered; 432 (54%) transfused and 363 (46%) returned to supply after having been cross matched whilst NBSZ data from the electronic system showed that 1,302 units were ordered, 833 (64%) transfused and 469 (36%) returned. Although Blood requisition forms from the hospital were complete with all critical information, clinicians only filled in 5% of the forms, the rest were filled in by nurses. Patient profile data received from the hospital showed that blood transfusion was mostly for medical indications (55%) followed by surgery and trauma at 35%. Obstetrics, Gynaecology and other usages constituted 3% each followed by Oncology and Paediatrics and others at 1%. Data on blood utilisation could not be extracted from the NBSZ database system due to non-capturing of critical information as it was observed that this information was appearing on the forms.

Conclusion: Blood requested and cross-matched for patients at this hospital is much greater than the real level of consumption. Clinicians need guidelines to help determine the number of units required so as to reduce artificial shortages caused by unjustifiable ordering patterns & unnecessary costs incurred by patients for cross-match. There is need to strengthen monitoring of the transfusion process as the hospital system reflects under reporting. There is need for NBSZ to capture all critical data pertaining to blood transfusion in the system to facilitate enhanced data management and analysis on blood utilisation.

P-054

ANALYSIS OF RATE AND REASONS FOR DISCARDING BLOOD & IT'S COMPONENTS IN SHARJAH BLOOD TRANSFUSION CENTER

MZ Rabeh Zouaoui[1], M Raouf[2], M Sulaiman[1], HE Alnajar[1], S Ahmad[1], K Abdallah[1] and N Bani Yas[1]

[1]*Sharjah Blood Transfusion and Research Centre, Sharjah, United Arab Emirates*
[2]*Dubai Blood Donation Centre, Dubai, United Arab Emirates*

Background: The availability of blood and blood components plays a major role in modern medicine and as there is no substitutes for human blood; proper utilization of blood and blood components is essential to ensure availability and safety.

Aim: Is to identify the rate,causes and trend for discarding blood and components in Sharjah Blood Transfusion Center that will assist us in improving performance through education and training of staff and introducing new measures in order to minimize the number of discarded blood to a reasonable rate.

Methods: This a retrospective analysis based on daily records for discarded blood and components for the period between 2011 and 2015 in Sharjah Blood Transfusion Center. A standard form has been formulated for this reason and KPI has been selected to measure the performance of this process (KPI for expired packed RBCs & single units Platelets). Least Squares regression has been used for the Statistical analysis and correlation study between variables.

Results: The total of blood units collected was: 77,028 including (56.5%) first time donors & (43.5%) repeat donors, 6,118 whole blood and packed cell units has been discarded that equals to 7.9% of the total collected units. Reasons for discarding includes: infectious disease testing (IDT) (76.23%) of discarded units, defect in collection or blood unit processing or storing incidents (14.9%), outdated blood unit (1.05%) and others reasons including: positive for Red Cells unexpected antibody test,CUE, lipemic and sickle cell positive test (7.8).

The most common IDT reactive marker was the HBc antibodies (71.5%) which was inversely correlated with the frequency of donation ($P < 0.0001$).

Discarding blood for expiratory date was monitored as KPI. The Red Cells expiratory rate target was <2% and for platelets expiratory rate <6.4% .A proper blood collection,storing and issuing process was implemented where RBC units expiratory target was met in most of the time. Concerning Platelets expiratory rate target; it was very difficult to meet this rate as the use and requests for platelets was unpredicted.

Conclusion: This study shows that the highest cause for discarding blood in our center is for IDT with HBc Ab is the highest; that was as expected higher in first time donors.

The trend analysis showed that the implementation of quality system including donor selection and education has improved our blood donor pool (repeat donors vs first time donors) which has a positive impact on the decrease of infectious disease markers prevalence and hence the rate of blood units discarded.

Proper management of the collected blood units and it's supply can lead to proper utilization of blood and would reduce discard, cost and wastage of blood units.

P-055

REDUCING RED CELL WASTAGE IN LIVER TRANSPLANT UNIT: USE OF ELECTRONIC INVENTORY MANAGEMENT SYSTEM

D Acharya, AK Tiwari, RC Dara, D Arora, G Singh and V Vohra

Medanta the Medicity, Gurgaon, India

Background: At our centre, liver transplant anaesthetist, who manage the blood transfusion in operation theatres (OT) maintain a buffer of few 'extra' red cell units as precautionary measure since blood loss during liver transplant surgeries is not so predictable and there is a lag-time between ordering and receipt of red cell units because of physical distance [between the OT and blood transfusion services (BTS)], need for human courier to carry the order from OT to BTS and then red cell units back to OT, completion of necessary tests and documentation at BTS. Several of these red cell units issued remain unused and go waste since the cold chain is interrupted.

In an effort to reduce this wastage an intervention was undertaken which ensured that compatible red cell units were stored in temperature controlled refrigerator in the OT along with compatibility documents. The issue of red cells from OT refrigerator was controlled with help of electronic inventory management system. The effectiveness of this system in reducing red cell unit wastage was studied.

Materials and method: The study was conducted by department of Transfusion Medicine in coordination with Liver Transplantation team. This was a prospective trial conducted over 2 month's period (March and April 2016) and compared with previous 2 months (January and February 2016). The intervention was limited to red

		January–February			March - April		
		Recipient	Donor	Blood Track	Recipient	Donor	P value
Number of patients		32	32		29	29	
RBC units	Issued	303	129		290	112	
	Transfused	204 (67.3%)	32 (24.8%)		128 (44.1%)	13 (11.6%)	
	Wasted (un-used)	83 (27.4%)*	72 (55.8%)*		0	0	<0.001
	Retrieved	16 (5.3%)$	25 (19.4%)$		162 (55.9%)^	99 (88.4%)^	<0.001

* Cold chain interruption more than 30 minutes
$- Cold chain interruption less than 30 minutes
^ - no interruption in cold chain

Table 1. Blood utiisation in liver transplants

cell units issued in elective cases through the electronic inventory management system (BloodTrack, Haemonetics Corporation, USA). It consisted of three components: the central server in Information Technology (IT) department, Manager installed in a desk-top computer at BTS, and a kiosk (BloodTrack Courier) attached to standard 2–8°C blood bank refrigerator (Helmer Scientific, Germany) located in the liver OT. Controlled access linked to hospital identity cards was provided to all users with different access levels.

Requests for RBCs were received at blood bank 1 day prior to surgery. Red cells compatibility tests were carried out, units were issued through the manager in the BTS and physically transferred to the OT refrigerator through the kiosk. RBC units were available for use in the OT refrigerator for a period of 24–36 h after which unused units were retrieved by the BTS and re-inventoried. Data collection involved number of transplant procedures, red cells stored, transfused and retrieved in the manager. The 't-test' was applied to compare the red cell unit's wastage before and after intervention.

Results: A total of 402 red cells were issued for 29 liver transplants in 2 months which was comparable with 432 red cells that were issued in 32 liver transplants before the intervention. Red cell wastage was 155 (35%) which was significantly reduced to nil after intervention ($P < 0.001$; Table 1). During the study period few 'alerts' were raised in the manager; temporary disruption of server hosting for couple of hours that led to refrigerator lockdown and on an occasion liver anaesthetist forgot to scan the bags while removing. However, these issues were promptly resolved.

Conclusion: Intervention was found to be very effective saving 220 units during the study period. Moreover, unnecessary transfusions were also reduced.

P-056

ASSESSMENT OF A BLOOD ESTABLISHMENT RED CELLS AGE ON DISTRIBUTION, WASTAGES AND NATIONAL BLOOD STOCK LEVEL

MCT Muon, CMR Caeiro, A Pinheira, JM Gomes and IMC Pires

Portuguese Institute of Blood and Transplantantion, Coimbra, Portugal

Background: The blood supply management (BSM) is the top mission carried out by Blood Establishments, supplying blood components to make safe blood supply available for patients. BSM have mainly focused on inventory management, but effective experiences of real BSM, coordinating activities from donors to patients, have still remained limited.

The ability to adequately predict the number of units of blood required throughout the year and to ensure that do not overstock and therefore increase wastage.

Aims: Understand the correlation between blood establishment red cells age on distribution and the outdated Red blood cells (RC), accordingly to the national blood stock level.

Methods: All data concern the year of 2015.

The Portuguese Blood and Transplant Institute maintain online a web based tool where Blood Establishments and Hospital Blood Services can access daily in real time the following data:

National blood stock inventory (Blood establishments and Hospital Blood Services)

Red blood cells (RC) units use in patients (Hospital Blood Services).

RCs national stock level in days is calculated based on the national daily stock inventory divided by the average daily RC use in the previous week

The Regional Blood Centre keep track on supplied RCs and RCs wastages due to validity expiration.

Age of supplied RCs is calculated based on collection date.

The correlation of the variables RCs age on distribution, wastages and national blood stock level were conducted in R Software (R Development Core Team, 2013).

Results: The 2015 data shows a correlation between blood establishment red cells age on distribution, RC outdate (wastages) and national blood stock level.

National blood stock level: mean = 17.18 days SD ± 2.97; Red cells age: mean = 10.02 days SD ± 2.72; RC outdate mean = 27.9 units/week SD ± 17.95

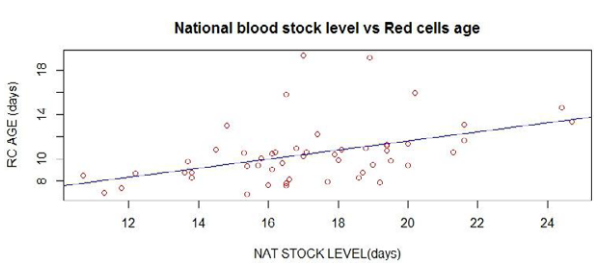


Figure 1. National blood stock level vs Red cells age

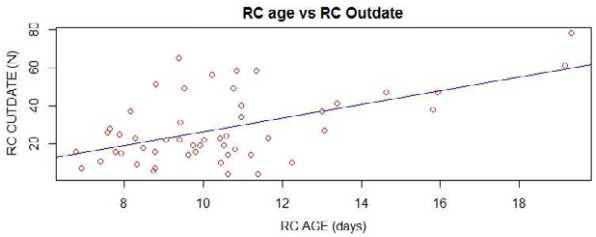


Figure 2. Red cells age vs RC outdate

National blood stock level vs Red cells age $r = 0.44$; $P = 0.0011$ (Fig. 1)

National blood stock level vs RC outdate $r = 0.34$; $P = 0.016$

Red cells age vs RC outdate $r = 0.54$; $P < 0.0001$ (Fig. 2)

Conclusions: The findings show that in our Blood Establishment, the high National Blood Stock Level increases Blood Establishment Red Cells Age on distribution (16%). The RC age increases RC outdate (25%).

It suggests the importance of monitoring the National Blood Stock Level to know its pattern in order to make appropriate blood collection annual planning. It may increase productivity and avoid wastages with regular planning adjustments.

The recommendation is the need of a major focus on a national coordination in the annual planning collection with at least one blood establishment being a buffer to correct the increase or decrease of the national blood stock level.

P-057

REDUCING THE FESTIVE EXCESS: STRATEGIES TO REDUCE RED CELL WASTE IN VICTORIA, AUSTRALIA

L Bielby[1], JC van Diemen[1], C Akers[1], B Glazebrook[1], P Beard[1], A Harper[1] and C Hogan[2]

[1]Blood Matters, West Melbourne, Australia [2]Australian Red Cross Blood Service, Melbourne, Australia

Background: Good inventory management promotes the best use of available resources and minimises any unnecessary use or waste. Australian Governments support minimising unnecessary blood waste through a wastage reduction strategy, National Stewardship statement and in the National Safety and Quality in Health Service Standards. National red blood cell (RBC) wastage targets were set to reinforce the strategy. In the financial year 2013–2014, 712,122 RBC units were issued nationally, 191,850 (27%) in Victoria. Nationally 37,264 (5.2%) RBC units were wasted, 11,746 (6.1%) of these in Victoria.

Victorian RBC wastage targets for the 2015–2016 financial year were 2.5–4.0%. Individual health service/pathology provider's targets vary depending on the size and throughput of the laboratory.

In Victoria, all RBC wastage is recorded via BloodNet, a web-based system that allows staff to order blood and blood products quickly, easily and securely from the Australian Red Cross Blood Service. When a RBC is discarded laboratories are required to document this, along with the discard reason in BloodNet.

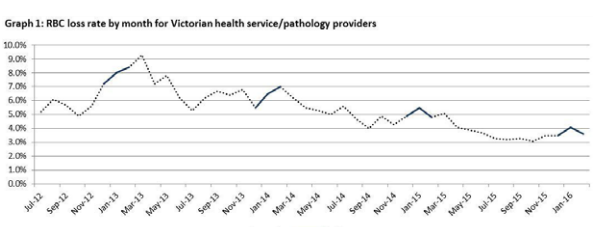
To support and assist health services/pathology providers to meet wastage targets, Blood Matters established and funded the RBC wastage reduction project in July 2014.

Data from November 2012 to February 2015 showed that waste is decreasing; however, it highlights a consistent 'festive spike' each year in January and February, (Graph 1). A 'stop the waste' festive season campaign was developed and disseminated to health/pathology services in Victoria.

Aim: To raise awareness of, and reduce the increased waste over the festive season.

Method: A 'stop the waste' festive season checklist was developed and circulated to key Victorian health service and laboratory stakeholders in October 2015, for their action.

Planned ward closures, or reduced services that could influence demand



Staff leave for key personnel such as waste champions, or fridge monitoring/maintenance staff
Potential inventory adjustment in line with expected demand
In April 2016, a follow-up survey was sent to laboratories (n = 72) exploring how the checklist was used, if it had been helpful, and to seek suggestions for improvements.
Results: The overall trend for RBC waste data in Victoria continues to decrease with a peak during the festive season.
While only a small number of survey repsonses have been received to date (n = 5), they indicate that the campaign was well received. One comment suggested it may have been more effective to launch the campaign earlier to allow more time for implementation. The checklist was useful, with over half indicating it was discussed at health services' blood management committee meeting. Four sites indicated they had adjusted the red cell inventory over the period.
Summary: Communication is the key to change. Involving key stakeholders such as the blood transfusion committee, laboratory staff and where available transfusion nurses/trainers help build collaborative working relationships to address all aspects of waste. Survey feedback will identify the strengths and potential areas for improvement to inform strategies to further reduce waste in 2016–2017.

P-058

PREVALENCE OF HIGH TITRE IGM ANTI A/B AMONG VOLUNTARY BLOOD DONORS AT REGIONAL BLOOD CENTRE, CHILAW – SRI LANKA

PAPM Arewatte[1], SP Vithanage[2], MAMDJ Meththasinghe[2] and WD Peris[1]
[1]Regional Blood Center, Chilaw, Sri Lanka [2]District General Hospital, Chilaw, Sri Lanka

Background: The Regional Blood Centre- Chilaw is the Puttalam district headquarters of the National Blood Transfusion Service and it contributes to 71% of total annual whole blood collection of the Puttalam district. Platelet concentrate is prepared either from Buffy coat derived or PRP method from 51% of the whole blood collected by the Regional Blood Centre- Chilaw.
Providing ABO compatible platelets at times become a difficult task because of the increasing demand for platelets and its limited shelf life. One of the biggest challenges to blood safety particularly in developing countries is accessing safe and adequate quantities of blood products particularly in developing countries. One strategy geared towards the optimum utilization of donor units is the use of blood and blood products against ABO blood group barrier by switcing groups. The aim of this study was to determine the prevalence of anti A and anti B haemolysins among blood group A, B and O donors in Regional Blood Centre, Chilaw – Sri Lanka.
Methods: A descriptive study was carried out for 6 months from May 2015 to October 2015. Sera from 140 blood donors were screened for anti-A and anti-B haemolysins using the standard tube technique at 37 C for 1 h. Titre of ≥1:64 was regarded as positive for high titre haemolysin.
A total of 364 donor samples tested by stratified sampling method. The first 10 EDTA blood samples (negative for all infectious disease markers) were obtained. Blood groups of each individual sample were obtained from blood grouping register. All the group AB samples in the particular batch were removed. The anti A titre of group B, anti B titre of group A and anti A and anti B titre of group O donors was tested. The titer was recorded as reciprocal of the highest dilution giving 1+ clumping.
Results: A total of 364 both male and female blood donors *t* aged 18–60 years were screened for anti-A and anti-B haemolysins. Study population included 189 blood group O donors (51.9%), 79 blood group- A (21.7%) and 96 blood group B (26.4%) donors. Out of the 364 blood donors screened, 16 (2.5%) were positive for high titre haemolysin (titre ≥64) while 348 (97.5%) were negative (titre <64). The prevalence of haemolysin was compared based on blood group of donors. The prevalence of high titre haemolysin was significantly higher among blood group O donors (5.8%) compared to 3.1% and 1.1% respectively for group B and group A donors respectively.
Conclusion: This study has shown that the prevalence of high titreanti A and anti B haemolysin is low particularly among blood donors in Chilaw,Sri Lanka. Prevalence of high titreanti A and anti B haemolysin is relatively high among blood group O donors. We recommend that all group O blood donors whose platelet concentrate intended for use against ABO blood group barrier should be tested and found negative for high titre anti-A and B haemolysin.

Prevalence of High Titre IgM Anti A/B among voluntary blood donors at Regional Blood Centre, Chilaw - Sri Lanka.

	A Donors with B antibody		B Donors with A antibody		O Donors with A & B antibody			
					with A antibody		with B antibody	
TITRE	Male	Female	Male	Female	Male	Female	Male	Female
Nil	47	25	61	26	Male 131, Female- 37			
1/2					1		2	
1/4	2			1	2		2	
1/8			2		6		3	
1/16		1	2		4		1	
1/32	2			1	2	1	2	
1/64	1		1	1	5		3	
1/128	1		1		1		2	
Total	53	26	67	29	Male -151	Female 38		
Total	79		96		189			

Picture 1.

P-059

IMPROVEMENT OF QUALITY INDICATORS FOLLOWING IMPLEMENTATION OF REAL-TIME AUTOMATED RED BLOOD CELL ORDERING ALGORITHM BASED ON RETROSPECTIVE AND PROSPECTIVE HOSPITAL DATA

J Quinn[1], C Campbell[1], T Covello[2], A Gomez[1], A Kumar-Misir[3], D Liwski[1], C Doncaster[4], J MacLeod[3], N Chisholm[3], I Sadek[1] and C Cheng[1]
[1]Dalhousie University, Halifax, Canada [2]University of Alberta, Edmonton, Canada [3]Nova Scotia Health Authority, Halifax, Canada [4]Canadian Blood Services, Ottawa, Canada

Background: Blood bank inventories must balance adequate supply with minimal outdate rates. The day-to-day practice of ordering red blood cell (RBC) inventory usually involves manually comparing current inventory levels with pre-determined thresholds, calculated from historical usage, and ordering the difference. To date, there are no published automated methods for ordering RBC inventory based on a combination of historical and future utilization based on laboratory characteristics of admitted patients.
Aims: We sought to address the problem of inconsistent and manual ordering practices for the RBC inventory, which historically have resulted in a larger inventory with increased outdate rates. We designed and implemented a blood ordering algorithm to provide a more accurate measure of predicted RBC utilization in our institution to standardize, and make the ordering process more efficient. Our algorithm uses system-wide hematology data to create a real-time 'anemia index' in combination with historical inventory data to create a real-time RBC order to the blood supplier.
Methods: Cerner Command Language (Cerner Millennium) was used to extract and combine historical RBC unit usage, current inventory levels, and system-wide hematology values and blood groups. A query is done twice daily and a report is automatically printed for the technologists; queries can also be done on an ad-hoc basis. This report contains a suggested order based on current inventory, historical inventory data, ABO group, and the current 'anemia index' for the institution. Data was collected for 6 months pre- and post- implementation.
Results: The mean daily total RBC inventory was significantly reduced following implementation (401.67 vs 308.96 units, $P < 0.0001$). There was a significant reduction in monthly red cell outdates in this time period (17.67 vs 9.33, $P < 0.009$) and a reduction in median time units spend in transfusion inventory (7.73 vs 5.75 days, $P < 0.0001$).
Summary/Conclusions: We developed a novel algorithm that automatically generates a suggested RBC inventory order using real-time hospital wide survey of patient ABO-typing, hematology values, and historical data. Following implementation of the algorithm we demonstrated a significant reduction in daily inventory levels, RBC outdate rates, and reduced the latency of red cell units in inventory. This methodology is reproducible and could be broadly applicable to large blood banks.

P-060

KNOWLEDGE IN BLOOD TRANSFUSION AMONG RESIDENTS IN A PORTUGUESE HOSPITAL: AN EDUCATIONAL PROGRAMME

C Vaz, F Vasconcelos, M Lopes and M Koch
Centro Hospitalar de São João, Porto, Portugal

Background: Blood is of vital importance in patient care all over the world. Blood transfusion is one of the five most overused medical procedures. Education in Transfusion Medicine (TM) is necessary to ensure appropriate and safe transfusions. In March 2012, the Hospital created a Transfusion Committee formed by a multi-disciplinary team which aims to promote an optimal use of blood. In 2015, this Committee, in partnership with the Hospital Education Department and the support of internship Director, developed a mandatory education programme about TM practices for all resident doctors in any year of residency.

Aims: Evaluate the blood transfusion knowledge among our residents in pre and post an TM educational programme.
Methods: The São João Hospital Centre is a university hospital and a tertiary care centre with 1,105 beds. Around 4.800 patients and 50.000 blood components or derivative are annually transfused.
Each educational session was conducted by two doctors of the Transfusion Medicine and Blood Bank Department, during a 1.5-h talk covering issues related to optimal blood transfusion, blood components indications, as well as transfusion complications and their management. The residents were given information about the hospital transfusion manual, which includes the MT hospital policy, and the electronic prescription of blood components using the J-One software. Clinical cases were discussed and participants were given a pocket size leaflet containing the key messages.
An anonymous assessment questionnaire with four multiple-choice questions, focusing on the indications of blood components and transfusion reactions, was performed by each participant immediately before and after the session.
Participants were asked to rate their subjective educational experience satisfaction.
Data underwent statistical analysis to determine the significance of TM educational sessions. A P value was calculated using T test and $P < 0.05$ was considered statistically significant.
Results: A total of 37.5 h sessions were organised over a 5 months period from October 2015 to February 2016.
199 residents took part in the educational programme, 130 (68.4%) of which were females and 69 (34.6%) male. The participants had a mean of 3,06 years of work experience. 80.4% were from medical departments and 19.6% from surgical departments.
A total of 199 residents participated in the TM sessions, and all answered the questionnaires.
The mean score in pre-assessment questionnaire was 1.92 out of four (48% correct answers), while in the post- assessment questionnaire was 3.06 (76.5% correct answers). The difference between the mean scores was statistically significant ($P < 0.05$).
About 53% of the participants rated the session as very satisfying. No participants considered it less satisfying or unsatisfying.
Conclusions: Residents from our hospital had a mean score of 1.92 points (48% of correct answers) before the educational session. These results are consistent with published findings of poor transfusion knowledge among clinicians in other hospitals.
There was a significant improvement of the mean score after the educational session, demonstrating a positive impact on the participants' knowledge of TM.
TM education is an effective measure for improving the quality of health professional performance, which is crucial for appropriate utilization of resources and maintaining patient safety.

Quality management

P-061

MONITORING BLOOD BANK QUALITY INDICATORS – DATA FROM A DEVELOPING COUNTRY

IA Siddiqui and FK Karim

Aga Khan University Hospital, Karachi, Pakistan

Introduction: Quality indicators are specific performance measurements designed to monitor one or more processes during a defined time and are useful for evaluating service demands, production, and adequacy of personnel, inventory control, and process stability. It is widely recommended that blood banks routinely monitor specific statistical data such as the ratios of cross matched to transfused (C:T) red blood cell (RBC) units, percentages of RBC units that expire (outdate) before those units are utilized, blood component wastage rate, adverse donor reaction rate, adverse transfusion reaction rate, transfusion transmitted infections etc. Monitoring of quality indicators is a quality management tool which could be utilized for improvement of blood bank services.
Objective: The aim of this study was to monitor quality indicators of our blood bank. This data provides insight into the level of quality maintained by our blood bank.
Material and methods: The data of quality indicators of blood bank of Aga khan university hospital, Karachi was analyzed over a period of 1 year from January 2015 to December 2015. The indicators monitored in the study included wrong transfusions, cross match/transfusion ratio (CT Ratio) for elective surgeries, packed red blood cell unit wastage rate and expiry rate. The target goal for CT ratio was <2.5. For RBC wastage and expiry rate, the goal was <0.5% and <1% respectively. For wrong transfusions, the number should be nil.
Results: During the study period, an aggregate of 60,331 units of blood/blood components were transfused. For transfusion reactions, the rate of all transfusion reactions was 1.3/1,000 blood products transfused. There were two cases of wrong transfusions for which intense root cause analysis was done. The cause was error in final bedside check (n = 1) and wrong blood in tube sent for compatibility testing (n = 1).
Data of cross match to transfusion ratio of elective surgeries revealed that total number of packed red blood cell units cross matched during the study period was 6,158 out of which 6,128 units were transfused. The CT ratio was 1.01. Red blood cells unit wastage rate was 0.06% whereas RBC expiry rate was found to be 3.75%.
Conclusion: The CT ratio (1.01) and RBC wastage rate (0.06%) met the target goal. The desirable CT ratio indicates efficient blood ordering practices in our institution. The RBC expiry rate (3.75%) and number of wrong transfusions (n = 2) exceeded the target limit. We identified that there is need to effectively manage our inventory to reduce RBC expiry rate. Monitoring of wrong transfusions and root cause analysis highlighted major areas of weakness. This prompted us to intervene and take measures to minimize incidence of wrong transfusions. Monitoring of quality indicators is thus a very effective tool which can be used to improve our practices.

P-062

ANALYSIS OF THE CAUSES OF DISCARDED BLOOD PRODUCTS

N Bujandric, N Milosavljevic Knezevic and R Jovanovic

Blood Transfusion Institute Vojvodina, Novi Sad, Serbia

Background: In order to improving processes, products and services, Blood Transfusion Institute could be implemented reliable quality indicators for systematically monitoring and evaluating the laboratory's performance. Proportion of discards of blood and blood products that is neither inevitable nor appropriate is defined as wastage. Monitoring wastage of blood product during collection, testing and processing of blood could be used as a quality indicator.
Aims: The aim of this study was to identify the causes of discarded blood and blood products and to determine its rate so that the blood transfusion institute can develop plan to minimize the number of discarded blood to a reasonable rate.
Methods: We retrospectively analyzed data of the rates and reasons for discarded blood products in Blood Transfusion Institute of Vojvodina from January 1st 2015 to December 31st 2015.
Results: A total of 26,486 blood units were collected during the study period. Out of them: 402 (1.52%) blood units were discarded because a vasovagal reaction, slow blood flow, weak veins, mislabeled and misidentified specimens during the donation process; 724 (2.73%) donations were discarded during testing process (because of seropositivity for transfusion transmissible infectious diseases 496 (1.88%), risky behavior 27 (0.1%), positive direct antiglobulin test 29 (0.1%), positive screening test for unexpected antibodies 172 (0.65%). A total of 4,697 (6.83%) blood products were discarded against 68,750 blood products prepared during the study period. Most common discarded products were Fresh Frozen Plasma 57.74% (2,712) and platelets 22.78% (1,070). The main reasons for discarded blood products were highly lipemic plasma, leakages, technical errors, etc.
Summary/Conclusions: We identified the reasons for discarding blood and the factors that need to be evaluated when searching for options to reduce discard levels. Blood Transfusion Institute has developed a plan to improve a performance in order to minimize the number of discarded blood. The plan includes education and training of staff.

P-063

This abstract has been withdrawn.

P-064

EVALUATION OF QUALITY CONTROL OF BLOOD COMPONENTS AT OUR BLOOD BANK

A Arshad, M Borhany, N Anwar, I Naseer, S Boota, R Muhammad, N Fatima, N Muhammad and T Shamsi

National Institute of Blood Diseases and Bone Marrow Transplantation, Karachi, Pakistan

Background: Quality Control is the backbone of Quality assurance programme. As part of it, quality control of blood components ensure availability of a sufficient supply of blood, blood components of high quality with maximum efficacy and

minimum risk to both donors and patients, thus ensures maximum safety of blood. The main objective of the study was to evaluate quality control of blood components preparation at our Blood Bank.

Aims: The aim of the study was to analyze the quality control of blood components in our blood bank.

Methods: An observational cross sectional study was done at blood bank of National Institute of Blood diseases and Bone Marrow Transplant Karachi from January 2014 to June 2015 to retrospectively analyze the quality control of blood components preparationand see the performance of our blood bank. Total of 100 units of each blood components were randomly selected during the study. Packed red cell units were evaluated for hematocrit and culture, random platelets and mega units were evaluated for pHby paper method and yield, fresh frozen plasma (FFP) and cryoprecipitate (CP) was evaluated for unit volume, factor VIII, fibrinogen concentration, and cryosupernatant (CS) was evaluated for unit volume and factor XI concentration.

Results: Among the blood products, mean packed red cells hematocrit was 74 with SD ± 2.84, mean platelet countwas 845 ± 76.43, pH was ≥6.2 in 98% of platelets units and platelet yield was found to be 5.8 ± 3.11. Mean FVIII and fibrinogen level was found to be 89.6 ± 5.2 and 2.8 ± 0.49 for FFP respectively. For cryoprecipitate, mean FVIII and fibrinogen level was found to be 108 ± 11.98 and 3.2 ± 0.34 respectively. The mean factor IX in cryosupernatant was found to be 86.9 ± 5.91.

Conclusion: The quality of blood components preparation at our blood bank is well meeting the standards. All those who are involved in blood transfusion related activity must be aware of the importance of quality management for its successful implementation. Good record-keeping, documentation and audit, use of standard operating procedures, laboratory worksheets, and implementation of safety guidelines can improve the quality performance of the services and ultimately patient's safety.

P-065

COMPARISON OF QUALITY PARAMETERES OF PLASMA SUSPENDED RED CELL CONCENTRATE AND RED CELL CONCENTRATE BUFFY COAT REMOVED IN ADDITIVE SOLUTION AT DISTRICT GENERAL HOSPITAL HAMBANTOTA SRI LANKA

M Adikarama, DKN de Silva and GMP Kumara

Blood Bank, Hambantota, Sri Lanka

Background: The Cluster Centre of District General Hospital (DGH) Hambantota recently started production of Red Cell Concentrate Plasma Suspended (RCC-PS) and Buffy Coat Removed in Additive Solution (RCC- BCR-AS) and monthly quality monitoring of 1% or minimum of 4 units of randomly selected blood products prepared. Washed cells of both these types are also issued under special circumstances and were not included in this study. 75% pass rate is required for each parameter as defined by the National Blood Transfusion Service of Sri Lanka (NBTS/SL).

Aims: To compare quality parameters of RCC-PS and RCC-BCR-AS produced at Cluster Centre DGH-Hambantota with the aim of providing better health care by issuing quality red cell products to the patients.

Methods: A retrospective study was carried out using monthly quality monitoring data of Cluster Center, DGH Hambantota for a period of 02 months from 1st of February 2016 to 31st of March 2016. The production of RCC-PS and RCC-BCR-AS for the above period was 186 and 168 respectively. Eight units were randomly selected for each category. NBTS/SL Specifications are shown in Table 1. For RCC-PS there was no specification defined regarding the WBC count but was analyzed in the study to test for significant difference compared with that of RCC-BCR-AS.

Results: In the present study the mean volume of RCC-PS and RCC-BCR-AS was 286.125 and 289 respectively. The mean HCT of RCC-PS and RCC-BCR-AS was 62.1375% and 58.7875% respectively. The Hb content per Unit of RCC-PS and RCC-BCR-AS stood at 56.76 and 50.00 while the WBC count per Unit of RCC-PS and RCC-BCR-AS stood at 2.3677 and 0.2626 respectively. There is no significant difference between RCC-PS and RCC-BCR-AS in terms of the mean values of volume ($P = 0.324$), and HCT ($P = 0.223$).There is significant difference ($P < 0.05$) regarding Hb content ($P = 0.0292$) which was higher in RCC-PS and regarding WBC count per Unit ($P < 0.00001$) which was much lower in RCC-BCR-AS. The 75% pass rate was achieved at 100% for both types in each month for all parameters except in HCT of RCC-PS which was 75% in March, and HCT and Hb content of RCC-BCR-AS which was 75% for both parameters in March.

Conclusions: Both types of red cell products have required pass rate in relation to NBTS/SL standards with regard to volume, HCT and Hb content. The WBC count of RCC-BCR-AS also met with the NBTS/SL specifications. Comparing the two types, there was no significant difference in volume and HCT. There was significant difference in Hb content and WBC count per Unit. Hb content is significantly higher in PS-RCC and therefore more suitable in raising Hb levels. However as WBC content is significantly very low in RCC-BCR-AS, it is ideal to be used whenever required, and specially in patients who undergo frequent routine transfusions. This study also highlights the importance of routine quality monitoring.

Blood Component	Parameter	Specifications
Red Cell Concentrate Plasma Suspended (RCC-PS)	Volume	230-330 ml
	Haematocrit(HCT)	55-75 %
	Haemoglobin (Hb) content	> 45 g/ Unit
Red Cell Concentrate Buffy Coat Removed in Additive Solution (RCC-BCR-AS)	Volume	230-330 ml
	Haematocrit(HCT)	50-70 %
	Haemoglobin (Hb) content	> 43 g/ Unit
	WBC Count	< 1× 10^9 g/ Unit

Table 1. RCC Specifications

P-066

QUALITY INDICATORS FOR TRANSFUSION PRACTICE – AN EVALUATION OF TRANSFUSION PROCESS FOR CONTINUOUS QUALITY IMPROVEMENT

D Kaur and M Kandwal

Max Super Specialty Hospital, Dehradun, India

Background: Quality indicators (QI) are essential tools of quality management system for driving continuous quality improvement. There is ongoing need for monitoring blood utilization and auditing transfusion practices to identify key areas of concern in blood component usage and inappropriate component use, wherein corrective actions can be planned. The LEAN approach is an effective blood utilization management strategy focusing on Waste, Value and Peril.

Aim: To evaluate the transfusion process by determining the quality indicators at our hospital.

Method: The data was collected and analysed for the year 2015 in the Department of Transfusion Medicine using blood donor, blood component and patient records. Specific indicators for blood utilization were crossmatch to transfusion ratio (CT ratio), transfusion probability (TP), transfusion index (TI) and maximum surgical blood ordering schedule (MSBOS), which were calculated using patient transfusion request forms, crossmatch and issue records. Furthermore, other quality indicators such as donor deferral rate, rate of seroprevalence of transfusion transmitted infections (TTI), adverse donor reactions, turnaround time (TAT), blood discard rate, blood utilization rate, adverse transfusion reaction rate and component quality control failure rate were evaluated each month during the study period.

Results: A total of 7,754 blood components were requested for 2,434 patients in the hospital during the study period. The overall CT ratio, TP and TI were observed to be 1.57 [highest at the departments of cardiothoracic surgery (1.92) general surgery (1.84) and neurosurgery (1.79)] 79% and 1.18 respectively. From a total of 2,400 blood donations, 280 were deferred (deferral rate is 11.7%). The combined seroprevalence of all the TTI markers was 1.29% and adverse donor reaction rate was 0.29%. The discard rate for red cells, fresh frozen plasma and random donor platelet were 2.7%, 1.20% and 1.03%, respectively. The adverse transfusion reaction rate was 0.08%.

The blood utilization rates were 66.7% (PRBC), 89.88% (RDP) and 36.8% (FFP). The TAT value for the issued blood components was within the specified time fixed by the organization and the QC failure rate was 3.57%.

Conclusion: The CT ratio, Transfusion probability and Transfusion index demonstrated significant blood utilization at our hospital. The assessment of the transfusion practice at our centre is found to be encouraging in terms of blood utilization. The QIs address important aspects on improving quality of the therapeutic transfusion processes.

P-067

QUALITY INDICATORS: PERFORMANCE TOOLS OF BLOOD TRANSFUSION SERVICE

L Varshney, S Gupta, U Maheshwari and S Biyani

MGM Medical College and Hospital, Navi Mumbai, India

Background: The primary goal of transfusion medicine is to promote high standards of quality in all aspects of patient care and related products and services. So, blood transfusion has become an integral part of patient management in modern medicine. BTS can reach the highest levels of efficiency in terms of quantity and quality of blood & blood components through implementation of quality management systems in all phases of blood collection, processing and storage. Quality indicators are performance measures designed to monitor & evaluate the quality of transfusion process.

Aims and objectives: To evaluate and analyse quality indicators of BTS.

Materials and methods: This is a retrospective study conducted for a period of 2 years (2014–2015) in Dept of IHBT at MGM Medical College & Hospital, Navi Mumbai. The data was captured for eight quality indicators defined by NABH, namely;

TTI%

Adverse transfusion reaction rate

Wastage rate for each component

TAT for blood issues

Component QC failure

Adverse donor reaction rate

Donor deferral rate

% of components issued

In addition to these, C: T ratio, Transfusion Index and Transfusion probability were also calculated.

Outcomes of the data were analysed and charted.

Result: A total of 8,444 blood bags were collected of which 7,849 units were separated into components and rest were either kept as whole blood or were discarded. Upon analysing the quality indicators, TTI% was found to be 0.85% and ATRR was 0.15%. Wastage rate of PRC was 5.64% while that of platelet, it was 24.5% and for FFP, it was 7.32%. TAT for routine issues were 64.57 min and for emergency issues, it was 13.3 min. The QC failure rate of PRC, platelet, FFP and SwPC was 7.69%, 10.20%, 10.41% and 6.38% respectively. ADRR was found to be 1.18% while DDR was 9.3%. Percent component issue was 98.18%. C: T ratio was found to be 1.77 while Transfusion Index (TI) was 0.56 and Transfusion probability was 56.46%.

Conclusion: Blood and blood components play an essential role in patients' management and a well-structured BTS contribute towards better healthcare in a hospital which is reflected by quality indicators. Blood components are frequently ordered and utilized in many hospitals without proper analysis of the real needs, thus wasting a very important resource. Quality indicators are only one tool for evaluating blood transfusion practice. However, if used in the right way they act as an efficient tool for continuous monitoring of blood transfusion process which will improve the quality of blood transfusion service in the organization. The quality improvement enable an organization to attain higher levels of performance by creating new or better standards or removing deficiencies in products, processes or services.

P-068

EVALUATING TRANSFUSION DEPARTMENT PERFORMANCE WITH IMPACT IN PATIENT CARE AND SATISFACTION

S Casimiro, A Spínola, L Gonçalves, S Pinheiro, M Sarmento, M Duarte, C Alves, C Brás, C Martins, R Lopes, M Ponte, C Soares, F Mota, P Nogueira, S Faria and S Sousa

Serviço de Imuno-hemoterapia, Unidade Local de Saúde de Matosinhos, Matosinhos, Portugal

Background: Adoption of Quality Indicators (QIs) has prompted the development of tools to measure and evaluate the quality and effectiveness of each Department's performance towards hospital setting and patient care. Results on the evaluation of these QIs are to be internally reported periodically and to the organization at large, along with an explanation of the follow-up actions to be taken to improve performance. The end goal of improving processes is to guaranty high quality care standards.

Aim: This abstract describes the evaluation and measurement of one performance QI, waiting time for a blood transfusion in a ambulatory setting, which was developed specifically to meet patient's satisfaction.

Methods: In order to reduce waiting time for a transfusion the standardized procedure was review and changed in late 2014. A comparison before and after the changes performed was made. Information about the elapsed time of the following processes was obtained: (i) complete blood count (CBC) request-CBC entry in pathology laboratory; (ii) CBC entry in pathology laboratory-analytical validation; (iii) CBC request- transfusion request; (iv) Transfusion request-discharge of the blood units from transfusion laboratory; (v) CBC request-discharge of the blood units.

Results: One hundred and fifty-one transfusion episodes from hospital ambulatory care unit were analyzed, 53% (80) were in 2015 after the changes in the standardized process. Mean time between CBC request registered in the laboratory database and CBC entering in pathology laboratory was 21 min and 31 min in 2014 and 2015 respectively, $P < 0.001$. Mean time between CBC request registered in the database and analytical validation was 26 and 29 min in 2014 and 2015 respectively, $P < 0.001$. Mean time between CBC request and transfusion request registered in transfusion database was 1 h and 17 min in 2014 and 44 min in 2015, $P < 0.001$. Mean time between transfusion request and the discharge of the blood units from transfusion laboratory was 1 h and 20 min and 1 h and 19 min in 2014 and 2015 respectively, $P = 0.843$. Overall procedure, from CBC request until the discharge of the blood units from the laboratory, toke 2 h and 17 min in 2014 and 1 h and 32 min, $P < 0.001$.

Discussion: The changes performed in transfusion request and unit deliver process for patients in ambulatory care improved the overall patient's waiting time. Design health care QIs that involve administrative, medical and laboratory performances are tools that can support quality improvement as it allows monitoring and evaluating routine practices. Our quality system instead of focusing on the 'find a problem, fix a problem' philosophy analyzes the underlying process that created the problem. To make significant improvements in transfusion department performance, systematic approaches need to be considered and a patient satisfaction survey should be validated and implemented to assess the impact of the amended measures.

P-069

THE IMPACT OF IMPLEMENTING CHANGE CONTROL MANAGEMENT SYSTEM UPON RATE OF ERROR OCCURENCE

M Fagelnour and S Shabayek

National Blood Transfusion Center, Giza, Egypt

Background: National Blood Transfusion service is a network with the head quarter in Giza & 23 regional blood transfusion centers in different governorates of Egypt.

The National Blood Transfusion center (headquarter) acquired the international accreditation of the American Association of Blood Banks as a donation center. During the phase of self assessment, blood collection using blood bags supplied by a sampling pouch if platelets are prepared from whole blood was required from our facility.

Aim: To demonstrate the impact of implementating a Change Control Management system upon the rate of occurrence of errors.

Methods: One of the requirements during the Self Assessment phase of the AABB accreditation was to use blood bags supplied by a sampling pouch if platelets are prepared from whole blood.

JMS & Complflex quadruple blood bags with sampling pouch were introduced in our Donation center for all our blood collection.

Since then, There was marked increase in the errors reported to our Quality department in 2014 (from May till July).

Root analysis for the cause of these errors was performed which was due to sampling errors & overweight blood units as follows: Compoflex quadruple (63 ml) resulted in errors due to increase rate of overweight units. By investigations, it was found to be of different dimensions from the double & triple blood bags that our staff used to use. The increase in overweight discards rate reached 4.26% in 2014.

Serology department complained from diluted samples. By investigations,it was found that JMS blood bags contains anticoagulant in the tube line leading to the sample port.

So, while sample withdrawal at the beginning of donation, anticoagulant passes in the samples.

Quality department performed an immediate preventive action after the root cause analysis & introduced change control management to our facility. Quadraple blood bags were stopped from being used on first of August 2014 & returned to use double & triple blood bags in blood collection.

A wide technical & theoretical 7 days training course was held in August 2014 for all our nursing staff.

Gradual re-introduction of quadruple blood bags to be used in blood collection after completing the training course.

Change control form was created & introduced in our donation department to be fulfilled upon reintroducing the quadruple blood bags with pouch & a copy was delivered to Quality department.

Results: Post training assessment was performed to evaluate & assess the trainees which had a success rate of 98%.

Using shaker balance during blood collection became mandatory to avoid overweight units.

According to manufacturer's instructions of the supplier (JMS),it was recommended to collect samples from the sample pouch during & not at the beginning of blood collection to avoid diluted samples.
Overweight discards rate reached 0.56% in 2015. Error analysis for errors related to quadruple blood bags with 10% in 2015 compared to 28% in 2014.
Conclusion: Implementing change control management system reduced the rate of error occurrence by 18%.
Any change in processes must be preceded by a change plan to train staff & gradually introduce this change.

P-070

This abstract has been withdrawn.

P-071

CAN QUALITY INDICATORS IMPROVE THE SUCCESS OF A HEMATOPOIETIC TRANSPLANTATION PROGRAM?

AI Spínola, F Bordalo, F Amado, S Lopes, S Ferreira, C Pinho and S Roncon

Therapy Cellular Departement, Instituto Português de Oncologia do Porto, Porto, Portugal

Background: Implementation of a quality management system (QMS) has become a standard practice for health care providers when their activities are associated with significant risks to human safety. Quality Indicators (QIs), as a tool to support continuous quality improvement, should identify areas where preventive/corrective actions can be focused; afterwards, the effectiveness of the implemented measures are submitted to further evaluation. Reviewing and assessing QIs can identify risks and determine whether a program is meeting accepted standards.
Aim: Measure and evaluate the quality of cellular therapy products distributed to patients through a specific set of performance QIs (pQIs).
Methods: This retrospective observational study analyses the outcomes of five independent pQIs that indirectly provide information about the routine activity of a Cellular Therapy Department during a 5 year's period (2011–2015): collection and processing of peripheral blood stem cells (PBSC), bone marrow (BM) and cord blood (CB) grafts for autologous (Auto) and allogeneic (Allo) hematopoietic transplantation. We determined the percentage (%) of high-quality products through the following criteria: fungal and bacterial sterility; cellular viability ≥40% after thawing (by trypan blue exclusion test); good functional capacity (PBSC: ≥1.0, BM: ≥0.1, CBU: $\geq 0.15 \times 10^5$ CFU-GM/Kg of patient body weight); elevated purity and potency (for Auto PBSC: $\geq 2.5 \times 10^6$ CD34+ cells, BM: $\geq 1.0 \times 10^8$ Nucleated Cells/Kg; for Allo PBSC: $\geq 4.0 \times 10^6$ CD34+ cells/kg, BM: $\geq 3.0 \times 10^8$ Nucleated Cells/kg, CB: $\geq 3.0 \times 10^7$ Nucleated Cells/Kg of patient body weight). Clinical efficacy was determined by the engraftment timing which was established according to Center for International Blood and Marrow Transplant Research (CIBMTR) criteria. For each year the intended targets were 95%, 99%, 99%, 80% and 95%, respectively. Data were analyzed using Excel 2013.
Results: Through the 5 years period, a mean of 333 grafts (283–363) were produced in our facility per year and for each pQI, the variation obtained was of residual significance: mean of 88% (81–92%) of the grafts had the minimum cellular content, with a number of bad mobilizers identical to that described in the literature; bacterial contamination occurred in 5–9 cases per year; overall, a steady 99% of the products presented an acceptable viability range; also, 90% of the grafts had their functional capacity maintained. Clinical efficacy showed a minor difference through the years, with no primary graft failure in 2012. Table 1 resumes the results (expressed as mean) achieved each year.
Conclusion: Measuring, monitoring and managing quality in our Department has been undergoing considerable development. Hence, not only we participate in an institutional quality program but we consider of most importance to be recognized for our value, quality of products and services by an accredited external program. In our opinion a reference QMS shall be addressed to all phases of cell collection, processing and distribution in order to guarantee high-quality products to all our patients.

	Purity and potency	Sterility	Cellular viability	Functional capacity	Clinical efficacy
2011	81%	100%	99%	84%	98%
2012	87%	98%	99%	90%	100%
2013	90%	99%	99%	85%	95%
2014	91%	99%	99%	90%	95%
2015	92%	99%	99%	90%	95%

Table 1. Summary of the pQIs results obtained per year

P-072

BACTERIAL CONTAMINATION OF PLATELET CONCENTRATE: AN INCIDENT OF NEAR-MISS

CG Kohombange and KS Kuruppu

National Blood Center, Colombo, Sri Lanka

Background: The risk of transfusion transmitted bacterial infection is becoming a major cause of transfusion related morbidity and mortality in worldwide. This is partly due to the reduction of other risks associated with transfusion, due to the stringent adoption of quality systems to the transfusion services. It is more frequent in platelet concentrates than in red blood components as it is more favorable for bacteria to grow in 20–24°C.
According to literature, clinically implicated bacterial contamination range from 1: 70,000 to 1:118,000 transfused platelet units. This largely depends on the dose and the pathogenicity of the bacteria. Some incidents are overlooked by the clinicians, attributing the signs and symptoms for a pre-existing clinical condition. Most of the signs and symptoms are being masked by the antibiotics.
Bacterial contamination is mainly exogenous and related to contamination by commensal bacterial flora present on the donor's skin.
Case Study: National Blood Center performs platelet culture as a part of its blood component quality monitoring program. A unit of random donor platelet was reported as positive for bacterial culture in BactAlert aerobic culture system. The co-components of the donation were traced and bacterial culture was performed by both BactAlert and manual blood culture systems. The RCC of the implicated donation was also found to be culture positive in both BactAlert and manual culture. Implicated FFP unit was negative in both culture methods.
Culture positive bottles were sent to the Microbiology Reference Laboratory for subculture and species identification. Culture report of the reference laboratory reveled a coagulase negative *Staphylococci* in both platelet and red cell samples. It was commented by the Consultant Microbiologist that, an accidental contamination of the blood pack at the time of venipuncture, probably due to inadequate antisepsis as the most likely cause for the incident.
The donor was called up and inquired to identify any infective foci which could have been present at the time of blood donation. There had not been a significant clinical condition triggering the bacterial contamination. In past medical history he has been on treatment for Diabetes Mellitus and discontinued treatment 6 months back. A blood sample was drawn from the donor for blood culture and Random Blood Sugar testing. Blood culture was negative in BactAlert culture system and the RBS was 605 mg/dl, which revealed an uncontrolled diabetes status.
Discussion and recommendations: According to literature the normal skin flora will be increased in patients with Diabetes Mellitus. In this case history, the skin disinfectants practiced has not been adequate to eliminate the excessive skin flora on the donors' arm.
The practiced technique seems to be adequate for routine disinfection in normal healthy donors. Assuring personal hygiene of the blood collecting staff, diversion of the initial 30 ml of blood to the diversion pouch, educating donors to report any symptoms of infections occurred after donating blood are the main strategies to prevent bacterial contamination which has to be adopted during whole blood collection.

P-073

MAINTENANCE OF THE QUALITY MANAGEMENT SYSTEM IN THE STEM CELL COLLECTION FACILITY IN THE INSTITUTE FOR TRANSFUSION MEDICINE

R Grubovic, S Useini, E Velkova, O Damevska Todorovska, N Veljanovski, E Petkovic, F Raka and M Grubovic

Institute for Transfusion Medicine, Medical Faculty, Skopje, Macedonia

Background: The Stem Cell Collection Facility is part of the Institute for Transfusion Medicine of the Republic of Macedonia (ITM). This Facility is operational since 2001 year with 653 collections of stem cells (527 procedures in 255 patients and 126 procedures in 74 sibling donors) till now. In 2012, in order to improve our overall service and to put it to the highest level, a quality management system (QMS) in Stem Cell Collection Facility was developed. Our aim is to show our experience in implementation and maintenance of the quality management system and our first steps in achieving the JACIE accreditation in order to provide our patients and donors the best possible care.

Materials and methods: The maintenance of QMS was obtained through establishing of the ISO standardization for the whole institution (ITM) and it was our first step leading to JACIE accreditation. The two of our colleagues became the JACIE Inspectors and the standard operating procedures (SOPs) were developed, followed by regular meetings, trainings and self-evaluation of the personnel. The orientation visit from the independent JACIE inspector was performed in order our institution to come one step closer to the JACIE accreditation and to improve our overall QMS.
Results: There is a national regulatory framework in place and WHO and World Bank initiatives in Macedonia which support quality in health care and accreditation. The Institute for Transfusion Medicine of RM was a part of the IPA project 'Strengthening the Blood Supply System'. This project aimed to ultimately bring the Blood Transfusion Service to European Union standards allowing the exchange of blood components and all other types of collaboration with other European Union countries in future. The project put the basis for unification of blood transfusion standards and operating procedures in the whole country as well as set up essential education of blood transfusion personnel. Although a lot of strengths were found during the JACIE's inspector orientation visit, there are still a lot of areas for improvement. Our strengths are motivated team and supportive institutional leadership including Macedonian Ministry of Health. Areas for improvement are: labeling of cellular therapy products and lack of laboratory for quality control.
Conclusions: Our institution has in plan to implement ISBT 128 standards for labeling of cellular therapy products and to establish a laboratory for quality control of cellular therapy, as well as to meet all the requirements to become JACIE accredited facility. Working by standards, following the rules and regular self-evaluations will help us to maintain the strong quality management system.

P-074

COMPARISON OF QUALITY PARAMETERES OF PLATELET RICH PLASMA DERIVED PLATELET CONCENTRATE AND BUFFY COAT DERIVED PLATELET CONCENTRATE AT DISTRICT GENERAL HOSPITAL HAMBANTOTA SRI LANKA

M Adikarama, DKN de Silva and GMP Kumara
Blood Bank, Hambantota, Sri Lanka

Background: The Cluster Centre of District General Hospital (DGH) Hambantota recently started production of Platelet Concentrate-Platelet Rich Plasma Derived (PC-PRPD) and Buffy Coat Derived Platelet Concentrate (PC-BCD) and quality monitoring of 1% or minimum of 4 units of randomly selected blood products. 75% pass rate is needed for each parameter according to the Standard Operative Procedure (SOP) for quality monitoring of the National Blood Transfusion Service of Sri Lanka (NBTS/SL) according to specifications defined for a said blood product. Currently Single Donor Platelets (SDP) or Aphaeresis Platelets (PC-AP) is not produced at this centre.
Aims: To compare quality parameters of PC-PRPD and PC-BCD produced at Cluster Centre DGH-Hambantota with the aim of providing better health care by issuing quality platelet products to the patients.
Methods: A retrospective study was carried out using monthly quality monitoring data of Cluster Centre DGH- Hambantota for a period of 4 months from 1st of December 2015 to 31st of March 2016. The production of PC-PRPD and PC-BCD was 106 and 0 in the first 2 months respectively. The production of PC-PRPD and PC-BCD was 0 and 168 in the latter 2 months respectively. Eight units were randomly selected for each category (4/month). NBTS/SL Specifications are shown in Table 1.
Results: In the present study the mean volume of PC-PRPD and PC-BCD was 57.5 and 55.5, the mean platelet count of PC-PRPD and PC-BCD was 79.115 X 109/U and 78.825 X 109/U respectively. The mean RBC Count per Unit of both PC-PRPD and PC-BCD stood at 0.00. While the mean WBC count per Unit of PC-PRPD and PC-BCD stood at 0.0236 and 0.0118 respectively. There is no significant difference ($P < 0.05$) between PC-PRPD and PC-BCD in terms of the mean values of volume ($P = 0.209$), platelet count per Unit ($P = 0.489$), WBC count per Unit ($P = 0.285$) and RBC count per Unit. The 75% pass rate was achieved at 100% in the relevant 2 months for both products in all said parameters.
Conclusions: Both types of platelet products have required pass rate in relation to NBTS/SL standards and no significant difference when means were compared with regard to volume, platelet count, WBC count and RBC count per unit. There is no or minimum contamination of red cells in both platelet concentrates as it was not measurable by the laboratory technique used. This study also highlights the importance of routine quality monitoring.

Blood Component	Parameter	Specifications
Platelet Concentrate Platelet Rich Plasma Derived (PC-PRPD)	Volume	45-75 ml
	Platelet Count	> 55 × 10^9 / Unit
	RBC Count	< 1 × 10^9 / Unit
	WBC Count	< 0.2× 10^9 / Unit
Platelet Concentrate Buffy Coat Derived (PC-BCD)	Volume	45-75 ml
	Platelet Count	> 55 × 10^9 / Unit
	RBC Count	< 1 × 10^9 / Unit
	WBC Count	< 0.05 × 10^9 / Unit

Table 1. PC Specifications

P-075

THE AUTOMATION OF QUALITY DOCUMENTATION SYSTEM IMPLEMENTATION OF E-DELPHIN IN NATIONAL BLOOD TRANSFUSION SERVICES

MM Nicola and S Shabayek
Egyptian National Blood Transfusion Services, Giza, Egypt

Introduction: Since the Memorandum of Understanding was signed in 1997 between the Egyptian Ministry of Health and Swiss State Secretariat for Economic Affairs for the establishment of the National Blood Transfusion Services, it was agreed that a comprehensive National Information Management System shall be developed.
Upon the implementation of E-Delphyn Blood Management System, there will be a necessity to make major changes in NBTS' documents and records.
Aims: To study the Quality documentation system improvements upon the implementation of E-Delphyn.
Methods: Error Management System and Customer Complaints: The implementation of E-Delphyn Blood Management System is expected to cut time and effort needed for the investigations. The current error reporting form can be updated to include a new section for information retrieved from the electronic system. Also, new versions of the corresponding Standard Operating Procedures will be generated to be used in any center that applies E-Delphin: A new step will be added before the verbal investigations with involved departments, so that system information checking will be a major step in the management of errors.
Auditing System and Quality Management Review: Currently an audit check every 3 months: two by the National Quality Team, alternating with two by the Head of Regional Quality Department.
The suggested modification is to create a new checklist for a quick monthly check only depending on the system. These will be independent from Quality Department's regular visits, but the auditor will have to review them before his scheduled audit.
Key Performance Indicators: Two major changes will be done.
Full KPIs profile will be mandatory for centers using E-Delphyn.
There will be a change in how the statistical information is retrieved: the current manual transcription on Excel workbooks will be upgraded to automatic retrieval from the system. Information Technology Department's assistance may be needed at the beginning until Quality team can fluently deal with E-Delphyn statistical reports.
Stock Management System: Conventional hard copies of blood (whole and components units) stock cards will be substituted with the electronic records. The same will be done for the empty blood bags.
Document Control System: This will be the system the most widely affected, as most of the departmental manuals will be updated in compliance with the new procedures after automation.
Validation and assessment of the new versions must be done to continuously improve the Quality Department's performance and flow of work.
ResultsCutting down the time required for the completion of different Quality Department tasks.
Close monitoring of all aspects of technical work, leading to more accurate behavior of technical staff.
Prompt error detection, faster actions of Quality Department Staff therefore speedy error correction.
Minimizing clerical and computational errors in statistical datasheets.
Better synchronization between different departments and between different Blood Transfusion Centers; due to the fluency of transmission of information to the Quality Department and between Regional Centers' departments and National ones.

Conclusion: New versions of the Quality Department forms and documents shall be of a great value if synchronized with the technical implementation of E-Delphyn Blood Management System. Five major systems will be updated, and assessment of the performance with the new versions will be done to make necessary modifications for optimal work flow within the Quality Department.

P-076

IQC TRENDING WITH UNITY SOFTWARE AND EVALUATION OF THE BIO-RAD EQAS BLOOD TYPING SYSTEM

T Harden

Royal Derby Hospital, Derby, United Kingdom

Derby Teaching Hospitals NHS Foundation Trust provides both acute hospital and community based health services, serving a population of over 600,000 people in and around Southern Derbyshire.

The Royal Derby Hospital, which incorporates the Derbyshire Children's Hospital, and is a busy acute teaching hospital.

The Trust treats a million patients each year and more than 6,000 babies are born in its maternity unit annually. 72,000 elective operations take place every year in the hospital's suite of 35 modern operating theatres, an average of more than 280 operations per day. The hospital has a total of 1,100 beds.

Pathology at the Royal Derby Hospital consists of three departments, Blood Sciences (Incorporating Haematology, Biochemistry, Immunology and Blood Transfusion), Cellular Pathology and Microbiology.

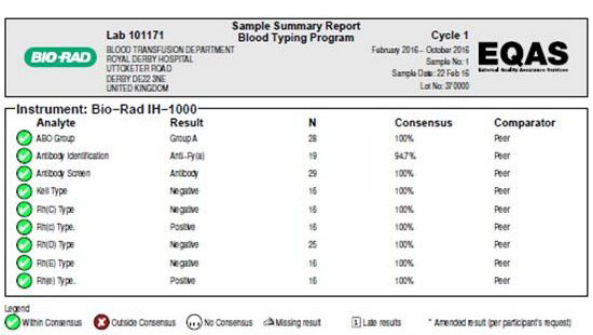
The Blood Transfusion Laboratory is CPA Accredited, MHRA compliant, meets current BCSH Guidelines and participates in all relevant Quality Assurance schemes. The department processes around 60,000 blood group and antibody screens annually utilising two Biorad IH-1000 analysers and Biorad column technology. Routine Internal quality assurance is performed daily using the Biorad Basic QC package. Other tests performed are Antibody identification, Compatibility testing including serological crossmatch and electronic issue, Neonatal sample testing, Kleihauer testing and Direct Antiglobulin testing is undertaken. The department provides an Antenatal Screening Service and a routine Antenatal Anti-D prophylaxis (RAADP) programme.

In January 2016 Bio-Rad installed a software package called Unity onto the Bio-Rad analysers for evaluation. Unity can be utilised to provide trend analysis/monitoring of sensitivity of the internal quality control material being processed routinely on the analysers.

It is a requirement of the International Standard ISO 15189:2012 (E), 5.6.2.3 that:

'Quality control data shall be reviewed at regular intervals to detect trends in examination performance that may indicate problems in the examination system'.

Initial results show that the Basic QC 1 & 2 cell samples in routine use on both the analysers appear to be stable throughout the period they are used maintaining consistent strengths of reaction for both grouping and antibody screening. Reports can be generated from the Unity software as PDF documents which are reviewed at local Quality meetings. Remote access for managers to the results on Unity via the hospital network is also a useful feature.


Lab 101171
BLOOD TRANSFUSION DEPARTMENT
ROYAL DERBY HOSPITAL
UTTOXETER ROAD
DERBY DE22 3NE
UNITED KINGDOM

Sample Summary Report
Blood Typing Program

Cycle 1
February 2016 - October 2016
Sample No: 1
Sample Date: 22 Feb 16
Lot No: 37 0000

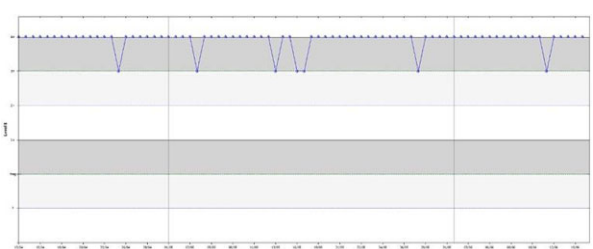
EQAS

Instrument: Bio-Rad IH-1000

Analyte	Result	N	Consensus	Comparator
ABO Group	Group A	28	100%	Peer
Antibody Identification	Anti-Fy(a)	19	94.7%	Peer
Antibody Screen	Antibody	29	100%	Peer
Kell Type	Negative	16	100%	Peer
Rh(C) Type	Negative	16	100%	Peer
Rh(c) Type.	Positive	16	100%	Peer
Rh(D) Type	Negative	25	100%	Peer
Rh(E) Type	Negative	16	100%	Peer
Rh(e) Type.	Positive	16	100%	Peer

Legend
Within Consensus | Outside Consensus | No Consensus | Missing result | Late results | * Amended result (per participant's request)

Caption 1. EQAS result report cycle 1 tests



Caption 2. IQC sample 1 Group A trending

In addition to evaluating the Unity software for IQC we were also involved in trialling the new Bio-Rad EQAS blood typing system. In February 2016 we received the first cycle of EQAS material which consisted of four blind samples, three samples suitable for Blood Grouping, phenotyping, antibody screening and antibody identification plus a further sample to act as a donor sample for compatibility testing. The samples were run routinely on the IH-1000 analysers and the results subsequently submitted before the closing date on the dedicated EQAS online portal via the Bio-Rad QCnet website.

An EQAS online account was set up for the Derby Blood Transfusion Laboratory, which allowed us access to the portal to set up the instrument requirements (IH-1000) and test requirements.

Once the system was configured we could then enter the results for each test performed and submit the results accordingly. The report being made available 48 h later, which provided a comparison with other EQAS users using similar and alternative technologies.

Further evaluation of the EQAS blood typing system will take place in Cycles 2 and 3 available in June and October 2016.

P-077

DETERMINATION OF RESIDUAL LEUKOCYTES USING A PORTABLE AUTOMATED MICROSCOPY SYSTEM COMPARED TO FLOW CYTOMETRY

HS Ahlzén, LM Larsson, L Daher, S Larsson, M Kaarlenkaski and B Diedrich

Karolinska Universitetssjukhuset, Stockholm, Sweden

Background: More than 90% of blood components for patient use in Sweden and all blood components for patient use produced at Karolinska University Hospital are leukoreduced, with a maximum of 1×10^6 leukocytes allowed per transfusion unit, which equals approximately 4–5 leukocytes/µl. Therefore, determination of the amount leukocytes in blood components is a corner stone in quality control. Since the concentration of leukocytes is very low, an accurate detection system with sufficient detection levels and reliable results is essential. Until today, determination of residual leukocytes has been performed using flow cytometry (FCM) or manual counting in a Nageotte chamber. However, recently the new portable automated microscopic system ADAM-rWBC (ADAM) emerged as an interesting alternative. It detects leukocytes after staining with propidium iodide.

Aim: The aim of this study was to evaluate ADAM as a method for routine determination of residual leukocytes in RBCs, plasma and PLTs. This evaluation was comprised of two parts: *(i)* the accuracy, precision and robustness of the method compared to FCM and *(ii)* the cost and time efficiency of the method compared to FCM.

Method: *(i)* We analyzed dilution series to evaluate the linearity and detection limits of ADAM. We furthermore compared results from ADAM with FCM, which is our standard method. This was done for additional dilution series, 20 units of PLTs from pooled buffy coats, 10 units of aphaeresis PLTs, 20 units of leukoreduced plasma, 5 units of non-leukoreduced plasma and 50 units of RBCs. In addition, repeated measures were performed for determination of reproducibility of analysis by ADAM. *(ii)* We compared the expense of analyzing residual leukocytes in blood components using ADAM and FCM. The time per analysis was also evaluated for the two methods.

Results: *(i)* The dilution series showed good linearity and accuracy, especially around our quality control limit of 4–5 leukocytes/µl. Results from ADAM and FCM were very similar for all tested types of blood components. However, the results from ADAM were consistently higher than the results from the FCM. Moreover, the reproducibility of ADAM was shown to be better than for FCM. *(ii)* The expense for performing analysis of residual leukocytes in blood components was 20% lower for ADAM than for FCM. In addition, the time used for analysis using ADAM was less than half of the time used for analysis by FCM.

Summary: In comparison to our standard method for determining remaining leukocytes (FCM), ADAM was found to be more sensitive than FCM. This is probably due to the larger sample volume of ADAM, which is about 2.3 times the sample volume of FCM, which increases the probability of finding very rare cells. Furthermore, both the cost of analysis and the time used for analysis is lower for ADAM than for FCM, which is of great benefit for any blood bank organization. Therefore, we conclude that using ADAM to determine residual leukocytes in leukoreduced blood components is suitable for routine quality control as well as for method development applications.

P-078

TIMELY RH IMMUNISATION OF POTENTIAL CANDIDATES WITH ANTI D IMMUNOGLOBULIN – A CLINICAL AUDIT

N Nasir

Aga Khan University Hospital, Karachi, Pakistan

Background: Before the availability of anti-D immunoglobulin (anti-D Ig), the incidence of Rh D alloimmunisation in D negative women following deliveries of D positive, ABO-compatible, infants was approximately 16%, and thereforehemolytic disease of the fetus and newborn (HDN) was a significant cause of morbidity and mortality. This incidence has greatly reduced following routine post-partum administration of anti-D IgG. According to the British Committee for Standards in Hematology (BCSH), anti-D IgG should be administered as soon as possible and always within 72 h of a potentially sensitizing event. Following birth, ABO and Rh D typing should be performed on cord blood and if the baby is confirmed to be D positive, all D negative, previously non-sensitized, women should be offered at least 500 IU/ 300 μg of anti-D within 72 h following delivery.
Objective: To evaluate timely administration of Anti Dwithin 72 h of delivery to all Rh negative mothers who gave birth to Rh positive babies.
Material and methods: This study is a retrospective clinical audit that took place atThe Aga Khan University Hospital, Section of Hematology, Department of Pathology and Laboratory Medicine. The study duration is 1 year and sample sizeis 111 patients. We retrieved information of the mother's blood group, date of delivery, baby's blood group, time of administration of anti D and reason for admission from our database. A Performa was then filled and the data on the questionnaire was audited. Statistical package for social sciences 21 was then used for data entry and analysis.
Results: All111 mothers were Rh negative and thus potential candidates for RhIgG administration. For the babies born to these Rh negative mothers, 73% (n = 82) were Rh positive and 26% were Rh negative (n = 29). All the mothers (100%) who gave birth to Rh positive children (n = 82) were immunized within 72 h. The mean time for immunization was 13.5 h. Seventy-four (90%) of mothers who gave birth to Rh positive babiesreceived a dose of 300 μg, seven mothers (8.5%) received 600 μg and one mother (1.2%) received 900 μg. Forty nine (59.7%) of females who received immunization were electively admitted, 29.3% were admitted withlabour pain (n = 24), 7.3% (n = 6) were admitted due to leaking liquor and 3.6% were admitted due to decreased fetal movements, vaginal delivery and pregnancy induced hypertension (n = 3).
Conclusion: This study demonstrates 100% compliance of our obstetricians with the BCSH guidelines for timely administration of Rh immunization.

P-079

This abstract has been withdrawn.

P-080

EXTERNAL QUALITY ASSESSMENT SCHEME FOR TRANSFUSION TRANSMISSIBLE INFECTIONS AMONG BLOOD SERVICE FACILITIES IN THE PHILIPPINES, 2015

KA Punzalan, RT Yu, BM de Vera and IMSC Chamen

Research Institute for Tropical Medicine, Muntinlupa City, Philippines

Background: The TTI-NRL has previously conducted external quality assessment schemes (EQAS) for blood service facilities (BSF) since 2003 with inconsistencies due to funding and implementation issues. The TTI-NRL established in 2015 a 20-sample HIV, Hepatitis B & C, Syphilis serology (HVHT4320) and a 5-smear malaria (MLRA415) EQAS designed for BSF. Results analysis provides informative data on assay performance and the performance of each laboratory using the same assays (peer groups). These results generated will be used by the BSF as a requirement for renewal of their license to operate.
Aims: The (EQAS) for Blood Screening Serology provided by the Transfusion Transmissible Infections – National Reference Laboratory (TTI-NRL) aims to raise standards and assess the phases of laboratory testing on blood units to determine interlaboratory comparison.
Methods: Participating BSF were given two sets of EQAS panel. HVHT4320 consists of 20 plasma samples and the MLRA415 consists of five blood smears. Participants were instructed to test the samples as they would test their donor samples. Results were entered through OASYS, an online informatics system. These were compared the TTI-NRL reference results and a quantitative comparison among their peer groups.
Results: A total of 147 participants participated in the EQAS, with 119 (81%) reported concordant results with the NRL. One participant used an expired reagent for testing one analyte and 10 other participants have failed to indicate the expiry dates of their assay reagent kits. 28 (19%) of the BSF reported aberrant results for HVHT4320. Out of the 11,760 total number of results entered by the BSF, 99.68% were correctly identified and 0.80% and 0.14% were entered as false negative and false reactive respectively. Six of these participants were identified to have reported results that were due to data entry error or clerical error. For the MLRA415 panel, 12% of participants reported aberrant results and out these, 9% reported false detection of human Plasmodia and 3% reported having false negative slides.

PLATFORM	HIV		HBV		HCV		Syphilis		Total Aberrant
	FALSE NEGATIVE	FALSE REACTIVE	FALSE NEGATIVE	FALSE REACTIVE	FALSE NEGATIVE	FALSE REACTIVE	FALSE NEGATIVE	FALSE REACTIVE	
CNLIA	3 (7.89%)	2 (5.26%)	3 (7.89%)	4 (10.53%)	2 (5.26%)	2 (5.26%)	0 (0.00%)	1 (2.63%)	17 (44.74%)
EIA	1 (2.63%)	0 (0%)	1 (2.63%)	4 (10.53%)	2 (5.26%)	3 (7.89%)	1 (2.63%)	0 (0.00%)	12 (31.58%)
RDT	0 (0.00%)	0 (0.00%)	5 (13.16%)	0 (0.00%)	0 (0.00%)	1 (2.63%)	2 (5.26%)	0 (0.00%)	8 (21.05%)
RPR	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	1 (2.63%)	0 (0.00%)	1 (2.63%)
Total Aberrant	4 (10.53%)	2 (5.26%)	9 (23.68%)	8 (21.05%)	4 (10.53%)	6 (15.79%)	4 (10.53%)	1 (2.63%)	38 (100.00%)

Caption 1. Number of aberrant results per transfusion transmissible infections testing platform (HVHT4320 1st Panel)

Summary/Conclusions: After the assessment, several factors were determined in order to improve concordance rate of the BSF. This includes the following: Compliance with regulations provided by the Department of Health for recommendations, strategies, methodologies and algorithms for testing blood units for transfusion transmissible infections. The testing staff of the BSF must be theoretically and technically proficient in testing for TTI including identification of malaria parasites. BSF should examine their assay performance to identify aberrant results and perform appropriate corrective actions. An additional person should check the assay results independently prior to reporting to resolve data entry errors. STD/AIDS Cooperative Central Laboratory (SACCL)-evaluated assay reagent kits, recommendations by the National Blood Program, adherence to manufacturer's protocols, strict internal quality control procedures and critical supervisory review are measures to avoid technical deficiencies. Rapid test kits for blood screening are of inadequate sensitivity compared to third generation kits. EQAS plays a vital role in the improvement of efficiency of BSF that improves the overall quality of the National Blood Program.

[Correction added on 30th August 2016: Abstract P-080 was deleted in error from the online files and has now been reinserted]

P-081

THE IMPACT OF QUALITY CHECKS AT BLOOD RECEPTION LABORATORY; A CASE STUDY AT NAKASERO REGIONAL BLOOD BANK

W Wambi, D Kyeyune and G Otekat

Uganda Blood Transfusion Service, Kampala, Uganda

Background: Uganda blood transfusion Service is a semi-autonomous organization mandated to collect, process, provide safe and adequate blood and blood products, and promote safe blood transfusion practice in Uganda. The institution is composed of seven regional blood banks, six collection/distribution centers and over 25 blood collection teams. Blood collected from the field is processed & screened for Transfusion Transmissible Infections (TTIs) before it is deemed safe for transfusion. In addition to the TTIs screening, more quality checks have been introduced to further improve on the safety of blood transfusion.
Aims: To find out the most prevalent parameter causing non-conformance at blood reception.
To evaluate the impact of Quality checks & training on the level of non-conformance.
Methods: Data for 3 months (December 2015, January 2016 & February 2016 was retrospectively reviewed and analyzed using Microsoft excel and word computer programs. Results for December 2015 and January 2016 were analyzed and shared with all the teams/collection centers which supply blood to Nakasero blood bank followed with subsequent training. Data for February 2016 was analyzed and the results compared to the previous 2 months to assess the impact of training.

Results: For the months of December 2015 & January 2016 a total of 7,352 blood units were received from all the collection teams compared to 2,923 blood units for the month of February 2016. The comparison for the two batches of results showed an increment of conforming blood units in five teams (A, B, D, DR & Jinja) by 2.4% (from 95.8% to 98.2%). The other three teams (C, AMB & Jinja) however deteriorated slightly instead by 2.7% (from 97.1% to 94.1%). The most prevalent parameter was volume (inadequate & overfilled) with inadequate (2.5%) for the first 2 months which reduced to 1.5% for the month of Feb & overfilled blood units (1.2%) however increased to 1.7%. No cases of haemolysis, mislabeling, leaking & temperature outside range were identified for all the teams.
Summary/Conclusions: The reduction in the number of inadequate blood units was attributed to members adhering to the resolutions made during the meeting (like training & provision of calibrated equipment) when the first batch of results were shared with the team members. In the event of correcting the issue of inadequate blood units however some teams instead collected overfilled blood units contributing to the increment of the rate of non-conformance due to overfilled units from 1.2% for the month of December 2015 & January 2016 to 1.7% for the month of Febuary 2016.
Quality checks at blood reception laboratory therefore had a positive impact on the rate of non-conformance as members became more careful after sharing with them the first batch of results and training hence improving the quality & safety of blood.

P-082

COMPARISON OF QUALITY INDICATOR RATES IN PLASMA DONORS AND WHOLE BLOOD DONORS

ME Lund and K Titlestad
Odense University Hospital, Odense C, Denmark

Background: Although blood and plasma donations are generally safe, a variety of complications related to the collection process exist. Donors experiencing complications during collection are less likely to make repeat donations. In our blood establishment several quality indicators are monitored, such as hematoma, puncture failure, vasovagal reactions and reduced flow. The data are evaluated each quarter and calculated as a percentage of the total donations.
Aims: Until 2 years ago, only whole blood was collected at the blood bank. During the last 2 years, a shift has been made to mainly plasma donations. Thus the aim of this study was to make more detailed comparisons of the rate of different quality indicators in whole blood- and plasma donors.
Method: At the Hospital blood bank, whole blood donations as well as plasma donations are carried out by the same staff. Complications related to the collection process are registered in our donation database system (ProSang, Databyrån AB, Stockholm, Sweden). All donation data from January 2014 to April 2016 were extracted from the database.
Results: The data showed a difference in the rate of the different quality indicators registered for blood and plasma donors. See Table 1. In general the rate of registered quality indicators was higher for plasma donations than for whole blood donations. Age and gender also seems to influence the complications related to donations.
Conclusion: The rates of different quality indicators in whole blood- and plasma donors were compared. The rate for puncture failures, hematoma and vasovagal reactions are all higher for plasma donors than for whole blood donors. The data also showed that gender influences on the quality indicators rate. Female donors have higher risk of puncture failures for both whole blood and plasma donations. Corresponding to this the rate of reduced flow is twice as high for female donors. For vasovagal reactions no difference in male and female donors are observed in whole blood donations. However for plasma donations the rate is twice as high for female donors. This may be due to lower body weight of female donors, but unfortunately the body weight is not registered. The quality indicators in relation to gender and age will be further investigated.

	FEMALE	MALE	Total	FEMALE%	MALE%	Total%
BLOOD, all donors	13.170	15.318	28.488	100%	100%	100%
No events registered	12.379	14.798	27.177	94.0%	96.6%	95.4%
Puncture failure	518	324	842	3.9%	2.1%	3.0%
Vasovagal etc.	41	48	89	0.3%	0.3%	0.3%
Hematoma	39	13	52	0.3%	0.1%	0.2%
Reduced flow	193	135	328	1.5%	0.9%	1.2%
PLASMA, all donors	6.190	11.589	17.779	100%	100%	100%
No events registered	5.665	10.984	16.649	91.5%	94.8%	93.6%
Puncture failure	295	351	646	4.8%	3.0%	3.6%
Vasovagal etc.	86	82	168	1.4%	0.7%	0.9%
Hematoma	41	66	107	0.7%	0.6%	0.6%
Reduced flow	103	106	209	1.7%	0.9%	1.2%

Table1: Quality indicators registered for blood and plasma donors

Table 1

P-083

NONCONFORMITIES REPORTING AS PART OF THE QUALITY MANAGEMENT SYSTEM

T Makarovska Bojadzjieva, V Velkova, M Blagoevska, J Samonikov Tosevska, B Todorovski and L Mitevska
Institute of Transfusion Medicine, Skopje, Macedonia

Background: In January 2011, Macedonian blood transfusion services were integrated in one national transfusion establishment. The integration enabled standardization of all the processes and laboratory procedures through the implementation of SOPs and Quality management system which mandates reporting of product nonconformities and laboratory errors.
Aim: To evaluate the nonconformity reporting forms (number and type of nonconformities), as well as the reporting system in general in the National Institute of Transfusion medicine in the period from 2014 to 2015.
Methods: The reported nonconformities concerning the collection, processing and testing of blood were collected, systemized, analyzed and documented by the Quality control department.
Results: The data concerning the total number of collected, tested and produced blood components, as well as the reported nonconformities in 2014 and 2015 are shown in Tables 1 and 2.
Conclusion: The implementation of quality management system and the reporting of the nonconformities on regular basis enabled spotting the weak points of the blood chain and undertaking corrective measures which is critical for the quality and safety of the transfusion treatment. In the past few years we have managed to transform the meaning of notifying adverse events from being an occupational duty with related consequences to an opportunity to increase knowledge that can be applied to prevent them and to provide better health care.

Blood Donation: Blood Donor Recruitment

P-084

FREQUENCY AND REASONS OF DONOR DEFERRAL PRIOR TO BLOOD DONATION PROCESS – A SINGLE CENTRE EXPERIENCE

N Fatima, S Khurram, M Borhany, N Anwar, M Nadeem and T Shamsi
National Institute of Blood Disease an BMT, Karachi, Pakistan

Background: Transfusion is an irreversible event that carries potential risks as well as benefits to the recipient. Therefore, donor selection prior to blood donation is one of the most important steps in ensuring the safety of blood and blood products.
Aim: The aim of the study is to determine the frequency and reasons for donor deferral prior to the blood donation process in our population.
Methods: A cross-sectional study was carried out at the blood bank department in our hospital from January 2012 to December 2014. All the blood donors who visited our department in the study period were included in this study.
Results: A total of 25,901 potential donations were recorded during the study period, comprising 24,309 (93.8%) replacement and 1592 (6.2%) voluntary donations. Females accounted for only 222 (0.9%) of potential donations. Deferral occurred in 3156 (12.2%) of attempts; 280 (1.1%) were permanently deferred while 2876 (11.1%) were deferred temporarily. The most common reason for permanent deferral was a history of Hepatitis B infection (n = 147, 4.7% of all deferrals). Major reasons for temporary donor deferral were low levels of haemoglobin (n = 971, 30.76%), low levels of platelets (n = 611, 19.35%) and previous history of jaundice (n = 192, 6.1%).
Conclusion: This study reported a fairly similar pattern of donor deferrals as in other regional studies. Low haemoglobin levels and a history of hepatitis B infection were the most common factors for temporary and permanent donor deferrals, respectively.

P-085

ANALISYS OF BLOOD DONORS DEFERRAL REASONS

N Milosavljevic Knezevic, M Krga Milanovic, N Bujandric and R Jovanovic
Blood Transfusion Institute Vojvodina, Novi Sad, Serbia

Background: Contemporary transfusion strategy attempts to improve the health of blood donors (BDs) and recipients of blood products. The donor selection was done by the questionnaire, physical examination and hemoglobin estimation. BDs who is not able to meet the donor selection criteria should be deferred on a temporary or permanent basis.
Aims: This paper is aimed to analyze the rate and reasons for deferrals of BDs in Blood transfusion Institute Vojvodina (BTIV).
Methods: A retrospective study was carried out for the 12-month period from January 1st to December 31st 2015. Data gathered from the blood donors questionnaires and information system of BTIV. Data for statistical analysis included first time and regular BDs.
Results: A total of 26,485 blood unit were collected. 3.550 BDs were deferred during the study period. Out of them regular BDs were 3.168 (89.24%) and first time BDs were 382 (10.76%). 77 (2.17%) blood donors were deferred on permanent basis. Blood donors were deferred for various reasons: Low hemoglobin level 1.595 (44.93%), Hypotension 279 (7.86%), Drugs 276 (7.77%), Hypertension 170 (4.79%), Medical examination 120 (3.38%), Colds and rhinitis 85 (2.39%), High cholesterol 84 (2.37%), Herpes 67 (1.89%), Risk behavior 66 (1.86%), Fatigue and lack of sleep 61 (1.72%), Low weight 60 (1.69%), Surgery 59 (1.66%), Asthma 47 (1.32%), Allergy 45 (1.27%), Medical examination 33 (0.93%), Tattoo 32 (0.90%), Menstruation 31 (0.87%), Bad veins 30 (0.84%), Alcoholism 28 (0.79%), Other reasons 382 (10.77%).
Summary/Conclusion: The deferral rate was higher for the regular blood donors than first time blood donors. The most common reasons for deferral were: low hemoglobin levels, use of drugs/antibiotics and hypotension. To protect blood donors and recipients, stringent donor selection criteria are necessary. The blood transfusion services should be able to decrease unnecessary deferrals. Also deferred donors should be helped to overcome their problems.

P-086

BLOOD DONORS DEFERRAL PATERN IN FIXED AND MOBILE SITES, UAE: EXPERIENCE

M Raouf[1], T Khalid[2], R Jarkas[2], M Mahmoud[2] and Z Saeed[2]
[1]*Dubai Health Authority, Sharjah, United Arab Emirates* [2]*Ministry of Health, Sharjah, United Arab Emirates*

Background: Deferred donors are those whom are deferred from giving blood regardless of the reasons for deferral which is either temporary or permanent. Blood collection centers aim to collect blood from safe donors by implementing proper selection criteria and meets patients demand concerning availability; which can be affected by un necessary deferral for donors.
Aims: Studying the different causes for donors deferral in different collection sites and evaluating the importance of having donors deferral rate as KPI that can help collection centers in achieving appropriate blood donor selection and avoid unnecessary deferral.
Methods: This is a Four years retrospective study conducted in the main blood donation Center in Ministry of Health in United Arab Emirates to study the different reasons for deferral taking into consideration two sites for collection:fixed (center) and mobile units (Mobile vehicle). Different deferral reasons and deferral rate are monitored on daily basis and deferral rate has been assigned as KPI.
Results: From 74,087 person presented for blood donation, 59,678 (80.6%) has been accepted for donation while 14,409 (19.4%) has been deferred. 12,524 (86.9%) were temporarily deferred and 1,885 (13.1%) were permanently deferred. Deferral for low hemoglobin was the highest reason for temporary deferral constituting 18.2% followed by travel history which was seen in 16.9%. Comparing the deferral reasons in different collection sites (fixed VS mobile); this study shows significant difference in deferral rate for Low hemoglobin Level that constitutes 25.3% of deferred donors in fixed sites compared to 14.4% for those in mobile sites. In addition;the deferral rate for travel history to malaria risk areas shows 6.5% in fixed sites compared to 22.4% in mobile sites which was statistically significant. Donor Deferral rate of <25% was selected as KPI and found to be a useful indicator to monitor the trend of donor deferral over time.
Conclusions: Proper donor selection is the cornerstone for safe blood transfusion and unnecessary donors deferral leads to losing blood donors while patients are in need for their blood. Having donor deferral rate as quality indicator, monitors the trend of donor deferral over time. Deviations from the predicted frequency may indicate the need for the investigation in order to verify if deferral criteria are consistently and uniformly applied. This study shows that regular donors are more frequently deferred for low hemoglobin compared to first time donors and less regular donors. When proper educational material is given to regular donors it will reduce deferral rate for certain reasons that will reduce the load on the collecting facility and enhance safety.

P-087

THE MOST COMMON REASONS FOR DEFERRAL THE BLOOD DONORS AT THE REGIONAL CENTER FOR TRANSFUSION MEDICINE IN SHTIP, MACEDONIA (FYROM)

J Vitlarova[1], N Vitlarov[2], N Ivanova[1], M Shorova[1] and T Timova[1]
[1]*Institute of Transfusion Medicine, Regional Center Stip, Stip, Macedonia* [2]*Institute for Pathology, Academic Teaching Hospital Feldkirch, Feldkirch, Austria*

Background: The basic process of blood donor's selection plays a crucial role in the safety of blood recipient and blood donor itself. However, according to the established national standards all volunteer donors must fulfill donor questionnaire and they must undergo for medical examination, Hemoglobin blood level's control as well as evaluation for temporary or permanent contraindication for blood donation. The insufficient eligibility for blood donation due to various causes can lead to withdrawing the process at any point during the collection or blood testing.
Aim: The aim of this study was to evaluate the leading reasons for deferral the blood donors at the Regional Center (RC) for Transfusion Medicine in Stip in the year 2015.
Materials and methods: In the past year (2015), 4,959 Volunteers have came for non-remunerated blood donation at our RC. Moreover blood donation was performed in 4,349 cases from the total 4,959 Volunteers. The successfully conducted 4,349 donations were achieved in our Department for blood donation (n = 2,740) as well as from our mobile Teams (n = 1,609). The total number of deferral donors was 607. Furthermore, from the 607 rejected donors first time donors were 437 and repeated donors were 166. The male/female ratio was estimated as 372:235.
Results: Leading reasons for deferral were low value of Hemoglobin n = 335 (55.18%), irregular blood pressure high (>160 mmHg systolic and >110 mmHg diastolic)-n = 63 (10.37%) and low (<110 mmHg systolic and <70 mmHg diastolic) n = 41 (6.75%) also the presence of infection (without antibiotic therapy n = 51 (8.40%); with antibiotic therapy n = 26 (4.28%). The other registered deferral reasons in our data basis included: operative intervention in last 12 mounts n = 16 (2.63%); short time for donation (<3 months for man) n = 12 (1.97%), or (<4 months for women) n = 5 (0.82%); usage of hormone therapy n = 9 (1.48%), tattoo and piercing n = 11 (1.81%); pervious history of exposure of Hepatitis B Virus n = 6 (0.98%); cardiovascular and chronic respiratory diseases n = 8 (1.31%), younger than 18 n = 10 (1.64%) years or older than 65 n = 5 (0.82%), low body weight (<50 kg) n = 4 (0.65%) and recent vaccination n = 1 (0.16%), or serum receptions n = 3 (0.49%).
Conclusion: Our analysis has shown that the leading causes for blood donor's deferral at our RC have remained the same in comparison with the previous 3 years. Thus, the low Hb levels especially in women in the reproductive period continued to be the core reason for rejection. Additionally, some studies have shown positive association between the shorter interval of donation and the higher Hb deferral rates, which was not analyzed in our Study. On the other hand we have remarked, that the number of deferrals in 2015 (n = 607) was smaller in comparison with the previous years (n = 736 (2013), n = 683 (2014). The new Hepatitis B Vaccination awareness policy in Macedonia as well as the decreased number of piercing and tattoo trends in our high school blood donors could have an effect on the decreasing deferral rates, which requires further observational research in this field.

P-088

DEFERRAL OF BLOOD DONORS ON THE BASIS OF PRE-DONATION HISTORY AND EXAMINATION: 1 YEAR EXPERIENCE AT THE BLOOD BANK OF A TERTIARY CARE HOSPITAL IN A DEVELOPING COUNTRY

H Muhammad and F Karim
Aga Khan University Hospital, Karachi, Pakistan

Background: Donation of blood is to give the gift of life. But this process should be made safe up to the maximum for both the donors and recipient. To ensure this, certain measures are taken for proper donor selection including pre donation history

and examination and screening of blood for transmissible infections. Donors who do not fulfill the criteria are deferred from blood donation temporarily or permanently. Implementation of these deferral criteria influences the quality of blood supply in a population. Thus proper selection and screening of donors is an important part of haemovigilance and plays a major role in safe blood transfusion.

Aims: The aim was to observe the reasons of deferral of blood donors on the basis of their pre-donation history and examination.

Methods: This was a retrospective cross sectional descriptive study. We reviewed 'pre-donation history and examination' charts of the donors who visited blood bank of the Aga Khan University Hospital, for blood donation during January 1st 2015–December 31st 2015. Charts were reviewed for the gender, age, type of donor (voluntary or exchange), reason for deferral and type of deferral (temporary or permanent). Statistical Package for Social Sciences version 21 was used for data entry and analysis.

Results: Total number of donors who visited blood bank during study period for blood donation was 25,495 of which 2012 (7.8%) donors were deferred on the basis of history and examination. Among the deferred donors, male to female ratio was 9.9:1. The median age of male donors was 28 years while that of females was 29 years. Number of donors who donated voluntarily was 83 (4.2%), of which 26 were female and 57 were male. Those who donated in exchange/replacement were 1,929 (95.8%), of which 157 were female and 1,772 were male. Total 30 different indications for donor deferral were identified, of which 25 were on the basis of history and five were based on donor examination. The most common indication based on donor examination was hemoglobin lower than 12.5 g/dl in 880 (43.7%) donors followed by high blood pressures in 131 (6.5%) donors. The most common indication based on donor history was recent or ongoing use of certain medications/antibiotics in 387 (19.2%) donors, followed by history of malaria during past 3 years in 148 (7.3%) donors. Only 22 (1.1%) donors were deferred permanently while 1,990 (98.9%) were deferred temporarily. The reasons for permanent deferral were: Tested positive for Hepatitis B or C in past (n = 7), known diabetics using insulin (n = 5) and donors with some chronic diseases (n = 10).

Conclusion: Percent of donors deferred were 7.8%. Various reasons of deferral were identified. Low Hemoglobin and use of certain medications were the most common reasons identified. Most of the donors (98.9%) were deferred temporarily. Among permanent deferral presence of chronic diseases was the most common cause.

P-089

ANALYSIS OF BLOOD DONOR DEFERRAL PATTERNS IN ISLAMABAD, PAKISTAN

R Hafeez[1], U Waheed[2], IS Kayani[2], A Maqbool[2] and HA Zaheer[2]

[1]Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan [2]Islamabad Blood Transfusion Authority, Ministry of National Health Services, Islamabad, Pakistan

Background: The increasing demand of blood and blood products in developing counties like Pakistan reflect the need for mobilization of more voluntary blood donors. The donor selection process requires continuous monitoring to ensure that it achieves its objectives of ensuring donor and recipient safety and providing a sufficient supply of blood and blood components. The blood donors are deferred from donating blood for numerous reasons, either permanently or temporarily. Knowledge of the reasons for donor deferral can help in planning more effective recruitment and retention campaigns aiming at the availability of safe donors. Blood donor deferral leads to loss of available blood units for transfusion.

Aims: The current study evaluated the blood donor deferral data from Islamabad collected by IBTA.

Material and methods: This was a retrospective study conducted through the data reported to IBTA from the 19 licensed blood banks of Islamabad during the period January–December 2015. Data were entered into SPSS (version 20) and frequencies of each causes of deferral were calculated.

Results: Data of 68,317 blood donors who came to donate at the 19 licensed blood banks or the camps organized by these blood establishments of Islamabad were analyzed. Among these donors, 65,376 (95.7%) were selected for blood donation after physical and behavioral screening. A total of 2,941 (4.3%) blood donors were deferred (temporary n = 2,923/permanent n = 18) from blood donation at this stage. A total of 3,988 blood donors were deferred permanently after post donation serological screening on the basis of Transfusion Transmissible Infection reactivity. The pre-donation defer−ral rate was considerably higher among females (60.5%) compared to (39.5%) males. The total percentage of deferred donors (pre and post donation) was 10.14% with majority of them (57.6%) being permanently deferred.

The causes of pre-donation deferral were Anemia 37.9% (n = 1,116), Jaundice 6.6% (n = 193), Under Weight 22.5% (n = 663), Inappropriate Height Weight Ratio 1.4% (n = 40), Menstruation 2.0% (n = 58), Low Blood Pressure 5.1% (n = 149), Taking Medicines 3.3% (n = 98), H/O Fainting/Vertigo 1.1% (n = 33), Recent Vaccination 0.9% (n = 26), Recent Blood Donation 1.5% (n = 45), Underage/Overage 5.9% (n = 173), Flu/RTI 0.2% (n = 7), Gastroenteritis 0.5% (n = 14), Typhoid% 0.3 (n = 9), Hypertension 0.6% (n = 19), Diabetes 0.3% (n = 8), Arthritis 0.31% (n = 9), Chest Pain 0.03% (n = 1), Difficult Vein/Obesity 1.4% (n = 42), Blood Phobia 0.5% (n = 15), Fever 1.2% (n = 35), Fasting 0.4% (n = 12), Fear of Needle 0.5% (n = 14), Without Breakfast 3.1% (n = 90), Known H/O Hepatitis B and C 0.4% (n = 11), Asthmatics 0.14% (n = 4), High BP 1.8% (n = 54) and Heart Disease 0.1% (n = 3). The causes of post-donation deferral included HCV Positive 69.4% (n = 2,765), HBV Positive 27.1% (n = 1,083), HIV Positive 1.1% (n = 45), Syphilis 1.8% (n = 71) and Malaria 0.6% (n = 24).

Collected units	Whole blood	RBC	PLT	FFP	CRYO
100.729	3787	96942	46424	96457	12540

Table 1. Number of collected and produced blood components (2014–2015)

Type of nonconformities	Number (%)		
Unsuccessful venipunctures	145 (0,14)		
Inadequate amount of blood	1151 (1,14)		
Nonconformities of blood processing	RBC	PLT	FFP
	63 (0,06)	212 (0,2)	83 (0,08)
Nonconformities of blood donor samples	Mislabeling	Fat	Hemolysis
	126 (0,12)	1414 (1,4)	86 (0,08)
Nonconformities of product labeling	56 (0,05)		

Table 2. Type and number of nonconformities as a % of the total of 100.729 collected blood units

Conclusion: Credible scientific data of an important urban centre, Islamabad, generated by a regulatory authority indicates that about 10% of the blood donors who presented for blood donations were unfit for donations temporarily or permanently. This study therefore underlines the need to focus on retention of suitable voluntary as well as replacement donors in addition to further strengthening donor management systems.

P-090

A RETROSPECTIVE ANALYSIS OF TEMPORARY DEFERRALS OF BLOOD DONORS IN THE REGIONAL BLOOD DONOR CENTRE IN POZNAN IN THE YEARS 2010–2015.

R Klupiec, K Olbromski and H Skalisz

Blood Center in Poznan, Poznan, Poland

Background: In the last few years there has been an increase in the number of transfused blood components which makes it necessary to increase continuously the number of blood donations. In Poland, the only authorized entities for the collection, processing, storage and distribution of blood and its components for treatment are Blood Donor Centres. The Regional Blood Center in Poznan is one of the largest institutions in Poland that supplies blood components to 36 Hospitals in Poznan and the surrounding area.

Aims: The analysis of temporary deferrals of blood donors in the Regional Blood Centre in Poznan in the years 2010–2015, including the most common reasons for deferrals in order to develop corresponding strategy preventing this occurrence.

Methods: The analysis of reports of temporary deferrals of donors in the years 2010–2015 carried out by qualified doctors from the Regional Blood Donor Centre in Poznan.

Results:

1. Total number of temporary deferrals in the years 2010–2015 (Table 1).
2. The most frequent reasons for temporary deferrals in the years 2010–2015 (Table 2).

Summary/Conclusions: In the Regional Blood Center in Poznan in the years 2010–2015 98,375 donors were temporarily deferred out who 34,319 were first-time donors, 64,056 repeat donors.

1	low Hb	30 285
2	other morphological abnormalities (leukocytes, platelets)	12 835
3	abnormal blood pressure	6 211
4	taking medicines	4 046
5	inflammatory and allergic skin diseases	3 886
6	major surgeries	3 573
7	flu, flu-like infections or acute respiratory diseases	2 957
8	no proper access to peripheral veins	2 923
9	tattoos or piercing of body parts in 6 months prior to donations	2 561
10	exposure to infection of transfusion transmitted diseases (due to conduct or activities of the so-called "risky behavior")	1 933
11	Not sufficient body weight or excessive disproportion between body weight and height	1 541
12	acute allergic, exacerbation of chronic allergic diseases, allergy desensitization	1 500
13	menstruation	1 081
14	lipemic serum	1 008
15	Minor surgery, including dental surgery and dental treatment	972
16	endoscopy	934
17	abnormal heartbeat	704
18	infection / exposure to WNV	588
19	exposure to the risk of infection due to close contact with patients with viral hepatitis in their home	571

Table 1

	Temporary deferral Repeat donor	Temporary deferral First time donors
2010	7495	6759
2011	9482	6029
2012	8695	8337
2013	10056	6134
2014	7290	5553
2015	21038	1507

Table 2

The vast majority of deferrals among multiple donors indicates the need to implement measures of prevention especially for this particular group of donors.
First of all, increased the range and methods of donors education should be increased:
1. Each donor should be informed about the most common reasons for temporary deferral and ways to prevent them, in particular about:
   the impact of blood donation on the level of Hb, the benefits of a diet rich in iron or preventive intake of iron supplementation,
   drugs the intake of which is a reason for the deferral and the time necessary to discontinue using them before the planned donation,
   the regular control of blood pressure,
   the type of diet before donation,
   the possibility of controlling the date of donation in connection with the planned trip to the areas of occurrence of certain diseases (eg. WNV), planned surgery, dental treatment or performing a tattoo.
2. Information should be communicated in different ways: verbal, brochures, educational films, information on the website.

P-091

FIRST TIME BLOOD DONORS: ARE THEY MOTIVATED? AN ANALYTICAL STUDY FROM A DEVELOPING COUNTRY

A Sadagopan

Sri Ramachandra University & Hospital, Chennai, India

Background: Blood donors are the backbone of transfusion services. Despite being a country with a population of 1.2 billion, India faces a blood shortage of 3 million units. If this problem can be addressed and an additional of two percent of Indians donates blood, it can solve the crisis. It is noted that only 9 million units are collected annually, while the need is for 12 million units. India being a highly populated country, there is always a great demand for blood and blood products but the supply is limited. In order to bridge the gap between demand and supply, we need to recruit donors and effectively retain them as regular repeat donors.
Aim: To analyze the myths and facts regarding blood donation among first time donors in South India.
Method: This study was conducted in the Department of Transfusion Medicine of a tertiary care hospital in South India during the period October 2015 to March 2016.
On an average, 40–50 donors donated per day. Among them, first time donors were very few when compared to repeat donors. A separate questionnaire was formulated in English and Regional language, and administered to the first time donors after donating blood. The data was collected and analyzed on the following lines:
Why have they not donated so far?
What made them to donate now?
Are they willing to become repeat donors?
Results: This study was carried out on 512 first time blood donors.
The study reveals that, the reasons for not having donated blood so far were:
246 (48%) of the donors did not get the opportunity,
60 (12%) of the donors were not aware of blood donation
26 (5%) of the donors did not have accessibility
109 (21%) of donors were not approached
23 (4%) of the donors were afraid to donate blood
48 (9%) of the donors did not have time to donate
The reasons which made them to donate now were
84 (22%) donors-obligatory need for family members.
38 (10%) donors were motivated by blood bank staffs
170 (45%) donors were approached by friends
87 (23%) donors turned up due to a sense of altruism
After their first blood donation,
486 (95%) donors were willing to become repeat blood donors
26 (5%) donors were not willing to donate blood due to personal reasons.
Conclusion: Donors should be ensured of a pleasant experience during blood donation-the first time and every time. First time donors will turn up for repeat donation only if this is ensured. Donor motivation is the key to a successful blood donation drive.

P-092

HOW FREQUENT DOES LOW PRE-DONATION HAEMOGLOBIN OCCUR AMONG FIRST TIME DONORS IN CHINESE?

CK Lee, CCY Chu, VWY Ho, CSY Li and JNS Leung

Hong Kong Red Cross Blood Transfusion Service, Hong Kong, Hong Kong

Introduction: Blood donor safety is important in recruitment and retention. As such all prospective donors have to go through pre-donation health screening and haemoglobin check. However, it is well known that failed haemoglobin check is commonly seen in many countries, which is mostly related to some degree of iron deficiency due to dietary factors, menstrual loss in female, haemoglobinopathy in South East Asia and after frequent blood donations. As they usually exhibit no anaemia related symptoms, donors are often surprised by the deferral and if not properly managed, lead to drop out.
In this study, the pre-donation haemoglobin results in Chinese first time blood donors are reviewed and the frequency of failed haemoglobin check are determined
Materials and methods: All first time blood donation records between 2014 and 2015 were retrieved and reviewed. Those deferred from reasons other than failed

Table 1: Proportion of Chinese first time donors who failed pre-donation haemoglobin check

	Male (cut off 13.0 gm/dl)		Female (cut off 11.5 gm/dl)		Total	
	Successful	Failed	Successful	Failed	Successful	Failed
16 - 20	19,978	1536 (7.14%)	23,856	3,270 (12.05%)	43,834	4,806 (9.88%)
21 - 30	8,876	759 (7.88%)	9,227	1,549 (14.37%)	18,103	2,308 (11.31%)
31 - 40	4,059	378 (8.52%)	5,638	1,121 (16.59%)	9,697	1499 (13.39%)
41 - 50	2,037	257 (11.20%)	3699	922 (19.95%)	5,736	1,179 (17.05%)
51 - 60	1,078	213 (16.50%)	1,776	314 (15.02%)	2,854	527 (15.59%)
61 -70	39	18 (31.58%)	28	15 (34.88%)	67	33 (33.00%)
Total	36,067	3161 (8.06%)	44,224	7,191 (13.99%)	80,291	10,352 (11.42%)

Picture 1

haemoglobin check were excluded. In Hong Kong, the requirements for blood donation are aged between 16 to 70, with a minimum body weight of 41 kg and pre-donation Hb of 11.5 and 13 gm/dl for female and male respectively. Donors are allowed to donate either 350 ml or 450 ml based on body weight cut off of 50 kg.
Result: Among 90,643 first time blood donors, there were 39,228 male and 51,415 female. Majority of them were aged below 30 with aged 16–20 (53.66%) and 21–30 (22.52%). 36,067 male and 44,224 female made successful donation amounting 80,291 (88.58%) or 11.42% deferral due to failed pre-donation haemoglobin check. A consistently higher proportion of female first time donors among all the age groups were having low pre-donation haemoglobin (<11.5 g/dl) than male (13.99% vs 8.06%).
Discussion: 11.42% Chinese first time donors were deferred from blood donation due to failed pre-donation haemoglobin check. An increasing incidence with age was observed and there was female predominance. Although the underlying cause of low haemoglobin is not known in this observation study, the findings raise an important public health message and prompt a more detailed investigation.

P-093

VOLUNTARY BLOOD DONATION AMONG COLLEGE YOUTH: AN INDIAN PERSPECTIVE 'HIGH TIME TO MAKE A MOVE IN INDIAN COMMUNITY'

N Mohanan and J Innah
Jubilee Mission Medical College, Thrissur, India

Background: Being a nation of over 1 billion population, India is unable to tap blood requirements for 8 million. Quality and safety of blood transfusion is still a concern. Blood will be safer if collected from voluntary non-remunerated donors and used rationally. Well motivated college students can be great source of quickly accessible quality blood.
Aim: To assess knowledge, attitude& practice of voluntary blood donation among college youth of Kerala, India.
To compare results among medical and non-medical students.
Materials and methods: Pilot study conducted among randomly selected college students of Kerala using a pretested questionnaire followed by an awareness session. SPSS software was used for analysis, P value of <0.001 was considered significant.
Results: The study had 1,091 participants (337 males, 754 females; 265 medicos, 826 non-medicos). Age ranged from 18–25 years. 93.6% knew their blood groups but 82% never donated blood. Among donors, 61% donated once, 77% donated voluntarily. Most common reason for not donating across all groups revealed 'not being asked' (25%) followed by 'fear of pain', significantly higher among females and non-medicos. Attrition rates among donors due to deferrals were significantly higher among females (22%) and medicos (22.3%).91% were interested in future donations, 'fear of pain' being the major deterrent. 50% knew blood can't be artificially manufactured, significantly high among medicos. 18% (23% males; 13% females) believed that blood is into commercialization. Significantly higher number of non-medicos believed work of Indian blood banks is excellent as opposed to medicos. Only 54% ever attended motivation classes for blood donation against 80% for organ donation.
Only 37.9% (59.5% medicos; 31% non-medicos) correctly knew amount of blood withdrawn. Only 18.4% (17.4% medicos) knew upto four lives could be saved by donating once. 21.4% believed that only one life can be saved. Significantly higher number of non-medicos believed the cost of blood products is for 'profit-making', whereas 33% medicos believed it was for blood tests. 31% medicos correctly knew the shelf life of blood components ($P < 0.001$).98% knew HIV is spread through blood, 6% wrongly believed nutritional deficiencies can spread through blood.
Conclusion: The study reflects that youth is definitely interested to donate further, but there is a lacunae tapping available blood resources. Some misconceptions are the setback. Our target must be to clear these, especially the easily resolvable like 'fear of pain' which has been highlighted in other KAP studies too. 'Deferral' of blood donation is a threat to sustain enthusiastic voluntary donors. Change of attitude from our part like providing evaluation and treatment for anemia and pain alleviation can boost donor recruitment and retention. Knowledge of medicos were at a comparatively higher level however when it comes to attitude and practice,both groups converge to a similar pattern. Blood donation needs to be organized and sustained as blood bank machinery in India is of the, for the and by the people!

P-094

TRANSFUSION TRANSMITTED INFECTIONS REACTIVITY RATE IN REPEAT AND FIRST TIME BLOOD DONORS: A 5 YEAR TREND ANALYSIS

S Patil, V Kale, N Tated, A Jain and R Kasodkar
Arpan Blood Bank, Nashik, India

Background: Introduction of tests with early window period detection capability such as NAT testing facility has increased blood safety to a large extent. However, there are still NAT yield or breakthrough infections of TTIs. Voluntary non-remunerated repeat blood donors are perceived to be safer than replacement and first time blood donors.
Aim: To analyse the trend of TTI Reactivity rate in Repeat & First time Blood Donors at our centre.
Methods: The screening results of 1,20,067 blood donations between January 2010 and December 2014 were analyzed for prevalence of hepatitis B virus (HBV), hepatitis C virus (HCV) & human immunodeficiency virus (HIV) infections. The trend of TTI positivity among repeat and first time donors was studied.
Results: Increase in our Repeat Donation rate has been 36.88% in these 5 years. [53.33% (13,363/25,057) in 2010 to 73% (15,273/20,921) in 2014]. Overall TTI reactivity was 1.24% (1,498/1,20,067) amongst which TTI reactivity in First time donors was 2.78% (1,242/44,621) and in repeat donors was 0.33% (256/75,442). First time donor TTI reactivity rate was nine times greater than Repeat donor TTI reactivity rate. Over the period of 5 years overall TTI reactivity of our donors decreased by 11.6%. (1.37% in 2010 to 1.21% in 2014). On further analysis of TTI reactivity trend in the repeat donor subset, an increasing trend of TTI reactivity was seen over the period of 5 years. [0.1% (16/13,363) in 2010 to 0.48% (74/15,273) in 2014].
Conclusion: Repeat donation has increased over the period of 5 years. A decreasing trend of overall TTI reactivity was seen over the period. Overall sero-reactivity rate in repeat donors was lesser than that in first time donors. However repeat voluntary blood donors showed marginally increasing trend of TTI reactivity which is a matter of concern. Therefore it would be advisable to direct more focus & attention on implementation of proper donor counselling and a proper system to prevent the TTI reactive repeat donors from donating again and again.

P-095

REPEAT DONORS, BUT NOT FIRST TIME DONORS, MORE OFTEN CONSENT TO REGISTER WITH ARDP (AMERICAN RARE DONOR PROGRAM) AND INCREASE THEIR DONATION FREQUENCY WHEN INFORMED THEY HAVE A RARE BLOOD TYPE

W Anani[1], M Schanen[2], CF Piefer[2], K Bensing[2], J Gottschall[2] and G Denomme[2]
[1]*Blood Center of Wisconsin, Milwaukee, Wisconsin, United States of America*
[2]*Diagnostic Laboratories and Blood Research Institute, Blood Center of Wisconsin, Milwaukee, Wisconsin, United States of America*

Background: The continual screening and identification of rare blood types among donors is critical to support national programs like the American Rare Donor Program (ARDP). Part of the Blood Center's process is to obtain consent from donors to be registered with the national registry. We sought to determine if a donor's willingness to register is associated with a change in donation behavior.
Aim: To identify the effect of donation frequency after requesting donors to register as a rare donor for the ARDP.
Methods: Rare donors identified by molecular typing (Lancet Haematol 2015;2: e282–9), were sent a letter requesting their permission to be registered with the ARDP. Each donor received at least one phone call to set up an appointment to donate after the letter was mailed. Information regarding the donor's age, gender, ethnicity, frequency of donations, response to the ARDP letter, and rare antigen(s) identified were collected from January 2010 to March 2015. The average number of donations per year was compared for each donor prior to receiving the ARDP letter and after the letter was mailed. Donors asked to enroll in the ARDP were categorized as either accepting or declining the offer. Donors not returning the letter were considered the same as donors declining the request. Paired t-tests comparing the statistical significance within each category were calculated. Inter-group comparisons and the mean baseline donation frequencies from each category were compared with a Mann-Whitney test.
Results: A total of 270 molecularly-typed rare donors received letters over 5 years (Tables 1 and 2). A total of 132 (49%) donors agreed to participate in the ARDP with an average of 2.0 (range 0.40–4.5) donations per year before and 2.3 (range 0.5–5.7)

Table 1. Rare Donor Demographics			
		ARDP Donors	Donors Declining ARDP Participation
Mean Age (range)		41 (18-92)	41 (17-92)
Gender			
	Male	56	70
	Female	76	68
Ethnicity			
	African American	39	103
	Asian	2	2
	Caucasian	83	17
	Hispanic	6	8
	Other	2	7
Blood Group			
	O	64	66
	A	49	45
	B	16	23
	AB	3	4
Total		132	138

Table 1. Rare Donor Demographics

Table 2. Rare Antigen Summary	
Antigen	Number
hrB-	59
Yt(a-)	35
k-	26
R1 Fy(a-b-)	23
Js(b-)	22
Lu(b-)	21
Co(a-)	18
U-	15
R2 Fy(a-b-)	13
Lu8	9
U Var	7
Jo(a-)	6
Hy-	4
r'r'	3
Cr(a-)	2
Kp(b-)	2
hrB-/hrS-	2
Jo(a-)/hrB-	1
Di(b-)	1
U Var/Lu(b-)	1

Table 2. Rare Antigen Summary

donations per year after the letter was sent. The average number of donations per year before and after responding were statistically significant ($P < 0.0001$). Only eight (6.0%) were first-time donors. A total of 138 (51%) donors declined to participate with an average of 1.1 (range 0.6–4) donations per year before and 1.2 (range 0.60–3.3) donations per year after the letter was sent. The number of donations were not statistically significant before and after responding ($P = 0.52$). Just over half of the donors (70, 51%) were first-time donors. The baseline pre-donation donation frequencies the between the two groups and intergroup donations were statistically significant ($P < 0.0001$).

Conclusions: A donor's willingness to be enrolled in the ARDP was associated with a post-response increase in donation frequency. Individuals declining or not responding to the ARDP letter did not change the frequency of their donations. Donors who have donated more than once before receiving the ARDP request to register were more likely to consent. Interventions to increase first-time donors to donate again may be a prerequisite to increase donation frequency or the willingness to be a rare blood donor.

P-096

THE IMPACT OF CHANGES IN POPULATION ON THE NUMBER OF REGISTERED DONATIONS IN 2012–2015 IN REGIONAL BLOOD CENTER IN POZNAN, POLAND

R Lacny and K Olbromski

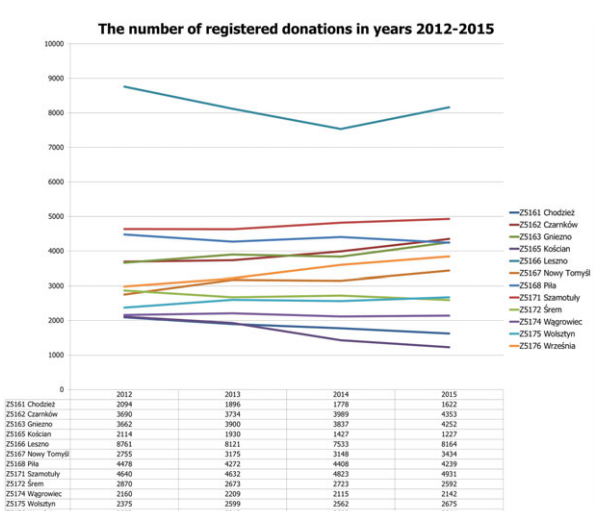
Blood Center, Poznan, Poland

Background: Fluctuations in the number of donors registering to donate blood are a major problem for all people working in blood donor centers. What does the number of donors depend on? Is it just the result of marketing and educational actions? Can it also depend on the growth/decline of the population?

Aims: The aim of this work was to analyse how the decrease/increase of the population in the counties and cities where Blood Collection Centers of Regional Blood Center in Poznan are located affects the number of registered donors. Donors do not necessarily live in a particular city or county, but their donations are registered in these branches. The analysis also included the issue in which areas there has been an increase in registered donations and what steps should be taken to encourage donors in the areas with observed decline in registered donations despite an increase in population. The questions is if increase/decrease of the population really has an impact on the number of donations.

Methods: The data was obtained from the computer system 'Blood Bank' by Asseco and the from the Central Statistical Office of Poland (CSO). The source data regarding donations was obtained using the Analysis system by the company BMM SP. z o.o. which is used for creating reports in Regional Blood Center in Poznan. The number of the population in the counties and cities as well as other statistics is provided by the CSO on the websites http://stat.gov.pl/ and http://www.polskawliczbach.pl. The data was compared in spreadsheets in the form of tables and graphs.

Results: The growth of population is not proportional to the increase in number of donations e.g in Koscian population growth was observed with accompanying decrease in the number of registered donations (in %) – an upward trend was observed in 2015 in the district: 1.55%, in the city: 5.13%. In Czarnkow the number of inhabitants in the city falls whereas the number of registered donations increases. In another location of the satellite branch Wrzesnia according to the CSO there was growth of population in the city and county with accompanying increase in the number of donations.



Graph 1

Summary/Conclusions: Having analyzed the data regarding the fluctuations in the population and in the number of registered donations in satellite branches we can draw the conclusions that population growth does correlate to the increase in the number of registered donations. Similarly, the decrease in the population does not correlate to the decrease in the number of donations. According to my opinion, the increase/decrease in the population does not have a significant impact on the number of donations. That is why it is essential to keep educational activities already on the level of high school to raise the awareness of the importance of donating blood.

P-097

SOCIO-DEMOGRAPHIC CHARACTERISTICS AND ATTITUDINAL FACTORS IN FIRST-TIME VOLUNTARY AND FAMILY REPLACEMENT BLOOD DONORS IN SOUTHERN GHANA

L Asamoah-Akuoko[1], OW Hassall[2], I Bates[2], PB Adongo[3], IC Bygbjerg[4] and H Ullum[5]

[1]National Blood Service, Accra, Ghana [2]Liverpool School of Tropical Medicine, Liverpool, United Kingdom [3]University of Ghana, Accra, Ghana [4]University of Copenhagen, Copenhagen, Denmark [5]Copenhagen University Hospital, Copenhagen, Denmark

Background: Repeating Voluntary Blood Donors are the key to an adequate, sustainable blood supply. In Southern Ghana, only 15.2% of first time donors return to donate. Understanding the socio-demographic and attitudinal factors that influence blood donation behaviour provides a basis for developing targeted intervention strategies for increasing the proportion of repeat blood donors.

Aim: To identify socio-demographic characteristics and attitudinal factors that influence blood donation in first time voluntary and replacement donors in Southern Ghana.

Methods: This is the second phase of a two-phased mixed method study. A total of 250 first-time Family Replacement Donors and 255 first-time Voluntary Blood Donors in Southern Ghana were surveyed using a structured questionnaire in Census and Survey Processing System (CSPro) software. Attitudinal factors in both groups were measured with five point Likert-type questions, compared, and differences tested for statistical significance using the Mann-Whitney two-sample rank-sum test. Data was analyzed with SPSS version 22. A coding scheme based on the taxonomy of blood donor motivators by Bednal and Bove, but modified to capture the perceptions of blood and blood donation found in Ghanaian blood donors was used for reporting the results.

Results: There were statistically significant associations between type of blood donor and age, gender, marital status, having children, means of transport, employment category, level of income, and religion. Although the factors that had mostly influenced participants' perceptions of blood and blood donation were education (59%), religion (25.5%) and cultural environment (11.3%), there was no significant association between education and type of blood donation. Of the Family Replacement Donors that were surveyed, 72.5% perceived themselves as Voluntary Blood Donors. Tables 1 and 2 show scores for attitudinal factors, with a higher score positive for the factor and a lower score negative. For the Voluntary Blood Donor group, the scores for perceptions that encourage blood donation behaviour (Table 1); altruistic motivators; collectivism and downstream, indirect reciprocity (Table 2) were statistically significantly higher than the Family Replacement Donor group. Conversely, for the Family Replacement Donor group, the scores for perceptions that discourage blood donation (Table 1); cash and non-cash incentives (Table 2) were higher than the Voluntary Blood Donor group. There were no significant differences between the scores in the two groups for the perceptions that 'Blood is life', 'Blood is Sacred' and 'Blood is used for rituals and sacrifice to deities' (Table 1). However, 97.2%, 84.9%, and 70.3% of respondents either agreed or completely agreed with these respectively.

Motivators	Mean Rank		Difference between Scores	P-Value
	Replacement Donors	Voluntary Donors		
Ease of access to donation site	237	270	33	0.006
Save lives	234	272	38	0.001
Help community	237	269	33	0.005
Available when needed for family / friends	240	266	25	0.028
Available when needed for self	239	267	28	0.016
Help Blood Bank	236	270	34	0.004
Help Ghana	233	273	40	0.001
Blood drive at my school or workplace	236	270	35	0.004
Cash gifts	267	238	-29	0.022
Incentives such as milk, milo, T-shirts	273	233	-40	0.002
Friends/family are blood donors	270	236	-33	0.008
If asked by friends, family or co-workers	268	238	-29	0.017
Deterrents				
Not enough time donate blood	277	229	-48	<0.001
Blood collection times are not convenient	273	232	-41	0.001
Long queues	270	236	-35	0.006
Not called or asked to give	279	226	-53	<0.001
Motivational items are not good enough	265	241	-24	0.043
Not aware of need for blood	277	228	-49	<0.001
Not treated well by Blood Bank staff	265	240	-25	0.048

Table 1. Motivators and Deterrents to Blood Donation

Perceptions of Blood	Mean Rank		Difference between Scores	P-Value
	Replacement Donors	Voluntary Donors		
Blood is life	254	251	-3	0.778
Blood is Sacred	261	245	-17	0.157
Spiritual significance	266	240	-26	0.033
Can transfer behaviour to recipient	269	237	-32	0.009
Used for rituals and sacrifice to deities	260	246	-14	0.250
Used medically to save lives	240	266	26	0.012
Used for covenants with other persons	266	240	-27	0.031
Used to link with the supernatural	285	220	-65	<0.001
Can be used to cause spiritual harm	281	225	-56	<0.001
Means pain or physical injury	279	226	-53	<0.001
Unique should not be given it out	267	239	-28	0.019
Unique for each tribe/kin	267	239	-27	0.028
Perceptions of Blood Donation				
Harmful to the donor's health	282	223	-59	<0.001
Important for saving lives	242	265	23	0.033
A waste of time	289	216	-73	<0.001
Reduces the donor's physical strength	273	233	-41	0.001
Makes a women unable to menstruate	285	220	-65	<0.001
Can cause impotence	272	233	-39	0.001
Give away part of donor's life	277	229	-48	<0.001
Creates a bond/covenant with recipient	278	228	-51	<0.001
Can cause the donor to die	279	226	-53	<0.001
Reminds me of pain	280	226	-54	<0.001
Harmful to the donor's health	282	223	-59	<0.001

Table 2. Perceptions of Blood and Blood Donation

Conclusion: The findings of this phase of the study support the findings of the first qualitative phase. They confirm that a high proportion of donors perceive that blood is related to spiritual power; donated blood can be used for rituals; and that both altruism and incentives were potential strong motivators for donation. Family Replacement Donors seeing themselves as volunteer donors supports the idea that these donors may also be willing to donate again for others. Findings from this study will be used to develop culturally-sensitive targeted interventions to increase repeat blood donation.

P-098

ATTITUDE TO BLOOD DONATION AMONG A TERTIARY HOSPITAL WORKERS IN NIGERIA

OM Adegoke

Olabisi Onabanjo University Teaching Hospital, Sagamu, Nigeria

Background: Adequacy of donor blood to meet demand is no more a problem in the developed countries of the World, which have established organized means of collection and distribution. The story is however different in most of sub-Saharan Africa where illiteracy, poverty and negative cultural beliefs remain obstacles to donation.

The inadequate donor blood supply in response to recent terrorist attacks in Nigeria has further highlighted the problem. Members of staff of hospitals who are close to blood transfusion centres and are expected to be better informed has been targeted in this study to assess willingness to donate blood as well as factors affecting their willingness or otherwise.

Aims: To assess willingness of health workers to blood donation as well as factors affecting their willingness or otherwise.

Method: Questionnaires were administered to consenting hospital staff from various departments of a tertiary health care centre with a blood transfusion unit, Respondents included staff of departments (Account and Finance, Administration, Catering, Health Information, Laundry, Medical, Nursing, Laboratory). Descriptive statistics (SPSS Version 17) were used to analyse data collected.

Result: Responses were received from two hundred and forty six (246) health workers, 76 male and 170 females, aged 20–60 years.

In Table 1, Columns A and B showed that the percentage of health workers who were willing to donate blood is uniformly higher than those who have been

Factors	Proportion
Culture	1/246
Religion	9/246
fear	32/246

Table 1

Staff Dept	n	Recruited	willingness	Desire To help	Known person	Voluntary donation	Past donation
		A (%)	B (%)	C (%)	D (%)	E (%)	F (%)
Accounts	18	60	72	94	50	17	67
Admin	28	32	64	86	25	36	25
Catering	8	38	25	75	38	13	75
Health Information	14	43	64	100	28	07	36
Laboratory	21	71	95	100	28	71	62
Laundry/Tailoring	5	60	20	20	0	0	20
Medical	31	74	81	93	31	58	58
Nursing	101	28	81	88	22	33	21
Security	11	73	64	91	18	36	73
Social Welfare	7	57	86	100	57	14	57
Total	246	45	73	88	28	35	37

Table 2

approached for donation, except for those in laundry and tailoring sections. Only a small proportion of Nurses (28%) who constitute the largest population of health workers has been approached for blood donation. Except for catering and Health Information staff, there is an identity between willingness to donate blood and the desire to help someone in need (columns B & C). The willingness therefore does not translate to actual donation. This is why the percentage that have donated closely approximates the percentage that have been invited to donate (Columns A & F), and not those who express willingness. Columns D and E showed that more people will rather donate for those they know than do it voluntarily. This is consistent with experiences in reports involving organ donation.

More people avoid blood donation out of fear than out of cultural or religious considerations (Table 2).

Summary/Conclusions: Blood donation is still based largely on primordial Motivation of helping those we know, and Voluntary donation is relatively unpopular. All these are evidence of ineffectiveness of current local and national blood transfusion services. There is a need to improve awareness and advocacy with respect to blood donation among health workers and the general population.

P-099

STUDY OF KNOWLEDGE AND ATTITUDE AMONG COLLEGE GOING STUDENTS TOWARDS VOLUNTARY BLOOD DONATION FROM NORTH INDIA

N Marwaha, SK Mishra, S Sachdev and A Avasthi

Postgraduate Institute of Medical Education and Research, Chandigarh, India

Background: Knowledge and attitude survey is a representative study of a specific population to collect information on what is known, believed and done in relation to a particular topic. Knowledge and Attitude study with the subject of blood donation could reveal myths, barriers and beliefs about blood donation and could vary across the socioeconomic and demographic zones. Conducting such a survey in our region would provide insight into the attitude and knowledge about blood donation and this could further be utilized in formulation of strategies to augment voluntary blood donation.

Aims: The objectives of the study were to assess college going students' knowledge and attitudes regarding voluntary, non-remunerated blood donation and to find out the reasons both for donating and not donating blood.

Methods: A self-administered pre-validated structured questionnaire was given to 1,000 college going students, 500 blood donors and 500 non-blood donors. The first section of the questionnaire incorporated twenty five questions that assessed the knowledge level. The second section assessed the attitude. The third section assessed the reasons for not donating blood. The fourth section assessed the reasons for blood donation. The study was approved by the Institutional Ethics Committee and informed consent of the participants was taken.

Results: Assessment of the level of knowledge among blood donors revealed excellent scores in 36 (7.2%), good in 222 (44.4%), average in 152 (30.4%), and poor in only 90 (18%). Among non-donor students 299 (59.8%) had poor level of knowledge, 108 (21.6%) had average knowledge and only 93 (18.6%) had good knowledge. Average knowledge score of all the twenty five knowledge questionnaire provided by blood donor students were 293 (58.6%) whereas by non blood donor students were 235 (47.0%) and this difference was statistically significant with P value of <0.001. Amongst donors there were students with previous donations and it was seen that participants with higher numbers of previous donation scored significantly more, P value <0.001. Assessment of attitude among blood donor students revealed that 92% of blood donor students had favourable attitude and only 8% had unfavourable attitude whereas among non-blood donors 81% had favourable and 19% had an unfavourable attitude towards blood donation. Most common reasons revealed by the donor students for donating blood was moral responsibility (74.2%) followed by altruism (12.2%). Most common reasons for not donating blood as revealed by non-donor students were fear of needle pain (27.4%) followed by the thought that they were not fit to donate blood (26.8%).

Conclusion: Blood donor students had more knowledge regarding blood donation. Fear and misconceptions were more prevalent among non-blood donor students. The latter group needs more attention in motivational campaigns. The awareness and education strategies could even begin in high school so as to help mitigate fears and myths before becoming eligible for blood donation.

P-100

ANALYSIS OF SOME CHARACTERISTICS AND ATTITUDES TOWARDS BLOOD DONATION AND DONOR RETENTION STRATEGIES AMONG VOLUNTARY NON-REMUNERATED BLOOD DONORS DONATED BLOOD TO THE NATIONAL BLOOD CENTER, SRI LANKA

IP Rupasena

National Blood Centre, Colombo 08, Sri Lanka

Background: The blood donation programs face challenges in recruiting and retention of blood donors and maintaining their blood supplies without any shortages throughout the year. These challenges make the exploration of factors affecting blood donation in order to identify effective strategies for increasing the donor base and fulfilling future blood needs.

Aims: The objective of the study is to analyze some characteristics which are difference of attitudes according to the gender of donors, according to the age of donors, according to the educational level and difference of attitudes of donors regarding the blood donation incentives as a donor retention strategy.

Method: A prospective community based study was carried out. Total numbers of 3,629 donors were participated in this study. All donors who donated blood two times or more (n = 3,981) in to the National blood centre within 1st January 2015 to 31st March 2015 were eligible for this study. Out of 3,981 regular donors, 151 were refused to participate in this study while 201 were not responded.

Results: Among the total study population 71.1% (n = 2,579) were males and 28.9% were females. Nearly one fourth (25.7%) of the donors were motivated by their friends while about 14% were motivated by the community group and printed media each. Majority of donors donated blood for self satisfaction (64.1%) and nearly quarter (24.4%) of the donors thought the blood donation is a responsibility. Majority of donors thought the effective ways for advertising was displaying banners and posters in the vicinity of blood donation area. Telephone calls or SMS was the second most preferred method for advertising (14.8%).

A higher percentage of female and male donors are having opinion that incentives are not helpful in motivating donors and there is no need for it. (73% vs 74%) When considering the blood donation incentives, majority of donors thought about non monitory incentives (95%). Majority of donors preferred free blood tests (58.4%) and Certificate or Medals were selected as second common non monitory incentives (31%).

Conclusions: The number of female donors was a long way behind the males. First time motivation factor of blood donors was more due to influence by friends and majority of donors started their blood donation as a young donor. Vast majority of blood donors donated blood for self satisfaction and as a responsibility. Advertising the mobile drive by displaying the posters and banners is the most popular way. Vast majority of blood donors thought that the blood donation incentives were not help to motivate donors. Almost all the donors preferred non monitory incentives, if organizers wished to give them. Among non monitory incentives, majority of donors preferred free blood tests.

P-101

BLOOD DONORS AWARENESS AND ATTITUDES TOWARDS BLOOD DONATION RISK

S Bogdanovic, N Bujandric and R Jovanovic

Blood Transfusion Institute of Vojvodina, Novi Sad, Serbia

Background: Blood safety depends on the recruitment and retention of blood donors who are at low risk of transfusion-transmissible infection (TTI). Blood donor selection is the first step in the process of ensuring blood safety as it helps to identify individuals with low risks. The health history screening and self-deferral process are blood bank unique instruments to curtailing donations from individuals with very recently acquired infections that cannot be detected by laboratory assays. Increase level of positive awareness and attitude towards blood donation risk is the highest priority of all blood transfusion centres.
Aims: This paper is aimed to present the awareness and attitude regarding blood donation risk amongst blood donors of the South Backa district of the Autonomous *Province of Vojvodina*.
Methods: A prospective multicenter cross-sectional study was conducted among 526 blood donors during the period from October 2012 to April 2013. Blood donors filled out a specially designed anonymous questionnaire. The questionnaire contained questions about demographic characteristics and five statements about awareness and attitudes related to TTI and risk factors. The 5-point scale of agreement/disagreement (Likert scale) was used to indicate how much they agreed with the statement. Variables explored during the analysis of the data were sex, age, the number of blood donations, education, marital status, place of blood donation, place of residence and agreement/disagreement with the statements related to TTI and risk factors.
Results: The study sample consisted of 392 males (74.5%) and 134 females (25.5%). Out of them: 73.4% were 21 to 50 years old; 91.8% were repeat donors; 70.3% had high school educated; 48.9% were married; 55.9% donated blood at the institution; 86.5% had the permanent place of residence. Average ratings for correct agreement/disagreement with statements were 89.2% (80.1–96.2%). 96.2% of blood donors strongly agreed and agreed with the statement 'I think my blood is safe for the recipient'. 80.1% blood donors strongly agreed and agreed with the statement 'Blood donor needs to notify the transfusion service if he develops any illness after donating until 6 months'.
Summary/Conclusions: The findings showed positive awareness and attitudes related to TTI and risk factors amongst blood donors in the South Backa district of Vojvodina. However, there are a small but significant number of blood donors without awareness about their own impact on the blood safety. Information, education and communication activities about the impact of blood donors on blood safety can be helpful in reducing blood transfusion risk as well as encouraging regularly blood donors to continue their active participation in the selection process.

P-102

MOTIVATION, RETENTION AND RECRUITMENT OF EGYPTIAN BLOOD DONORS

HA Nasr

Egyptian National Blood Transfusion Service, Cairo, Egypt

Background: With an ever-increasing demand on blood supplies worldwide, there is an immense need to ensure a safe and sufficient supply of blood products. However, recruiting and retaining blood donors remain key challenges for blood agencies.
Aim: In Egypt where the blood donor system depends on a combination of voluntary and replacement donors, this study was conducted in an attempt to determine what factors influence a person's choice to give blood. The aim of the study is to explore the attitudes, beliefs and motivations of both blood donors groups toward blood donation and the strategies for recruiting both blood donors groups.
Methods: A cross sectional study was carried out among 500 blood donors in which 50% were voluntary non-remunerated donors and 50% were replacement donors. Each donor completed a questionnaire in Arabic language which investigated sociodemographic characteristics, attitude and motives for blood donation as well as factors which enhance retention and recruitment of blood donors. Data was processed using SPSS statistical analysis package.
Results: There is male predominance among both donor groups. Replacement donors included older and less educated individuals than voluntary non-remunerated donors. The main motives of voluntary donors for donation were religious motive (52%), followed by altruism and empathy (44%).The main motive for replacement blood donors was intimate person need (88%) whereas religious motive comprised (8%). The main reasons for donor retention among voluntary donors were feeling of self-esteem and being useful to the community 48.9%, religious motive 14.6%, need of the country 15.6% and satisfaction by the service 19.9%. The main reasons for donor retention among replacement donors were feeling of self-esteem 49%, intimate person donation 36.3%, religious motive 7.6% and health improvement 5.2%. On analysis of donor recruitment, awareness about blood donation was the most suggested by both groups showing 55.6% among voluntary donors and 57.2% among replacement donors. It was followed by emphasizing the religious impact (20.4% among voluntary donors vs 14% among replacement donors), ensuring the safety of blood and instruments (13.6% in voluntary donors vs 2.8% among replacement donors %) and proximity of blood donation site (10.4% in voluntary donors vs 16.8% among replacement donors).
Conclusion: Our data clearly showed that the choice of blood donation is affected by increasing awareness about blood donation, satisfaction by the service, emphasizing the religious impact and proximity of donating site. We recommend continuous education concerning blood donors and donation among the society as a whole; this will create awareness on motivational factors for repeat donations. It is also crucial to focus donor recruitment strategies on the transformation of replacement donors into voluntary donors with emphasizing the importance and the need of their blood donations to the community.

P-103

UNDERSTANDING MOTIVATORS FOR BLOOD DONATION AMONG MEN: A REVIEW OF THE EVIDENCE

A Carver[1], K Chell[2], TE Davison[1] and B Masser[3]

[1]Australian Red Cross Blood Service, Melbourne, Australia [2]Queensland University of Technology, Brisbane, Australia [3]University of Queensland, Brisbane, Australia

Background: The effective recruitment and retention of male donors is of increasing importance to blood collection agencies to ensure the ongoing provision of blood and plasma products. Compared with females, male donors are less likely to be medically deferred, to experience a vasovagal reaction or have unsuitable veins, and are typically preferred for conversion to plasmapheresis. However, among donors aged under 40 years, who potentially have long future donation careers, males are outnumbered.
Aims: The aims of this systematic review were to synthesise existing evidence and identify the key motivators for blood donation among male donors, in order to inform targeted recruitment and retention campaigns.
Methods: Databases (CINAHL, MEDLINE complete, PsychInfo, Health Business Elite and Web of Science) were searched using the following terms: (dona* OR dono*) AND (blood OR aphaeresis OR apheresis OR plasma* OR platelet* OR platlet*) in the title AND (male OR gender OR sex OR female) AND (motivat* OR intention OR attitude OR behavi* OR predictor OR barrier OR deter*) NOT (organ OR sperm OR tissue OR autologous OR oocyte) in the text.
The following inclusion and exclusion criteria were applied: selected articles had to be full-text, scholarly peer-reviewed journal articles, published in the English language between 1990 and 2015. Articles had to describe quantitative studies that examined males/females separately with at least of the following outcome measures: actual blood donation (objective/self-report) or self-reported intention to donate. Studies conducted in remunerated settings were excluded. All identified titles were screened independently by two authors (AC, KC) who subsequently reviewed all abstracts considered to be potentially eligible for inclusion. All full-text articles were reviewed (by AC and KC) with input from two further authors (TD, BM) to resolve any queries or discrepancies.
Results: Among 25 identified articles, inconsistencies in measurement precluded a meta-analytic approach. Despite this, our review identified several key themes. Prosocial motivations such as altruism and helping family/friends were key motivators for male donors. However, altruism was less pronounced among males compared with females and was combined with 'warm glow' (i.e. feeling good about oneself') in novice male donors (impure altruism). Perceived social pressure or

encouragement from family/friends was cited frequently. Perceived health benefits and incentives (e.g. gifts such as t-shirts and movie tickets; certificate of recognition for milestone donations; health checks and screening for infectious diseases) were stronger motivators of males than females. Males were more likely to become donors in response to a natural disaster or emergency, but females who did so were more likely to make a further donation.

Summary/Conclusion: Campaigns to recruit and retain male donors should focus on empirically identified motivators of this group rather than take a 'one-size-fits-all' approach.

P-104

UNIVERSITY STUDENTS AWARENESS AND ATTITUDE TOWARDS BLOOD DONATION IN YAZD, IRAN

H Javadzadeh Shahshahani and F Akhavan Tafti

High institute for Research and Education, Yazd, Iran

Background: The most important aim of all blood centers is to recruit blood donors from low-risk groups of society to donate blood voluntarily and regularly to have a safe and continuous blood supply. In Iran, all required blood obtained through voluntary blood donation. Because of population increase and doing specialized surgical procedures, blood and blood consumption is increasing. The healthy, active and interested student population is potential blood donors to meet safe blood requirements. However, there are not many studies on awareness and attitude among university students on voluntary blood donation.

Aims: The aim of this study was to determine the level of knowledge, attitude and practice regarding blood donation in university students of Yazd, Iran.

Methods: In this cross-sectional study, 1,306 students were selected by clustering sampling method and asked to fill a specially formatted questionnaire. Data were analyzed by analysis of variance, Sheffe test and t-test.

Results: A total of 703 (54%) of students were females and 599 (46%) were males. The mean age of students was 22.7 $\pm$ 4.4 years. Level of knowledge was weak in more than half of students. Younger students had the least levels of knowledge and performance. Paramedical students had higher level of knowledge than other students ($P < 0.001$). There was a direct relationship between knowledge and attitude ($P < 0.001$). Although there was no difference between knowledge level of females and males, female performance was lower. 18% of the population under study (25% of men and 12% of women) had donated blood at least once in the past. Less than half of the population under study was aware about the appropriate age for blood donation and certain deferral criteria.

Conclusions: Increase in the level of knowledge of students should be the topmost priority. Barriers to donation of blood by female students should be studied and evaluated, and steps must be taken to remove or decrease them as far as possible. Advertisements should be with the goal of increasing the level of knowledge of the students concerning factors of blood donation and keeping fresh the idea of regular voluntary blood donations in their minds.

P-105

THE ANALYSIS OF REASONS TO DONATE BLOOD AND FACTORS TO BOOST DONORS' MOTIVATION

M Sokolowski and K Olbromski

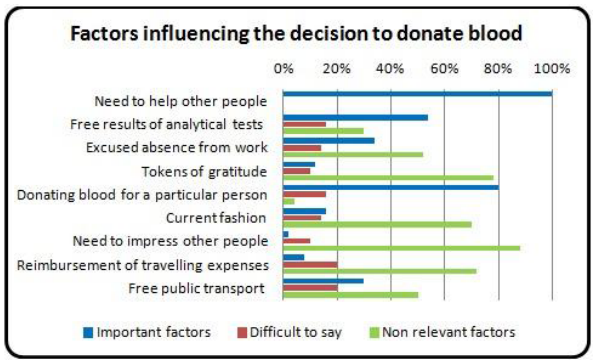
Blood Center, Poznan, Poland

Background: Blood service in Poland is based on voluntary and non-remunerated donations. Regional Blood Donor Centre in Poznan as well as other regional centres are the only entities authorized to collect, process, store and distribute blood and its components to hospitals in the region of their activity but they are also responsible to provide sufficient amounts of blood and its components. Hence, it is critical to introduce suitable measures to increase the number of donors.

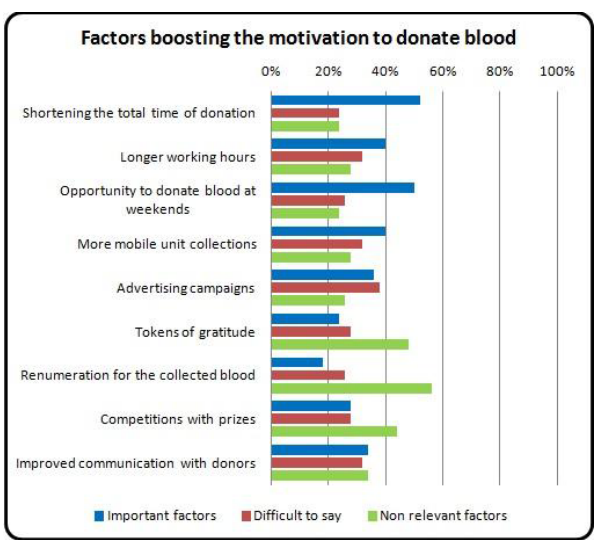
Aims: The aim of the survey was to analyse the most common reasons to donate blood as well as factors that could potentially boost donors' motivation and subsequently modify the current marketing strategy of the Regional Blood Centre in Poland.

Methods: A survey was carried out in March 2016 in group of 100 donors and 100 people that have never donated blood. They were asked questions regarding the factors influencing the decision to donate blood and factors that may positively increase the level of their motivation to donate blood.

Results: Important factors influencing the decision to donate blood: fulfilling the need to help other people (100%), necessity to donate blood for a particular person



Graph 1



Graph 2

(80% in general, in group of not-donors totalling 96%), possibility to obtain results of performed analytical tests free of charge (55%), excused absence from work (34%)

Factors completely non-relevant for decision to donate blood: need to impress other people (88%), tokens of gratitude i.e. little presents, gadgets (78%), reimbursement of travelling expenses (72%) and current fashion (70%)

Factors boosting the motivation to donate blood: shortening the total time needed for donation (52%), improving accessibility i.e. possibility to donate blood at weekends (50%), longer working hours of blood centre (40%), increasing the number mobile unit collections (40%)

Factors completely non-relevant for increasing the motivation of donors: remuneration for donated blood (56%), tokens of gratitude (48%), organizing competitions with prizes (44%)

Conclusions: The complete process of collecting blood should be reviewed and optimised in order to keep the time necessary for the donation as short as possible.

Large group of motivating factors were actions improving accessibility. It needs to be emphasized that the survey regarded the donors perception and not the actual state. Recently Regional Blood Donor Centre has introduced several in order to improve the accessibility for the donors that is why it is essential to publicize these positive changes and include relevant information in the marketing activities.

As the group of minor factors included psychological barriers, special measures targeted at potential donors should be taken, such as: relevant training of the medical staff including supplying them with necessary knowledge allowing to break the stereotypes regarding donating blood; ensuring for the constant availability of well designed, concise educational material on the premises and on the website (articles, downloadables etc.)

In terms of the projected image of the company, current policy regarding the transparency of the functioning of the Blood Donor Centre must be kept.

P-106

LIMITATIONS OF USING ONLY HAEMOGLOBIN LEVELS FOR SCREENING BLOOD DONORS

PD Chavan, S Ojha, M Poojary, V Bhat, BG Pillai, M Ganage, S Pal and M Tiwari
Actrec-Tata Memorial Centre, Navi Mumbai, India

Background: Blood and aphaeresis donors are screened for fitness based on their haemoglobin count. A haemoglobin value of 12.5 gm/dl is considered acceptable for blood donation. However, donors with acceptable haemoglobin levels may have low mean corpuscular volume (MCV) with high total Red Blood Cell (RBC) Count. The cause for these low levels of MCV may be iron deficiency or haemoglobinopathy.
Aim: To assess the low MCV values and total RBC count and its correlation with haemoglobin levels in blood and aphaeresis donors found fit for donation.
Method: Pre-donation blood samples from 751 (M: 558; F:193) blood and aphaeresis donors found fit for donation were processed for Complete Blood Count (CBC). CBC was processed on Advia 2120i by Siemens Healthcare Ltd. Serum Ferritin levels of donors with MCV levels below the reference range (83–101 fl) were analysed. Serum Ferritin levels were analysed on Architect i1000 by Abbott Healthcare. Serum Ferritin levels below the reference range (M: 21.8–274.6 ng/ml; F: 4.63–204 ng/ml) were defined as iron deficiency. Mentzer index (MCV/RBC count) was calculated for all samples. Mentzer index below 13 was considered suggestive of haemoglobinopathy and that above 13 was suggestive of iron deficiency anemia. Reference range for total RBC count was M: 4.5–5.5 $\times$ 10^{12}/l; F: 3.8–4.8 $\times$ 10^{12}/l.
Results: From the 751 samples analysed 27 (M: 19; F: 8) samples (3.6%) showed MCV levels lower than 83 fl. Of these, 58% (11/19) male donors and 50% (4/4) female donors had Mentzer index of <13 suggesting evidence of haemoglobinopathy. The rest (M: 8; F: 4) had Mentzer index more than 13 suggesting iron deficiency anemia. Three male donors showed low serum ferritin levels of these two had Mentzer index <13 suggesting coexistence of both hemoglobinopathy and iron deficiency anemia. RBC count was above the upper limit of reference range in all donors with low MCV count.
Conclusion: Screening the blood and aphaeresis donors for haemoglobin alone is not a reliable method for selecting healthy individuals fit for donation. A complete CBC count, with emphasis on MCV and RBC count, should be done while screening to select donors fit for donation. This will help exclude those individuals who have either hemoglobinopathy or iron deficiency anemia, but have higher haemoglobin levels due to high RBC count. A detailed study with further investigation for iron deficiency as well as haemoglobinopathies is required in such cases.

P-107

DEFECTIVE PLATELET FUNCTION IN QUALIFIED WHOLE-BLOOD DONORS DETECTED BY PLATELET FUNCTION ANALYZER PFA^{TM}100

M Belkacemi
University Djillali LIABÈS Sidi bel Abbès Faculty of medicine, Oran, Algeria

Background: Platelet transfusions are effective for the prevention and treatment of bleeding in patients with disorders of platelet number and/or function. In our blood transfusion center, in addition to plateletpheresis concentrates are prepared standard platelet concentrates. Therefore, the platelet quality of individual donation is increasingly important.
Aim: The aim of this study was to investigate the prevalence of defective platelet function among qualified blood donor.
Methods: Citrated Blood samples were obtained from 275 donors following routine blood donation. PLT function was evaluated by measurement of the closure time (CT) in a platelet function analyzer (PFA-100^{TM}) using both collagen/epinephrine (CEPI) and collagen/ADP (CADP) cartridges.
Results: 22% of donors (61 of 275) had anormal platelet function of whom 10.18% had prolonged closure time with two cartridges, 6.9% and 5% with only the CEPI and the CADP respectively. No closure (>300 s) has been reported in 6.54% qualified donors from whom 1.45% with the CEPI, 2.9% with CADP and 2.18% with both cartridge CEPI and CADP.
Conclusion: Impaired PLT function may have other causes than intake of ASA or NSAIDs therefore the donor questionnaire is currently insufficient for the selection of platelet donors. Platelet function screening within donors by platelet function analyzer PFA would provide a simple means to identify donors with diet-related platelet dysfunction or with poor recollection of aspirin use in order to eradicate defective PLTs from being clinically utilized.

P-108

IRON DEFICIENCY AND IRON DEFICIENCY ANAEMIA IN BLOOD DONORS AT A TERTIARY CARE HOSPITAL IN ISLAMABAD, PAKISTAN

U Waheed[1], R Hafeez[2], IS Kayani[1], Q Jabeen[3], M Arshad[4], A Arshad[3] and HA Zaheer[1]
[1]Safe Blood Transfusion Programme, Ministry of National Health Services, Islamabad, Pakistan [2]Department of Blood Bank, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan [3]Department of Zoology, PMAS Arid Agriculture University, Rawalpindi, Pakistan [4]Department of Biotechnology, International Islamic University, Islamabad, Pakistan

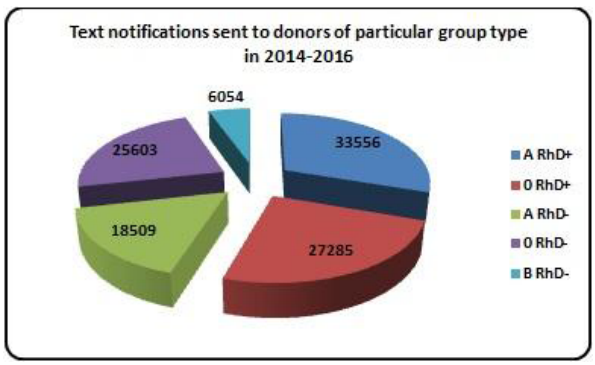
Background: Iron deficiency is the most widespread form of malnutrition affecting the developing nations. Iron deficiency and iron deficiency anaemia decrease the work capacity of the affected, resulting in adverse socio-economic consequences. Iron deficiency anaemia is an important restrictive factor for blood donations and especially for regular donors. Each blood donation is linked with 200–230 mg iron loss which is not replenished instantly. Many studies have documented that the incidence of iron stores depletion increases with an increase in the number of donations. Not many studies have been conducted in Pakistan about the prevalence of iron deficiency anaemia in blood donors.
Aims: To assess the incidence of iron deficiency anaemia in blood donors using a combination of haemoglobin and iron status parameters.
Material and methods: This was a prospective, single centre cross sectional study conducted at the Department of Blood Transfusion Services, Shaheed Zulfiqar Ali Bhutto Medical University (SZABMU), Islamabad, Pakistan, from September 2015 to February 2016. 528 blood donors who donated blood during the study period were included in the study. These donors were screened through physical examination also. All of the donors were males and gave informed consent to participate in the study. 50% (n = 264) belonged to regular voluntary donor category while rest were first time replacement donors. Six haematological parameters (haemoglobin, red cell count, packed cell volume, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration) and five biochemical iron parameters (ferritin, iron, total iron binding capacity, transferrin saturation, unsaturated iron binding capacity) were tested. WHO haemoglobin thresholds were used to categorize individuals as anaemic. Ethical approval of the study was taken from the Ethical Review Board of the SZABMU. The statistical analyses were conducted using SPSS (version 20.0) software.
Results: The prevalence of anaemia only (Hb <12 g/dl, RBC count <4.6 $\times$ 10^6/µl, PCV <41%, MCV <80 fl MCH <31 Pg, MCHC 31 g/dl) was 11.1% (6.6% regular donors and 5.5% replacement donors). The prevalence of isolated iron deficiency (serum ferritin <20 ng/ml, iron <59 µg/dl, TIBC >400 µg/dl, transferrin saturation <20%) was 19.6%. The iron deficiency was more prevalent in regular voluntary donors (11.2%). The incidence of iron-deficiency anaemia (haemoglobin <12 g/dl + serum ferritin <20 ng/ml) was 8.52% (n = 45) and was more linked with regular donation (5.6% prevalence in regular voluntary donors).
Conclusion: Among the two categories of the donors, the regular donors were found to be most adversely affected as shown by the reduction in mean values of both haematological and biochemical iron parameters. The blood bank management needs to review the screening criteria used at the time of donation. One key suggestion is to include serum ferritin measurement in the assessment of regular blood donors.

P-109

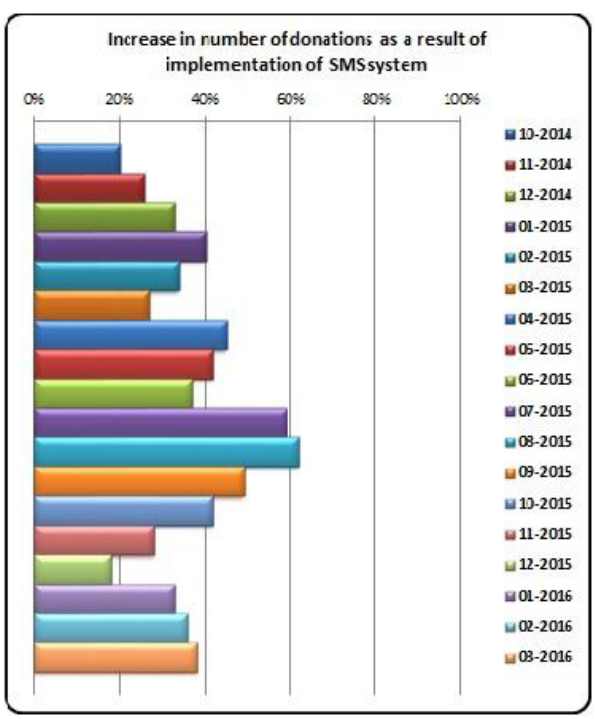
AN ANALYSIS OF LEVEL OF BLOOD FOR EACH GROUP AND D ANTIGEN IN REFERENCE TO THE OPERATING SYSTEM OF SENDING TEXT NOTIFICATIONS TO DONORS IN THE REGIONAL BLOOD CENTER IN POZNAN IN 2014–2016

L Gulinski, M Sokolowski and K Olbromski
Blood Center, Poznan, Poland

Background: In Poland, the Blood Service is based on voluntary, non-remunerated donations. Regional Blood Centers are the only authorized entities for collection, processing, storage and distribution of blood and its components. There are 21 Regional Blood Centers, one Military Blood Center and one Blood Center of the Ministry of the Interior Affairs that are all substantively supervised by the Institute of Hematology and Transfusiology in Warsaw (IHIT). The Regional Blood Center in Poznań is one of the largest institutions in Poland. One of the main tasks of the Regional Blood Center in Poznan, as well as other blood centers in Poland, is supplying blood and its components to all medical entities (hospitals) from the particular region of the country. Due to periodic shortages of blood of particular group and D antigen from October 2014 Regional Blood



Graph 1



Graph 2

Center in Poznan has been operating the system of text notification to donors (further: SMS system) inviting them to donate blood in order to optimize the stocks of blood.

Aims: The analysis of the effectiveness of the implementation of the donor text notification system (SMS system) inviting donors to donate blood of the desired group and D antigen in the Regional Blood Center in Poznan.

Methods: The study involved data regarding 'critical' and 'emergency' levels as part of management of the stocks of blood in the Regional Blood Center in Poznan from October 2014 to March 2016. The analysis included the documentation related to the activities conducted after the implementation of system of sending text notifications to donors and also reports on the stock of blood before and after the interventions were carried out. The data was obtained from the computer system 'Blood Bank' by Asseco.

Results: Using the software in operation it was possible to generate reports including following parameters regarding donors: gender, age, blood type (including D antigen), deferral, first-time donor, repeat donor, next possible time of donation, area by postal codes of targeted donors.

The effectiveness of the SMS system i.e. the increase in the number of registered donors from October 2014 to March 2016 (compared to the similar time period prior to the implementation of the system) is presented in Graph 1.

The number of sent notifications in the investigated period totaled 111,007 (Graph 2). Since the implementation of the 'Donors SMS invitation system' Regional Blood Centre in Poznan has observed no need to purchase the necessary units of blood from other blood centers.

Conclusion: There has been a significant increase in the efficiency of obtaining donors with desired blood group after the implementation of the SMS system.

The implementation of the system proved to be successful and effective.

In order to obtain a more accurate feedback from the SMS system there is need to introduce a precise tool enabling registration of the blood donation by the particular donors that were invited using the SMS system.

The analysis of the positive effects of the implementation of the SMS system proved it to be a useful tool for the management of donor flow and subsequently stocks of blood in the Blood Collection System.

P-110

THE CHARACTERISTICS OF BLOOD DONORS IN TIRANA

I Seferi, ZH Abazaj and A Metka

NBTC Tirana, Tirana, Albania

Background: NBTC Tirana is the biggest blood transfusion centre in Albania. Together with five hospital blood banks of Tirana, NBTC collects 70% of the total of whole blood donations collected at national level. In the last years there has been a constant increase of donations but nevertheless we still do not manage to cover all needs in blood and components therefore we still need to intensify our efforts in increasing donations. The number of donations is actually 10 donations/1,000 inhabitants.

Aims: Evaluation of the characteristics of whole blood donors. This evaluation helps us to identify donor groups that donate less and based on these data we can determine the appropriate approach to attract that group and increase donations which remains our major challenge.

Methods: Voluntary non-remunerated blood donors are all collected in mobile sessions from NBTC. Family replacement donors are collected from five hospital blood banks of Tirana and paid blood donors are collected from NBTC mostly during summer shortages. All donors that donated blood in Tirana during the period 2011–2013 have been analyzed regarding: age, sex and donor status (first time donor or multiple donor). Total number of donors was 54,103.

Results: The 98% of donors were first time donors. 70% were family replacement donors, 28.5% were voluntary non-remunerated donors, and 1.5% paid donors. All characteristics of our donors are shown in Tables 1 and 2. There is a predominance of male donors. This predominance of male donors is more expressed among family replacement donors (77% vs 23%). In VNRBD donors there is no significant difference between males and females in donation (54% vs 46%). There is a high percentage of young donors in our donor population (70% of them are <40 years old). This occurrence is more predominant in VNRBD where 67% of donors are <30 years old.

Summary/Conclusion: There is a very high number of first time donors. A well-defined retention strategy is needed. If we manage to make our first time donors donate at least twice per year we will achieve our objectives for sufficiency and quality of blood. The high number of young donors among VNRBD is a good

Year	Number of donors	First time donors	Multiple donors	Males	Females	18-40	41-65
2011	16475	16119	356	11477	4998	11451	5024
2012	18031	17656	375	12905	5126	12905	5712
2013	19597	19201	396	13664	5933	13719	5878
Total	54103	52976 (98%)	1127	38046 (70%)	16057	38075 (70%)	16614

Table 1. Donor characteristics in Tirana

	Males	Females	Total
VNRBD	8338 (54%)	7124 (46%)	15462
Family replacement	29328 (77%)	8718 (23%)	38046
Paid donors	249 (61%)	158 (39%)	407

Table 2. Gender and donor type

premise for long term sufficiency by implementing an accurate and clearly defined retention policy. Efforts should be intensified also for the conversion of family replacement donors in VNRBD donors.

P-111

THE POTENTIAL INFLUENCE OF ABO/RH BLOOD TYPES ON REPRESENTATION RATES AND BLOOD DONATION BEHAVIOR AMONG NEW VOLUNTEER BLOOD DONORS

B Hermundstad, SA Mousavi and AH Llohn

Akershus University Hospital, Lorenskog, Norway

Background: The extent to which representation rates among potential blood donor or blood-donation behavior might be influenced by ABO/Rh blood types is unknown.

Aims: The aim of this study was to assess the potential influence of ABO/Rh blood types on the representation rates among volunteer potential donors and to examine the effect of ABO/Rh blood types on blood donation behavior among prospective donors. We also assessed whether the likelihood of attending to donate for the first time associated with the index-to-first-time presentation interval.

Methods: All potential donors who attended at our blood bank for eligibility determination from 1/5-2013 to 30/12-2014 were included in the analysis. Information on relevant variables was collected from the donor database. Potential/prospective donors were categorized according to their blood types as those with common types (A+ and O+ combined) and those with rare types (all other types combined). Prospective donors were followed for 19 months from the date they were accepted as blood donors.

Results: During the study period, 1,000 potential donors were eligible for inclusion in the analysis. The population study comprised a higher proportion of females (62.5%). Almost three-quarters were aged 18–39 years. Potential donors with rare ABO/Rh types were overrepresented relative to their proportion in the general population ($P < 0.001$). Of the potential donors 305 individuals with rare blood types were observed, while 241 individuals were expected ($P < 0.001$). There were 193 (19.3%) permanent deferrals.

Of the prospective donors (n = 807), 636 (78.8%) donated at least once; the remaining 171 did not donate during the study period. Females were 32% less likely than males to donate at least once ($P = 0.032$). Prospective donors with rare ABO/Rh blood types were about 2.6 times more likely to donate for the first time compared to those with common blood types ($P < 0.001$). Logistic regression analysis determined independent associations with gender and blood type status.

Among first-time donors who still were able to donate, significantly more with rare ABO/Rh blood types than those with common blood types returned for a subsequent donation. First-time donors who returned for a second donation had a shorter index-to-first-time presentation interval compared to those who gave only once. Adjustment for age and the index-to-first-time presentation interval eliminated the association between blood types and subsequent donation among first-time male donors, probably because of small sample size, but not among female donors.

Conclusion: Our study is the first that specifically has analyzed the relationship of ABO/Rh blood types and representation rates/donation behavior among new volunteer blood donors and also the first that has examined the effect of the index-to-first-time presentation interval on the return behavior among first-time donors. The observed overrepresentation of individuals with rare blood types among potential donors may be related to prior knowledge about ABO/Rh blood types that are often requested. Anecdotal evidence suggests that new donors with rare ABO/Rh blood types receive more encouragement from our interviewing staff to donate, potentially increasing their likelihood of donating for the first time.

P-112

A COMPARATIVE ANALYSIS OF THREE NON-INVASIVE SYSTEMS (SOFTWARE 2015–2016) FOR MEASUREMENT OF HAEMOGLOBIN WITH THE HEMOCUE SYSTEM HAVING COULTER UNICEL DXH800 AS REFERENCE VALUE

AL Pajares Herraiz, G Sanchez Moreno, C Coello de Portugal, MV Flores Sanz, C Torrades, MA Bejerano, LM Morillo, V Fernandez Ramirez and JD Rodriguez Gambarte

Centro Regional de Transfusión Toledo-Guadalajara, Toledo, Spain

Background: Pre-donation hemoglobin (Hb) screening is mandatory in Spain for blood donation, and it is determined with capillary methods at a large number of blood transfusion centers (BTC). In the last few years, transcutaneous non-invasive methods of Hb (Hb-NIM) appeared as a very promising alternative, more comfortable and painless, and different studies have proposed that these systems could develop better software and algoritthms in order to use them regularly in a safe way.

Aims: Analyze the feasibility and the last software of three Hb-NIM [Pronto-7 (P7) Software b99e8000004fc131 (2.4.17); HaemoSpect (HS) 01.83.100 & OrSense NBM200 47001610] compared with an invasive method [Hemocue 301 (HC)], using the venous Hb analyzed in Unicel DxH 800 as reference method or gold Standard.

Methods: A prospective study was performed with 176 blood donors who were atended at our BTC in Toledo. All blood donors underwent an interview and medical examination according to our protocols, and all of them were informed on a naciona-l regulation basis and they consented the procedure. We measured the Hb with the three Hb-NIM and Hemocue according to the supplier's instructions and were done at room temperature between 21 to 25°C. Before the collection of whole blood

TABLE 1				
Method N (176)	Mean±SD (g/dl)	Max	Min	Correlation Index (Pearson)
Hb DxH	14,69±1.42	18	11,4	1
HC	15,13±1.34	18	12	0.898
P7	14,27±1.24	17	10,5	0.652
HS	14,3±1.18	17	11	0.555
NBM	14,61±1.41	18	10,5	0.581

Table 1

TABLE 2	Rejected Falsely (n/%)			Accepted Falsely (n/%)		
METHOD	Male	Female	Total	Male	Female	Total
HC	0/0	1/1,79	1/0,57	2/1,67	8/14,29	10/5,68
P7	7/5,83	7/12,5	14/7,95	2/1,67	12/21,43	14/7,95
HS	8/6,67	5/8,93	13/7,39	1/0,83	14/25	15/8,52
NBM	5/4,17	10/17,86	15/8,52	1/0,83	11/19,64	12/6,82

METHOD	Accepted Correctly (n/%)			Sensitivity	Specificity
	Male	Female	Total		
HC	118/98,33	48/85,71	166/94,32	99,43	94,32
P7	111/92,50	37/66,07	148/84,09	92,05	92,05
HS	111/92,50	37/66,07	148/84,09	92,61	91,48
NBM	114/95	35/62,5	149/84,66	91,48	93,18

Table 2

unit we performed an extraction of venous blood sample to study with Unicel DxH.A questionnaire was done by the operators who used the Hb-NIM in order to evaluate the usability, time to readiness, clearing, maintenance, intrinsic errors, sensitivity to ambient light and handling, this survey was made with an evaluated scale from 0 (unsatisfactory) to 5 (excellent). All data were recorded into a database Excell 2007 version and the statistical anális and Bland-Altmand of data were performed with IBM SPSS Statistics 19 and MedCalc 12.2.1.0.

Results: A total of 176 blood donors were tested [120 men (41.91 ± 10.59 years old) and 56 women (38.59 ± 13.64)]. Descriptive statistics are exposed in Table 1. The best correlation index with DxH as reference value was obtained with HC, and the worst result, althougt acceptable, was with HS. Regarding sex we found worst results in women than in men. Donors rejected and acccepted falsely, accepted correctly and the sensitivity and specificity of the diferent methods are exposed in Table 2. The HC showed the best results of usability (4.86), although the Hb-NIM was evaluated with a satisfactory level (4.04HS; 4.14NBM; 4.21P7). A Bland-Altman 95% limits of agreement analysis yields lower and upper limits of −1.7 and 0.86 g/dl (HC); −1.8 and 2.6 (P7); −2 and 2.8 (HS) and −2.4 and 2.6 for NBM.

Conclusion: Hemocue is better method for Hb screening compared with Hb-NIM. Hb-NIM are more comfortable and have better correlation index compared with previous analysis (Pajares et al, Hematology & Transfusion International Journal, 2015) and they can be used as screening test but we must have in consideration the differences between men and women, and adjust the evaluation algorithms accordingly, and that their ability to determine an acceptable donor is greater that their ability to determine an unacceptable being the NBM the best of Hb-NIM in this item.

P-113

THE EFFECT OF FREQUENT BLOOD DONATION ON IRON STORES IN BLOOD DONORS

M Raouf[1], M Rabeh[2], S Joseph[1], A Amer[2], S Al Shamsi[2], A Al Ali[1], H Al Najar[2], R Sharma[1] and A Yousef[2]

[1]Dubai Blood Donation Center, Dubai, United Arab Emirates [2]MOH, Sharjah, United Arab Emirates

Background: Regular blood donation can lead to preclinical and clinical iron deficiency anemia. Due to increase demand on blood supply; donors are encouraged to donate regularly to ensure sustained and safe national blood supply. Hence, Iron stores evaluation by screening serum ferritin level in regular blood donors in required for donors safety and availability.

Aims: To determine the effect of blood donation on iron stores in regular, voluntary blood donors in United Arab Emirates that can determine the need to review the guidelines on acceptance of donors (eligibility Criteria) and wither there is a need to add serum ferritin level to blood donors screening tests after certain frequency of donation.

Methods: This is a multicenter study (Dubai Blood Donation Center and Sharjah Blood Transfusion Center). 2,152 blood donors included. Lower hemoglobin level accepted pre donation is 12.5 gl dl in females & 13.5 g/dl in males. Blood donors were divided into three groups according to serum ferritin level .Group I are those with ferritin level ≤12 ng/ml, Group II with ferritin level >12 ng/ml, <200 ng/ml, and group III are those with ≥200 ng/ml ferritin. Pre donation Hemoglobin,HCT, MCH, MCV. & MCHC were done using automated blood counters. ferritin Level was measured using ARCHITECT Ferritin assay (CMIA) for the quantitative determination of ferritin in plasma. Other variables included: Sex, frequency of donation (within last 2 years), smoking status and diet habits.

Results: Males constitutes 86.1%, females 13.9% of the studied group. Group I constitute 3.7%, Group II = 86.8% and Group III = 9.4%. Group I are considered as donors with depleted iron stores. The frequency of donation in that group showed 27.8% as first time donors,49.4% has donated 3–4 times and 22.8% >5 times. Depleted Iron stores was significantly related to frequency of donation >3 times: $P < 0.05$. Females constitutes 38% of group I compared to 13.9% of the total studied group reflecting the higher incidence of depleted iron stores in female donors. The average hemoglobin level in group I was 13.8 g/dl, and an average HCT of 42.8%. This study shows no significance relation of smoking and vegetarian food with depleted iron stores in our studied group. Iron supplement was taken in 2.5% of donors in group I and 0.9% of group II.

Conclusions: Frequent blood donation could lead to depleted iron store in regular blood donors. Whole blood donors acceptance criteria should consider the effect of frequent donation on iron stores and therefore accept blood donors whom hemoglobin level is higher than lower normal level taking gender in consideration. Considering the policy for screening regular donors for ferritin level after 4th donation within 1 year could be a tool to identify blood donors with low iron store where iron supplement could be considered.

P-114

CHARACTERISTICS OF BLOOD DONORS IN THE DEPARTMENT OF TRANSFUSION MEDICINE – STRUMICA

T Timova, L Maninska, D Stambolieva, E Kocovska, T Malinova, V Gjorgjevska and A Baldzieva

Institute of transfusion medicine of R.Macedonia, Strumica, Macedonia

Background: Socio-demographic profile of blood donors can provide valuable information and can contribute to an increase in the number of voluntary donors and in the preparation of relevant plans for promotional activities for recruiting and retaining blood donors.

Aim: The aim of the study was to determine the characteristics of blood donors who donated blood in Department of transfusion medicine -Strumica, as well as to prepare plans for dealing with the target groups that are under-represented among blood donors.

Methods: In this study carried out between January 2015 and December 2015 were included 1,976 blood donors who donated blood in the Department of transfusion medicine and in the mobile teams outside the Department. The aim of the study was to find out if they were: voluntary donors or donors who donate blood for family members, male or female, first time or regular donors, employed or unemployed, university students, high school student, pensioners, Macedonians and other ethnic groups.

Results: During this 1 year period were collected 1,976 units of blood, of which 1,275 (64.5%) were donated in the Department of Transfusion medicine and 701 (35.5%) in the mobile team campaigns. The majority of the blood donors 1,900 (96.2%) were voluntary and non-remunerated, the rest of them 76 (3.8%) donated blood for family members. 1,587 (80.3%) were males and 389 (19.7%) were female donors. The number of blood donors who donated blood for the first time were 267 (13.5%). 1,233 (62.4%) blood donors were employed, 410 (20.7%) were unemployed. 129 (6.5%) were university students, 202 (10.2%) were high school students, four were pensioners, 1,872 (94.7%) were Macedonians and 104 (5.3%) were donors from other ethnic groups.

Conclusion: The majority of the donors are voluntary donors who donate blood regularly. There are more male than female donors. Most of the donors are employed. In relation to the nationality most of the donors are Macedonians. We need to make plans for promotional activities for voluntary blood donation by university students, unemployed people, and females in order to increase the number of blood donors in these target groups in the future.

P-115

DETERMINING BLOOD PRESSURE AND HEMOGLOBIN NORMAL RANGES IN OMANI BLOOD DONORS, A COHORT STUDY

AZ Al Riyami[1], A Al Zakwani[1], S Murthi[2] and H Al-Sabti[3]

[1]Sultan Qaboos University, Al Khod, Oman [2]Oman Medical Speciality Board, Muscat, Oman [3]Sultan Qaboos University Hospital, Muscat, Oman

Background: Voluntary blood donation is critical to the health system worldwide. Negative donation experiences, like donor deferral or experiencing an adverse reaction might affect donors' retention rate. Healthcare professionals responsible for blood collection have a moral obligation to protect donors from harm and not to unfairly discriminate against them. The measurement of blood pressure (BP) and Hemoglobin (Hb) levels are generally regarded as an indicator of good health and is required by many guidelines in donor screening and selection. However, acceptable levels for donor acceptance vary between regulators.

The BP ranges in use at the Sultan Qaboos University Hospital Blood Bank to assess blood donors follows national guidelines, which recommend a BP range of 110–160 mmHg systole and 65–100 mmHg diastole. Capillary Hemoglobin (Hb) lower cutoff values are 12 g/dl for male donors and 11.5 g/dl for female donors. When compared to international guidelines, the BP thresholds for donor deferral appear to be higher than what is recommended elsewhere which potentially may lead to unnecessary donor deferrals. Moreover, the Hb cutoff for female donors is lower than what is recommended internationally, which may lead to acceptance of anemic females for blood donation.

Aims: This study was undertaken to verify the BP and Hb normal ranges in Omani whole blood donors in order to ensure donor safety and to avoid unnecessary deferrals.

Methods: Ethical approval has been obtained from the local ethics committee. The study undertook a prospective design between September 2015 and March 2016. Systolic and diastolic BPs were measured on enrolled donors by a validated

automated measuring device and by a manual sphygmomanometer. Capillary and venous samples were collected from each enrolled donor for Hb measurements.
Intra-class correlation coefficient analysis was used to assess the agreement between manual and automated BP and the capillary and venous Hb measurements. A non-parametric analysis was utilized to develop systolic BP, diastolic BP and Hb normal ranges in accordance with the CLSI guidelines. To derive gender specific normal ranges for the Hb, the Robust method was utilized. Donors with low ferritin levels were excluded form the analysis. All analyses were performed using MedCalc software (version 16.0).
Results: Total of 239 Omani healthy subjects were involved in the study (88 females, 151 males), with a mean age of 26 ± 8.0 and weight of 59.1 ± 12.3. There was 98% agreement between the manual and the automated BP measurements ($P < 0.001$). Normal ranges of manual systolic and diastolic BP were 100–149 and 60–91 respectively.
There was 96% agreement between the capillary and venous Hb measurements ($P < 0.001$). Capillary and venous Hb normal ranges were 11.8–16.4 and 12.1–15.9 g/dl respectively. Upon gender-specific sub-analysis, the obtained capillary and venous Hb normal ranges in male donors were 12.3–16.2 and 12.7–16.1 respectively. In comparison, female donors had capillary and venous Hb normal ranges of 11.4–13.7 and 11.7–13.8 respectively.
Summary/Conclusion: Our study reports population-specific BP normal ranges that can be used in donor assessment in Omanis. Moreover, it reports lower normal Hb ranges in female donors compared to international recommendations.

P-116

THE RETURN PATTERN OF FIRST-TIME BLOOD DONORS – THE AGE EFFECT

HH Wang and RC Jau
Technology, Taichung, Taiwan

Background: First-time donors are very important because they represent the continuity of blood supply. However, the age effect on return behavior for first-time donation is poorly described. The transform from first-time donors into regular donors is a primary target of donor management.
Aims: The purpose of this study is to examine the donation behavior of first-time-donors among different age group.
Methods: There were 8,297 first-time donors during the period from November 1, 2009 to February 28, 2010, and their donation behaviors were followed for 24 months. Returned and total yield rates among different age groups are compared. In addition, the age factor is grouped into following categories: 17–20, 21–30, 31–40, 41–50 and 51–65. In each category, the particular age 17, 18, 19 and 20, their blood donation behavior is also examined and discussed. A donor metric term 'yield rate' refers as the mean number of donation, it is calculated and used to describe the donation performance within an age group. Data was calculated to adjust ORs and 95% CIs by logistic regression.
Results: The youngest donor group, age 17, shows the highest return rate (51% during the following 12 months, and 58% for the following 24 months), then followed by the age 18 and group 41–50. All factors in the regression model were considered significant if the P value was <0.05. The yield rate for the 12 and 24 months period displays a very similar pattern (1.42–1.68, 1.78–2.24, respectively), the return rates at other age groups had lower ORs than 17-year-old for the 12 and 24 months period, male also shows the lower ORs than female during the same periods (OR = 0.79, 95% CI = 0.72–0.87; OR = 0.77, 95% CI = 0.70–0.84, respectively).
Conclusions: First-time 17-year-old donors had the highest return and yield rates, therefore the donation behavior starts at young age plays critical role for the future committed repeat donors and sustained donors return behavior.

	OR(95% CI)	p-value	OR(95% CI)	p-value
Factors	12 months		24 months	
Age group (years)				
17	1.00(reference)		1.00(reference)	
18	0.55(0.47 - 0.63)	<0.001	0.59(0.52 - 0.68)	<0.001
19	0.45(0.37 - 0.54)	<0.001	0.5(0.42 - 0.59)	<0.001
20	0.46(0.37 - 0.58)	<0.001	0.51(0.41 - 0.63)	<0.001
21-30	0.36(0.31 - 0.41)	<0.001	0.4(0.35 - 0.45)	<0.001
31-40	0.38(0.31 - 0.46)	<0.001	0.45(0.37 - 0.54)	<0.001
41-50	0.49(0.41 - 0.6)	<0.001	0.53(0.45 - 0.64)	<0.001
51-65	0.43(0.35 - 0.54)	<0.001	0.51(0.41 - 0.63)	<0.001
Sex				
Male	0.79(0.72 - 0.87)	<0.001	0.77(0.7 - 0.84)	<0.001
Female	1.00(reference)		1.00(reference)	

Caption 1. Logistic regression of factors associated with return rate

	12 months			24 months		
Age group (years)	Percent returning	Initial yield	Repeat yield	Percent returning	Initial yield	Repeat yield
17	51.47	1.68	1.41	58.17	2.21	1.23
18	37.19	1.48	1.74	45.67	1.9	1.4
19	32.88	1.44	1.89	41.21	1.9	1.44
20	33.67	1.47	1.82	41.71	1.92	1.44
21-30	29.19	1.42	1.99	36.28	1.78	1.56
31-40	30.86	1.46	1.9	40.76	1.97	1.43
41-50	37.27	1.58	1.66	45.09	2.24	1.32
51-65	32.84	1.49	1.82	43.14	2.05	1.38

Caption 2. Age distribution of First-Time donors

Blood Collection Including Apheresis

P-117

DON'T WE REQUIRE TO PERFORM PLATELET COUNT IN BLOOD DONORS BEFORE FIRST PLATELETPHERESIS PROCEDURE IN INDIAN POPULATION?

P Pandey
Jaypee Hospital, Noida, India

Introduction and aim: Significant reduction in platelet counts has been acknowledged in plateletpheresis (PP) donors. As per the directorate general of health services (DGHS), India and American Association of Blood Bank (AABB) Guidelines, pre donation platelet count is not required before the first plateletpheresis procedure. This policy is being routinely practiced in many centers but its outcome on quality of platelet product and post donation platelet counts (PC) has not been characterized yet.
Materials and methods: A prospective blinded controlled trial was performed to evaluate the effect of policy (no platelet count assessment in first time donor) on quality of platelet product and donor platelet counts. Study was conducted on 421 healthy, first-time donors over a period of 3 months. Study design is shown in Table 1. The plateletpheresis procedures were performed using a fully automatic cell separator (com.tec, Fresenius Kabi, Germany) and various pre- and post-donation hematological values were measured. Pearson correlation was used to assess the relationship between baseline platelet count and yield of platelets.
Results: Amongst the 421 selected donors, 34 (8.07%) were already thrombocytopenic, having pre donation PC below 150×10^3/mm. Post-procedure drop in mean PC was observed to be significant (baseline mean PC – 223.7 ± 67.16 while post-procedure mean PC – 169.12 ± 87.21, $P < 0.001$). Among the 34 thrombocytopenic donors, 21 donors showed post procedure PC below 100×10^3/mm with no clinical manifestations. The mean platelet yield was $2.8 \pm 0.46 \times 10^{11}$ which showed direct relationship between pre-donation PC and yield ($R = 0.45$, $P < 0.001$). Platelet yield was below 1.9×10^{11} in all 34 platelet products from thrombocytopenic donors. During the follow up among the 34 thrombocytopenic donors, 30 returned on day 5, none of the donors showed PC above 150×10^3/mm.
Conclusion: This policy masks the thrombocytopenic donors which may affect donor's health and quality of platelet product. Our study suggests that the platelet count should be assessed before every PP procedure to prevent developing thrombocytopenia in healthy blood donors.

First time platelet donors		
Counselled, screened and selected for platelet donation (pre-donation platelet count was not done)		
Kit was installed		
Baseline sample	Obtained from blood diversion pouch after phlebotomy and procedure was started	Hematological parameters were assessed • Platelet count (PC) • Hemoglobin (Hb) • WBC count • Platelet distribution width (PDW) and • Mean platelet volume (MPV)
Post donation sample	Obtained by repeat phlebotomy after 60 minute of completion of procedure	
Follow-up sample (post procedure day 5)	Obtained by phlebotomy	
Platelet Product	Quality control	Quality parameters were assessed • Swirling • pH • Platelet count • WBC count
*All results were kept blinded till the end of the study		

Table 1

P-118

AUTOMATED PLATELET COLLECTIONS AT ITM-SKOPJE

F Raka, S Useini, R Grubovic and K Kacarski

Institute for Transfusion Medicine, Skopje, Macedonia

Background: Platelet transfusions are routinely needed to support patients undergoing chemotherapy in treatment of malignant diseases, open-heart surgeries, organ transplantations, as well as patients with bleeding disorders. Due to the short shelf-life and the necessity to be transfused within 5 days, the blood banks face a constant need of replenishing the supplies.
Aims: To report on the annual number of platelet apheresis procedures using TRIMA ACCEL version 6.0 at our Institute.
Methods: A retrospective study, involving analysis of a 1 year period of time (from January 2015 through December 2015).The data were obtained from the Department of Apheresis Donations at Institute for Transfusion Medicine- Skopje.
Results: The Apheresis department of the ITM-Skopje has performed 113 plateletpheresis procedures using TRIMA ACCEL version 6.0 customized based on the donor's physical size, preyield platelet count as well as donation frequency. Five failed procedures (4.4%) were recorded due to venipuncture problems as well as technical ones. Other 108 successful procedures were performed due to requirements from the university clinic of adult hematology (37.31%), pediatric hematology (17.91%), pediatric oncology (10.7%), radio-oncology (9.7%), gynecology (8.95%), gastroenterohepatology (3.73%), as well as other clinics such as cardiosurgery, cardiology, orthopedics, urology and neonatology (11.7%).Platelet yield was ranging from 0.8×10^{11}/l in one case to 6.5×10^{11}/l with the average yield of 4.4×10^{11}/l.
Summary/Conclusions: All the platelet concentrate units were satisfactory according to recommended yield norm. No donor adverse reaction was reported as well as post transfusion reactions.

P-119

SAFETY AND QUALITY OF PLATELET CONCENTRATES IN RUSSIAN FEDERATION

AV Chechetkin

Research Institute of Haematology and Transfusiology, St. Petersburg, Russian Federation

Background: Platelet concentrates is demand for the treatment of patients in haematology, cardiac surgery, oncology. Need of clinics in donor's platelets increases annually. To ensure the safety and quality of platelet concentrates the modern technologies used in blood service establishments, but the degree of their implementation remains unstudied.
Aims: The aim was the investigation of organizational aspects of platelet concentrates collection, safety and quality ensuring in blood service establishments in Russian Federation.
Methods: The parameters of platelet concentrates collection and processing were researched using the reports from blood service establishments in Russia during 2007–2014 followed by a statistical analysis and questionnaire survey.
Results: For the period 2007–2014 the amount of the collected platelet concentrates has enlarged in 1.8 times. The percentage of platelet obtained by the apheresis in 2014 amounted to 67.9%. The implementation level of plateletapheresis in different regions of Russian Federation differs significantly. In Urals federal district 76.5% platelets concentrates was collected by the apheresis and 48.1% – in Southern federal districts in 2014. These regional differences depended on technical equipment of blood service establishments and the medical organizations demand in platelet concentrates. To improve the safety of platelets leukoreduction methods were used. For the period of 2007–2014 the percentage of leukocyte-depleted platelets has increased from 17.0% to 32.7%. The leukoreduction technology for platelets was widely used in blood service establishments in Ural federal district, where more than 63% of platelets was subjected to leukoreduction in 2014. The percentage of pathogen reduced platelets has increased from 2.1% to 12.4%. The pathogen reduction of platelets was widely used in blood service establishments in North-Western и Central federal districts. In these districts, 16.4–17.4% of the collected platelets was subjected to inactivation of pathogens in 2014. The system of quality control of platelets and the automatic methods of control of bacterial contamination used. In some regions, the additional methods of control of viral infections apply, including anti-HBcore antibodies and anti-CMV antibodies screening.
Conclusion: For the period 2007–2014, the safety and quality of platelet concentrates was provided in blood service establishments in Russia. The degree of using modern technologies in different regions varies considerably.

P-120

IMPACT OF APHAERESIS PLATELET DONATION ON DONOR HEMATOLOGICAL PROFILE AND HEALTH

G Patidar[1], R Sharma[2], N Marwaha[2] and J Kaur[2]

[1]Adlakha Medical Center, Amritsar, India [2]Post Graduate Institute of Medical Education and Reaserch, Chandigarh, India

Background: Plateletpheresis is the specific procedure to remove platelets from the donors by automatic cell separators. Although automated cell separators have undergone a lot of technical refinements, however, attention has been focused more on the quality of platelet product than on donor safety. Thus we planned a study to understand the effect of plaeletpheresis donation on donor hematological profile and health.
Aims and objectives: To Study the effect of plaeletpheresis donation on donor hematological profile and health using two different cell separators.
Material and methods: A total of 500 plateletpheresis donors were studied. All donors were selected after fulfilling the basic criteria for plateletpheresis donation. Pre and post-donation hematological profiles were assessed in whole blood samples collected in EDTA vials just before and within 30 min after the procedure. The comparative analysis was done for normally distributed data by Paired t test (inferential statistics) and descriptive statistics.
Results: The aphaeresis platelet donation showed a statistically significant ($P = 0.0001$) decline in hemoglobin (5.29%), hematocrit (6.22%), platelet count (27.59%), & lymphocyte count (9.88%) and non-significant decline in monocyte count (0.5%). While total leukocyte count (1.43%) & neutrophil count (5.68%) showed a statistically significant ($P = 0.0001$) rise post donation. Cell separator wise [Trima (Terumo BCT) and Amicus (Fresenius Kabi)] analysis of pre and post donation hematological profiles were comparable. The observed changes in the hematological profiles were within the physiological limits, hence, no clinical evidence of anemia, thrombocytopenia and leukocytosis observed in donors.
Conclusion: In view of the statistically significant post donation decline in Hemoglobin and hematocrit of the donor, careful donor selection approach has to be followed in donors with lower cutoff values (Hb – 12.5 to 12.8 g% & Ht – 36 to 38%) and reassess them post donation.

P-121

COMPARISON BETWEEN MCS+ AND TRIMA ACCEL MACHINES (OPTIMISATION OF PLATELET COLLECTION AT BLOOD SERVICES GROUP)

H Xin Xia Avelina, J Hiok, NF Jamil, SN Saiman, N Rahamat, YL Ang and M Sim

Health Science Authority, Singapore, Singapore

Background: Plateletpheresis is performed by two cell separators- MCS+ and Trima Accel at Blood Services Group (BSG), Singapore. Adhering to strict quality control in accordance to AABB standards, platelet components collected

through the Apheresis method are required to have a yield of at least 3.0×10^{11}.

Prior to 2015, historical full blood counts were used in both MCS+ and Trima Accel. However, since 2015, we began using current full blood count for Trima Accel as recommended by vendor. Trima Accel platelet yield performance improved marginally with donors' current full blood count when compared to using historical data. Nevertheless, its performance was unmatched to MCS+ blood cell separators which had been consistently achieving a lower failure rate.

Aims: The aim of this study is to make comparisons between MCS+ and Trima Accel in order to find out the best practices in order to optimise platelet collection of each machine. Three main areas were explored in this study: (i) platelet yield obtained by the two cell separators; (ii) correlation between blood processed volume and platelet yield, and (iii) relationship between donor platelet count depletion and platelet yield.

Methods: In order to achieve a platelet yield of at least 3.0×10^{11}, all cell separators at BSG are required to pre-set a target yield of 3.6×10^{11}. Retrospective data of single plateletpheresis were obtained from MCS+ and Trima Accel over a period from January to December 2015. Platelet donation data such as platelet yield, blood volume processed, pre and post platelet counts were documented, sorted and analysed. Influence of blood volume processed and donor platelet count depletion against the platelet yield was evaluated using Pearson Correlation ('*r*' value).

Results: Trima Accel obtained platelet yield of $4.0 \pm 0.5 \times 10^{11}$ while MCS+ had a yield of $4.3 \pm 0.6 \times 10^{11}$. When compared against the platelet yield and total blood processed volume, there was no relationship for MCS+ (n = 100; $r = 0.14$; P = 0.2) or Trima (n = 100; $r = -0.16$; P = 0.1). Similarly, donor platelet count depletion and platelet yield did not reach statistical relationship for both MCS+ (n = 50; $r = 0.1$; P = 0.22) or Trima (n = 50; $r = 0.09$; P = 0.5).

Summary/Conclusions: Platelet yield attained from Trima Accel was closer to the targeted yield, whilst MCS+ platelet yield was vastly varied and skewed by comparison. Our results highlighted key differences in the collection efficiency settings for Trima Accel and MCS+.

The lack of statistical correlation between total blood processed volume and donor platelet count depletion against the platelet yield showed that neither factors impede optimal yield collection.

Having efficient cell separators that function optimally is ideally desired; it is important to work closely with machine vendors to continuously regulate and monitor blood cell separators' performance and the corresponding practice in order to achieve department's objectives.

P-122

This abstract has been withdrawn.

P-123

COST-EFFICIENCY ANALYSIS ON OBTAINING DOUBLE AND SINGLE OUTPUT FROM PLATELETPHERESIS WITH THE AMICUS SYSTEM 3.21 (FRESENIUS KABI)

JD Rodríguez Gambarte, AL Pajares Herraiz, C Coello de Portugal, G Sanchez Moreno, MV Flores Sanz, E Rodriguez Segura, MA Bejerano, C Torrades, LM Morillo and V Fernandez

Centro Regional de Transfusiones, Toledo, Spain

Background: The production of platelets by plateletpheresis is a very widespread methodology, with different benefits and outomes according to the cell separator used. Obtaining double product in a single process with a high platelet-count donor optimises resources and the stock of a transfusion centre.

Aims: Carry out a savings analysis (cost-efficiency) after implementing the double-dose plateletpheresis with the Amicus System 3.21 (Fresenius Kabi) in high platelet-count donor, compared with obtaining a single product, in compliance with correct implementation of European and Spanish regulation cell standards.

Methods: All multicomponents processes – both double and single plateletpheresis and plasma – were reviewed regarding donors between 1 January 2015 and 31 March 2016. We registered information regarding donors (sex, age, body surface, blood type, blood volume, pre-process cell count), process (volume processed, time, programmed output, adverse reactions) and final outcome (final net volume, platelets/microl, final output platelets/bag, volume of additive solution). We established two pair groups to both processes with same number of componentes. We quantified direct costs (apheresis kit, additive solution, time/cost of healthcare workers, no overlapping of samples for analytic processing) and indirect costs (journey times, parking and refreshments). All data were recorded into a database Excell 2007 version and the statistical analysis and Bland-Altmand of data were performed with IBM SPSS Statistics 19 and MedCalc 12.2.1.0.

	Single Dose	**Double Dose**
Direct technical costs:		
- Kit:	€ 160.4355	€ 160.4355 / 2 = € 80.22
- Additive solution:	€ 8.6	€ 6.53
- Analysis:	€ 78.5	€ 78.5 / 2 = € 39.25
Direct personnel costs (* adjusted to average time for each process):		
-Nursing	€ 16 /h x 0.87* = 13.92 €	€ 16 /h x 1.2* / 2 = 9,6 €
- Doctor:	€ 22.42 /h x 0.87* = 19.5 €	€ 22.42 /h x 1.2* / 2 = 13.45 €
Indirect costs (donor) (food,transport,parking)		
- Miscellaneous	€ 20	€ 20 / 2 = € 10
Cost final product/s:	€ 300.95	€ 159.05
Component saving:		- € 141.9
Products obtained:	114	114
Final process cost:	€ 34308,3	€ 18131.7
Final saving (total processes):		- € 16176,6

Table 1

	Donor				Process		
	Age	BS (m^2)	Platelet ($x10^9$/L)	Blood vol. (ml)	Program Platelet ($x10^{11}$)	Time (min)	Plasma Vol (ml)
S.D	45.87 ± 9.64	1.9	238,52± 44.27	4797.83 ± 818.2	3.45 ± 0.37	52.77 ± 7.80	318.73 ± 16.46
D.D	44.95 ± 7.24	1.98	291,63± 30.74	4999.23 ± 620.9	6.08 ± 0.23	72.58 ± 9.21	321.35 ± 19.54
p	NS	NS	<0,0001	0,1035	-----	-----	NS

Product			
Net volume (ml)	Platelets / μL	Plat./ bag ($x10^{11}$)	Vol Intersol (ml)
311.12 ± 10.76	1214.37 ± 195.71	3.78 ± 0.63	234.35 ± 22.72
308.68 ± 11.57	1058.47 ± 142.93	3.28 ± 0.38	233.76 ± 26.75
NS	-----	<0,0001	NS

Table 2. Estimated saving €

Results: In the period analysed, 547 and 57 processes were carried out, single and double respectively. We selected 114 final outcomes from each group, 41 men and 16 women in double plateletpheresis; and 81 men and 33 women, in single process. We also detected one (double) and five (single) adverse effects in each group. The results in donors, donations and processes are showed in Table 1. Significant differences were detected in Platelet pre-process and number of platelets in final bag. Collects platelets and plasma complying with the European Guidelines and Spanish CAT rules. Estimated saving obtained per double-dose plateteletpheresis dose was €141.9 and the total for the period analysed was € 16.176,6 (details in Table 2).

Conclusions: Donors selection with high count of platelets allow us to perform Double Dosis process so we could save an important amount of money compared with the Single process, complying with the European Guidelines and Spanish CAT rules. Like we expected we have confirmed significant differences in some variables. These results push up to promote the donation of Double Dosis process in donors with high count of platelets vs Single process in the future with better cost-efficiency of our procedures.

P-124

EVALUATION OF A NEW APHERESIS SYSTEM FOR PLATELET COLLECTION

B Case[1], S Wagner[2], J Gottschall[3], D Peckenpaugh[1], P Whitley[2] and S Graminske[3]
[1]Fresenius Kabi, Lake Zurich, Illinois, United States of America [2]American Red Cross, Rockville, Maryland, United States of America [3]Blood Center of Wisconsin, Milwaukee, Wisconsin, United States of America

Background: The AmiCORE Apheresis System (Fresenius Kabi) utilizes centrifugation separation technology to produce plateletpheresis products. The system uses a single needle apheresis kit capable of collecting single and double dose plateletpheresis products.
Aim: This investigational study evaluated the safety of the AmiCORE Apheresis System and platelet quality characteristics after storage for 5 days of 15 single units and 2 double units from 17 unique subjects.
Methods: Procedures were performed under an IRB approved protocol at three sites. Subject pre- and post-donation parameters, procedure characteristics, and platelet *in vitro* quality assays during 5 days of storage were measured. Platelets were collected in 100% plasma and stored under standard blood banking conditions.
Results: Pre- and post-procedure subject vital signs and cell counts remained stable. No adverse events were reported from any subject and no significant hemolysis was observed in the plasma post-procedure as demonstrated by the low reported plasma Hgb concentration of 8.7 mg/dl (lower level of sensitivity for the assay). The average subject height was 179 ± 9.3 cm and weight was 85.5 ± 12.8 kg. Prior to apheresis collection, subjects had average Hct of 42.8 ± 3.1% and average Hgb of 14.9 ± 1.3 g/dl. Red cell loss from each procedure averaged 21.3 ± 1.5 ml. The AmiCORE predicted post-procedure platelet count was consistent with reported laboratory values. Collection times averaged 48.8 ± 10.8 min and collections had an average platelet collection efficiency of 78.4 ± 8.1%, producing a total average platelet yield of $3.8 \pm 1.0 \times 10^{11}$. The average actual to targeted platelet yield ratio was 0.9 ± 0.1. On Day 5 of storage, pH levels were well maintained at an average of 7.47 ± 0.08. Total WBCs averaged $0.07 \pm 0.11 \times 10^6$/unit; all collections were $<1 \times 10^6$/unit. Other average *in vitro* measures of platelet quality are given in the Table 1.
Conclusion: In this clinical investigation, the AmiCORE Apheresis System safely and efficiently collected leukoreduced platelets. Leukocyte reduction and stable pH during storage met European Union requirements. The platelet products had acceptable *in vitro* platelet quality characteristics at the end of 5 day storage, similar to data from other plateletpheresis systems.
Note: The AmiCORE Apheresis System is CE marked for distribution in the EU. It is not FDA cleared for marketing in the United States of America. Regulatory approval has not been obtained in United Arab Emirates.

Assay	Day 1	Day 5
PLT Count (x10³/µL)	1247±190	1246±200
MPV (fL)	6.7±0.6	6.6±0.6
Morphology Score (0-400)	332±23	299±27
Hypotonic Shock Response (%)	76.4±17.2	64.6±14.7
Extent of Shape Change (%)	34.2±7.4	28.3±6.8
CD62p (% positive PLT)	19.8±19.0	32.8±11.4

Table 1. Average *in vitro* measures of platelet quality

P-125

IMPLEMENTATION OF ITRACE® DATA COLLECTION SOFTWARE WITH AMICUS®: IMPACT IN ACHIEVING SELF-SUFFICIENCY OF APHERESIS PLATELETS IN A PORTUGUESE BLOOD BANK

F Robles de Vasconcelos, C Vaz, F Cardoso, I Costa, A Silva, G Regalado, L Ricca Gonçalves and C Koch
CH São João, Porto, Portugal

Background: Portugal produces around 5,000 Single Donor Platelets (SDP) per year by apheresis. These SDP are collected in 10 Sites: three belong to the Portuguese Institute of Blood and Transplantation (Instituto Português do Sangue e Transplantação – IPST) and the other seven correspond to National Hospitals in different geographic areas.
Centro Hospitalar São João (CHSJ) is a University Hospital and a tertiary care centre with 1,105 beds making it the largest hospital in northern Portugal, with a high degree of differentiation between services. On average 50,000 blood components are transfused yearly in this Hospital. Over the last years, the Department of Transfusion Medicine and Blood Bank has been able to achieve self-sufficiency in platelets both derived from whole blood – Random Donor Platelets (RDP) – and from apheresis – Single Donor Platelets (SDP). This Hospital implemented Platelet Apheresis in 2011, however, in order to better fulfill the demand of Single Donor Platelets (SDP) for particular patients with high need of platelet transfusions, some actions were taken in the beginning of 2015. The target is 1,000 SDP per year and one of the most impacting actions was the implementation of iTrace®.
iTrace® is an advanced software application from Fresenius Kabi® for Data Management that allows wireless transfer and storage of apheresis data automatically from the Amicus® Cell Separator Platform in a computer. This application also allows the operator to input relevant information as additional observations or quantitative data.
Aims: Evaluation of the impact of iTrace® software implementation in the management and number of Platelet Concentrates collected by Apheresis with Amicus®.
Methods: Relevant items were evaluated before and after iTrace® implementation, especially the number of platelet collections on a routine basis (by month).
The workflow during Platelet Collection Procedure was adjusted and the process was optimized. The ratio of human resources was not changed.
Paper records were replaced by electronic records, ready for easy and fast search.
Donor profile was analyzed and recruitment was done using the database available: paper records before iTrace® implementation and Computer Database after iTrace® implementation.
Results: The number of collections on a monthly basis switched from 65 to 80 apheresis procedures, comparing the periods before and after iTrace® implementation. This evolution represents an increase of more than 20%, without human resources investment.
Summary/Conclusions: The iTrace® application associated with the Amicus® Cell Separation Platform (Fresenius Kabi®) offers summary *reports*, event and entry reports, search function, query module and data export. These functions have a major positive impact on staff productivity reducing the time spent on paperwork and data retrieval. The immediate access to historical donation records contributes to efficient donor scheduling.
As an interesting fact we confirmed that there was no increase of assigned errors despite the increasing number of apheresis collections.
iTrace® not only contributes to streamline the process but it also provides key features that enhance traceability for regulatory compliance.
Among all the strategies we have developed in order to increase the number of platelet donations by apheresis, we believe that the implementation of an advanced data management software like iTrace® was an essential added value to be successful in achieving that goal.
If the trend persists, the intended goal of 1,000 SDP per year will be fully accomplished until the end of 2016.

P-126

WHOLE BLOOD AGITATION IMPACT ON THE PALATELET COUNT OF THE PLATELET CONCENTRATES

C Amaouz
Blood Transfusion Center – CHU Mustapha, Algiers, Algeria

Background: Standard guidelines for blood transfusion recommend, during the whole blood levy, a constant gentle agitation to avoid onset of coagulation and clots forming. However, some blood transfusion centers don't have any blood bag agitators, so the nurses are obliged to agitate them manually, which isn't really efficient and doesn't respect the standard guidelines. Because, even if we can't see any clot in those bags, it doesn't mean that there are no micro clots inside. One of the inconvenients of micro clots forming in the whole blood, is the consumption of platelets, which can have an impact on the quality of the Platelet Concentrates.
Aim: The aim of this work is to evaluate the Whole Blood agitation impact on the platelet count of the Platelet Concentrates.
Methods: Whole bood was collected from healthy donors for whom a platelets count was done. 410 bags were collected in 2 months, for 210 of them it was under a gentle agitation, using blood bags agitators. For the other 210, a manual agitation was performed. Whole bood was collected using a triple blood bag system. Few hours after processing, simples were drawn aseptically from the platelets concentrates bags for a platelet count. A comparative study was carried out, according to the type of the whole blood agitation.

We hypothesized that gentle agitation using blood bags agitators, would improve quality of the platelet concentrates regarding to their platelets count.
Results: Comparing the two groups, there were no significant differences between the conformity rates of the platelets concentrates volume (59.04% vs 61.9%) $P > 0.05$. For the general aspect (89% vs 77%) *there was a significant difference* $P < 0.005\%$. Many bags weren't conform because of macro blood clots. These units were destroyed.
Even for the platelets count, there was a significant difference (60.67% vs 19.53%) with $P < 0.001\%$ which confirm our hepothesis.
Conclusion: Our study has proved statistically that collecting the whole blood must be done under an automatic agitation, for the quality of the red cells concentrate and the platelets concentrates. Otherwise, the blood products would be dangerous (micro clots in the red cells concentrate) and inefficient (low platelet count in the platelet concentrates). That's why we should advice each blood transfusion center to use the blood bag agitators.

P-127
EVALUATION OF INVITRO CHANGES AND TRANSFUSION EFFICACY OF APHERESIS PLATELET CONCENTRATES

A Tendulkar and P Jain
Tata Memorial Hospital, Mumbai, India

Background: Platelet concentrates (PC) collected by apheresis form an integral part of supportive care in hemato-oncology patients. The requirement is high in oncology centres, however due to a 5 day shelf-life, the platelets are in short supply. The *in-vitro* parameters related to morphology, viability and metabolism predict the *in-vivo* survival of platelets. Donor characteristics, cell separator type, storage container, type and frequency of platelet agitation may affect the *in-vitro* parameters. This study helped us to strengthen our quality assurance programme.
Aim:
(1). To study the *in-vitro* quality parameters in platelet concentrates stored over a period of 5 days.

Table 1: Quality parameters of Apheresis Platelet Concentrates (n=50)

Parameter	Day 0			Day 4			P-value
	Mean	Median	Range	Mean	Median	Range	
Volume (ml)	293.98	293	276-320	NA	NA	NA	
Yield (x10e11)	4.29	4.38	2.52-6.27	4.22	4.3	2.45-5.90	0.09
Platelet Concentration (/µl)	1459	1464	871-2150	1448	1487	870-1899	0.616
Sterility check	B/C negative			B/C negative			
Swirling (score)		4	2-4		3	1-4	
Morphology Score (kunicki)	310	312	260-354	265	280.5	188-310	<0.001*
Mean Platelet Volume (fL)	7.9	7.8	5.6-12.5	8.2	7.9	5.9-12.7	<0.001*
pH	7.114	7.121	6.88-7.31	6.984	7.023	6.64-7.17	<0.001*
pO2 (kPa)	14.11	14.077	10.4-17.2	15.25	15.30	11.8-20.88	0.004*
pCO2 (kPa)	5.343	5.303	3.03-7.97	3.233	3.232	1.25-4.55	<0.001*
OCR	0.483	0.486	0.21-0.76	0.421	0.440	0.03-0.66	0.01*
Bicarbonate level (mmol/L)	17.50	17.85	10.90-21.8	8.75	9.55	5.1-13.1	<0.001*
Glucose level (mmol/L)	15.305	15.485	10.27-18.2	9.9	10.3	2.55-14.93	<0.001*
GCR (day 0to4)(mmol/day/10^{12}platelets)	0.317	0.286	0.11-0.64	NA	NA	NA	NA
Lactate level (mmol/L)	5.25	4.4	2.1-13.2	14.17	13.95	7.1-21.5	<0.001*
LPR(day 0 to 4) (mmol/day/10^{12}platelets)	0.53	0.53	0.05-0.89	NA	NA	NA	NA

Caption 1. Quality parameters of apheresis platelet concentrates

Table 2: Clinical Efficacy of Apheresis Platelet Concentrates

Patient Parameters	Mean	Median
Weight (kg.)	59.30	58.5
Height (cm.)	158.44	158
Body Surface area (m^2)	1.6135	1.6125
Pre-transfusion Counts (/µl)	17540	14500
Platelet Dose	4.23	4.30
CI 1hr (/µl)	31500	27000
CCI 1hr (/µl/m^2)	13525	11319
CCI 24hr (/µl/m^2)	7893	6165
PPR 1Hr (%)	36.6	30.55

Caption 2. Clinical efficacy of apheresis platelet concentrates

(2). To evaluate the clinical efficacy of transfused platelets in in-house patients.
Material and methods: Single Donor Platelets (SDP, n = 50) were prepared from voluntary apheresis donors on Amicus® (version 3.2; Fenwal Inc., USA) cell separator which provides a 3 log leukodepletion. The units were studied on day of collection and day 4. The parameters studied were: Volume, yield, sterility, Mean platelet volume (MPV), Swirling, Kunicki score, pH, partial pressure of O2 and CO2, bicarbonate levels, Glucose levels, rate of glucose consumption (GCR), lactate, rate of lactate production (LPR), Oxygen consumption rate (OCR). The units after being studied on day of collection (day 0) and day 4, were transfused to the adult inpatients presenting with thrombocytopenia. The clinical parameters studied post transfusion were Count Increment (CI), corrected count increments (CCI) and Percentage platelet recovery (PPR). The statistical analysis was performed using paired t test for parametric data and Kruskal- Wallis test for non-parametric data.
Results: The mean volume and yield of the PC on the day of collection was 298 ml and 4.29×10^{11} respectively. The bacterial culture of PC was negative on day 0 and day 4. Swirling was maintained throughout the storage duration. Significant differences were observed in Kunicki's morphology score, MPV, pH, pO2, pCO2, glucose, bicarbonate and lactate levels, oxygen and glucose consumption rate and lactate production rate when measured on day 0 and day 4. There was no significant difference in platelet concentration over the storage duration. The height, weight and Body Surface area (BSA) of the patients receiving platelet transfusions was documented. The mean CI was 31,500/µl. The mean CCI at 1 h and at 24 h was 13,525 and 7,893/µl respectively. The PPR was 36.6%. No adverse reaction was reported in any patient secondary to transfusion of these apheresis platelets.
Conclusion: Invitro testing of quality indicator parameters for morphology, viability and metabolism were performed and were well maintained over a 5 day storage period. The clinical efficacy was evaluated and our patients responded well to the platelet therapy indicating that quality at various steps of apheresis platelet processing were in place.

P-128
EFFECT OF ORAL CALCIUM SUPPLEMENTATION IN PLATELETPHERESIS DONORS

P Pandey
Jaypee Hospital, Noida, India

Introduction and aim: Earlier, only few blood centers in India had the facility of plateletpheresis (PP) but as a result of periodical dengue epidemics and increased awareness among the clinicians, now SDP is the product of choice in present clinical practice. It is well known that citrate induces ionized hypocalcaemia by the chelating effect during plateletpheresis procedures. The aim of the study was to observe the effects of oral calcium (Ca) supplementation before and during plateletpheresis on various parameters related to calcium metabolism.
Materials and methods: This prospective observational study was performed at a tertiary healthcare center in the national capital region of India on 200 Plateletrpheresis donors. All the PP procedures were performed using the closed system kits and the ACD to Whole Blood ratio was kept in the range of 1:9–1:10, throughout the procedure.
These 200 donors were divided into two categories. In first category of the donors (n = 100), no prophylactic oral calcium supplementation was given. In second

Parameters	Pre-plateletpheresis		Post-plateletpheresis		Difference	T value	P value
	Mean±SD	Range	Mean±SD	Range	Mean±SD		
Total Ca (mg/dl)	8.49±1.33	4.9-10.3	8.27±1.27	5.1-9.8	0.22±1.17	1.29	0.1970
Total Mg (mg/dl)	2.04±0.49	1.1-4.2	1.94±0.43	1.5-4.4	0.10±0.32	2.07	0.0384
Serum Alb (mg/dl)	4.16±0.69	2.6-5.8	4.02±0.62	3-5.6	0.15±0.46	2.13	0.0332
Ionized Ca (mmol/l)	1.25±0.12	1.09-1.5	1.12±0.10	0.85-1.5	0.13±0.09	9.72	<0.01*

Table 1. The effect of plateletpheresis on Biochemical parameters of donors with calcium supplementation (n = 96)

Parameters	Pre-plateletpheresis		Post-plateletpheresis		Difference	T value	P value
	Mean±SD	Range	Mean±SD	Range	Mean±SD		
Total Ca (mg/dl)	9.02±0.60	7.8-11.1	8.23±1.28	5.1-12.2	0.79±1.01	5.29	<0.01*
Total Mg (mg/dl)	1.92±0.27	0.99-2.3	1.79±0.26	1.09-2.2	0.13±0.17	5.23	<0.01*
Serum Alb (mg/dl)	4.21±0.58	3.3-5.6	4.01±0.58	3.2-5.6	0.20±0.32	4.16	<0.01*
Ionized Ca (mmol/l)	1.14±0.13	0.9-1.5	0.91±0.13	0.47-1.12	0.23±0.09	16.64	<0.01*

Table 2. The effect of plateletpheresis on Biochemical parameters of donors without calcium supplementation (n = 93)

category of the donors (n = 100) 1,000 mg of prophylactic oral calcium carbonate was given 1 h before the start of the procedure and 500 mg (total = 1,500 mg) during the procedure.

Biochemical parameters like serum total calcium (T Ca), serum total magnesium (T Mg), serum albumin level (Alb) were analyzed on Vitros 5,600 (Ortho Clinical Diagnostics, Johnson and Johnson, USA) and ionized calcium level (iCa) on ABL800Basic (Denmark). All the analyses were done using the manufacturer's instructions and the SOP of the concerned department.

Results: In PP donors who did not receive prophylactic oral calcium carbonate supplementation as per the study protocol, we did observe seven donors (7/100, 7%) who presented with signs and symptoms suggestive of hypocalcemia in form of perioral tingling, feeling of vibrations in extremities and mild shivering and finally they required calcium supplementation. Among the PP donors who were administered calcium before and during the procedure, only four of them presented with above symptoms (4/89, 4.5%) and required oral calcium supplementation above the dosage protocol of the study. Thus, for the final analysis of biochemical parameters there were only 93 PP donors in the 'no oral calcium carbonate supplementation category' while there were only 96 donors in 'prophylactic oral calcium carbonate supplementation category'.

Amongst the PP donors, who did not receive prophylactic calcium before and during the procedures, there was a statistically significant fall in total calcium, ionized calcium level, total magnesium and serum albumin level (Table 1). Likewise, in prophylactic calcium intake group, we did observe a significant drop in total magnesium, serum albumin and ionized calcium level. We did observe a drop in total calcium level also in these donors; however, this observation was not statistically significant (Table 2).

Those donors who were not administered prophylactic calcium before and during the procedure showed a relatively higher risk (RR = 1.75) of citrate toxicity.

Conclusion: Prophylactic oral calcium carbonate supplementation would help in to reduce the risk of citrate toxicity in these donors. Therefore, we suggest for prophylactic administration of elemental calcium carbonate to make PP procedures uneventful. It will also help in future retention of these donors.

P-129

This abstract has been withdrawn.

P-130

THE EVALUATION OF PLATELET FUNCTION, SERUM IRON AND PLASMA PROTEIN IN PLATELETPHERESIS DONORS IN CHINA

Q Zhou

Beijing Red Cross Blood Center, Beijing, China

Background: In 2012 July, China Health Ministry issued the whole blood and component donor selection requirement (GB18467-2011). The apheresis platelet donation interval shortened from 28 days to 14 days. But there is less paper to evaluate if frequency donation will affect the platelet function, serum iron concentration, or plasma protein concentration in plateletpheresis donators, because the blood residual in each disposable, pipeline is usually 50 ~ 100 ml per residual blood.

Group	pre-apheresis	30min	7d	14d
20 times	62.43±3.67	59.23±3.77	60.89±3.77	61.88±3.32
15 times	61.33±4.58	57.31±2.61	60.63±3.82	62.89±4.27
10 times	62.1±3.11	58.9±2.98	61.9±3.67	62.3±4.21
5 times	63.0±3.65	60.2±2.11	63.7±1.98	63.2±2.14
control group	61.3±4.12	61.3±4.12	61.3±4.12	61.3±4.12

Table 1. The change time of in high-frequency plateletpheresis donors

Group	Total protein	Serum Albumin	Serum iron
20 times	73.46±3.75	44.92±2.56	24.73±2.09
15 times	74.8±3.64	50.58±3.26	23.58±6.06
Reference range	60~80	40~55	11~30

Table 2. Plasma protein level and serum iron concentration in plateletpheresis donors

Aim: To evaluate the changes of PLT function, serum iron concentration, or plasma protein concentration before and after plateletpheresis, and the effect of donation frequency for plasma protein, serum iron and blood cell counts.

Methods: The study subjects were divided into four groups, 20 donations times as group1, 29 people; 15 times as group2, 12 people; 10 times as group 3, five people; 5 times as group 4, five people; and the establishment of healthy non-blood donors as control group. Two consecutive plateletpheresis donation were required, and donation interval was 14 days. The blood samples were collected after 30 min, 7 days, and 14 days for testing PLT function, plasma protein level, serum iron concentration.

Results: The function of PLT decreased after 30 min of Blood collection, the coagulation time was prolonged. But PLT functions will recover to the original level of pre-collection after 7 days of collection. In addition, for annual blood donation is 5 times or more than 20 times, no signification between blood coagulation time and PLT function. The plasma protein levels in 20 times group and 15 times were higher than control group, and no difference found for the serum iron levels compared with the control group. PLT content was maintained at stable fluctuations in 24 times donation per year, no blood donors were anemia.

Conclusion: The frequency blood donation will not affect PLT function, plasma serum iron and protein protein level in China. China can imprve the clinical platelet management from more double procedure.

P-131

IRON REPLACEMENT FOR DONORS: BENEFICIAL IMPACT OF A COMBINATION OF LONGER COURSE OF ORAL IRON SUPPLEMENTS PLUS TARGETED DIETARY ADVICE AND FOLLOW-UP REVIEW OF COMPLIANCE

JWM Chay, PH Thant, NA Nwe and HH Tan

Blood Services Group, Health Sciences Authority, Singapore, Singapore

Background: Despite oral iron supplements and information on dietary modification, low haemoglobin (Hb) level is currently the commonest cause of deferrals among female regular blood donors. An estimated 12% of all donation visits are unsuccessful because of a low pre-donation Hb value, with female donors accounting for approximately 90% of all donors deferred due to low Hb.

Aims: To assess the effectiveness of providing a combination of intensive advice over iron supplements, dietary modification and follow up compliance review on reducing low Hb deferral rates among female regular blood donors.

Methods: A pre and post intervention study of female blood donors was conducted in Outram blood bank, Singapore from 2012 to 2014. 67 regular female blood donors aged younger than 50 years who had donated whole blood at least two times and failed Hb test (Hb below 12.5 g/dl) at least two times in the last 12 months before

		Baseline n=67			Repeat n=37	
Serum Ferritin, mean(SD) mcg/l		13.1(9.4)			13.6(13.1)	
n=67	1 Yr before intervention			1Yr after intervention		
	Median	Mean(SD) No		Median	Mean(SD) No	
Visit	6	6.2(1.9)		4	4.1(2.1)	
Donation	3	3.0(.87)	51% of visits result in successful donation	2	2.1(1.2)	54% of visits result in successful donation
Deferral	2	3.2(1.9)		2	2.0(1.5)	
Low Hb deferral	2	3.1(1.8)		2	1.8(1.4)	

Picture 1

study period were enrolled and followed up for 1 year. Donors from this sub-set were selected because female regular donors are known to be at higher risk of developing anaemia from reports in the literature, as well as analysis of our own donor deferral data. Our current incidence of low Hb deferrals for female donors is 8.87% in those donating once a year and 23.95% in those donating 3–5 times a year.

During follow up, 100 mg of elemental iron per day for 10 days course after each successful donation or 20 days course after a low hemoglobin deferral were given. Intensive donor health education on importance of iron supplements and dietary intake of iron was provided during interviewing. Post-visit follow up calls were made to check and encourage compliance to both completing the course oral iron replacement and following dietary intake of iron.

Results: Pre and post intervention serum ferritin level, rates of successful donations and rates of low Hb deferrals, were compared.

Serum ferritin level were mean (SD) of 13.1 (9.4) μg/l and 13.6 (13.1) μg/l respectively before and after intervention; the difference was not statistically significant (P = 0.16).

Even though average total visits decreased, the average rate of successful donation increased from 51% to 54% and average deferral rates decreased from 49% to 46%.

Low Hb accounted for 98% of all deferrals prior to intervention; low Hb accounted only for 78% of deferrals post-intervention.

Conclusion: There was only a slight increase in the mean serum ferritin level from 13.1 μg/l prior to intervention and 13.6 μg/l post-intervention. However the 24% decrease in mean deferral rate due to low haemoglobin is promising and suggests a combination of donor care measures (iron supplements, dietary advice and compliance aftercare/check) can be effective in managing regular female blood donors who fail haemoglobin test frequently.

P-132

A RETROSPECTIVE STUDY OF LOW HAEMOGLOBIN AND IRON DEFICIENCY IN NEW AND REPEAT DONORS OVER A PERIOD OF 1 YEAR

S Afzal and T Hwee Huang

Health Sciences Authority, Singapore, Singapore

Background: With continuous development in medicine, the demand for blood is also ever increasing and the ultimate goal of any blood transfusion services (BTS) to maintain an adequate and safe blood supply becomes difficult to achieve. Among the challenges faced by BTS, the most important are donor recruitment, donor retention and the problem of iron deficiency and low haemoglobin among the donors. Frequent donations together with iron losses are the predominant factor of iron deficiency anaemia (IDA). Significant number of repeat donors is deferred owing to low haemoglobin. As donor retention proves to be more cost effective than donor recruitment, it is our aim to understand the causes behind the low haemoglobin to maintain our regular donor pool and implement ways to address these issues.

Aims: This study was carried out to determine the percentage of new and repeat donors who were deferred from blood donation owing to low haemoglobin, and to suggest ideas to help such donors increase their haemoglobin levels.

Method: This is a retrospective study carried out at the donation centre of Blood Services Group at Health Sciences Authority, Singapore. New as well as repeat male and female donors were included in this 1 year study period. A regular donor in Singapore can donate every 12 weeks if the haemoglobin is 12.5 g/dl or above. Based on their haemoglobin levels, the donors are deferred under three categories. Each category assigned a deferral code, 12–12.4 gm/dl (0006). 11.5–11.9 g/dl (0007) and <11.5 g/dl (0009). Females under code 0006 were not included as for females 12–13 g/dl is considered normal.

Results: Total female donors registered were 37,896 with new donors numbering 7,794 and repeat female donors counting to 30,102. New female donors with low haemoglobin (11.9 g/dl and below) were 2,305 whereas, total females with low haemoglobin were 10,565.

Total male donors were 50,244, out of whom, 11,548 were newly recruited and 38,696 were repeat donors. New male donors with low haemoglobin (12.4 gm/dl and below) were 119 whereas, total males with low haemoglobin were 1,881.

Percentage of female donors with low haemoglobin among both new and repeat donors was much higher than the male donors.

Conclusion: Iron deficiency anaemia (IDA) and low haemoglobin among donors is a major cause of concern. Donor selection is critical to blood transfusion safety and low haemoglobin deferrals guarantee the required standards of Haemoglobin content in the donated units. As repeat donors can better assure the reliability of blood supply and safety of blood and blood products, it is even more important to retain our repeat donors. However, our regular donors show higher prevalence of low haemoglobin. This may be because of blood loss especially during childbirth, menses, or even low nutritional iron intake in females and improper diet, blood loss or minor blood disorders like B-Thalassemia trait (BTT) in males. Referring such male donors to hospitals for a thorough check up may help pick up these traits. Performing a complete blood count for such donors and analysing their ferritin and haemoglobin A2 may help to differentiate between IDA and BTT. The problem of prevalence of low haemoglobin among donors can be brought to the attention of Health Promotion Board as efforts in prevention at the community level.

P-133

THE IRON PROFILE OF SOUTH AFRICAN DONORS

K van den Berg, C Ingram and R Swanevelder

South African National Blood Service, Port Elizabeth, South Africa

Background: The Blood Transfusion Services (BTS) of South Africa (SA) are dependent on a healthy, regular donor population to ensure a safe and sustainable blood supply. Iron deficiency, the most common nutritional disorder worldwide, impacts on donors' eligibility to donate. Regular blood donation can increasingly contribute to iron deficiency. Low haemoglobin (Hb) is one of the most common causes for deferral of SA donors, however, the status of these donors' iron stores is not known.

Aims: This study aimed to determine the prevalence of iron depletion and iron deficiency among SA donors and to identify associated risk factors.

Methods: We performed a cross sectional study among blood donors in SA. Each operational area within the two BTS, with active donor populations ranging between 35,000 and 105,000, were allocated 2 days during which enrolment occurred. Using consecutive sampling, specimens were collected from all eligible whole blood donors. Specimens only were collected from donors deferred for low Hb who consented to participate in the study. Each donor had a full blood count, serum-ferritin, iron and transferrin saturation performed. Locally determined normal reference ranges for s-ferritin were used in the analysis (males: 20 μg/l; females: 12 μg/l).

Results: In total 4,412 donors were included in the study. Of these, 202 (5%) were deferred donors and 2,323 (53%) were male. The race and age distribution mirrored the donor population with 60% White, 24% African, 8% Coloured and 6% Asian donors. The majority of the donors were repeat donors (70%) and aged 41 to 65 (42%). Mean Hb for females was 13.7 g/dl and for males 15.5 g/dl. Mean s-ferritin was 41.6 μg/l and 85.4 12 μg/l for females and males respectively. Mean s-ferritin levels differed by age, race, donor type, 2-year donation count and donation interval (Figure 1). Overall, 17% of the donors (18% of males and 16% of females) had low ferritin levels with or without low Hb. Low s-ferritin with a normal Hb were more common among males (16%) than females (9%), while 7% of females and 2% of males had low Hb and low s-ferritin (Table 1).

In multivariable analysis the odds ratios (OR) for low ferritin and/or low Hb was 9.05 (95% CI: 6.46–12.69) in females compared to males; 0.23 (95% CI: 0.17–0.31) for donors who donated 1 to 2 units vs 7–8 units in the preceding 24 months; 3.46 (95% CI: 2.61–4.61) in Black vs White donors and 2.79 (95% CI: 2.03–3.80) in donors aged 18 to 20 vs donors aged 41 to 65.

Conclusions: Iron depletion and deficiency are common among SA blood donors with male donors affected more than female donors. This finding differs from other studies, but is in part due to the use of different ferritin cut-off levels for males and females. Iron profiles improved with age and increasing donation interval, but deteriorated with increasing donation counts. Although more likely to have low iron stores, female donors maintained ferritin levels within a tighter range compared to male donors.

	Normal Hb & Normal Ferritin N(%)			Low Hb & Normal Ferritin N(%)			Normal Hb & Low Ferritin N(%)			Low Hb & Low Ferritin N(%)			P-value
	All	Male	Female	All	Male	Female	All	Male	Female	All	Male	Female	
All	3457 (78)	1863 (79)	1594 (76)	138 (3)	4 (0)	134 (6)	569 (13)	385 (16)	184 (9)	191 (4)	37 (2)	154 (7)	
Race													**< 0.001**
Asian	196 (71)	130 (73)	66 (65)	14 (5)	(0)	14 (14)	50 (18)	41 (23)	9 (9)	17 (6)	6 (3)	11 (11)	
African	739 (70)	356 (71)	383 (68)	78 (7)	2 (0)	76 (13)	159 (15)	124 (25)	35 (6)	79 (7)	14 (3)	65 (12)	
Coloured	291 (80)	142 (81)	149 (78)	12 (3)	(0)	12 (6)	44 (12)	26 (15)	18 (9)	13 (4)	3 (2)	10 (5)	
White	2187 (83)	1215 (84)	972 (81)	29 (1)	2 (0)	27 (2)	306 (12)	188 (13)	118 (10)	81 (3)	13 (1)	68 (6)	
Unknown	44 (72)	20 (71)	24 (72)	5 (8)	(0)	5 (15)	10 (16)	6 (21)	4 (12)	1 (2)	1 (4)	(0)	
Age													**< 0.001**
18 to 20	265 (69)	122 (78)	143 (63)	18 (5)	(0)	18 (8)	50 (13)	28 (18)	22 (10)	46 (12)	4 (3)	42 (19)	
21 to 30	851 (76)	429 (81)	422 (72)	41 (4)	(0)	41 (7)	148 (13)	87 (1)	61 (10)	58 (5)	7 (1)	51 (9)	
31 to 40	785 (81)	402 (83)	383 (79)	32 (3)	1 (0)	31 (6)	105 (11)	68 (14)	37 (7)	31 (3)	7 (1)	24 (5)	
41 to 65	1476 (80)	850 (79)	626 (81)	45 (2)	2 (0)	43 (5)	250 (14)	189 (17)	61 (8)	53 (3)	17 (1)	36 (5)	
65+	80 (79)	60 (79)	20 (80)	2 (2)	1 (1)	1 (4)	16 (16)	13 (17)	3 (12)	3 (3)	2 (3)	1 (4)	

Table 1. Haemoglobin and ferritin analysis by race and age.

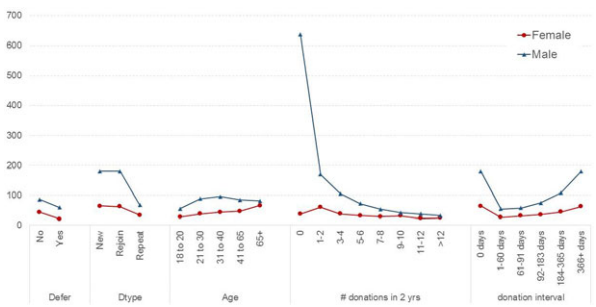


Figure 1. Ferritin levels by deferral status, donor type, age, 2-year donation count and donation interval.

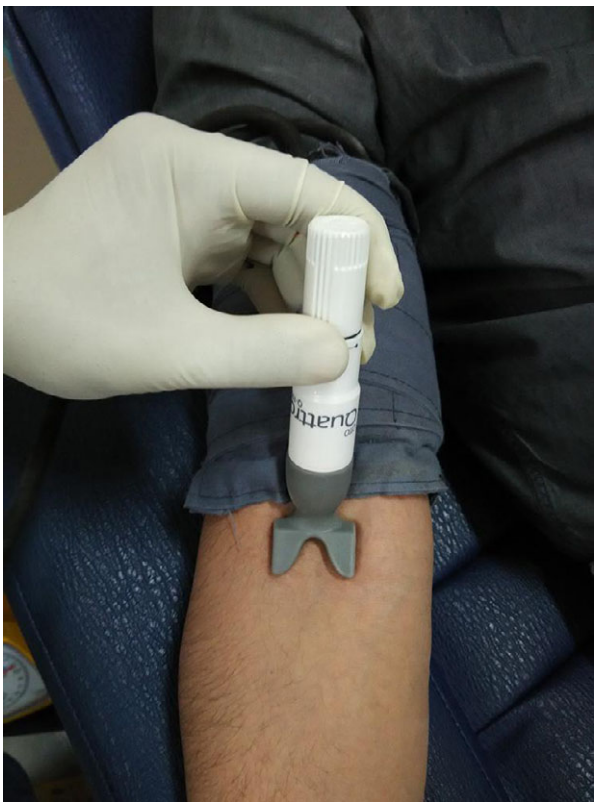


Figure 1. Vibrator device applied near phlebotomy site on donor arm

P-134

EFFECT OF VIBRATORY ANESTHESIA ON PAIN DUE TO PHLEBOTOMY IN BLOOD DONORS

N Saini, RK Bedi, K Mittal and T Sood

Government Medical College and Hospital, Chandigarh, India

Background: Fear of pain due to phlebotomy during blood donation is one of the major reasons for not donating blood especially among first time blood donors. The vibratory sensation can reduce the pain during venepunctures, various dermatologic and oral procedures in patients which is based upon the gate control theory of pain hypothesized by Melzack and Wall.

Aim: To study the effectiveness of vibratory anaesthesia in reducing pain due to phlebotomy in blood donors.

Method: A total of 74 voluntary blood donors (who had donated blood at least three times were included in the study because first time blood donors may report higher pain during phlebotomy due to anxiety and lower pain on subsequent blood donation visits) were recruited for this prospective study. We performed a routine phlebotomy procedure on all blood donors and rating to amount of pain during phlebotomy procedure was given by blood donors based upon 0–10 Numeric Pain Rating Scale (Figure 1). After 3 months all the donors were called again telephonically and requested for a repeat blood donation but only 40 came for repeat donation (34 donors did not turn up for repeat donation because of various reasons like busy schedule, out of station, donated somewhere else). This time while performing phlebotomy, a compact battery operated Quattro Q-100 massager (Figure 2) was used to provide vibratory stimulus near the phlebotomy site and rating to amount of pain during phlebotomy procedure was given by blood donors based upon 0–10 Numeric Pain Rating Scale. The amount of pain experienced by blood donor during phlebotomy on first visit acted as control and was compared with the amount of pain on subsequent visit with vibratory stimulus. The phlebotomist was kept same, phlebotomy was conducted on same side of arm on both occasions and the blood bags from same manufacturer (to ensure similar needle sharpness) were used in the study to minimise the effect of these factors on the study.

Results: Out of 40 blood donors, 11 (27.5%) gave the same rating to pain due to phlebotomy without and with vibrator device. One (2.5%) donor gave higher rating to pain due to phlebotomy with vibrator device than without it. 28 (70%) donors gave less rating to pain due to phlebotomy with vibrator device than without it. There was significantly ($P < 0.0001$) lower amount of pain due to phlebotomy with vibrator device than without it in blood donors. The mean pain experienced by blood donors during phlebotomy was 3.95 (SD $\pm$ 1.22) and with application of vibrator device was with 2.22 (SD $\pm$ 1.42) with effective reduction in pain by 43.8%.

Conclusion: A vibratory device like massager is effective, easy to use, economical, reusable and safe method to decrease the pain experienced by blood donors during phlebotomy. The disadvantage of using vibration is that two operators are required during phlebotomy. This advantage can be overcome if instead of using massager the vibratory device could be included in the tourniquet band itself with an on/off switch. So far such a device is unavailable.

P-135

EFFECTIVENESS OF DONOR ARM DISINFECTION WITH ONE-STEP CHLORHEXIDINE-ALCOHOL BASED ANTISEPTICS

KY Choi[1], SJ Park[1], DJ Oh[2] and JH Kim[1]

[1]*Blood Transfusion Research Institutue, Korean Red Cross, Wonju, South-Korea*

[2]*Blood Services Headquaters, Korean Red Cross, Wonju, South-Korea*

Background: Transfusion-associated bacterial infection remains significant problem in transfusion medicine, and major source of bacterial contamination is donor arm skin derived. Optimal skin disinfection of the phlebotomy site is important in minimizing bacterial-contaminated risk of blood products.

Aims: In this study, efficacies of two-step skin disinfection methods at Korean Red Cross Blood Services and two one-step methods produced by different type of application were compared.

Methods: Two one-step application of 2% chlorhexidine gluconate/70% isopropyl alcohol (2% CHG/70% IPA) formulation and two-step application of 10% povidone-iodine (10% PI) and 70% isopropyl alcohol (70% IPA) for skin disinfection in 120 healthy volunteers were evaluated. A direct swabbing and plating techniques was used to enumerate bacteria present before and after disinfection. Staff experience of one-step disinfectants was monitored in three regional blood centers.

Results: Skin disinfection with CHG/IPA one-step disinfectants was shown 99.93% reduction of bacteria after disinfection, which is better disinfection efficacy as the two-step method showed 99.87% reduction of bacteria after disinfection. There are no difference in efficacy between types of applications for one-step disinfectants. Percentages of fraction defective and allergic skin reactions of two types one-step disinfectants were <2% and 10% respectively, which is satisfied evaluation criteria of appropriacy for one-step disinfectant based on staff experience.
Summary/Conclusions: In this study, the 2% CHG/70% IPA one-step disinfectants are more efficacious than 70% IPA/10% PI two-step disinfectant. The one-step application of 2% CHG/70% IPA formulation could replace the currently used sequential application of 70% IPA and 10% PI in predonation skin disinfection.

P-136

BLOOD DONATION PROCESS IN R. MACEDONIA IN THE PERIOD OF 3 YEARS (2013–2015)

M Blagoevska, L Mitevska, T Makarovska Bojadzieva and E Velkova
National Institute of Transfusion Medicine, Skopje, Macedonia

Background: R. Macedonia has 70 years tradition in blood donation. National Institute of Transfusion Medicine (NITM) is a unique public health care organization at national level, integrated as one institution since the year 2011, with the Institute of Transfusion Medicine in Skopje (ITM-Skopje), 3 Regional Centers (RC) and 18 Blood Transfusion Services (BTSs) all over the country. NITM provide blood and blood components for public and private healthcare organizations. With its 2 million citizens, the country has reached self-sufficiency in red blood cells, fresh frozen plasma and platelets, but immunoglobulin, albumin and coagulation factors are still importing.
Aim: To analyze the blood donor's structure at NITM, by different demographic characteristics: men/women, voluntary/family donors, pupils/students, deferred donors, in order to estimate is there a need for targeted activities for recruitment of new donors and retention of regular ones.
Material and method: Retrospective study was used to evaluate data from the registry of blood donations at national level, for the period of 3 years (2013–2015). Were analyzed different categories: total number of donations by BTSs, number of donations from voluntary and family blood donors, pupils (17–18 years old) and university students, first time and regular donors, as well as deferred donations with reasons for deferral.
Results: During the 3 years period were collected 153.984 blood units, with the biggest number of collection in the year 2013 (54.018). At mobile sites were collected 83.910 units (54.5%), while at BTS 70.074 units (45.5%), mainly from voluntary donors (152.800, 99.2%) vs family donors (1,284, 0.8%). Men donated more often blood than women (126.159 units, 81.9% vs 27.825 units, 18.1%). Total number of deferred donors is 18.029 (11.9% in 2013, 12.3% in 2014 and 10.7% in 2015), which represent 10.5% of all registered donations. Pupil's donations represent 10.1% of all donations vs students 6.5% donations in total. The temporary deferred donations were 4,542 (82.6%). The most often reasons for deferral were: low hemoglobin level (31% for women and 24.6% for men), infections (11.2%), hypotension (9.3%), hypertension (9%) etc. for the analyzed period.
Conclusions: There are significant discrepancies between men vs women donations; number of donations among university student's population is lower that among high school pupils, that can be accepted as a negative indicator in blood donor recruitment and a challenge for future activities. The blood donor population is getting old, which confirms the results of this study. In R. Macedonia there is a yearly national plan for donor sessions, but what is needed is a national strategy for blood donor recruitment and retention. Therefore, it is needed to take proper measures: targeted groups, adequate motivation, social marketing for blood donor recruitment and retention etc.

P-137

RATIONALIZATION AND OPTIMIZATION OF THE THERAPEUTIC PLASMA EXCHANGE APPLICATION

Z Kirov
Blood Transfusion Institute Nis, Nis, Serbia

Background: Therapeutic plasma exchange (TPE) is a nonselective,nonspecific method used for the separation of circulating agents, an automated apheresis procedure with extracorporal circulation aimed at reducing the concentration or elimination of pathogen from the blood plasma, to reimburse the removed volume with suitable liquid. TPE is used in a number indications, which are currently classified in to four separate categories. The category I includes diseases in which the TPE is standard – the primary treatment option. For disorders of category II, TPE is an additional, supportive therapy, usually in combination with other treatment modalities. In the category III are pathological conditions, in which the exact role of apheresis is not stil safely defined – validity of TPE application, as well as the achieved effects are individual. The category IV includes diseases in which performing of TPE has not been proven effective.
Aim: Continuous introduction of new and more effective immunomodulatoty and other medications, the indications for performing TPE and achieved effects require continuous critical re-evaluation. For this reason, the aim of this work was analisysis of the feasibility of this type of treatment, with the categorization of indications, are essential for understanding the place and role of TPE in the treatment of these patients group.
Methods: Retrospective analysis of indications for the total of 1,075 TPE during the period from 2010 to 2015, as well as categorization according to criteria AABB at the Blood Transfusion Institute Nis, Serbia, has been done. TPEs are performed using the blood cell separator Haemonetics MCS+, according to the applicable standards and recommendations about the number and frequency of procedures, optimized time of application and the amount of extracted plasma.
Results: All of 1,075 TPE procedures are analysed and categorized, and the following results were obtained, category I was represented in 44.2%, 31.8% category II, category II -III in 20.2% and category III in 7.8%, while category IV was not represented. The most common indication was myasthenia gravis (MG) 55 patiens,205 TPE, followed by Guillain – Barre syndrome with 34 patients,141 TPE, multiple sclerosis 29 patients, 109 TPE, hiperbilirubinemia 21 patients, 72 TPE, CIDP 13 patients, 49 TPE, and thrombotic thrombocytopenic purpura (TTP) was represented in eight patients, 35 TPE, and odher in I, II, III category.
Conclusion: The results suggest that the indications for TPE predominantly belong to category I and II, while disorders in the category IV were not presented. This can be interpreted as rationalization and optimization of the TPE application, as a special form/modality of the treatment. The positive therapeutic effect of TPE in the treatment ofpatients depended upon the nature of the basic disease, its stage, general condition, volume of plasma removed and additional therapy.

P-138

THE EFFECT OF A SINGLE WHOLE BLOOD DONATION ON COMPLETE BLOOD COUNT VALUES AND LYMPHOCYTE SUBSETS

M Almohammadi and A Borai
King Abdulaziz Medical City, National Guard, Jeddah, Saudi Arabia

Background: While many studies has studied the effect whole blood donation on parameters like complete blood count values, iron status and others. The effect of transfusion on the immune system theat is established and studied on transfusion recipient under the term (TRIM: transfusion related immunomodulation) many parameters in. The effect on the whole blood donors is less studied and established.
Aim: This study aimed to investigate the impact of blood donation on hematological and the immune system by measuring the values of complete blood count (CBC) and lymphocyte subsets by flowcytometry.
Methods: A total of 45 male subjects were recruited for whole blood donation to the study. CBC and WBC categories were measured before (visit A) and after the blood donation (1 day, visit B; 1 week, visit C; 3 weeks, visit D; and 10 subjects at 3 months, visit E). 16 male subjects were recruited for T-lymphocyte subsets from visit A to D only. Repeated measures ANOVA was used for comparisons of quantitative variables between different visits.
Results: Blood hemoglobin (Hb) concentration declined significantly (−7.7%, $P < 0.001$) 1 day after whole blood donation and returned to the same level after 3 months of donation. The reduction in hematocrit (Hct) levels extended up to 3 months of donation (−3.4%, $P < 0.001$ respectively). Platelet increased 8.7% ($P < 0.001$) up to 3 weeks of whole blood donation (5.0%, $P < 0.05$). Lymphocyte subsets with CD3, CD4, CD16–56 and ratio were increased significantly (2.6%, 6.3%, 16.2% and 9.2%, $P < 0.05$ respectively) 1 week after donation. The increased in ratio was extended up to the third week (13.7%, $P < 0.05$).
Conclusion: After whole blood donation, the transit changes in the counts of lymphocyte subsets and hematological indices reflect a critical change in the immunological status of healthy blood donors which can extends up to 3 weeks. To arrive at a final conclusion, additional studies looking specifically at the changes in the immune sysyem of whole blood donors are needed.

P-139

ANALYSIS OF BLOOD COLLECTION AT NATIONAL INSTITUTE OF HEMATOLOGY AND BLOOD TRANSFUSION, VIETNAM DURING 10 YEARS (2006–2015)

N Manh Quan, TH Le, HH Ha, TD Pham and AT Nguyen

National Institute of Hematology and Blood Transfusion, Ha Noi, Vietnam

Background: Founded in 1984, the National Institute of Hematology and Blood Transfusion, Viet Nam (NIHBT) has been known as first ranking and national blood transfusion center of the network on blood transfusion services in Viet Nam. In 1994, Viet Nam started to launch blood donation program in order to ensure safe and adequate supply of blood and blood components to meet growing patient's needs. After more than 20 years, positive results of blood donation programs have promoted the development and centralization of Vietnamese blood services.

At NIHBT, blood donor recruitment and blood collection service has been improved gradually, contributing to the increase in blood collection and rate of voluntary non-remunerated blood donor.

Aim: This paper aims to review the situation and results of blood donor recruitment and blood collection at the Institute of Hematology and Blood Transfusion during 10 years (2006–2015).

Methods: A retrospective study was conducted on statistical records of blood donor recruitment and blood collection at NIHBT in the past 10 years (2006–2015).

Results: In 2006, NIHBT collected 65,015 blood units; 69.9% of them were voluntary non-remunerated blood donation. In 2008, centralization for blood transfusion service of the nation was successfully implemented (blood for hospitals has served by centralized blood centers). Moreover, the completion of Regional Blood Transfusion Centers Project resulted in the new buildings and higher capacity of NIHBT. In 2015, a total of 251,632 blood units were collected; the rate of voluntary non-remunerated blood donation reached 98.3%. This collection accounted for 22.8% of blood collection of the whole country; meanwhile this percentage was 15.5% in 2006.

In the past 10 years, there has been significant expansion of platelet donations and platelet usage for treatment. The figure showed that units of platelet apheresis increased from 857 units in 2006 to 23,660 units in 2015.

Like other countries, hemoglobin test is compulsory for blood donors in Viet Nam. Since 2007, NIHBT has applied this kind of test; the rate of blood donors who cannot donate due to low hemoglobin ranging from 3.4% to 6.3%. Hepatitis B virus has been estimated to infect approximately 8% of Viet Nam's population where belongs to the regions with highest prevalence rates; therefore, quick test for hepatitis B virus must be done for blood donor screening. It was found that this deferral rate tends to decrease in recent years, from 8.1% to 5.2%.

Conclusion: A steady increase of blood collection in 10 passing years has proved the effectiveness of blood donation campaigns as well as centralization for blood transfusion service. This is an important base to improve the safety and sufficiency of blood products.

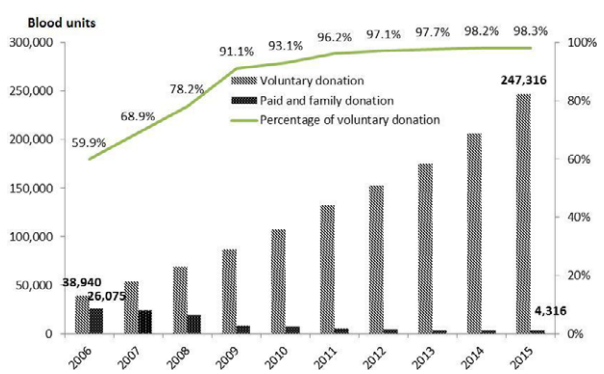


Chart 1. Result of blood collection at NIHBT during 2006–2015

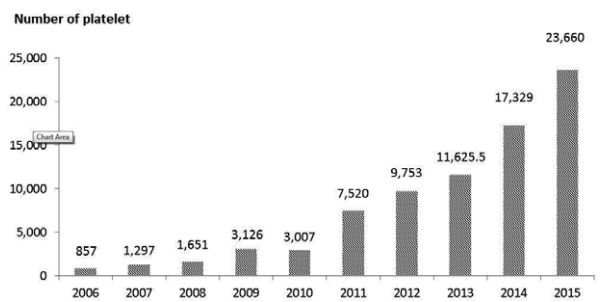


Chart 2. Platelet apheresis collection at NIHBT during 2006–2015

P-140

FREQUENT PLASMA DONATION REJUVENATES LYMPHOCYTES

EC van der Schoot, R Groenland, G Vidarsson and MA Berkowska

Sanquin, Amsterdam, The Netherlands

Background: Selected groups of donors undergo hyperimmunization to produce plasma rich in immunoglobulins (Ig) of a required specificity. Among these are anti-D donors immunized with RhD to produce Igs for anti-D immunoprophylaxis. To cover high demand for anti-D Igs many donors donate frequently for multiple years. Although frequent plasma donation is known to reduce serum protein levels, including proteins involved in lymphocyte homeostasis, effects of plasma donation on circulating B- and T cells have never be thoroughly investigated. Furthermore, long-term effects of hyperimmunization remain poorly investigated.

Aim: To study the effects of plasma donation and hyperimmunization on the immune system.

Methods: Peripheral blood samples were obtained 54 high-titer (HT) anti-D donors, who were immunized on average ~9 times in their donor career, a control group of 40 healthy age-matched individuals, who did not donate plasma. To correct for the effect of hyperimmunization, we included a cohort of 40 plasma donors, who were age-matched (58.5 y.o) with the HT donors, and donated plasma with a similar frequency (7.5 times/year vs 7.9 times/year in HT donors). Finally, we included a cohort of 49 low-titer (LT) anti-D donors to correct for the effect of frequent plasma donation. To study the direct effect of plasmapheresis on T- and B-cells subsets a detailed flow cytometric analysis of circulating B- and T cells was performed. Additionally, in all these groups we measured serum levels of Igs and BAFF.

Results: Significant differences were found between the different groups, and most of these differences were found to be dependent on the frequency of plasma donation. Only elevated level of BAFF was the most discriminative factor between hyperimmunized and regular plasma donors. Frequent plasma donation was associated with significantly higher frequency of naive B cells (CD27−, IgM+) and naive T cells (CCR7+ CD45RO−) on expanse of IgA+ and IgG+ memory B cells ($CD38^{dim}CD27+$), plasmablasts ($CD27+ CD38^{high}$) and effector memory T cells (TemRO, CCR7− CD45RO+ and TemRA, CCR7− CD45RO−). For most of the donors, these values remained within a normal range.

Conclusions: Frequent plasmadonation results in rejuvenation of lymphocytes. The mechanism underlying this juvenation remains elusive, but our data suggest increased loss of memory cells during plasmapheresis, and an influx of naive cells a few hours after the procedure. Further studies are required to evaluate the impact of lymphocte rejuvenation on donors health.

Donor Adverse Events

P-141

DO BLOOD DONORS WITH VASOVAGAL REACTIONS RISK DEVELOPING MORE THAN ONE REACTION PER DONATION?

G Facco, V Piccinini, L Catalano, G Marano, S Pupella and GM Liumbruno

National Institute of Health, Rome, Italy

Background: In recent years, studies from different countries have analysed the incidence and risk factors for immediate and delayed vasovagal reactions (VVR). To date, however, it is still not clear if donors with VVR might risk developing more than one reaction per donation.

Aims: The aim of the study is to investigate if donors, who experienced VVR, develop more than one reaction per donation when compared to donors who had other reactions (OR).

Methods: Data was retrieved from the national IT system that manages information related to all Italian blood activities, including Haemovigilance (HV). Blood donor

		First donors			Revisitors		
		Female	Male	total	Female	Male	total
Age (years)	16	1.55	0.54	0.96	0.80	0.31	0.52
	17	1.67	0.94	1.48	0.80	0.29	0.68
	P value	0.49	0.01	<0.001	0.99	0.85	0.06
Weight (kg)	< 50(60*)	2.79	0.84*	1.49	1.06	0.35*	0.6
	≥ 50(60*)	1.33	0.46*	0.95	0.75	0.27*	0.61
	P value	<0.001	<0.001	<0.001	0.09	0.43	0.92

Table 1. Frequency (/1,000 people) of vasovagal reaction after 320 ml whole blood collection for 2 years in 16- and 17-year-old donors

complications were classified following ISBT-IHN-AABB criteria. The records of 9,419,650 allogeneic whole blood and apheresis donations collected in 2012–2014, were assessed for VVR and OR. The incidence per 100,000 donations of more than one reaction other than VVR or OR per donation and their severity was evaluated.

Results: From January 2012 to December 2014, 16,081 VVR (170.7 per 100,000 donations), and 3,815 OR (40.5 per 100,000 donations) were reported. Another reaction during the same donation was reported in 233 donors who had VVR, and in 104 donors who had OR, of which 23.6% and 59.6% respectively were citrate reactions. The incidence of more than one reaction per donation, in donors who had VVR and OR, were respectively 2.4 and 1.1 per 100,000 donations ($P < 0.01$); of which, 0.55 per 100,000 donations were classified as moderate and 1.1 per 100,000 donations as severe.

Conclusions: The development of more than one reaction per donation seems to be a rare event, considering also that some of them are citrate reactions and thought to be related to collection by apheresis. The Authors believe that further data and sharing experience between different countries is necessary to confirm this finding.

P-142

FREQUENCY OF VASOVAGAL REACTIONS IN FIRST TIME WHOLE BLOOD DONORS IN KOREA

YA Lim[1], YI Seo[2], EJ Kim[3], JU Kim[4], K Na[5], J Oh[5], YS Choi[5] and JN Kim[5]

[1]Ajou University School of Medicine, Suwon, South-Korea [2]Blood Transfusion Research Institute, Korean Red Cross, Wonju, South-Korea [3]Daegu-Gyeongbuk Korean Red Cross Blood Center, Daegu, South-Korea [4]Headquarter of Korean Red Cross Blood Center, Wonju, South-Korea [5]Division of Human Blood Safety Surveillance, Osong, South-Korea

Background: It is well known that vasovagal reactions (VVR) frequently occur in young blood donors. The lower limit of age for whole blood donation (LLAWB) in Korea is 16-years. These donors represent about 12% of whole blood donors, and their donations correspond to 250,335 units of blood per year. However, in other countries, 17-years is defined as LLAWB; furthermore, there are weight and height restrictions for young donors, which are not used in Korea.

Aims: The aim of this study is to evaluate whether change of LLAWB as to 17 years could prevent VVR in first time young donors.

Methods: Data about weight, gender, donation frequency and history of VVR related to blood donations in 16-year-old and 17-year-old donors visited Korea Red Cross Blood Center during 2013 and 2014 were extracted from blood information management system. Low weight of donors was defined as <50 kg for female or <60 kg for male in this study. The data between age or weight groups were compared and analyzed using chi-square test.

Results: The frequency of VVR in 17-year-old first time donors (1.48) was significantly higher than that in 16-year-old first time donors (0.94, $P < 0.001$), especially in male donors (Table 1). Furthermore, the frequency of VVR in both female (2.79) and male (0.84) first time donors with low weight was significantly higher than those with normal weight ($P < 0.001$). There were no significant differences between the groups of age or weight within the revisitors group.

Conclusions: Our data suggest that the change of LLAWB as to 17-year-old years would not improve the prevention of VVR in first time young donors. However, as the frequency of VVR was higher in donors with low weight, the introduction of weight and height restrictions should be considered to reduce the occurrence of VVR for first time young donors in Korea.

P-143

SIGNS AND SYMPTOMS OF VASOVAGAL DONOR ADVERSE REACTIONS

H Kamel, MD Bravo and M Townsend

Blood Systems, Inc., Scottsdale, Arizona, United States of America

Background: The mechanisms underlying vasovagal reactions (VVR) in blood donors are thought to include the direct effects of removal of blood, the psychological stress of giving blood and the orthostatic effects superimposed on a hypovolemic state after the donation.

Aim: We report on the frequency of signs and symptoms of VVR associated with blood donations under operational environment.

Methods: We present data on needle-in allogeneic donations over 6-year period. Mild reactions are marked in the blood center computer system. Signs and symptoms are recorded for prolonged pre-syncope reactions (>30 min) and reactions associated with syncope. At any time during the reaction, rapid and slow pulses are defined as heart rate ≥100 and ≤50 bpm respectively, and hypotension is defined as systolic blood pressure (BP) that is >30 mmHg below and/or diastolic BP that is >20 mmHg below the pre-donation systolic and diastolic BP respectively.

Results: For 4.3 million venipunctures, there were approximately 56,600 VVR (rate of 1.3%). The majority (76%) were classified as mild. Signs and symptoms were recorded for 13,569 VVR (3,940 presyncope and 9,629 syncope). Syncope rate was 0.22% of all venipunctures and 17% of all VVR. More than 90% of falls and injuries were associated with syncope. Convulsions, other involuntary muscle movements, and incontinence were rare and mostly seen with syncope. Conversely, nausea/vomiting, chest pain, and difficulty breathing were more frequent in pre-syncope (Table 1).

Of the 2,164 syncopal reactions with hypotension, slow pulse was present in 15% and rapid pulse was present in 8%. Of syncopal reactions, 48% occurred before/during collections and 52% occurred post collection. Of syncopal reactions occurring before/during collections, more were associated with slow pulse than with rapid pulse (10% vs 6%). Conversely, of syncopal reactions occurring post collections, more were associated with rapid pulse than with slow pulse (11% vs 6%).

Signs and Symptoms	Percent of Pre-syncope (n=3940)	Percent of Syncope (n=9629)
Light-headedness	75	76
Pallor	60	75*
Nausea	58	34*
Sweating	53	69*
Vomiting	41	13*
Feeling warm	37	46*
Weakness	30	33**
Hypotension	19	22*
Anxiety	14	11*
Rapid pulse	13	8*
Irregular pulse	10	5*
Tingling	10	7*
Chills	8	5*
Muscle spasms, tetany	5	19*
Slow pulse	3	8*
Difficulty breathing	2	1*
Fall	2	15*
Continued vomiting	2	1**
Hyperventilation	2	1*
Incontinence	2	6*
Chest Pain	1	0.2*
Muscle twitching	1	7*
Injury	0.5	3*
Convulsions	0.03	2*

Two-way test of proportions comparing signs and symptoms in syncope and pre-syncope, Chi square test: * $p < 0.0001$, $p < 0.01$**

Table 1

Conclusions: Five signs and symptoms (light-headedness, pallor, sweating, feeling warm and weakness) were common to both types of VVR. Muscle twitching, spasms (tetany) were overrepresented in syncope. Nausea and vomiting were overrepresented in pre-syncope, suggesting that gastrointestinal manifestations may be protective against syncope. Syncopal reactions before/during collections are more likely to be vasovagal while those occurring post collection are more likely to be due to orthostatic effects superimposed on hypovolemia.

P-144

NO PROLONGED EFFECT ON COAGULATION DEFICITS AMONG REGULAR PLATELETPHERESIS DONORS

S Ahmed, WZ Abdullah, TH Tuan Mohammad and R Mustaffa

School of Medical Sciences/Universiti Sains Malaysia, Kubang Kerian/Kota Baru, Malaysia

Background: Plateletpheresis is a safe procedure that is routinely used nowadays for platelet donation. A handful of studies have evaluated the effects of plateletpheresis on platelet indices and coagulation parameters, mainly the pre and post donation effects of plateletpheresis. Immediate post-donation reductions in the levels of natural anticoagulant proteins have been reported. A possible association of the automated plateletpheresis procedure and the hypercoagulable state has been a major concern in regular plateletpheresis donors.

Aim: To investigate the effects of plateletpheresis procedure by measuring common coagulation parameters including the natural anticoagulant proteins prior to procedure during subsequent donations.

Methods: A comparative study was performed in a tertiary medical institution. Forty-one plateletpheresis donors with a frequency of five or more donations conducted within 18 months. For the control group, 58 healthy volunteers were selected for the study. Measurements included the common coagulation parameters including the natural anticoagulant proteins before and after the plateletpheresis procedure during subsequent donations and to compare the findings from the healthy non-blood donor volunteers (the control group). The rationale for the timing of blood testing is to study the recovery of the selected coagulation parameters particularly in the anticoagulant pathway which is known to be reduced following the procedure. The tests performed included those for prothrombin time (PT), activated partial thromboplastin time (APTT), protein C, protein S, antithrombin and von Willebrand factor (vWF) antigen assays. Blood samples were collected at pre-donation time and all the blood tests were run by Coagulometer (Diagnostica Stago, France).

Results: The results showed significant shortening of PT among regular plateletpheresis donors as compared to healthy controls. No significant differences with the other parameters were detected (APTT, protein C, protein S, antithrombin and vWF antigen) when compared with healthy controls.

Conclusions: The regular plateletpheresis procedure did not affect the common coagulation parameters tested in this study except for shortening of PT. This finding could be due to coagulation activation of unknown significance. A pre-analytical error is not very likely as the tests were performed according to the CLSI guidelines for coagulation tests. Future studies need to address this finding as it may be related to a hypercoagulable state. Regular health monitoring and proper selection of plateletpheresis donors should be exercised to ensure a life-long safety of this procedure among regular donors. This study however recruited a less intensive plateletpheresis donation program, which is one of its limitations. This effect requires to be confirmed in a larger scale study on intensive plateletpheresis donors.

P-145

HIGHER LEVELS OF THE INFLAMMATORY MARKER SOLUBLE UROKINASE PLASMINOGEN ACTIVATOR RECEPTOR (SUPAR) AMONG BLOOD DONORS WITH NON-O BLOOD TYPE – RESULTS FROM THE DANISH BLOOD DONOR STUDY

LW Thoerner[1], C Stilling[2], E Haastrup[1], AS Rigas[1], MH Nielsen[1], E Sørensen[1], MS Petersen[2], OB Pedersen[3], C Erikstrup[2] and H Ullum[1]

[1]Copenhagen University Hospital, Copenhagen, Denmark [2]Aarhus University Hospital, Aarhus, Denmark [3]Naestved Hospital, Næstved, Denmark

Background: Urokinase plasminogen activator receptor (uPAR) is a biomarker of inflammation and immune activation. It is a membrane-linked protein found in various cell types, including immunologically active cells and vascular endothelial cells. Elevated levels of its soluble form (suPAR) have been shown to reflect low-grade inflammation and are associated with lifestyle factors such as smoking, alcohol consumption, and sedentary lifestyle. Importantly, elevated suPAR levels are also associated with disease severity and mortality in several patient groups. Among those are patients with cardiovascular disease, type 2 diabetes, and cancer. In the general population suPAR is associated with premature mortality and assumed to reflect the state of chronic inflammation, similar to C-reactive protein (CRP), and elevated levels are associated with premature mortality.

	Non-smokers		
suPAR (ng/ml)	**Regression coefficient**	**95 % Confidence Interval**	***P***
ABO (Non-O)	1.01	1.00 – 1.03	0.008
Age (10 years older)	1.04	1.03 – 1.04	< 0.0001
Sex (women)	1.13	1.12 – 1.14	< 0.0001
Obesity (BMI≥ 30)	1.13	1.11 – 1.15	< 0.0001
Physical activity, work (high/low)	1.05	1.03 – 1.06	< 0.0001
Physical activity, leisure (high/low)	0.99	0.98 – 1.00	< 0.03
	Smokers		
suPAR (ng/ml)			
ABO (Non-O)	1.05	1.02 – 1.08	< 0.001
Age (10 years older)	1.08	1.07 – 1.09	< 0.0001
Sex (women)	1.12	1.09 – 1.15	< 0.0001
Obesity (BMI≥ 30)	1.07	1.02 – 1.11	0.004
Physical activity, work (high/low)	1.10	1.07 – 1.14	< 0.0001
Physical activity, leisure (high/low)	0.94	0.92 – 0.97	< 0.0001

Table 1

The ABO blood groups are associated with various disease phenotypes, particularly cardiovascular disease. Associations have been reported between non-O blood type and an increased risk of some cardiovascular disorders including thrombosis.

Aims: Given that suPAR levels are widely associated with lifestyle factors and diseases severity and outcome and that non-O blood type associates with lifestyle diseases, we aimed to explore the possible association between suPAR levels and ABO blood group.

Methods: Initiated in 2010, the Danish Blood Donor Study is a population-based epidemiologic cohort study and biobank with continuous enrollment currently encompassing >107,000 blood donors aged 18–67 years. At inclusion, blood donors complete a 4-page questionnaire, including questions on anthropometric measurements, smoking habits, diet, and self-perceived health including physical activity level. Additionally, DNA, plasma and data from Danish health registers are available. suPAR measurements were performed with commercially available ELISA kits (suPARnostic®, Virogates, Alleroed, Denmark).

Donors were stratified in two groups according to ABO blood group: One group with blood type O (36%) and another group (non-O) with the blood types A, B, and AB (48%, 11%, and 5%, respectively). Multiple regression analyses were applied to test for association between suPAR and blood type.

Results: Multiple regression analyses showed a higher suPAR level among blood donors with non-O blood type (n = 5,527) as compared with blood donors with blood type O (n = 6,883; P < 0.0001) adjusted for sex, age, body mass index, smoking status, and physical activity level. There was a significant interaction between ABO blood type and smoking (P = 0.02). Therefore, models were stratified for current smoking status. Among smokers the difference in suPAR level between non-O and O type blood donors was larger than the difference among non-smokers (Table 1).

Summary/Conclusions: Blood donors with non-O blood type have higher suPAR levels than donors with blood type O. This suggests that individuals with non-O blood type may have increased risk of low-grade inflammation associated with cardiovascular disease.

P-146

DECREASE INCIDENCE OF LOW IRON STORES IN BLOOD DONORS WITH ACCEPTABLE HEMOGLOBIN LEVELS FOLLOWING AN INCREASE IN INTER-DONATION INTERVAL: A SINGLE CENTER EXPERIENCE

M Gandhi, K Duffy, S Bryant, M Benike, JR Stubbs and R Singh

Mayo Clinic, Rochester, New York, United States of America

Background: Frequent blood donations may result in depletion of iron stores with normal hemoglobin as we reported previously. To mitigate this risk, we increased the inter-donation interval from 8 to 12 weeks at our blood donor center. To evaluate the effect on iron stores in our blood donors, we measured ferritin levels in a subset of our blood donors and compared this with the ferritin levels in a subset of donors prior to change in our policy.
Methods: One year after increasing our inter-donation interval from 8 to 12 weeks, over a 5 month period (August–December 2014), samples from 1,000 random whole blood donors (7% of donor pool) were tested for ferritin levels at the time of donation. Ferritin levels were evaluated using a two-site immunoenzymatic ('sandwich') assay on the Beckman Coulter UniCel DxI 800. Low ferritin was defined as <24 ng/ml for males and <11 ng/ml for females. Data was compared with the ferritin levels prior to change in our policy. Data was analyzed using the Wilcoxon rank sum test and Kruskal-Wallis test, as appropriate, using SAS 9.3 software, with significance defined as P-value < 0.05.
Results: Donor demographics and gender distribution was similar to previous study. Median age of the blood donor was 51 years (range: 17–87 years) with equal gender ratio. 96% of the donors were Caucasian. 272 (28%) donors had low ferritin as compared to 36% of the donors prior to policy change. Increase in the ferritin levels was seen in both male and female donors. Median ferritin levels for female donors was 21 ng/ml (range 3–792 ng/ml) as compared to 19 ng/ml (3–228 ng/ml) prior to change. Similarly median ferritin levels for male donors was 31 ng/ml (range 3–802 ng/ml) as compared to 24 ng/ml (3–228 ng/ml) prior to change. In addition there was an 18% decrease in hemoglobin deferral rate after implementing the increased inter-donation interval.
Conclusions: Increasing the inter-donation interval resulted in increased iron stores in regular blood donors with decrease in low hemoglobin deferrals.

Table

	8 week inter-donation interval	12 week inter-donation interval
Donor Median Age	53 years	51 years
Males	501	498
Females	499	502
Median Ferritin ng/mL		
Males	24	31
Females	19	21
Low Hemoglobin Deferral	12.5%	10.1%

Picture 1

P-147

IRON DEFICIENCY DOES NOT AFFECT THE TIME TO NEXT DONATION IN BLOOD DONORS – RESULTS FROM THE DANISH BLOOD DONOR STUDY

MH Larsen[1], AS Rigas[1], E Soerensen[1], OB Pedersen[2], MS Petersen[3], H Hjalgrim[4], K Rostgaard[4], C Erikstrup[3] and H Ullum[1]

[1]Copenhagen University Hospital, København Ø, Denmark [2]Naestved Hospital, Naestved, Denmark [3]Aarhus University Hospital, Aarhus, Denmark [4]Statens Serum Institut, Copenhagen, Denmark

Background: Iron deficiency is a well characterized challenge in a blood donor population. In The Danish Blood Donor Study (DBDS) we previously reported 38% of frequently donating young female blood donors to be iron deficient. Indeed the strongest predictors of iron deficiency are sex and number of previous donations. Although we have previously reported that iron deficient donors do not have worse self-perceived mental and physical health compared to iron replete donors, it is of interest if iron deficiency affects the return time to the blood donor center among the iron deficient donors. Theoretically, a subclinical tiredness not reflected in the self-perceived mental and physical health scores could lead to prolonged time to next donation among the iron deficient donors.
Aims: We aimed to explore if iron deficiency affects time to next donation among blood donors.
Methods: DBDS is a nation-wide study currently comprising more than 100,000 participants. At enrollment into the study participants fill in a questionnaire about diet, exercise, and anthropometric measurements. For the first 18,000 included participants in DBDS ferritin levels were available. Information on the date of the next donation after ferritin levels were measured was retrieved from the Scandinavian Donation and Transfusion Database. Survival analysis (follow-up time approximately 1,000 days) was done in order to determine if iron deficient donors were more likely to return for donation at a later stage. The analyses were adjusted for body mass index, age, smoking status, and number of previous donations. Analyses were performed stratified by sex.
Results: In all, 8,518 men and 7,572 women were included in the study. After 3,517 and 3,136 person-years of follow-up 7,690 and 6,449 men and women, respectively, returned to the blood bank for donation at a later stage. A total of 22.4% women and 3.2% men were iron deficient (ferritin <15 ng/ml). We found no association between iron status and time to next donation.
Conclusions: Iron deficiency does not affect the time to next donation among blood donors. Thus, there is no indication that iron deficiency results in major health problems on the short term. Iron deficiency is prevalent in a blood donor population and further investigations into possible health-related outcomes due to iron deficiency are warranted.

P-148

INCREASED RISK OF IRON DEFICIENCY AMONG INDIVIDUALS WITH NON-0 BLOOD TYPE – RESULTS FROM THE DANISH BLOOD DONOR STUDY

AS Rigas[1], OB Pedersen[1], K Rostgaard[2], E Sørensen[1], LW Thørner[1], H Hjalgrim[2], C Erikstrup[1] and H Ullum[1]

[1]Clinical Immunology, Copenhagen, Denmark [2]Department of Epidemiology Research, Statens Serum Institut, Copenhagen, Denmark

Background: Iron deficiency is prevalent among blood donors. In blood donor populations sex, menopausal status and number of previous full blood donations are the most important predictors of iron levels. The ABO blood group system has been associated with cardiovascular disease with the non-O blood group being associated with an increased risk of both venous and arterial thromboembolic events. Markers of inflammation, such as C-reactive protein, have been associated with cardiovascular disease risk and mortality. Recent genome-wide association studies have reported alleles in the ABO locus to be associated with both C-reactive protein and ferritin levels. These studies warrant the investigation of a possible association between ABO blood group and ferritin levels, and whether any such association might be mediated through an increased inflammatory level.
Aims: We aimed to explore if ABO blood type is associated with iron levels measured as ferritin levels.
Method: The Danish Blood Donor Study (DBDS) is a nationwide blood donor cohort with questionnaire-based information on anthropometric measures, exercise, diet and smoking status. Ferritin levels had been determined once for 30,595 DBDS participants. Linear regression analyses were performed on all participants with ABO blood group as explanatory variable and adjusted for age, number of donations in 3 years prior to the ferritin measurement, and time since latest donation. In addition, a sub-analysis was performed on 15,280 individuals whose ferritin levels had been determined at inclusion allowing adjustment for body mass index, age, smoking status, donation history, and C-reactive protein levels (CRP). Furthermore, logistic regression analyses were performed in order to determine if ABO blood group was associated with iron deficiency (ferritin <15 ng/ml).
Results: Iron deficiency was seen in 2.2% of 15,865 male and in 16.2% of 14,730 female donors. Non-O blood type donors had lower iron levels than blood type O donors, regardless of sex. Accordingly, risk of iron deficiency was statistically significantly increased for individuals with non-O blood type compared with O blood type (Men: OR 1.42; 95% CI 1.14–1.76; women: OR 1.13; 95% CI: 1.04–1.24). In sub-analyses adjusted for body mass index, smoking status, and CRP similar associations were observed, albeit in women the association between blood group and risk of iron deficiency did not reach statistical significance. Ferritin levels and CRP levels were only weakly correlated; $r = 0.004$.
Conclusion: Donors with non-O blood type have lower ferritin levels and an increased risk of having iron deficiency compared to donors with O blood type. These results warrant further investigation into the possible relationship between iron stores and ABO blood type.

	n	OR (ferritin<15ng/ml)	95%CI	p
	Men			
Avs0*	13,667	1.31	1.04-1.65	0.020
Bvs0*	8,590	1.58	1.08-2.30	0.018
ABvs0*	7,702	2.33	1.48-3.68	<0.001
Non0vs0*	15,865	1.42	1.14-1.76	0.001
	Women			
Avs0*	12,518	1.12	1.02-1.24	0.017
Bvs0*	8,060	1.19	1.02-1.39	0.026
ABvs0*	7,142	1.10	0.88-1.38	0.40
Non0vs0*	14,730	1.13	1.04-1.24	0.007

*Adjusted for age, time since latest donation, and number of donation in the previous 3 years.

	n	OR (ferritin<15ng/ml)	95%CI	p
	Men			
Avs0**	6,937	1.35	1.03-1.78	0.029
Bvs0**	4,276	1.63	1.03-2.59	0.037
ABvs0**	3,856	2.13	1.24-3.68	0.006
Non0vs0**	8,047	1.44	1.11-1.86	0.006
	Women			
Avs0**	6,156	1.10	0.97-1.24	0.13
Bvs0**	3,867	1.09	0.90-1.33	0.38
ABvs0**	3,406	1.13	0.85-1.52	0.40
Non0vs0**	7,233	1.10	0.98-1.23	0.10

**Adjusted for age, BMI, smoking status, time since latest donation, and number of donation in the previous 3 years, and CRP.

Caption 1. Logistic regression analyses

P-149

EVALUATION OF THE CELLULAR IMMUNE STATUS AMONG REGULAR MALAY MALE WHOLE BLOOD DONORS

S Ahmed, N Fauzi, R Mustaffa, WZ Abdullah, WH Wan Mahmood, CM Che Hussin and JAH Mat Hasan

School of Medical Sciences/Universiti Sains Malaysia, Kubang Kerian/Kota Baru, Malaysia

Background: Blood donations are an essential part of a healthcare system and it is crucial to ensure sufficient and safe supplies of blood from blood donation. However, it is important to ensure that the donor's health is not compromised by the donation of blood or blood components. Whole blood donors may lose approximately 1×10^9 lymphocytes in a single whole blood donation, with no significant drop in the peripheral blood lymphocyte count. No known immediate adverse effects are associated with the slight decreases in peripheral blood lymphocyte counts following whole blood donations. Few studies have addressed long term reductions in the counts of some immune cells as a consequence of regular blood donations. Thus, the possibility of blood donation causing changes in the immune status requires a close assessment.

Aim: To evaluate the levels of immunological cellular activity in regular blood donors.

Methods: A comparative cross sectional study was done at Hospital USM from May 2015 to April 2016. Peripheral blood was taken from 38 regular Malay male whole blood donors and from 38 non-blood donors Malay males. The standard hematological parameters and immunological cell counts were measured. Those included total white blood cells with neutrophils, lymphocytes, monocytes, eosinophils and basophils counts by using XE 5000 Sysmex hematology analyzer. Flow cytometry measured the absolute counts of CD3, CD4, CD8, CD16, CD56 and CD19-positive cells. The CD4:CD8 ratio was calculated using BD FASCANTO II.

Results: The entire cellular immune markers tested showed no statistical differences between the non-blood donors and the regular donors. The only significant finding was the monocyte absolute count. The mean monocyte absolute count for the non-blood donors and regular whole blood donors were 0.4939 ± 0.12925 and 0.5921 ± 0.15936, respectively. Using the independent t-test, the mean distance from monocytes absolute counts were different between non- blood donors and regular whole blood donors ($P = 0.004$). This result showed that regular whole blood donors had higher means than non-blood donors.

Conclusions: Regular blood donation showed no significant effects on cellular immune markers when compared with the non- blood donors' counterpart. The difference in monocytes absolute count between the two groups is probably related to variation of immune activity of no clinical significance. This study supports that there are no added risk to the regular blood donors as a result of immune changes.

P-150

VEIN SUITABILITY SCORING TO PREDICT PHLEBOTOMY OUTCOMES IN YOUNG WHOLE BLOOD DONORS

PJ Mondy, A Thijsen, T Brama, D Waller, DO Irving, J Fisher, G Shuttleworth, P Diaz and B Bell

Australian Red Cross Blood Service, Sydney, Australia

Background: Donor adverse events and failed phlebotomies remain challenges for Blood Services internationally. Within our recent vein visualisation trial donor veins were assessed and scored by phlebotomists. The trial did not show an improvement in donor adverse events or phlebotomy success with the use of vein visualisation technology. Vein assessment and scoring may however be useful in predicting phlebotomy outcomes.

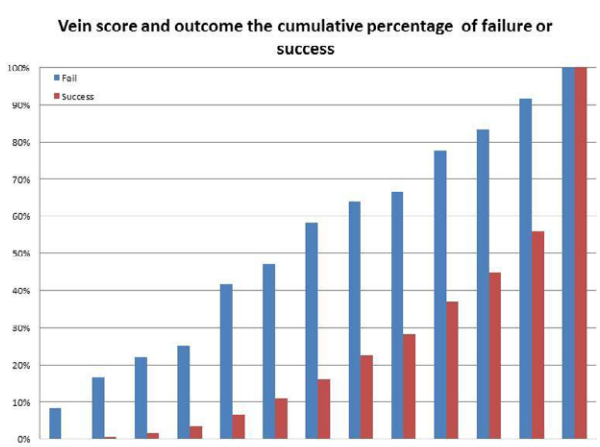
Aim: To review the value of a simple vein score system in predicting the risk of blood donation phlebotomy failure.

Methods: A *post hoc* analysis was performed on vein score data from the vein visualisation trial. The trial included 285 new and 587 returning whole blood donors aged 18–30 randomised to standard or device assisted whole blood donation. Phlebotomists completed four sets of 5-point vein suitability measures (0–4 scores: measuring vein visibility, palpation, size and volume). This vein suitability measure was devised following a review of vein score scales used in clinical medicine for predicting cannulation difficulties. The data on all study participants in the VVT was pooled and comparisons made between donor vein scores and phlebotomy success. The data was considered suitable for pooled analysis since no differences in the initial study outcome measures were detected between the device and control groups.

Results: Vein volume was found to be highly concordant with vein size and was removed from analysis. Scores (0–4) for vein visibility, ease of vein palpation and vein size allowed a total vein score (0–12). The majority of donors had high vein suitability scores, 364/857 receiving the maximum possible score 12. The median vein score for the whole group was 11 and the median was also 11 in those with successful phlebotomy. In contrast the median score in the phlebotomy failure group was 6. A Kruskal-Wallis H test showed this as a statistically significant difference in total vein score between the different phlebotomy outcomes, $X^2(1) = 36.808$, $P < 0.001$, with a mean rank vein score of 194.00 for those with a failed phlebotomy and 439.30 for donors with a successful phlebotomy.

Donors with the maximum score of 12 recorded phlebotomy fail rates of <1% (3/361) vs 4.2% (36/857) in the overall group. The lowest 8% of vein scores (69/857), with score 4 or less accounted for 41.67% (15/36) of the failures and this group had a phlebotomy failure rate of 21.7% (15/69 fails with score 4 or less). Most phlebotomy failures 63.9% (23/36) occurred with vein score 7 or less within the donors with the lowest quarter (24.30%, 208/857) of vein scores. The scale did not appear effective in predicting vasovagal risk with the majority of recorded vasovagal reactions occurring in association with successful needle insertion and commencement of blood flow.

Conclusion: A simple scale completed by phlebotomists shows promise in helping predict phlebotomy success. The scale requires further validation but does appear to have potential utility for predicting phlebotomy outcomes in blood donation.



Caption 1. Vein score and outcome the cumulative percentage of failure or success

P-151

FREQUENCY OF ADVERSE REACTIONS AFTER BLOOD DONATION IN CHINESE BLOOD DONORS

CK Lee, VWY Ho, CCY Chu, IYM Lee, SC Ng and JNS Leung

Hong Kong Red Cross Blood Transfusion Service, Hong Kong, Hong Kong

Background: Despite stringent pre-donation screening, some donors may suffer from adverse reactions during and after blood donations. Albeit being mild and transient in most situations, serious reaction does occur occasionally. Nevertheless, these reactions would not only have medico-legal consequence in donor management, but also could potentially affect blood donor return in the future. As such many attempts have been made to minimize their occurrences. Besides, it is necessary to have a mechanism in monitoring of the incidence and severity of adverse reactions so that follow up actions could take place.
Materials and methods: A period of 2 years, 2014 and 2015 of all blood donation records was reviewed in Hong Kong. Adverse reactions as recorded by the donor vigilance system were analysed. In Hong Kong, the requirements for blood donation are aged between 16 to 70, with a minimum body weight of 41 kg and pre-donation Hb of 11.5 and 13 gm/dl for female and male respectively. Donors are allowed to donate either 350 ml or 450 ml based on body weight cut off of 50 kg. 515,213 donation records were reviewed.
Results: Among 515,213 whole blood and apheresis donations, there were total 12,208 adverse reaction (2.37%) reported. Vasovagal reaction (VVR) was the most commonly observed adverse reaction (1.41%), followed by haematoma (0.80%), local allergy (0.05%) and apheresis related citrate reaction (0.02%). No serious reaction was observed. Most VVR occurred immediately after donation (1.35%) and more frequent in female (2.19%) vs 0.79% in male. Among young donors aged between 16 and 20, VVR were three times more common in female (1.20% vs 0.41%). Besides, a similar trend of venipuncture related haematoma was seen in young female donors.
Conclusion: For blood donation in Chinese, the most common types of adverse reaction were immediate vasovagal reaction and haematoma. It is observed that younger age and female are found to have the highest rate of these adverse reactions. As the adverse reaction can decrease donor retention rate dramatically, resources should be focused on female on adverse reaction prevention and management program in the coming future.

P-152

IMPLEMENTATION OF DONOR HAEMOVIGILANCE SYSTEM IN ISLAMABAD, PAKISTAN

IS Kayani, U Waheed, R Hafeez, A Maqbool and HA Zaheer

Islamabad Blood Transfusion Authority, Ministry of National Health Services, Islamabad, Pakistan

Background: A blood regulatory authority has the responsibility of data collection, management and analysis. This work allows the decision makers in BTS to identify where systems are weak and corrective actions required. Accordingly the Islamabad Blood Transfusion Authority streamlined data collection from all registered and licensed blood banks on all aspects of blood safety including donor haemovigilance. Donor haemovigilance systems allow monitoring of donor safety and assessment of the success of interventions designed to further improve donor safety. The activities of prospective and continuous surveillance of donation related complications are largely voluntary in Pakistan but are mandated in the ICT through the ICT Blood Safety Ordinance.
Aims: The study was conducted to improve the safety standards of blood donation by monitoring all adverse events in blood donors visiting public and private blood banks of Islamabad.
Material and methods: This was a retrospective study conducted from January to December 2015. A standardized data collection tool (pre-tested) was designed by the IBTA for the collection of donor haemovigilance data from the 19 registered and licensed blood banks operating in Islamabad. The compliance to the submission of data was 100 percent and all the data gathered was analyzed through SPSS version 20.0 (for windows).
Results: The donor haemovigilance data shows that out of a total of 65,376 blood donations (91.75% family replacement and 8.25% voluntary) in 2015, 750 donors experienced adverse events. These adverse events included 1,262 signs and symptoms which were all mild in nature. The adverse events included slow pulse 18.9% (n = 206), low BP 18.89% (n = 205), sweating 17.2% (n = 187), fainting 8.2% (n = 89), pallor skin 13.6% (n = 115), nausea 7.9% (n = 86), drowsiness 1.4% (n = 15), vomiting 17.34% (n = 189), cold extremities 5.6% (n = 62), haematoma 0.5% (n = 5), multiple pricks 3.9% (n = 43), shortness of breath 2.3% (n = 25), vasovagal 0.5% (n = 5), headache 1.0% (n = 10), bruising 1.1% (n = 12), weakness 0.2% (n = 2), falling 0.6% (n = 6).
Conclusion: It is crucial that all blood banks adopt a systematic approach to monitor the rates of donor adverse reactions. The current practice of data collection and analysis must be adopted by provincial authorities also to improve donor care and satisfaction. This practice is also likely to increase the trend of voluntary blood donation by reducing the potential adverse events in blood donors.

P-153

This abstract has been withdrawn.

P-154

DONOR HAEMOVIGILANCE: AN EFFECTIVE TOOL FOR IMPROVING DONOR SAFETY AND SATISFACTION. MINISTRY OF HEALTH, UAE

M Raouf[1], M Mahmoud[2], R Jarkas[2], Z Saeed[2] and H Elnagar[2]

[1]Dubai Health Authority, Shj, United Arab Emirates [2]Ministry of Health, Sharjah, United Arab Emirates

Background: While blood donation is a safe and easy procedure for most eligible donors; there are recognized donor complications which can occur. The goal of donor haemovigilance is to continuously improve donor safety and satisfaction through monitoring, analyzing,and researching adverse events associated with blood donation before, during and after blood donation procedure.
Aims: This 3 years study aiming to monitor and analyze the incident related to whole blood donation process in fixed and mobile units. By monitoring the frequency and the trend of incidents it is possible to identify opportunities for improving donor safety and satisfaction.
Methods: Donor adverse reaction has been selected as KPI under blood collection process with a target rate of 1.2%. A standard forms has been created for reporting incidents related to blood donation process and the expected incidents has been clearly defined. Staff were trained and each unit was requested to report to higher management on monthly basis. Adverse reaction report include: donor age, sex, frequency of donation,-donation site: (fixed or mobile),reaction location: (registration area, sampling area, bedside, waiting area or off site), hemoglobin level and blood Pressure before donation, bleeding time,volume of blood collected and type of reaction (incident).
Results: During 2013–2015; a total of 44,740 whole blood donations collected in Shj.BTRC and were monitored for blood donation adverse reactions. There were 166 adverse reactions reported with the following results: location: 0.35% in fixed sites & 0.45% in mobile sites; Donors age: 45.8% in age group between 26 and 35 years followed by 32.5% in age group 18 and 25 years; Gender:among females the incidents = 6% of donors compared 0.32% in males; Weight: the average weight was 84.3 kg with range between 55–145 kg; Frequency of donation showed: 80% in first time donors and 20% in regular donors; Donation duration: the average time was 5.27 min with range between 2 and 14 min; Reaction type: vasovagal with no loss of consciousness constitutes 81.9%; Reaction site: 91.9% were at bedside followed by 5.6% at recovery area and concerning the volume collected: 62.5% was <300 ml & 1.4% >400 ml.
The rate of <1.2 25% was selected as KPI and found to be a useful indicator to monitor the trend of donors incident.
Conclusions: The haemovigilance rate was significantly higher in females and in first time donors. Assigned KPI rate was reached put personal variation was noticed among staff; indicating the need for staff training on unified SOPs. Vasovagal reaction with no loss of consciousness was the most come type of reaction. Absence of hematoma or bruises reporting was noticed; indicating the need for donors education and proper haemovigilance system that enhance reporting for most of expected post donation complications.

Blood Products: Blood Processing, Storage and Release

P-155

OPTIMIZATION OF PLATELET QUALITY AND PATIENT COMPLIANCE USING SINGLE DONOR PLATELETS STORED IN PLATELET ADDITIVE SOLUTION

SS Das

Apollo Gleneagles Hospitals, Kolkata, India

Background: Platelet additive solution (PAS) to store pooled buffy – coat prepared platelet concentrates have in practice since long time. Use of PAS not only increased the availability of plasma for transfusion or fractionation but significantly reduced ABO incompatibility issues and plasma related adverse events.
Aims: With the availability of PAS in India we have started its use for the storage of single donor platelet (SDP) or Apheresis platelet. Here we share our experience of using PAS, the quality outcome of products and patient compliance.
Methods: The prospective study was conducted in 25 healthy platelet donors from November 2015 to February 2016. All SDPs were collected using automated cell separator (Amicus, Fenwal, USA) and stored in solution containing 85% PAS (SSP+, Maco Pharma) and 15% donor plasma. Quality analysis of each SDP unit stored in PAS was conducted on the 4th day and 7th day of storage and documented. Donor demography and apheresis procedure details were obtained from respective screening and procedure registers. Patient post-transfusion details were obtained from patient file and hospital information system (HIS).SPSS statistical software (version 16, USA) was applied for all statistical analysis.
Results: The mean age, weight and pre-donation platelet count of donors was 34 years, 63 kg and 193 $\times$ 10^3/μl respectively. A mean total blood volume of 3,126 ml was processed in 87 min using a mean anticoagulant (Acid citrate dextrose – ACD) volume of 247 ml. The mean platelet yield and leukocyte content of platelet products on 4th day storage was 3.4 $\times$ 10^{11} and 2.7 $\times$ 10^6 respectively. The pH of all units tested was ≥6.9. Aliquots of all units tested on 7th day storage for mean platelet yield and leukocyte content was 3.16 $\times$ 10^{11} and 1.9 $\times$ 10^6 respectively. The pH of all units tested on 7th day was ≥6.78 with all units found sterile after 5 days of incubation. None of the patient had any transfusion reaction after platelet transfusion. A 1 h post-transfusion corrective count increment (CCI) and post-transfusion platelet recovery (PPR) in 13 Aplastic anemia patients were found to be 13,260 platelet $\times$ m^2/μl and 28.3% respectively.
Conclusions: We conclude that PAS is a useful storage medium of platelet concentrates that optimize the quality of platelets even after 7 days of storage with a better patient compliance. It should be available at all blood centres for its optimal uses in platelet storage.

P-156

PLATELET FACTOR 3 BASED-CLOTTING TIME ASSAY MAY SHOW BETTER CORRELATION WITH OTHER QUALITY MARKERS IN COMPARISON WITH PH DURING LONG-TERM STORAGE OF PLATELET CONCENTRATES

S Nasiri

High Institute for Research and Education in Transfusion Medicine, Tehran, Iran

Background: Platelets rapidly lose their qualities usually after 5 day storage. Standards to check the quality of platelets during storage have been published by various bodies and in this regard, a diversity of tests have been investigated to show better correlation with other quality markers during storage.
Aims: The purpose of this study was to demonstrate that platelet factor 3 (PF3) assay indicate higher correlation with other quality markers in comparison with pH during long-term storage of platelet concentrates (PC) up to 11 days.
Methods: Twelve random units of PC were prepared and were placed in a standard platelet incubator with under continuous agitation at 22–24°C for 11 days. Samples were taken on 1, 3, 5, 8 and 11 days and the related tests were performed. Correlation coefficient of PF3 and pH with other parameters were evaluated.
Results: The mean percentage changes of PF3, pH, glucose, LDH, platelet count, MPV and PDW on day 11 relative to the first day were found 61, 15, 52, 440, 9%, 18% and 39% respectively. After LDH, PF3 had higher trend relative to other markers. The PF3 demonstrated better correlation with glucose, platelet count, MPV and PDW in comparison with pH during long-term storage of PC.
Conclusions: It may be concluded that platelet factor 3 based-clotting time assay can be potential candidate for monitoring the quality of PC, due to apparent trend of its changes during storage with better correlation between the quality markers.

P-157

RELEASE OF SOLUBLE CD40 L AND SERUM AMYLOID A DURING PLATELET STORAGE: GENDER-SPECIFIC DIFFERENCE

F Cognasse[1], C Sut[2], H Hamzeh-Cognasse[2], CA Arthaud[1], MA Eyraud[1] and O Garraud[3]

[1]French National Blood System (EFS), St Priest en Jarez, France [2]EA3064 – GIMAP, Saint Etienne, France [3]Institut National de la Transfusion Sanguine (INTS), Paris, France, Paris, France

Background: Mammalian blood platelets (PLTs) have a specialized role in the haemostatic responses. This function has long been considered as the only role of PLTs. Recently, new functions for platelets have been identified. Indeed, these non-nucleated cellular elements participate in the process of inflammation, by liberating different substances capable of modulating an inflammatory response via interactions with endothelial cells and white blood cells (WBCs). Recent studies have demonstrated that platelets promote immunity and inflammation mainly by means of the CD40/CD40L pathway. Serum amyloid A (SAA) exhibits significant immunological activity and is an acute phase reactant that can become the predominant apolipoprotein in HDL during severe inflammatory conditions.
Aims: In view of achieving the Pla_TRIP *ex vivo* investigation data (please refer to the *ad hoc* summary), our objective was to examine the levels of Serum amyloid A (SAA) and soluble CD40 ligand (sCD40L) in apheresis platelets during storage (d1–d5) and to examine if there were donors' gender differences – Females (F) and Males (M).
Methods: Apheresis platelets were collected from regular blood donors (n = 3,445 – F 34%/M 66%). Blood was collected on acid citrate dextrose-A with Amicus or Trima cell separators. All collection procedures were performed in one of regional (EFS Rhône-Alpes-Auvergne) settings. The samples were immediately shipped to the unique processing platform of this blood establishment and distributed after qualification, days 1 and 5 after collection. Serum amyloid A (SAA) and soluble CD40 ligand (sCD40L) secretion were tested for by means of a Luminex® platform.
Results: As described previously, the levels of sCD40L increased during the platelet storage (d1–d5). For the first time however, we described a SAA secretion by stored apheresis platelets, that parallels sCD40L secretion, with a significant increase from Day 3. Interestingly, we noticed that donor gender might influence the evolution of the Serum SAA and sCD40L during storage. Male donors showed significantly higher sCD40L and lower SAA compared to female donors from Day 3 to 5 in stored PLTs.
Summary/Conclusions: sCD40L and SAA (possibly along other Biological Response Modifiers (BRMs), increase during platelet storage; this was expected regarding sCD40L but not for SAA. This study also evidences substantial heterogeneity of sCD40L and SAA between genders. Additional studies should investigate this sexual dichotomy and determine their clinical significance, especially with respect to anyassociation with adverse events.

P-158

EVALUATION OF POOLED BUFFY-COAT PLATELET PRODUCED FROM 350 ML OF WHOLE BLOOD IN JAKARTA BLOOD TRANSFUSION SERVICE

NK Ritchie, A Udyaningrum, U Bachrussani and S Salim

Jakarta Blood Transfusion Service, DKI Jakarta, Indonesia

Background: Jakarta Blood Transfusion Service collects more than 300,000 blood per year. Most of our blood donor were male, 30–50 years old. Most of blood collected were 350 ml of whole blood because the average weight of Indonesian male is 55.73 $\pm$ 2.56 kg and female is 49.67 $\pm$ 2.46 kg with the average height of Indonesian male is 160.90 $\pm$ 2.97 cm and female is 151.27 $\pm$ 2.01 cm. The platelet concentrate produced from 60% from platelet-rich plasma (PRP) method, 20% from buffy-coat removed method and 20% from platelet apheresis. Almost 90% of PRC was preserved in CPDA-1 (Citrate-Phosphate-Dextrose-Adenine) for 14 days of shelf life. Only 10% of PRC produced from 450 ml of whole blood using Top and Bottom Bag and preserved in CPD (Citrate-Phosphate-Dextrose)-SAGM (Saline-Adenine-Glucose-Manitol). This kind of PRCs were leukoreduced and had longer shelf life for 30 days. The clinicians were aware the benefit of these leukoreduced PRC so the demands were increasing. The donors preferred to donate 350 ml but in the other hand we had to increase the production of leukoreduced PRC. We also had an increasing demand of platelet apheresis. So, we decide to order special bag

350 ml with configuration Top and Bottom Bag from JMS and we try to utilize the buffy-coat for pooled platelet production in order to cover up the lack of platelet apheresis.
Aim: This study is aimed to see the quality of pooled buffy-coat platelet from 350 ml of whole blood.
Method: We separated the buffy-coat from 350 ml of whole blood using JMS HemoPress, an automated plasma separator then we connect the buffy-coat bags and plasma with sterile connecting device. We used train method to pool the buffy-coat and 1 plasma. The pooled buffy-coat were spun down and the platelets were transferred through Pall ATSBC filter. We counted the platelet yield, leukocyte residue and the volume of pooled-platelets. We compared the pooled of four buffy-coat and 1 plasma with five buffy-coat and 1 plasma. We also compared if we keep the whole blood overnight at 22°C and 8 h at 22°C. We did also comparison of buffy-coat with whole blood which kept overnight at 22°C.
Results: We expect the platelet yield of pooled platelet must exceed 2×10^{11}/unit, the leukocyte residue $<1 \times 10^{6}$/unit and the volume more than 200 ml. From total of 14 bags of pooled-platelet, we found that pooled of five buffy-coat and 1 plasma were more consistent and had higher platelet yields compared to pooled of four buffy-coat and 1 plasma. We did not find any significant difference in QC results of pooled-platelet which produced from whole blood overnight at 22°C and 8 h at 22°C and no significant difference between buffy-coat with whole blood which kept overnight at 22°C.
Conclusion: The pooled buffy-coat platelet could be produced from 350 ml of whole blood. Either whole blood or buffy-coat could be kept in 22°C overnight to increase the efficiency and effectiveness of the production. The pooled buffy-coat platelet could be a complement to platelet apheresis.

P-159

DONOR CHARACTERISTICS AND LACTATE PRODUCTION IN SINGLE-DONOR PLATELET CONCENTRATES: A PILOT STUDY

IJ Bontekoe[1], D Sijbrands[1], PF van der Meer[1], KK van den Hurk[1], AJ Verhoeven[2] and D de Korte[1]

[1]Sanquin Blood Bank, Amsterdam, The Netherlands [2]Academic Medical Center, Amsterdam, The Netherlands

Background: In retrospective studies, it has been shown that differences in pH and other parameters during storage of platelet concentrates (PCs) are partially donor dependent.
Aim: To study whether donor characteristics are associated with PC quality and could explain 'good' and 'poor' storage quality.
Method: From QC data of outdated apheresis PCs (Sept 2011–Aug 2014), 2 × 6 male donors were selected with at least 1 PC with pH >7.0 ('good') or 1 PC with pH <6.7 ('poor') on Day 8. These donors donated a PC (Trima Accel, Terumo) and completed a short questionnaire about their health and lifestyle. PCs were stored for 12 days under standard conditions, and analyzed at regular intervals for *in vitro* quality.
Results: Average donor characteristics such as age, body mass index and blood pressure on the day of collection were comparable. However, 0/6 'good' donors and 4/6 'poor' donors reported high blood pressure and/or high cholesterol/fat and/or use of medicines. 'Good' PCs contained significant more platelets (Table 1), probably due to different yield factors of the apheresis machines used. Lactate production in 'good' PCs was lower than in 'poor' PCs (0.09 ± 0.03 vs 0.13 ± 0.04 mmol/day/10^{11} platelets, $P < 0.05$) resulting in a higher pH from Day 5 onwards. At the end of storage, the 'good' PCs were also less activated (CD62P expression), showed less phosphatidylserine exposure (Annexin A5 binding) and a higher mitochondrial membrane potential as detected with the fluorescent dye JC-1. Functional properties of platelets such as the hypotonic shock response or support of coagulation [as measured by thromboelastography (TEG)], were only slightly different. Despite having lower pH, also the 'poor' PCs fulfilled European Guidelines during 7 day storage.
Summary/Conclusion: 'Good' PCs showed better storage characteristics than 'poor' PCs. This pilot study suggests that 'poor' PCs were more likely to come from donors with health issues, possibly due to different functionality of the mitochondria. More research is needed to establish the underlying causes and the implications for donors and blood products.

PCs	'good	'poor'	p-value
Day 1			
Volume, mL	295±6	294±15	n.s.
Platelets, x10^9	429±29	375±45	< 0.05
Day 12			
pH (37°C)	6.81±0.06	6.46±0.26	< 0.01
CD62P-expression, %	21.0±3.7	32.9±15.3	< 0.05
Annexin A5 binding, %	17.8±5.4	29.2±9.5	< 0.05
JC-1, ratio FL2/FL1	11.5±1.9	8.8±2.2	< 0.05
HSR, %	73±7	53±26	< 0.05

Table 1. Storage characteristics of good and poor PCs

P-160

PROVIDING OF IMMUNOLOGICAL AND INFECTION PLATELET SAFETY BY CRYOPRESERVATION

I Vysochin, EN Kobzeva, AI Kostin, MS Makarov and VB Khvatov

Sklifosovsky Research Institute of Emergency Medicine, Moscow, Russian Federation

Blood transfusion safety is one of the main requirements of the transfusion. Infectious transfusion safety of platelet concentrates (PC) is provided both by quarantining and virus inactivation.
Aim of research: To study the effect of immunological compatibility on the clinical efficacy of quarantinized cryopreserved platelets (QCP).
Materials and methods: We quarantined QCPs after twice donor analysis on HIV, HCV, HBsAg and RW, following 180 days after CP harvesting. Before transfusion QCPs were defrosted and resuspended in plasma. The number of platelets in the QCPs was determined on ABX Micros 60, platelet functional activity was studied by vital staining. Medical QCP transfusion were provided due to WHO recommendations to correct thrombocytopenia (the number of platelets in the blood $63 \pm 12 \times 10^9$/l) and bleeding stopping after: aortic (n = 6) and heart valves (n = 3) grafting with AIK; lung transplantation (n = 4); gastrointestinal bleeding (n = 5); concomitant injuries (n = 7). To assess the immunological compatibility of QCP with the recipients we used the apparatus of the CSW Immucor 100, as well as Capture Reagent-P® Ready-Screen – for the detection of anti-platelet antibodies (ATA) and Capture Reagent-P® – for individual selection of CCP. Determination of compatibility is based on identifying both ATA and Anti-HLA-A and HLA-B. Efficiency of transfusions QCP was assessed by corrected count increment (CCI) and corrected increment of functionally active platelets (CIFAP) in blood recipients through 1 and 12 h after transfusion. Median of CCI was estimated in different groups using U Mann-Whitney test.
Results: For transfusion we used 70% of previously quarantinized QCP. Defrosted QCP contained $280 \pm 80 \times 10^9$ platelets and $73 \pm 8 \times 10^9$% of functionally active platelets. 25 patients with QCV transfusions were studied due to anti-platelet antibodies analysis. 12 (48%) patients had identified anti-platelet antibodies; however, only 17 (68%) had transfusion after of HLA/HPA compatible QCP: 6 (50%) of 12 patients with ATA, and 11 (85%) without ATA. After QCP transfusion CCI parameter estimated 8.1×10^9/l after 1 h и 0 after 24 h of transfusion in patients with ATA and compatible to QCP; in patients with ATA and non-compatible to QCP – 9.2×10^9/l & 5.5×10^9/l; in patients without ATA and compatible to QCP – 9.5×10^9/l & 13.5×10^9/l; in patients without ATA and non-compatible to QCP – 2.5×10^9/l & 8.2×10^9/l, CIFAP dynamic was similar to CCI: in patients with ATA and compatible to QCP it was 29×10^9/l after 1 h and 20×10^9/l after 24 h of transfusion; in patients with ATA and non-compatible to QCP – 11×10^9/l and 10×10^9/l; in patients without ATA and compatible to QCP – 28×10^9/l and 27×10^9/l; in patients without ATA and non-compatible to QCP – 18×10^9/l & 14×10^9/l.
The most visible diversity and growth of CCI/CIFAP rate after 24 h maintained in patients without ATA and compatible to QCP. One could conclude, that single compatible QCP transfusion was adequate for bleeding stopping, whereas non-compatible QCPs needed one more act of transfusive therapy.
Conclusion: Individual compatibility to QCP enhances the efficacy of transfusive therapy and elongates time of donors' platelets circulation. The implement of individual QCP selection in the ambulance of Sklifosovsky Research Institute of Emergency Medicine ensured not only the infectious and immunological safety of transfusions, but also reduced the overall number of transfusions by increasing their effectiveness.

P-161

NOVEL BLOOD DONATION BAG SYSTEMS WITH SEPACELLTM R-S11, TO PREPARE RED CELL CONCENTRATES BY TB AND TT METHODS

T Yokomizo[1], T Kai[1], T Sözen[2], H Göktepe[2] and K Yajima[1]

[1]Asahi Kasei Medical, Oita, Japan [2]Kansuk Laboratuari, Istanbul, Turkey

Background: Leukocyte reduction is widely used to reduce the risk of non-hemolytic febrile transfusion reactions and alloimmunization by multiple blood transfusions. Around and after year 2000, universal leukocyte reduction was adopted in many developed countries. There are mainly two types of filter, one to filter whole blood and the other to filter red cell concentrates (RCCs) used during pre-storage blood processing.

Aims: In this study, we conducted the tests with the blood donation bag systems with Sepacell™ R-S11 for RCCs, manufactured by Kansuk Laboratuari.

Methods: Blood bag systems for top-and-top (TT) methods were tested with 450 ml of whole blood donation, and systems for top-and-bottom (TB) were with 450 and 500 ml of whole blood.

The methods are summarized in the Table 1.

Results: The test results are also summarized in the Table 2.

Conclusion: This study shows the capability of the novel blood bag systems with Sepacell™ R-S11 as the pre-storage leukocyte reduction filter systems for RCCs prepared by TB and TT methods, not only in leukocyte reduction performance but also in quality of RCCs.

Test Group	Test Methods			
	Bag system	Donation Volume (mL)	Blood Storage Time, temp. (hrs, temp)	Filtration Head (cm)
1	TT for PRP removal	450	2–6, RT	140
2	TB for Buffy Coat removal	450	2–6, RT	140
3		500	17–22, RT	140
4		500	17–22, RT	120

Table 1

Test Group		Results			
		WBCs in Filtrate (Log(cells/unit)	Filtration Time (min)	Volume loss by filtration (mL)	Hemolysys at 42 days (%)
1	n	10	10	10	10
	avg	4.78	36.2	25.5	all < 0.8
	sd	0.01	4.9	1.0	
2	n	10	10	10	–
	avg	4.69	24.4	24.3	
	sd	0.02	1.6	0.7	
3	n	10	10	10	5
	avg	4.83	25.1	27.3	all < 0.8
	sd	0.61	4.6	0.4	
4	n	10	10	10	5
	avg	5.11	30.3	28.4	all < 0.8
	sd	0.59	3.6	1.7	
Note		WBCs counted by Nageotte in Group 1&2, by FCM in 3&4.			

Table 2

P-162

AN *IN VITRO* COMPARISON OF CELLULAR DESTRUCTION AND METABOLIC EFFECTS OCCURRING IN STORED, LEUCO-REDUCED AND IRRADIATED RED BLOOD CELLS

F Adams[1], GRM Bellairs[1], AR Bird[1] and O Oguntibeju[2]

[1]Western Province Blood Transfusion Service, Cape Town, South Africa [2]Cape Peninsula University of Technology, Cape Town, South Africa

Background: Gamma irradiation of cellular components is the primary preventative measure to avoid transfusion-associated Graft-versus-Host disease (TA-GvHD) as it inhibits lymphocyte proliferation while maintaining component integrity. The most common red blood cellular products associated with TA-GvHD are whole blood and red blood cell concentrates (RBCC) despite the well- documented effects of gamma irradiation exacerbating storage lesions. The available literature regarding storage lesions occurring in RBCC and leucocyte-reduced RBCC is extensive although data regarding the effects of gamma irradiation on pre-storage leucocyte-reduced RBCC is considerably less since the trend of First World countries adopting the universal leucocyte reduction (ULR) policy. A study comparing the *in vitro* storage lesions of irradiated and non-irradiated red blood cell products had not previously been completed at a blood transfusion establishment in South Africa as most studies comparing the *in vitro* storage lesions of irradiated and non-irradiated red blood cell products have taken place in First World countries.

Aim: To evaluate and compare the biochemical and haematological changes that occur in gamma irradiated and non-irradiated red blood cellular products during the standard 35 to 42 day storage period at 1–6°C.

Materials and methods: The red cell products evaluated included whole blood, RBCC, leucocyte reduced RBCC and paediatric RBCC. The tested products were collected in citrate-phosphate dextrose anticoagulant and relevant products stored in saline-adenine-glucose-mannitol nutritive solution. Serial sample aliquots were taken of both non-irradiated and non-irradiated products on Days 1, 7, 14, 21, 28, 35 and 42 respectively and tested for haematological indices which included haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentrate levels and a full blood count. The biochemical indices evaluated included potassium, sodium, phosphate, glucose, lactate dehydrogenase, free haemoglobin (percentage haemolysis) and pH levels.

Results: The effects of gamma irradiation indicated potassium ion and lactate dehydrogenase leakage. A significant difference in plasma haemolysis was observed in the irradiated products with haemolysis concentrations higher than the recommended 0.8%.

Conclusion: Gamma irradiation causes cellular damage in red cell products as observed by the study results which have also indicated that our findings are comparable to previous studies.

P-163

IMPROVE WORKFLOW EFFICIENCY IN PACKED RED BLOOD CELLS (RBC) PROCESSING

H Wongso, MZ Lim, Z Huang, RB Che Omar, YB Yusof, KK Chia, KS Ng, PH Shu and S Lam

Health Sciences Authority, Singapore, Singapore

Background: The Blood Services Group (BSG) Singapore secures the nation's blood supply, ensuring all patients in Singapore have access to adequate and safe blood. To be able to keep up with the high demand of high quality blood products, there is a need for constant improvement on the blood component processing workflow.

BSG's centralized blood component processing laboratory processes whole blood (WB), collected from various collection centers, into blood components. Current processing workflow uses buffy-coat preparation method for separation of WB collected in various blood bag types with different minimum holding times ranging from 2 to 4 h. As such, with the different arrival time of WB from different sites (blood run time) and varying holding time, most of the blood could not be processed on the same day and have to be held in room temperature overnight and for next day processing. The amount of overnight hold WB will affect the Turn Around Time (TAT) for the RBC to be ready for use.

Aims: Our aim is to identify possible factors to reduce the RBC processing time and to improve work processes from start of component processing till component labelling.

Methods: Lean Six Sigma tool is used to identify the potential factors affecting TAT for workflow processes improvement. Simulated scenarios were performed to understand the factors before moving on with further data collection for analysis. Statistical analysis was performed on data collected using SigmaXL software.

Results: Four potential factors (manpower allocation, blood run time arrangement, equipment placement, and WB holding time) were identified. Results showed that current manpower allocation and the blood run time arrangement have no effect on reducing the TAT.
Equipment placement simulation presented the travelling time to the equipment was reduced from approximately 30 to 5 s per run ($P < 0.0001$). However, post implementation data showed that the improvement is not significant ($P = 0.4009$) in reducing the overall TAT.
Simulation on shorter WB holding time exhibited a significant effect on reduction of processing time. There is 32% increase in the amount of WB being processed on the same day of collection. As such, the overnight hold WB can be reduced by 57%, hence reducing TAT. Process validation was carried out and the result revealed that blood products made from WB with minimum 2 h holding time, standardized for all blood bag types, passed all product quality controls.
Conclusions: Shortening WB holding time significantly reduced the RBC processing time, hence improving workflow efficiency. However, more data is needed to prove that the product quality is not compromised. One-month pilot study on processing of WB with minimum 2 h holding time for all bag types is proposed to substantiate the finding before implementation.

P-164

IN VITRO EVALUATION OF DEHT (BIS (2-ETHYLHEXYL) TEREPHTHALATE) PLASTICIZED PVC BLOOD BAGS FOR RED BLOOD CELL STORAGE IN AS-1 AND PAGGSM PRESERVATIVE SOLUTIONS

S Graminske[1], KE Puca[1], AP Schmidt[1], SR Brooks[1], A Boerner[1], SR Heldke[1], MDA Indig[1] and MG Brucks[2]

[1]*Blood Center of Wisconsin, Milwaukee, Wisconsin, United States of America*
[2]*Eastman Chemical Company, Kingsport, Tennessee, United States of America*

Background: Di-(2-ethylhexyl) phthalate (DEHP) makes PVC film flexible and useful for blood bags. Use of DEHP also has a beneficial membrane-stabilizing effect on RBCs resulting in lower hemolysis levels. During storage, DEHP can leach from the bag film into solution and be metabolized. Studies in rodents have suggested that exposure to DEHP may be associated with adverse health effects. Both the FDA and EU laws allow the use of DEHP in medical devices. Nevertheless, there have been many attempts to find DEHP alternatives for blood bags, made difficult by DEHP's membrane-stabilizing effect. This study tests an alternative non-phthalate plasticizer bis (2-ethylhexyl) terephthalate (DEHT). Although structurally and functionally similar to DEHP, DEHT is very distinct from a metabolic and toxicological standpoint since DEHT can undergo complete hydrolysis. Introduced in 1975 DEHT has an excellent toxicological profile: it is not a cacinogen, mutagen, or reproductive toxicant.
Aims: The *in vitro* study objective was to evaluate RBC stability while stored in DEHT plasticized containers with AS-1 and PAGGSM preservative solutions compared to RBCs stored in conventional DEHP plasticized containers with AS-1 solution.
Methods: Thirty-six (36) whole blood units were collected into CPD anticoagulant, leukoreduced, centrifuged, and separated into RBCs and plasma. Twelve (12) pools of three ABO-identical RBCs were mixed together and then divided equally among three different plasticizer and preservative solution combinations: DEHP/AS-1 (Control), DEHT/AS-1, and DEHT/PAGGSM. *In vitro* RBC testing (CBC, rWBC, ATP, 2,3-DPG, hemolysis, pH, pCO_2, pO_2, RBC morphology score, and plasma hemoglobin, potassium, glucose, lactic acid) was done on Days 0, 35, and 42 of 1–6°C storage.
Results: Table 1 summarizes the key results from the study. No individual bag at Day 42 exceeded the EU 0.8% hemolysis criteria. ATP retention was above 70% and potassium levels were as expected regardless of plasticizer. Additional RBC parameters exhibited some statistically significant differences but were not viewed as clinically important.

Table1	Day 42 Results, (Mean ± SD), N=12 Bag and preservative type		
	DEHP-PVC with AS-1	DEHT with AS-1	DEHT with PAGGSM
Hemolysis (%)	0.32 ± 0.07	0.49 ± 0.13*	0.38 ± 0.10*
ATP(μmol/ghgb)	3.34 ± 0.46	3.13 ± 0.46*	3.03 ± 0.36*
ATP % of initial value	77%	71%*	73%*
Potassium (mmol/L)	51.8 ± 2.9	51.3 ± 2.8	52.9 ± 2.4

*p<0.05 from Day 42 control

Table 1

Summary/Conclusions: DEHT with PAGGSM provides similar hemolysis protection to that of DEHP. Although hemolysis values with DEHT and AS-1 are higher than that of DEHP, DEHT can be considered a viable replacement of DEHP in RBC storage bags.

P-165

RED BLOOD CELL COMPONENTS FROM PATIENTS WITH HEREDITARY HAEMOCHROMATOSIS AND PARAMETERS ALLOWING ISSUING TO TRANSFUSION RECIPIENTS

F Cognasse[1], C Sut[2], H Hamzeh-Cognasse[2], S Laradi[1], V Bost[1], C Aubrège[1], S Acquart[1], M Vignal[1], N Boutahar[3], CA Arthaud[1], MA Eyraud[1], B Pozzetto[2], P Tiberghien[1] and O Garraud[4]

[1]*French National Blood System (EFS), St Priest en Jarez, France* [2]*EA3064 – GIMAP, Saint Etienne, France* [3]*Département de Biochimie Hôpital Nord, Saint Etienne, France* [4]*Institut National de la Transfusion Sanguine (INTS), Paris, France, Paris, France*

Background: Red blood cells (RBCs) contain large amounts of iron, and periodic bloodletting is thus the main treatment for hereditary hemochromatosis (HH). HH is a genetic disorder of iron metabolism characterized by increased iron absorption and storage, resulting in progressive and multisystemic oxidative organ damage However, the donation of bloodletting products from asymptomatic patients remains controversial.
Aims: In this study, we compared the quality of red blood cell concentrates (RBCCs) obtained from HH patients with those of non-HH RBCSs, within the allowed 42-day storage period.
Methods: RBCCs were obtained from HH patient donors and random regular blood donors by whole-blood collection. RBCCs were stored for up to 42 days, according to national regulations and standard blood bank conditions in France. The following parameters were assessed: haematological and biochemical results; RBC membrane and soluble inflammatory markers; and the pro-inflammatory potential of HH RBCC supernatant towards endothelial cells in an *in vitro*.
Results: There were no major differences between the two groups in terms of biophysical, biochemical or soluble immunomodulatory factors. However, we observed small but significant differences in changes in RBC membrane proteins during storage, including increased annexin-V expression and decreased haemolysis in HH compared with normal RBCs. However, there were no differences in terms of bioactivity of soluble immunomodulatory factors in the RBC supernatant during storage between HH and control donors, determined by their effects on endothelial cells *in vitro*.
Summary/Conclusions: Given that a dedicated, prospective study of the possible toxicity of iron-overloaded RBCCs is unlikely to be feasible, we suggest that 'at risk' recipients (such as neonates and infants, patients undergoing extracorporeal circulation, etc.) should be identified and the use of HH RBCCs avoided in these individuals. This would require RBCCs to be tagged in some way, either physically or electronically, to allow their source to be identified within the inventory. RBCs from HH patients appear, while exhibiting subtle differences, to be suitable for transfusion purposes according to currently-accepted criteria.

P-166

RETROSPECTIVE STUDY OF TRANSFUSION PRACTICES (RED BLOOD CELLS) IN A TERTIARY CARE HOSPITAL – A PILOT STUDY

K Bains, N Shetge and A Deshpande

P D Hinduja National Hospital & MRC, Mumbai, India

Background: In hospital setting providing blood adequately for a patient is just not the balance between donors number and blood units transfused but also depends on certain important variables like Blood Ordering Schedule (BOS), Hold Time and Type & Screen policy (T&S). This study was conducted to assess efficient blood utilization using standard blood utilization indices and to look for any impact on blood inventory. In addition, need of modification in BOS, Hold Time (72 h) policy and T&S policy was assessed to further improve blood transfusion services in our hospital.
Aims: To analyze red cell transfusion practices against current available utilization indices and to modify the current Blood Ordering Schedule, T&S policy and Hold Time policy based on the findings.

Methods: A hospital based retrospective study was conducted in our institution over a period of 1 year from April 2015 to March 2016. Red cell utilization for admitted patients was evaluated by calculating crossmatch to transfusion ratio (C/T), transfusion index (TI), transfusion probability (%T), expiration rate and wastage rate. Number of red cell units being crossmatched (CM) against available stock and any hassle faced during crossmatch were also analyzed. In current practice, for each patient CM unit is reserved (on hold) for 72 h at our hospital. In relation to this hold time, various CM hassles were observed including less number of units CM than requested as per BOS, units released in <72 h of hold time, units arranged from other hospitals, CM done with alternate blood group and single unit CM for two patients. T&S policy for angiography and tissue biopsy was reviewed based on the results.
Results: A total of 7,150 red cells were prepared from 7,167 donors. For 8,744 patient requests (medical and surgical), 14,538 units were CM. 13,635 (93.7%) units were CM without hassle whereas 904 (6.3%) units with hassle (less no.of units CM = 79, released units = 784, units from other hospitals = 17 and CM with alternate blood group = 24). No single unit was CM for two patients. Only one surgery was postponed due to shortage of blood. 6,073 (42%) units from total CM were transfused to 2,885 patients. The overall ratios of C/T, TI, %T, expiration rate and wastage rate excluding TTI Positive were 2.3, 0.6 and 32.9%, 0.3%(25) and 0.05%(4) respectively.
Conclusions: All indices evaluated were comparable with standard values i.e. C/T ratio 2–2.5, TI ≥0.5, %T ≥ 30%, expiration rate <1% and wastage rate <0.5%. To overcome hassles during CM, current Hold Time policy of 72 h would be reduced to 48 h and the results analyzed. Implementation of T&S policy for medical conditions other than angiography and tissue biopsy cases would be recommended to hospital Transfusion Committee. In addition, revision of current Blood Ordering pattern is required for more efficient management. This study shows its not only the number of donors which determine the efficient blood utilization.

P-167

EVALUATION OF WHOLE BLOOD LEUKOREDUCTION FILTER INTEGRATED IN QUADRUPLE BLOOD BAGS SYSTEM OFFERED BY TERUMO BCT

M Scelsi[1], M Fiore[1], M de Michele[1], A Masciopinto[1], A de Cosimo[1] and L Buttarelli[2]
[1]Hospital Blood Bank San Paolo – Asl Bari, Bari, Italy [2]Terumo BCT Europe N.V., Zaventem, Belgium

Background: The implementation of pre-storage leukoreduction into the blood bank process reduces the number of transfusion complications such as Febrile Non-Hemolytic Transfusion Reactions (FNTR), Transfusion associated Graft-versus-Host disease (TA-GvHD), HLA alloimunization, CMV transmission, cytokine production and risk of infections (pathogens trapped by WBCs eliminated).
Aims: The goal of this study was to evaluate leukoreduction performance and the quality of resulting blood components produced with the quadruple blood bags system with an integrated whole -blood filter which was used in whole blood fractionation under routine conditions.
Methods: Whole blood (WB) donations (450 ml) were collected from regular donors using quadruple blood bags system with an integrated whole blood filter (BB*WGQ456G6 – Terumo BCT), kept for at least 2 h at room temperature before being subjected to filtration by gravity. The resulting leukoreduced whole blood was then centrifuged and fractionated (T-ACE II+ – Terumo BCT) into RBCs in SAGM and Plasma. Both blood components were stored at standard conditions. The relevant product characteristics for plasma and red cell concentrates, as volume, residual leukocytes and platelets contamination, as well as haematocrit, haemoglobin, % haemolysis, factor VIII, total proteins were recorded. Filter performance attributes like volume loss and filtration time were also assessed.
Results: WB donations (n = 30) were processed with quadruple blood bags system described (BB*WGQ456G6 – Terumo BCT). RBCs (n = 30, volume 273 ± 16 ml) showed normal values of haemoglobin (52.5 ± 6.5 g/unit).The percentage haemolysis also showed normal values at the day of collection and during storage so far. Moreover the residual leukocyte count was below 1×10^6/unit ($0.0106 \pm 0.018 \times 10^6$/unit, n = 30) in all produced units after leukocyte filtration (filtration time between 10 and 20 min). Plasma, pre-freezing (n = 30, volume 290 ± 18 ml) showed normal values of factor VIII (112.95 ± 4%) and total proteins (5.85 ± 0.3 g/dl).
Conclusions: Quality of blood components (RBCs and Plasma) originating from leukoreduced whole blood with quadruple blood bags system showed all quality parameters fulfilled European guideline requirements. Filter performances and easy handling of that system are suitable for current routine process.

P-168

EVALUATION OF THE REVEOS SYSTEM FOR WHOLE BLOOD PROCESSING

J Pineau[1], S Jadoul[1], M Oresti[1], M Cardoso[2], E de Schrijver[2], L Lecomte[1], C Megali[1], M Poffé[1] and JC Legrand[1]
[1]Asbl La Transfusion du Sang, Charleroi, Belgium [2]Terumo BCT Europe N.V., Zaventum, Belgium

Background: The ETS 'asbl La Transfusion du sang' of Charleroi wanted to automate the processing of whole blood (WB) to increase the production of platelet concentrates and improve quality of plasma. The Reveos system from Terumo BCT separates blood into two or three components: plasma, red cell concentrate (RCC) and one IPU (interim platelet unit) that may be pooled and leukoreduced resulting in one final platelet concentrate (PC). This system was primarily selected for evaluation because it makes possible to process automatically two manufacturing steps with a single device: centrifugation and fractionation into blood components.
Aims: To evaluate the quality of Reveos-processed blood components derived from WB and investigate the capability of the technology to improve productivity in routine conditions at the blood processing site.
Methods: The evaluation of the process was performed into phases. In phase I a total of 57 WB donations (450 ml) were separated into two components (2C protocol, n = 14), and three components (3C protocol, n = 43). A phase II evaluation followed: 198 WB bags were processed with the 3C and 225 bags with the 2C protocols. Both protocols were assessed in three different time-windows: 2–8 h after collection for 2C; 4–8 h for 3C, both named fresh; 14–18 h, named overnight (ON). The resulting blood components were assessed according to standard storage parameters. The system was also evaluated by professionals at the collection site in respect to user-friendliness of the kits and needle puncture performance, as well as process execution.
Results: Resulting RCCs showed in three out of the four protocols mean hemoglobin levels above 50 g, with the 3C/ON protocol showing a lower value of 49 g (range 44–57 g). Mean hematocrit value was 58.7% (range: 54.4–62.4%). All products were in accordance with EU requirements for white blood cell contamination and hemolysis at end of storage (mean value = 0.19%). Resulting plasma products had mean volume of 280–290 and 248 ml for 2C and 3C protocols, respectively. One single product showed slightly higher platelet contamination value (56 PLT/µl) but all products had $<1 \times 10^6$ WBC/unit. PC produced by the 3C protocol after pooling 5IPUs showed mean yields of $4.0–4.9 \times 10^{11}$/unit, depending on the mix of fresh and overnight IPUs. Swirl and pH were maintained at standard levels until day 7 of storage. Technicians considered the fact that there is no need of pre-folding and balancing of bags in the centrifuge, together with the full-traceability of the process major accomplishments. Needle puncture performance was considered optimal by nurses and blood donors. In a simulation of routine production it was concluded that this system allowed an increase from 18% to 60% in the production of WB derived PC.
Conclusion: This evaluation showed that the Reveos system is quickly adapted to the routine of our blood center. Validation work was easily processed due to pre-adapted protocols. The robustness of the system was confirmed. Blood component quality increased for all three products. The Reveos system has been gradually implemented and will be in full routine by May 2016.

P-169

EFFECTS OF CENTRIFUGATION PARAMETERS ON THE CHARACTERISTICS OF BLOOD COMPONENTS PRODUCED UNDER THE BUFFY COAT METHOD

AG Chartois-Leaute[1], Y Giraud[1], P Pale[2], E Rivery[1], N Sauvetre[1], L Grivault[2] and F Dehaut[1]
[1]Etablissement Français du Sang, Nantes, France [2]Fresenius Kabi, Brézins, France

Background: Since the 1980s, whole blood (WB) is centrifuged via hard spin to produce a separation of plasma (PLS), buffy-coat (BC) and red cell concentrate (RCC) by sedimentation of the blood components according to their relative density. PLS and RCC are expressed in dedicated transfer bags for further processing into leucocyte reduced PLS (LR-PLS) and leucocyte reduced RCC (LR-RCC) suspended in saline-adenine-glucose-mannitol (SAGM). BCs are stored in their primary bag for at least 2 h before pooling and processing into leucocyte reduced Pooled Platelet Concentrates (LR-PPC) suspended in platelet additive solution (PAS) which represent now a large part of the platelets products available for transfusion in France.

Aims: This study aims to determine the effects of centrifugation parameters such as Total Centrifugal Force (TCF), Relative Centrifugal Force (RCF) and acceleration on the characteristics of each blood components in order to meet labile blood products French specifications, and provide optimal blood components throughput.
Study design and method: Whole blood units (475 ml ± 10%) were collected with quintuple top and bottom disposables (NPT systems – MacoPharma) and centrifuged with Cryofuge 6000i (HERAEUS) after overnight storage at +22 ± 2°C. Three centrifugation parameters (or factors) with two modalities (low–high) were investigated: TCF (2.5–5.0 × 10^6 g s), RCF (3500–5314 g) and the acceleration (1–9). Eight factors combination were assessed with 12 WB units. Blood components were separated with an automated blood component separation device (Compomat G5 – Fresenius-Kabi) and 5 ABO identical BCs from each test were pooled and suspended in PAS and processed with TACSI system (TerumoBCT).
Samples from WB, RCCs before and after filtration, PLS before and after filtration, BCs and LR-PPC were taken and analyzed by standard QC lab assessment techniques to evaluate volumes, cell contents and hematocrit.
Results: The factor which provided the highest effect is TCF: in its low modality on platelet recovery, in its high modality on BCs Hct stability and plasma recovery. However, the best factors combinations are:
TCF 2.5 10^6 g*s, RCF 5314 g and acceleration 1 for platelets recovery (total duration spin: 21 mn)
TCF 5.0 10^6 g*s, RCF 5314 g and acceleration 1 for BCs Hct stability (total duration spin: 29 mn)
TCF 5.0 10^6 g*s, RCF 5314 g and acceleration 9 for plasma recovery (total duration spin: 23 mn).
Conclusion: Centrifugation parameters strongly affect the characteristics and throughput of blood components. Settings should be selected carefully to maximize the quality and consistency of blood components' characteristics and also maximize the efficiency of the processing laboratory.

P-170

THE INNOLIA CONFIRMATORY ASSAY IN THE EVALUATION OF FALSE POSITIVE RESULTS BY THE CMIA SCREENING ASSAY FOR HTLV

M Pape, V Bakaloudi, E Ntinopoulou, F Girtovitis, A Konstantinidou, M Chatzikyrkou, V Voulgaridou, D Pantelidou, P Chalkia, P Kaltsa, G Taliona and E Hasapopoulou-Matami

Ahepa University Hospital, Thessalon Iki, Greece

Background: Human lymphotropic viruses type I and II (HTLV-I/II) can be transmitted by transfused infected cellular blood components. Detection of antibodies against HTLV-I/II serves to diagnose HTLV infection and to protect the safety of blood supply. Routine blood bank screening for HTLV antibodies is obligatory in Greece. A major problem with the mass screening of blood for HTLV antibodies has been the relatively high rate of false reactivity associated with commercial HTLV immunoassays. This makes counseling of blood donors complex, even after repeat testing.
Aims: The aim of this study was to evaluate the rate of false positive results among blood donations reactive for HTLV-I/II by CMIA routine screening, by use of a serological confirmatory test (INNOLIA HTLV).
Methods: A total of 28,162 blood donors were tested for antibodies to HTLV-I/II at the AHEPA blood bank of Thessaloniki, during an 18 months period (June 2014 through December 2015). The Architect HTLV-I/II assay was used for initial screening. It is a two-step chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of antibodies to HTLV-I/II. Specimens with S/CO values ≥1.00 were considered reactive, while specimens with S/CO values 0.80–0.99 were considered 'indeterminate' (gray zone). All initially reactive and indeterminate samples were retested in duplicate. Samples that were either repeatedly reactive or in the gray zone, were further tested by a commercial INNOLIA HTLV Score assay (confirmation assay). The INNOLIA kit uses recombinant antigens and synthetic peptides derived from both HTLV-I/II protein sequences. We used the interpretation criteria suggested by the manufacturer. A sample was classified as positive if it reacted with at least one envelope antigen (gp21 or gp46) and one *gag* antigen (p19 or p24). Reactivity with two envelope antigens (gp21 and gp46) also indicated a positive sample. If an isolated band or no reaction appeared, the sample was considered negative. When two *gag* antigens were reactive, the sample was considered indeterminate. Discrimination between HTLV-I and HTLV-II was indicated by the corresponding specific antigens present on the same strip.
Results: CMIA detected 29 samples (0.10%) that were repeatedly reactive or in the gray zone. After testing with INNOLIA, 28 were resolved as negative and one as indeterminate. The latter one was INNOLIA retested and found to be negative as well.
Conclusions: The INNOLIA confirmation assay helped to classify as negative all 29 samples found reactive by commercial CMIA HTLV immunoassay. The very high rate of false reactivity (100%) by the screening method might warrant a change in policy. Complete abolition of screening for HTLV would be worrisome, while choosing to screen only first-time donors in countries with low HTLV prevalence, would substantially decrease unnecessary discarding of blood units and confusion about the need for counseling.

P-171

THE INFLUENCE OF TEMPERATURE AND TIME OF WELDING PLASTIC TUBES ON COMPONENT QUALITY

V Libek, A Kulic, A Strugar, M Milicev, J Djurdjevic, I Simic and I Gojkovic

CHC Zemun, Belgrade, Serbia

Background: Blood is stored in sealed plastic bags. The bags are filled trough plastic tubes which need to be sealed at the proper temperature, pressure and time to obtain standardization and GMP in component production. Special equipment provides reliable watertight closing by welding plastic tubes. These devices utilize the elementary welding theory. The tube has to be heated until it begins to melt. Then it has to be exposed to a pressure for a certain period of time sufficient to seal walls of tube together. This process can cause red cell hemolysis with a consequent production of low level free hemoglobin (LLFH). Thus LLFH can be used as an indicator of components quality.
Aims: In order to assess the impact of Sealmatic (LMB technologies), on the quality of components measured as LLFH under different temperature and duration of welding process itself, we investigated produced components.
Material and methods: The device Sealmatic (LMB technologies) utilizes this elementary welding theory. As tubes are made of a dielectric material, plastic, the most efficient way to heat it is to utilize dielectric losses caused by high frequency RF signals. Depending on particular purpose the power needed for the welding process ranges from a few dozen to several hundred watts.
Besides temperature and pressure the third parameter that controls the welding is time. The RF welding is needed to seal the tubes quickly and reliably and to keep the liquid from contamination and leakage. The required output power is 50 W at 50 Ω load. Besides, standards that regulate usage of radio frequency bands allow operating frequency of 40.68 MHz for medical appliances. The generator is loaded with an actuator that performs the welding by applying high electrical RF field on plastic material settled between two metal plates. During testing period we studied and compared LLFH in samples from 30 TT (top/top) quadruple blood bags (Blood bags CPD/SAG-M, with Buffy Coat Bag, TerumoBCT®). External tube diameter was 4.4 ± 0.1 mm, and the internal tube diameter was 2.95 ± 0.05 mm. The specimen for testing was taken after the sealing, after blood bags were centrifuged at +4°C at 3,500 rpm/20 min. LLFH was measured in plasma on Hemocue®. The adequate time of welding process was 2 s for this kind of tubes. Results were analyzed using descriptive statistic methods.
Results: All units met quality control of low level free hemoglobin between 0.06 and 0.00 g/dl. After sealing tubes with Sealmatic for 2 s, average value of LLFH was 0, 0216 g/dl. Such LLFH ensure the best quality of components.
Summary: Due to the fact that if welding causes less hemolysis, the better is component quality, the Sealmatic® ensures the constant and reliable sealing for all separations with possibility of connecting several units in a segment application. Sealmatic® allows to adjust the time of sealing process depending on tube diameter of different blood bags with no effect on component quality.

P-172

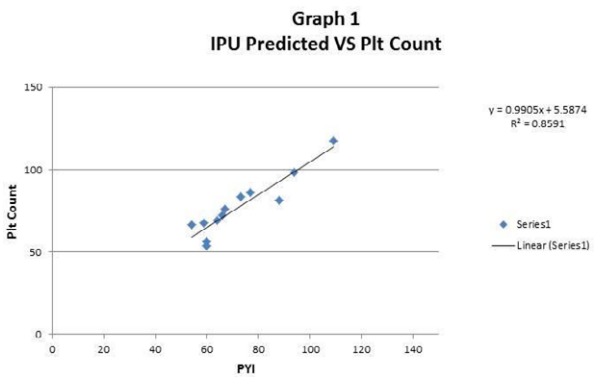
VALIDATION OF REVEOS TM SYSTEM USED FOR ROUTINE PROCESSING OF BLOOD COMPONENTS

E Lachert[1], J Antoniewicz-Papis[1], A Plodzich[1], M Gawrys[1], J Kubis[1], A Rosiek[1], Z Przybylska[2], D Piotrowski[2] and M Letowska[1]

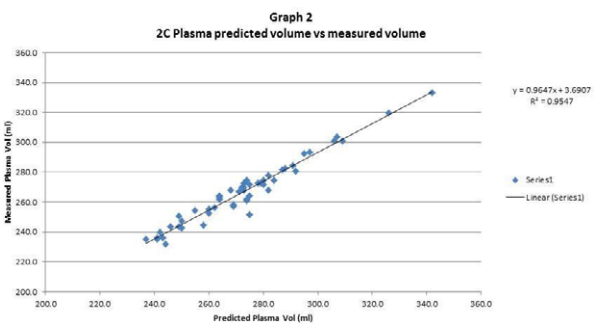
[1]Institute of Hematology and Transfusion Medicine, Warsaw, Poland [2]Regional Blood Transfusion Centre in Warsaw, Warsaw, Poland

Background: The Reveostm (Terumo BCT) is an automated system for processing up to four whole blood (WB) units in a simultaneously. Each blood unit can be processed into two components (2C protocol – plasma and red blood cell concentrate, RBCC) or three components (3C protocol -RBCC, plasma and leukoreduced platelet concentrate, PC). The Reveostm software presents the volume and platelet yield indicator (PYI) for each blood unit. PYI determines the platelet count in single PC units.

Aim: Evaluation of the quality of blood components obtained with Reveos™ system put to routine use.
Methods: For the purpose of the study 77 units of WB (450 ± 45 ml) were collected into special bags for the REVEOS™ system. Quality control (QC) parameters were evaluated forplasma, RBCCs and PCs. obtained with the Reveos™ system (3C) installed at the Processing Department of the Warsaw Blood Transfusion Center. The volume of each blood component was determined. The plasma quality control included: factor VIII coagulation activity, fibrinogen content as well as the number of residual leucocytes, erythrocytes and platelets. Each unit of leukoreduced RBCC was evaluated for hemoglobin and hematocrit values. Quality control of each leukoreduced PC included the number of white blood cells and platelet count.
Results: QC findings for blood components obtained with the Reveos™ system were compared with the parameters recommended in 'Medical Standards for collection, preparation and distribution of blood and blood components, 2014, edited by the Institute of Hematology and Transfusion Medicine'. We found that 96% (n = 74) of the evaluated plasma samples met the QC parameters for platelet contamination ($<50 \times 10^9$/l) and 100% (n = 77) met the QC parameters for leukocyte ($<50 \times 10^9$/l) and erythrocyte (6.0×10^9/l) contamination. Of the evaluated plasma samples 98.6% (n = 74) met the QC parameters for factor VIII coagulation activity (>70 IU/100 ml). The correlation between estimated (Reveos™ data) and the determined plasma volume was high (correlation coefficient – 0.83). The mean plasma fibrinogen content was 285.17 ± 47.5 mg/unit.
According to data analysis 100% (n = 77) of the leukoreduced RBCC units met QC parameters for both hematocrit (0.50–0.70) and hemoglobin (≥43 g/unit). The correlation between 'Platelet Yield Indicator', PYI and the real platelet count in pooled leukoreduced PCs was high (correlation coefficient – 0.75). Mean platelet content in leukoreduced PCs was $3.74 \pm 0.62 \times 10^{11}$.
Conclusions: Blood components obtained with the Reveos™ system met the QC parameters. The special Reveos™ software provides data on the volume of individual blood components and the platelet count in single units of blood (PYI) which contributes to better planning for further processing of pooled PCs.



Graph 1. IPU predicted VS PLT count



Graph 2. plasma predicted volume VS measured volume

P-173

INVESTIGATING THE FEASIBILITY AND EFFICIENCY OF THE REVEOS AUTOMATED BLOOD PROCESSING SYSTEM

N Oumeziane[1], J Genin[2], LB Haymour[1], M Joy[1], M Diab[1], C Vela[1], B Castro[1] and E Al Zaabi[1]

[1]Seha/SKMC, Abudhabi, United Arab Emirates [2]TERUMOBCT, Brussels, Belgium

Introduction: The Abu Dhabi Blood bank (ADBB) processes 30,000 units of whole blood per year using a semi-automated system into plasma, red blood cell concentrates (RBCC) and platelet concentrates (PC). In an attempt to optirnize the blood processing system, the ADBB decided to evaluate the performance of the Reveos automated system from Terumo BCT.
Aim: The aim of the study was to analyze recovery and quality of blood components processed out of whole blood using two different protocols: two components (2C) for whole blood separation into red blood cell concentrates RBCC and plasma and three components (3C) for production of RBCC, plasma and IPU (interim platelet unit). Additionally, user-friendly features like software tools for prediction of plasma volumes and platelet yields were evaluated.
Methods: For this study, 93 units of Whole Blood (450 ml ± 10%) were collected with REVEOS disposables and processed within 8 h of collection. Forty-two (42) units were processed using the 3C protocol and 51 units with the 2C protocol. After separation, the plasma units were blast frozen to −40°C. SAG-M was added to the red blood cell concentrates (RBCC) and leukoreduced using the inline integrated filter. IPU were rested for 1 h and stored on a shaker overnight, after which they were pooled by four in plasma and leukoreduced, resulting in a therapeutic platelet concentrate (PC). Standard *in-vitro* parameters to assess quality of blood components were performed.
Results: The 2C protocol produced plasma with a mean volume of 267 ml, contamination levels of white blood cell (WBC), platelets and red cell was far below the requirements of EU guidelines. Mean factor VIII and fibrinogen per unit were 1.39 IU/ml and 4.2 g/l, respectively. RCC produced by this protocol showed mean values for hemoglobin and hematocrit of 58 g/unit (47–71) and 58% (55–60), respectively.
Mean volume for plasma collected with the 3C protocol was 195 ml with minimal residual contamination of WBC, platelets and red cell. Mean values for factor VIII and Fibrinogen were 1.44 IU/ml and 5.9 g/l, respectively. Average RBCC hemoglobin and hematocrit were 59 g (55–65) and 57% (55–61), respectively. Residual WBC values were far below EU requirements. Leukoreduced platelet concentrates (PC) contained on average 3.3×10^{11} platelets/Unit. For both protocols, coefficient of determination R^2 between Reveos-estimated plasma volume and measured volume was ≥0.95 (Graph 2). Correlation between estimated and measured platelet yield in IPU (Graph 1) was also very high ($R^2 = 0.86$).
Conclusion: The Reveos automated system for processing of whole blood seems to solve many physical and operational problems faced by the ADBB: it requires less space in our facility due to the combined functions (centrifuge and fractionator), it produces standardized products of excellent quality and it is operator-friendly, demanding minimal staff training time. The estimated platelet yield provided by the Reveos system for each IPU allows for optimal pooling into therapeutic doses and minimal patient-donor exposure that may improve patient outcomes.

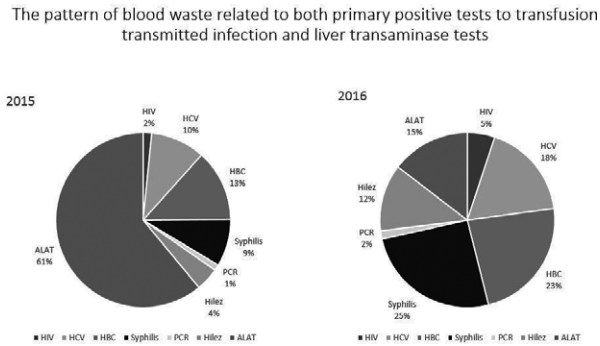
P-174

THE PATTERN OF DONOR BLOOD REJECTION AFTER IMPLEMENTATION OF PRE-DONATION LIVER TRANSFERASE SCREENING

S Abdrakhmanova

Republic Research Center of Transfusion, Astana, Kazakhstan

Background: The pre-donation liver transaminase screening was implemented since October 19, 2015 and 7 day business week was started since November 1, 2015.
Aims: To study how the implementation of pre-donation liver transaminase screening and 7 day business week make an effect to the pattern of donor blood rejection.
Methods: The donor blood rejection reporting has reviewed that is included the period from November 2015 to March 2016 and from November 2014 to March 2015. The data has treated statistically by EXCEL program.
Results: The total number of blood donations was 18,806 in 2015 and 15,376 in 2016. The donor blood rejection rate has improved due to its shrinking from 9.1% to 3.8%. The number of collected blood units with abnormal ALAT is decreased significantly from 1,048 to 48. Therefore blood rejection rate due to abnormal ALAT has decreased from 60.9% to 12.3% relatively.
The following results of pre-donation liver transaminase screening had revealed: 17,388 donors has underwent to screening where 877 donors had a high degree of ALAT which are withdrawn from blood donation that is showed as 5.0%. Among 973 donors intended to blood donation by the outward sessions, 58 donors had an abnormal ALAT (5.9%). The donor blood rejection rate due to lipidemia decreased



Picture 1

from 10.4% to 4.7%. The study of the donor rejection pattern by its ranking had revealed abnormal ALAT, hepatitis B virus, hepatitis C virus in 2015 and syphilis, hepatitis B virus, hepatitis C virus in 2016.

Summary: The rate of donor blood rejection had reduced up to 66%, including ALAT abnormality causes (93%). Therefore, discarding rate of blood had decreased up to 65% and discarding rate of blood related to abnormal ALAT had decreased up to 93% relatively. The retention of plasma products had decreased up to 49%.

Blood Components

P-175

THE EFFECT OF L-Carnitine on Platelet Oxidative Damage and Platelet Lipid Peroxidation During Storage of Platelet Concentrate

N Helmi Siasi Farimany, MR Deyhim, F Amiri and S Amini Kafiabad

High Institue for Education and Research in Transfusion Medicine, Tehran, Iran

Background: Platelet transfusion is used in various forms of medical care and hence, there is an increasing demand for platelet concentrates. As we may know, platelet concentrates require particular storage conditions. It has been shown that some biochemical,functional and morphological changes occur in platelet during storage called platelet storage lesion (PSL) which cause decreases in the functioning and efficacy of platelet concentrate for transfusion. It has been indicated that oxidative stress has a crucial role in formation of PSL. Some studies suggested that L-carnitine can modulate platelet oxidative stress.

Aim: To evaluate the antioxidant effect of L-carnitine on PSL.

Methods: Platelet concentrates were stored in blood bank conditions. Platelet units were sampled on the day of collection (first day), 3th and 5th day. We measured the total antioxidant capacity and the levels of Malondialdehyde (a marker of lipid peroxidation) in the platelet concentrates treated with 100 mmol L-carnitine and platelet concentrates which were treated with saline solution (as control groups) at day first, 3th and 5th of platelet storage and results compared in this two groups.

Results: According to results, we have observed that L-carnitine prevents decreasing total antioxidant capacity of stored platelet concentrates (total antioxidant capacity on day 5 of storage were 0.126 ± 0.013 mmol in control groups and were 0.195 ± 0.015 mmol in L-carnitine treated groups, $P < 0.05$).

Moreover, our result demonstrated that L-carnitine may protects platelet concentrates against lipid peroxidation during storage (Malondealdehyde levels on day 5 of storage were 7.5 ± 0.25 µmol in control groups and were 2.13 ± 0.43 µmol in L-carnitine treated groups, $P < 0.05$).

Conclusion: It seems that L-carnitine may have some protective effects against oxidative changes occur during platelet storage.

Variables	PC type	Day 1 mean ± SD	Day 5 mean ± SD
PLT count (x10^{10}/dose)	Plasma	7,83 ± 0,9	7,14 ± 0,7
	SSP+	7,33 ± 0,8	6,83 ± 0,7
	Intersol	7,3 ± 0,8	6,56 ± 0,7
MPV (fL)	Plasma	5,61 ± 0,2	4,82 ± 0,2
	SSP+	5,65 ± 0,3	4,82 ± 0,3
	Intersol	5,36 ± 0,1	4,54 ± 0,2
pH	Plasma	7,29 ± 0,03	7,49 ± 0,1
	SSP+	7,17 ± 0,03	7,32 ± 0,1
	Intersol	7,17 ± 0,03	7,21 ± 0,1
Glucose (mmol /L)	Plasma	16,3 ± 0,6	14,2 ± 0,4
	SSP+	6,4 ± 0,1	4,7 ± 0,2
	Intersol	6,3 ± 0,2	3,3 ± 0,2
Lactate (mmol /L)	Plasma	9,5 ± 1,2	12,7 ± 0,8
	SSP+	5,1 ± 0,6	8,2 ± 0,6
	Intersol	5,2 ± 0,7	11 ± 0,6
HCO^-_3 (mmol /L)	Plasma	17,5 ± 1,0	12,4 ± 0,7
	SSP+	8,2 ± 0,5	7,4 ± 0,5
	Intersol	8 ± 0,8	5,8 ± 0,5
pO_2 (kPa)	Plasma	13,6 ± 3,1	12,9 ± 2,6
	SSP+	14,7 ± 2,7	14,3 ± 2,4
	Intersol	14,5 ± 2,6	14 ± 1,9
pCO_2 (kPa)	Plasma	6,7 ± 0,5	3,3 ± 0,2
	SSP+	3,8 ± 0,2	2,3 ± 0,2
	Intersol	3,6 ± 0,4	2,6 ± 0,3
CD 62 (%)	Plasma	5 ± 1	4 ± 2
	SSP+	4 ± 2	4 ± 2
	Intersol	11 ± 4	11 ± 3
ADP (IU)	Plasma	49 ± 5	28 ± 4
	SSP+	31 ± 9	19 ± 4
	Intersol	33 ± 9	15 ± 3

Table 1. *In vitro* properties of platelets stored in plasma and additive.

P-176

IN VITRO PROPERTIES OF PLATELETS STORED IN PLASMA AND TWO DIFFERENT ADDITIVE SOLUTIONS

M Liker[1], M Strauss Patko[1], M Tomicic[1], J Gulan Harcet[1], K Gojceta[2] and I Jukic[1]

[1]Croatian Institute for Transfusion Medicine, Zagreb, Croatia [2]University Hospital Centre, Zagreb, Croatia

Background: The use of additive solution for partial substitution of plasma for storage of leucodepleted platelet concentrates can have advantages, such as reduction of allergic and febrile transfusion, facilitation ABO-incompatible platelet transfusion and availability of more plasma for fractionation.

Aims: Comparation *in vitro* properties of platelets stored in plasma and two different additive solutions (Intersol and SSP+) for 5 days. The storage of platelets concentrate in plasma is well-establish practice and provide a benchmark against which the performance of platelets stored in additive solutions can be compared.

Methods: Donor differences were avoided by applying pool-and-split design. Twelve buffy coats were pooled and divided into three parts. Each part was pooled with 240 ml of plasma or 250 ml of PASs (Intersol, Fresenius Kabi or SSP+, Macopharma) using pooling system of Haemonetics. On days 1 and 5 of storage samples were tested for platelets concentration, mean platelet volume (MPV), pO_2, pCO_2, glucose, lactate, bicarbonate, pH, CD62P, ADP induced aggregation.

Results: The results were summarized in the Table 1. All platelets concentrates met national and Council of Europe guidelines for platelet products. No significant differences were observed for pH, MPV, pO2 between PCs stored in plasma and PCs stored in platelet additive solutions. PCs stored in plasma showed higher platelets count, glucose and bicarbonate level and ADP induced aggregation. PCs stored in SSP+ showed better quality compared to PCs stored in Intersol as indicated by better platelets count, lower glucose consumption and lower platelets activation.

Conclusion: *In vitro* quality of PCs stored in both platelet additive solution is well maintained. However, storage of PCs in Intersol results in increased platelet activation and platelet metabolism compared with PCs stored in SSP+.

P-177

THE IMPACT OF PLATELET SOURCE, FROM WHOLE BLOOD OR COLLECTED BY APHERISIS AND OTHER TRANSFUSION CHARACTERISTICS, ON PLATELET TRANSFUSION EFFICACY IN ADULT AND PEDIATRIC HEMATOLOY-ONCOLOGY PATIENTS

E Lydaki

University Hospital of Heralkion, Herklion, Greece

Background: Platelet concentrates prepared either from pooled buffy-coats of the whole-blood donations (P-PCs) or by apheresis (A-PCs) are indicated to prevent or treat acute hemorrhage in patients with chemotherapy induced thrombocytopenia and there is an ongoing debate which is superior. Moreover, other factors affecting the quality of the product such as storage period, ABO compatibility, or platelet dose also have an impact on platelet transfusion efficacy.
Aim: The aim of the study was to evaluate the effect of specific platelet product characteristics {dose, source [apheresis (P-PCs) vs (A-PCs)], donor-recipient ABO compatibility, and duration of storage} on post-transfusional platelet increments and most likely on clinical bleeding in thrombocytopenic patients.
Methods: One hundred and thirty four platelet transfusions (80 P-PCs and 54 A-PCs) in 17 adult and pediatric patients with chemotherapy induced thrombocytopenia were studied. Patients included in the study did not present platelet refractoriness, sepsis, splenomegaly or severe hemorrhage. All patients were assessed for bleeding according to WHO scale system (Milller et al., Cancer 1981;47:2007–2014). Furthermore, for a more precise definition of bleeding manifestations and in order to evaluate the effect of transfusions, patients with grade I hemorrhage were subcategorized into two groups: grade IA and grade IB according to standard criteria. Transfusions in patient with ≤grade IA bleeding were categorized in the prophylactic platelet transfusion arm and 24 h Corrected Count Increment (24 h CCI) for platelets was calculated. All other patients were categorized in the therapeutic arm and were assessed daily for bleeding events. The following parameters were examined during the study: the source of platelet transfused (P-PCs ή A-PCs), the platelet dose (plt/m^2), donor-recipient ABO major mismatch and platelet storage days (1–3 or 4–5 days). Statistical analysis was performed using correlation, t-test and chi square test.
Results: The content of platelets (plt/m^2) per transfusion was strongly correlated with the 24 h CCI of the patients (r = 0.015). In P-PCs platelet dose was higher than in A-PCs in adults (P = 0.002) and as a result the 24 h CCI was found significantly higher (P = 0.033). However, the 24 h CCI was found higher in pediatric patients transfused with A-PCs than those with P-PCs (P = 0.024) although the platelet dose of A-PCs was lower than in P-PCs (P = 0.0009). Nevertheless, a higher number of pediatric patients are needed to clarify this finding. Duration of storage significantly affected the 24 h CCI (P = 0.047). ABO major mismatch was more often in A-PC transfusions (χ^2 = 0.032). In this study it was not proven to influence 24 h CCI, but ABO major mismatch sample was small because ABO-identical or minor-mismatch platelets were preferred. In 3/17 patients worsening of bleeding from grade I to grade II were observed with the platelet source not having an effect. Finally, major bleeding (>grade 2) was not observed in any of our patients.
Summary/Conclusions: Platelet source was not found to effect transfusion efficacy in adults. Other transfusion factors such as, platelet storage and platelet dose were found to play a role but further investigation is need with a higher sample number of transfusions to establish these findings.

P-178

TRANSFUSION-RELATED FACTORS INFLUENCING POST TRANSFUSION PLATELET INCREMENT IN THROMBOCYTOPENIC PATIENTS

S Ahmed, MZ Mohd Hayat and MN Hassan

School of Medical Sciences/Universiti Sains Malaysia, Kubang Kerian/Kota Baru, Malaysia

Background: Most clinicians are inclined to request platelet transfusion in many cases of thrombocytopenia where patients suffer from bleeding or would be at high risk of bleeding. Many studies had been performed to determine the factors that may contribute to post transfusion platelet increment. No documented data is available in our institution in this area of knowledge yet.
Aims: This study was carried out to determine the transfusion-related factors that may influence post-transfusion platelet increment.
Methods: A retrospective cohort study was set to include 80 patients who received platelet transfusions in Hospital Universiti Sains Malaysia. The study reviewed 283 of platelet transfusions within the years of 2013 to 2014. The pre and post transfusion platelet counts were measured by using Sysmex haematology analyzer XE5000 and the corrected count increment (CCI) was calculated at 1 and 24 h post transfusion. Poor increment was defined as CCI < 7,500 m^2/μl at 1 h and/or CCI < 4,500 m^2/μl at 24 h. The analyzed factors including the type of platelet concentrate (random vs apheresis), ABO blood group and age of platelet concentrate were obtained from the transfusion unit record. Simple (SLR) and multiple logistic regression (MLR) were used for statistical analysis and P-value of <0.05 was considered as significant.
Results: Majority of the patients were Malays (92.5%) with a mean age of 41 years. The number of males and females patients was equal. Majority of patients were O positive (40%) followed by A and B positive (27.5% each) and AB positive (5%). The mean and median of CCI was 15,087 m^2/μl and 11,037 m^2/μl, respectively. Majority of the patients had good CCI (66.8%). Simple and multiple logistic regressions showed that only platelet age was a significant factor influencing post transfusion platelet increment. Platelet age of >3 days had 2.4 time odds of poor CCI as compared to fresh platelet (P = 0.042, 95% CI = 1.03, 5.60) and had 2.2 time odds of poor CCI compared to fresh platelet (P = 0.004, 95% CI = 1.29,3.75) by SLR and MLR respectively. Other factors including ABO-identical, plasma compatibility, and type of platelet concentrate were not statistically significant.
Conclusion: Fresh platelet concentrate (age of ≤3 days) is highly recommended compared to older platelet concentrates in thrombocytopenic patients as it showed better platelet increment post platelet transfusion.

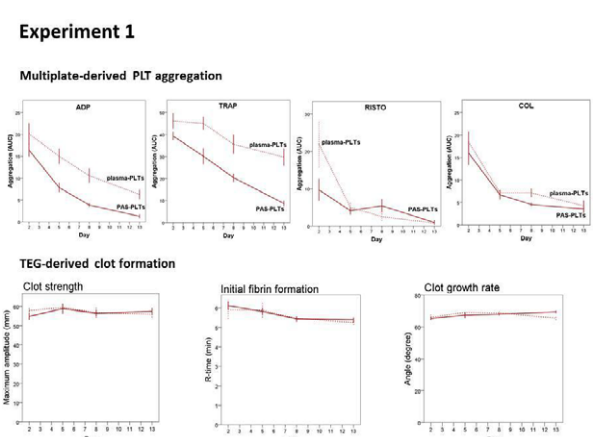
P-179

QUALITY OF DOUBLE-WASHED PLATELETS USING THE ACP215 AUTOMATED CELL PROCESSOR

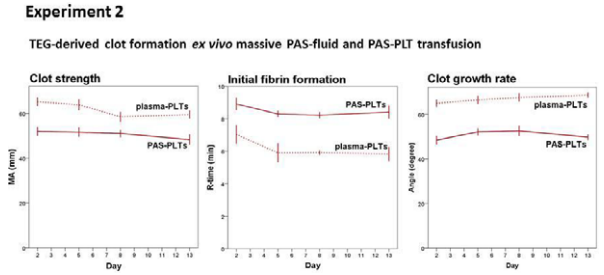
Y Hayashi, S Wakamoto, H Fuse, M Fujihara, M Endo, C Homma, T Yamamoto, H Ikeda, S Kino and S Takamoto

Japanese Red Cross Hokkaido Block Blood Center, Sapporo-shi, Japan

Background: Washed platelet concentrates suspended in additive solution (W-PC) are shown to be effective in preventing non-hemolytic transfusion reactions (NHTRs) which are often caused by plasma proteins. In the invalid cases with W-PC or the haptoglobin (Hp)- or immunoglobulin A (IgA)-deficient patients, further reduction of plasma protein by double or triple washing of PCs may be required. In fact, we reported that triple-washed PCs prepared manually (n = 47, residual plasma protein: 13.7 ± 5.6 mg/bag) were effective for preventing NHTRs in the Hp-deficient patient with anti-Hp antibody. Recently, it has been reported that the automated ACP215 cell processor (Haemonetics Corporation) is a feasible alternative to the manual method. The double washing by the ACP215 may be required for some recipients with Hp- or IgA-deficiency.
Aim: The aim of this study was to evaluate *in vitro* properties and platelet functions on double-washed PCs (2w-PCs) using the ACP215 in comparison with single-washed PCs (1w-PCs).
Method: Two bags of apheresis PC were pooled and split on the next day after collection from donor (n = 5). Split PC was washed either once or twice with the



Caption 1. Experiment 2



Caption 2. Experiment 1

platelet additive solution, termed BRS-A. Each washing process required 2 l of BRS-A, in which bicarbonate Ringer's solution and acid-citrate-dextrose formula A were automatically mixed with the ACP215 at ratio of 20:1. Total protein, IgA and Hp of the 1w-PCs or 2w-PCs were measured before and after washing. Platelet recovery was calculated from the number of platelets per bag of pre- and post-washed PCs. 1w-PCs and 2w-PCs were stored for 5 days after preparation, and compared on platelet functions (% HSR, aggregation (ADP 5 μM+Collagen 2.5 μg/ml), CD62p expression), pH, glucose, lactic acid and mean platelet volume (MPV) on 0, 1, 2, 3, 5 days.
Results: The levels of total plasma protein, IgA, and Hp in 1w-PCs were 120.7 ± 11.1, 3.93 ± 1.57, and 0.64 ± 0.29 mg/bag, and those of 2w-PCs were 13.5 ± 7.0, 0.022 ± 0.005, and 0.03 ± 0.02 mg/bag, respectively. Platelet recovery of 1w-PCs and 2w-PCs were 95.4 ± 2.2% and 82.3 ± 9.3%, respectively. Although % HSR of 2w-PCs during storage were significantly lower than those of 1w-PCs from day 2 to day 5, % HSR of 2w-PCs remained more than 70% from day 0 to day 3. Similarly, aggregation responses of 2w-PCs to agonists were significantly lower than those of 1w-PCs on day 0 and day 2, but aggregation responses of 2w-PCs were more than 75% from day 0 to day 3. The CD62p expression of 2w-PCs were significantly higher than those of 1w-PCs from day 0 to day 3. No differences in pH, glucose and lactic acid were found between 1w- and 2w-PCs. MPV of 2w-PCs was higher than that of 1w-PCs on day 1.
Conclusion: The residual plasma protein of 2w-PCs prepared with the ACP215 was comparable to that of triple-washed PC by manual. The platelet functions of 2w-PCs were maintained sufficiently for 3 days after preparation. These results suggest that 2w-PCs prepared with the ACP215 may be effective for preventing NHTRs in Hp- or IgA-deficient patients.

P-180

HEMSOTATIC FUNCTION OF PLATELETS STORED IN PLATELET ADDITIVE SOLUTION C COMPARED TO PLATELETS STORED IN PLASMA

FMA van Hout, I Bontekoe, L de Laleijne, JL Kerkhoffs, D de Korte, JG van der Bom and PF van der Meer

Sanquin, Leiden, The Netherlands

Background: Platelet (PLT) concentrates can be stored in plasma or platelet additive solution (PAS-C). PAS-platelets cause fewer allergic reactions than plasma-platelets. However, there are concerns about the hemostatic efficacy of PAS-PLTs.
Aim: The objective of this study was to compare the hemostatic function of PAS-platelets to plasma-platelets.
Methods: To compare the hemostatic function of the two platelet products independent of patient factors, an *ex vivo* model was created by reconstituting whole blood. This study was divided in two experiments.
In the first experiment the reconstituted blood samples were composed of red blood cells, solvent-detergent pooled (SDP) plasma, and either PAS-platelets (n = 7) or plasma-platelets (n = 7) in physiological ratio.
In the second experiment the potential influence of a massive transfusion of platelet storage medium (PAS or plasma) on hemostasis was studied. Thus, in the second experiment the SDP-plasma component was replaced either by PAS fluid or by plasma from the same platelet concentrates where the platelets came from. In other words, besides red blood cells the samples either contained PAS fluid and PAS-platelets (n = 7) or plasma and plasma-platelets (n = 7).
Hemostatic function was evaluated with impedance aggregometry (Multiplate) induced by four agonists: thrombin receptor-activating peptide (TRAP), adenosine diphosphate (ADP), collagen (COL) and ristocetin (RISTO) and thrombelastography (TEG) on days 2, 5, 8 and 13 following platelet donation. The influence of the product types, independent of storage day, was analysed with a linear model with correlated errors.
Results: In the first part of the study the samples with PAS-platelets showed significantly lower aggregation (Multiplate) than the samples with plasma-platelets when induced with ADP −5 U [95% confidence interval (CI) −6; −4] or TRAP −16 U (−22; −9). When activated with COL and RISTO there was no difference between the PAS-platelet and the plasma-platelet samples. In all samples the aggregability declined over storage time. The TEG derived maximum clot strength (maximum amplitude), initial fibrin formation (R-time) and clot growth rate (angle) of the samples with PAS-platelets were similar in the samples with plasma-platelets. All TEG parameters remained constant over time.
In the samples of the second experiment containing both PAS-platelets and PAS fluid all TEG parameters were less favourable than in the samples with plasma and plasma-platelets: maximum amplitude −11 mm (−16; −7), R-time +2.3 min (1.9; 2.7) and angle −17° (−20; −14).
Conclusions: PAS-platelets show less aggregability in response to ADP and TRAP. However the overall clot formation was not affected by these platelet receptor defects. Whole blood containing a considerable volume of PAS-fluid and PAS-PLTs shows significantly declined clot formation compared to whole blood containing plasma-PLTs and accompanying plasma.

P-181

IN VITRO ASSESSMENT OF RED CELL CONCENTRATES AFTER WARMING WITH A BLOOD WARMER

JWM Lagerberg[1], R Vlaar[1], B Teunissen[2], R van Oudenallen[2] and D De Korte[1]

[1]*Sanquin Research, Amsterdam, The Netherlands* [2]*The 37Company, Amersfoort, The Netherlands*

Background: A blood warmer is a device used in hospitals to prevent patients receiving large volumes of blood plasma, intravenous fluids or blood products from cooling down. Preventing hypothermia during surgery decreases the risk of complications and improves recovery. The 37Company has developed the Fluido® Compact, a blood and fluid warmer suitable for warming red cell concentrates (RCC) at low flow rates at bedside. The Fluido® Compact consists of a control module, a warming module and a disposable set. The disposable set includes a warming unit which warms the fluid as it passes through the unit.
Aim: To evaluate the *in vitro* quality of the RCC after warming with the Fluido® Compact.
Methods: To simulate worst possible conditions, both leukocyte depleted (LD) as well as non-leukocyte depleted (non-LD) RCC in SAGM, stored at 2–6°C for 35–50 days, unless otherwise indicated, were used. To test the capability of the warmer, in some cases pools of three RCC were used. The RCC were removed from the refrigerator just prior to warming. The disposable set was primed with a 0.9% NaCl solution. The Fluido® Compact has a fixed set temperature of 39°C and the RCC passes through the warming device at various flow rates. Prior to and immediately after warming, the cellular composition and haemolysis of the RCC were analysed.
Results: The results are summarised in the Table 1.
After warming, the temperature of the RCC was 30–37°C depending on the flow rate. The results of the RBC count and haematocrit indicate that some dilution of the RCC by the priming fluid occurs during the warming procedure (values are approximately 20% lower). RBC parameters such as MCV and MCHC were not affected by the warming procedure.
After warming, the number of platelets increased slightly, particularly in conditions 1 and 2, however the absolute values are quite low. The increase in platelets must

Test condition		RCC	Fluido®Compact settings		Hct (L/L)		Trombocytes (x10^9/L)		Haemolysis (%)	
			Flow	Temperature	Before	After	Before	After	Before	After
1	a*	LD	Maximum	39°C	0.538	0.460	2	9	1.15	1.25
	b				0.572	0.464	1	4	0.74	0.72
	c				0.567	0.465	0	6	0.19	0.17
2	a	LD	Gravity	39°C	0.554	0.469	2	10	0.39	0.34
	b				0.572	0.455	4	13	0.35	0.36
	c				0.565	0.420	2	12	0.30	0.28
3		LD	Maximum	off	0.597	0.515	2	1	0.19	0.14
4*		LD	Gravity	off	0.568	0.479	3	2	1.21	1.21
5#		LD	65 mL/min	39°C	0.529	0.525	1	3	0.32	0.30
6#		LD	gravity	39°C	0.677	0.597	2	1	0.26	0.29
7#		LD	50 mL/min, interrupted	39°C	0.663	0.616	1	3	0.15	0.18
8#		Non-LD	50 mL/min, interrupted	39°C	0.769	0.722	4	3	0.33	0.44

*: 64 days stored before heating, #: pool of 3 RCCs

Table 1. Cellular composition and haemolysis of RCC before and after heating using Fluido® Compact

be attributed to vesicles derived from RBC since no platelets are formed during the warming process. The RCC (1a) stored for 64 days and the pool of 35-day-old non-LD RCC (8) showed a slight (0.1%) increase in haemolysis caused by warming while there was no increase in haemolysis in all other RCC. RCC which met the requirement for haemolysis (<0.8%) prior to warming, also met the requirement after warming. Neither the increase in haemolysis nor the increase in number of vesicles was seen when the heating was switched off, indicating no mechanical damage.

Summary/Conclusions: Warming LD-RCC which has been stored for 35–50 days or non-LD RCC which has been stored for 35 days using the Fluido® Compact with a temperature of 39°C and various flow rates resulted in only minimal damage to the RCC. The warmed RCC fulfilled the requirement for maximum haemolysis (0.8%).

P-182

EVALUATION OF THE REVEOS AUTOMATED WHOLE BLOOD PROCESSING SYSTEM WITH REGARD TO THE HEMOGLOBIN YIELD IN RED BLOOD CELL CONCENTRATES

B Hermundstad, I Walbækken, AKB Flesland, AH Llohn and SA Mousavi

Akershus University Hospital, Lorenskog, Norway

Background: The Reveos processing system (Terumo BCT) represents an automated blood component processing system that combines all steps of whole blood processing, including balancing, centrifugation and pressing, in one system. The device has the capacity to process four whole blood units per run. The Reveos system offers two independent processing options. The first processing option is a two-component (2C) protocol for the production of a plasma unit and a red blood cell (RBC) concentrate and is designed to increase the amount of plasma and RBCs from each whole blood unit. The Reveos three-component (3C) protocol automates whole blood processing by providing, in addition to a plasma unit and a RBC concentrate, an interim platelet unit. Whole blood units can either be processed on the collection-day (2C-fresh and 3C-fresh protocols) or kept overnight at 22 ± 2°C (2C-overnight and 3C-overnight protocols) before processing.

Aim: The aim of this study was to evaluate volume and hemoglobin yields in RBC concentrates prepared by the Reveos protocols and to compare the results with volume and hemoglobin yields in RBC concentrates prepared by the 'Top-Bottom-system' by using Heraeus Cryofuge 6000i, T-ACE II Component extractor with Fenwal Quad Optipure 450 ml donation set.

Materials and methods: The units of whole blood were obtained from 120 volunteer donors, 30 units for preparation of RBC concentrates by each of the four protocols. Volume and hemoglobin yields in 30 RBC concentrates prepared by 'Top-Bottom-system' were used for comparison with those in concentrates prepared by each of the four protocols in the Reveos system. Hemoglobin levels were measured by Sysmex XT2000i.

The volume (ml) was calculated by weighing the content of storage bag.

Statistical analysis: Independent t-tests were used to determine significant differences in volume and hemoglobin yields in units prepared by Top-bottom and those prepared by Reveos. Differences were considered significant if the P value was <0.05.

Table: Comparison of volume and hemoglobin yields prepared by Reveos protocols with volume and hemoglobin yields prepared by Top-Bottom system

	Reference	Reveos system protocols			
Variable	Top-Bottom	2C-Fresh	2C-Overnight	3C-Fresh	3C-Overnight
Volume (ml)	247.2 ±10.4	272 ± 13.6	277 ± 18.2	281 ± 10.7	277 ± 16.2
Haemo-globin (g/unit)	47.2 ± 3.6	52.7 ± 3.9	54.4 ± 5.6	53.4 ± 4.7	53.8 ± 5.1

Results are reported as means ± SD

Results: The mean volume values of RBC concentrates prepared by all Reveos protocols were significantly higher than the mean volume value of RBC concentrates prepared by 'Top-bottom-system', with a mean volume value >272 ml per unit compared to 247 ml per unit, respectively (P < 0.001 for all comparisons). The mean hemoglobin concentration values in RBC concentrates prepared by all Reveos protocols were consistently greater than the mean hemoglobin concentration value in RBC concentrates prepared by 'Top-bottom-system', with a mean hemoglobin concentration >52 g/unit, compared to 47 g/unit, respectively (P < 0.001 for all comparisons; Table 1). The only difference between the four protocols in the Reveos was seen in the average volume value of the RBC concentrates prepared by 2C-Fresh compared to that prepared by 3C-Fresh (P = 0.032).

Conclusion: The Reveos system is satisfactory with regard to its performance in producing higher volume and hemoglobin levels compared to the 'Top-bottom-system'. Depending on the protocol used, up to 34 ml additional volume per RBC unit was obtained. The hemoglobin yield was also increased by about 6 g per RBC unit.

P-183

EVALUATION OF A NEW FLEXIBLE FILTER SYSTEM (BIOR FLEX) FOR LEUCODEPLETION OF RED CELL CONCENTRATES IN A WIDE RANGE OF PROCESSING CONDITIONS

F Cosenza[1], J Lagerberg[2], N Veera Sekaran[3], A Rosnizah[3], L Budzynski[4], E Embiger-Paprota[5] and U Schmidt[6]

[1]Fresenius Kabi, Mirandola, Italy [2]Sanquin Research, Department of Blood Cell Research, Laboratory for Blood Transfusion, Amsterdam, The Netherlands [3]Transfusion Medicine Department, University MALAYA Medical Center, Kuala Lumpur, Malaysia [4]Regionalne Centrum Krwiodawstwa i Krwiolecznictwaim, Poznan, Poland [5]Regionalne Centrum Krwiodawstwa i Krwiolecznictwa, Lublin, Poland [6]Fresenius Kabi GmbH, Bad Homburg, Germany

Background: Quality and consistency are pivotal features in the processing of blood components. Manifold separation procedures and blood storage conditions are applied all over the world, which affect Red Cell Concentrate (RCC) quality and clinical effectiveness. In order to fulfil increasing quality requirements, improve standardization and handling, Fresenius Kabi designed a new flexible filter (BioR flex) for the post-processing leukocyte depletion of RCC by gravity filtration. The new design promotes blood recovery and allows an easy handling, since no intervention is required at the filtration onset. The system includes an air venting device, to remove residual air from the leukocyte depleted RCC bag, while preserving sterility and store blood component till the expiry date.

TABLE I
Testing conditions
(data are reported as mean ± st.dev.)

	n	Whole Blood Volume (mL)	Procedure	RCC age @ filtration (day)	RCC Volume (ml)	RCC Hct (%)
Site 1	20	450	BC	≤ 14	275 ± 13	57 ± 2
Site 2	20	450	BC	≤ 4	279 ± 17	58 ± 3
Site 3	12	500	BC	≤ 14	250 ± 5	69 ± 1
Site 4	15	470	PRP	≤ 7	345 ± 32	53 ± 9

Table 1

TABLE II
Filtration parameters
(data are reported as mean ± st.dev.)

	Filtration time (mm:ss)	resWBC x 10E6 (cells/U)	Total Hb (g/U)	Hemolisys % @ day 42
Site 1	08:43 ± 01:45	0.02 ± 0.03	47.6 ± 4.4	0.34 ± 0.17
Site 2	05:05 ± 00:54	< 0.01*	44.4 ± 4.7	0.51 ± 0.17
Site 3	13:04 ± 01:05	0.07 ± 0.03	43.2 ± 2.7	0.32 ± 0.12
Site 4	08:03 ± 01:57	0.14 ± 0.13	56.7 ± 6.2	--

* under detection limit

Table 2

Aim: A multi-site study has been initiated to perform a comprehensive evaluation of the new BioR flex labside system under different RCC storage conditions and following different methods of blood processing [Buffy-Coat (BC) removal or Platelet-Rich-Plasma (PRP) removal]. Six sites in Europe and Asia were enrolled; up to now, four of them have completed the planned validation.
Methods: Sixty-seven SAGM-RCC units were prepared from CPD-Whole Blood donations (450–500 ml) in four different sites, following BC- or PRP-removal procedures. Blood separation was performed with CompoMat G4® or G5® (Fresenius Kabi). Test conditions were selected to handle blood components of different ages (within 14 days), after storage at 4°C. Blood temperature at the time of filtration was in a range between 10 and 19°C. System performance was evaluated by filtration times, residual leukocytes, hemoglobin content and hemolysis rate. One site performed a comparison on 12 RCC units processed after 1–4 days or 10–14 days of storage. Leukocyte depleted units were further stored at 4°C to perform quality tests at day 42 (pH at 37°C, Potassium, Glucose, Lactate, Haemolysis rate).
Results: RCC units prepared following different procedures showed wide ranges of volume and Hct values before filtration (Table 1). Overall filtration results are presented in Table 2. All tests met both European and AABB Guidelines of residual WBC $<1 \times 10^6$/unit. 65/67 haemolysis rate values were <0.8%, but all <1.0%. Filtration times correlated well with RCC Hct values, regardless of the RCC age. RCC units filtered after 1–4 days or 10–14 days of storage didn't show clinically relevant differences in metabolic parameters, when tested at day 42th.
Conclusions: Available results from this multi-site study showed good leukocyte depletion performance and hemolysis rates at the expiry date, both following BC- or PRP-removal procedures. Filtration times were influenced by the preparation method, regardless of the RCC age at the time of filtration. The system handling was found very convenient and efficiently reduced the risk of misuse. The BioR flex labside system performance consistently fulfilled all quality requirements from international guidelines, while improving usability and standardization.

P-184

EVALUATION THE WASTAGE OF BLOOD AND BLOOD COMPONENTS IN IRANIAN TRANSFUSION SERVICES IN 1393 (2014–2015)

S Sharifi, M Shahabi and A Balazadeh
Research Center of Iranian Blood Transfusion Organization, Tehran, Iran

Background: Economy is one of the most important concerns in every country, so cost-benefit analysis is a top priority in each process. Blood safety is the main issue in transfusion medicine and each cost-benefit study should be considered from this standpoint. Reducing blood wastage would save large amount of money for blood centers. Finding and correction of the causes is a way of wastage reduction. Although wastage is inevitable to some extent, but the majority is avoidable by proper planning and right management.
Materials and methods: This study was a retrospective. Data was extracted from donation software without the possibility of any change by operator. Data contained the information from 31 provinces for a 1-year period (1393).
Results: A total of 2,565,208 volunteers registered during the year 1393, of which 2,070,028 were accepted and donated blood. The others were deferred for different reasons. Blood components were prepared from these donations as follows:
Red blood cell concentrate 1,598,813 units
Platelet concentrate 1,102,372 unites
Fresh frozen plasma (FFP) 1,701,996 unites
Conclusion: Our findings indicate that: FFP has the highest rate of wastage among the other components (332,414 units). To reduce this amount, the following recommendations may be useful: making contract with fractionation company to manufacture plasma derivatives need assessment to prevent resources losing.
The number of expired components available in blood centers is much greater than those in hospitals. This does not make sound and emphasizes the need for the implementation of comprehensive software in hospitals to record data throughout the process.
The FFP component is much more vulnerable to physical damage in both the blood centers hospitals. This is quit logic and in accordance with global data.
Platelet wastage due to expiration is greater than other products. This is best explained by the short shelf-life of this component. It is recommended that proper measures to be taken with the aim of platelet releasing at the first day of donation. This will increase the availability of the component by 1 day.
Pricing the blood component, Corrective action:
1. Preparation of instruction manual for wastage management
2. Monthly monitoring of wastage of blood component
3. Holding Regular committee meetings to evaluate wastage in blood centers
4. Staff training regarding corrective and preventive action
5. Holding workshops for production and distribution departments
6. Correction of instruction manual for the storage of RBC component to reduce storage time in distribution department from 7 days to 5
All of these resulted in a 7% decrease in the wastage in 2 last years.

P-185

CHANGE IN BLOOD PRODUCTS TRANSFUSION REQUIREMENT BEFORE AND AFTER ROTEM BASED PATIENT BLOOD MANAGEMENT STRATEGIES (PBM) IN CHILDREN UNDERGOING CRANIOFACIAL SURGERIES

R Joshi, S Ganu, N van Niekerk, D Costi and P Anderson
Women's and Children's Hospital, Adelaide, Australia

Background and aims: Moderate to severe bleeding complicated with perioperative coagulopathy is a major problem in children undergoing craniofacial surgeries. We implemented patient blood management strategy in our institute based on ROTEM results from January 2015. Blood products transfusion requirements were analysed and compared pre and post ROTEM use in children undergoing craniofacial surgeries.
Methodology: Retrospective analysis of the data for consecutive children undergoing major elective craniofacial surgeries for cranial synostosis in Women's and Children's Hospital, Adelaide from January 2014 to December 2015 was performed. The blood product transfusion requirements were compared 1 year before and after the implementation of ROTEM assisted Patient Blood Management strategy (PBM).
Results: The data of the 70 children (38 pre-ROTEM, 32 post-ROTEM) undergoing craniofacial surgeries were analysed. 97.3% and 90% children in pre and post-ROTEM group respectively required Packed RBC (PRBC) transfusion. Fresh Frozen Plasma (FFP) was administered in 64.9% children in pre-ROTEM group, whereas only 16.7% children in post-ROTEM group. Cryoprecipitate was administered in 37% in post-ROTEM group and none in pre-ROTEM group. Only one patient received platelets, in post-ROTEM group.
Conclusion: We saw change in blood product usage with increase in use of Cryoprecipitate and declining use of FFP after implementation of ROTEM assisted PBM. The number of patients requiring PRBCs transfusion undergoing craniofacial surgeries has not changed significantly.

P-186

DONOR SELECTION FOR IGA DEFICIENT PLASMA PROVISION

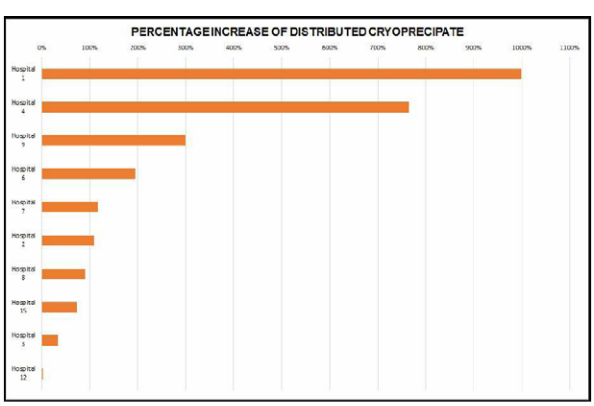
E Svirnouskaya[1], LV Novak[1], OV Klimovich[2], VV Pasiukov[1], FN Karpenko[1], AV Novik[1] and AI Svirnovski[1]
[1]Scientific and Practical Center for Transfusiology and Medical Biotechnologies, Minsk, Belarus [2]City Blood Center, Minsk, Belarus

Background: IgA deficient plasma products are the recipient-specific blood components for a patient with anaphylactic transfusion reactions secondary to anti-IgA. These patients need to receive blood components, which lack IgA and can be collected from an IgA-deficient donor. Anaphylactic reactions occur in approximately 27.1 or 10.7 in 1,000,000 components transfused to patient with a history of pregnancy or previous blood transfusion, or with autoimmune diseases. The incidence of IgA-deficiency in donors was found to occur at a rate of 0.26%/0.08% (1 in 372, 1 in 1,200).
Aims: We aimed to investigate a rate of IgA deficient donors and to evaluate both amounts of needed donors and plasma donations to provide IgA-deficient plasma collection sufficient for prevention of anaphylactic reactions in patients who require avoidance of transfusion with plasma-containing IgA.
Methods: The level of IgA in serum samples from 10,000 blood donors was studied in Republican scientific and practical center for transfusiology and medical biotechnologies during the year 2014 by using immunoturbidimetric assay. Data from the developmental studies were reviewed to calculate a demand in IgA deficient donors, involved in moderate frequency plasmapheresis program.
Results: In the present study of 10,000 blood donors the frequency of IgA deficiency (0,05 mg/dl) was 1 in 250 donors. The low level of IgA was found in four donors: 0.01 mg/dl and 0.04 ml/dl were detected in three donors and in 1 donor respectively. Donor selection for plasma donations must be based on the demand of IgA deficient plasma for clinical use which can be calculated as less as 2,000 units

of AB group apheresis plasma per year. The number of plasma units is done taking into consideration the rate of anaphylactic incidence (20 in 1,000,000) and therapeutic dose of plasma (5 units). Using for plasma collection developed in our center moderate-frequency plasmapheresis system (3 cycles and 6 weeks intervals between cycles per year, every cycle consists of 10 apheresis procedures at 1 week interval) there is necessary to select no more than eight apheresis donors.
Conclusions: According to the results only four donors in 10,000 (0.4%) may donate IgA deficient plasma for clinical use. The implementation of new plasmapheresis algorithm allows to obtain about 30 l of plasma from one donor and to create a stock of rare plasma products having small number of selected donors.

P-187
CRYOPRECIPITATE – PAST OR STILL PRESENCE?
L Budzynski, A Skrzypczak and K Olbromski
Blood Center, Poznan, Poland

Background: Cryoprecipitate is component of blood containing cryoglobulin fraction obtained from one unit of quarantine fresh-frozen plasma. It contains a major portion of the Factor VIII, von Willebrand factor, fibrinogen, Factor XIII and fibronectin. In cases of hypo-and afibrinogenemia or dysfibrinogenemia, disseminated intravascular coagulation (DIC) and deficiencies of factor VIII transfusion of cryoprecipitate (in the case of non-availability of plasma derived clotting factor concentrates-pdCFC) is recommended.
Aims: The aim of the work is analysis of cryoprecipitate distributed from the Regional Blood Center in Poznan in the years 2014–2015. The analysis includes evaluation of quality: the volume, the activity of Factor VIII and Fibrinogen content and the evaluation of quantity of distributed cryoprecipitate. This is due to observed increased production of this component (in comparison to the previous years) despite the wide availability of pdCFC as well as (recombinant coagulation factor concentrates) in Poland.



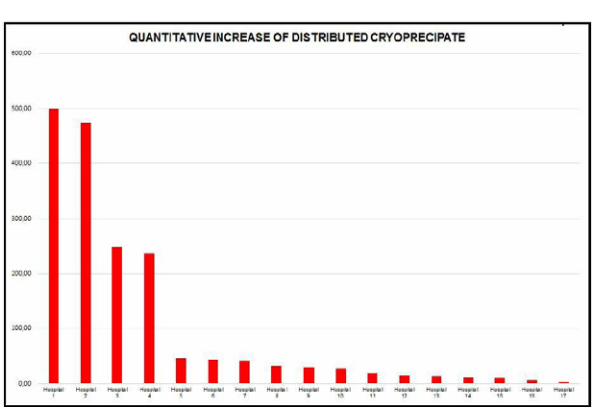


Chart 1

Year	n	Volume [ml]	Factor VIII [IU/unit]	% >70 IU/unit	Fibrinogen [mg/unit]	% > 140 mg/unit
2014	45	41	117	91	444	100
2015	71	37	98	79	404	100

Chart 2

Methods: Cryoprecipitate is obtained in the Regional Blood Center in Poznan using the centrifuge method. Out of 5,847 produced units in the years 2014–2015 116 units were analyzed. The study was carried out using the ACL Elite Pro's Instrumentation Laboratory. The level of fibrinogen was determined with von Clauss method using Fibrinogen reagent C, whereas the Factor VIII with coaguolometric method using of FVIII Deficient Plasma. In both tests calibrator Calibration Plasma by Instrumentation was Laboratory used.
Results: In 2014 45 units (out of 2,148 distributed) were tested (including the activity of Factor VIII in 22). In the year 2015 71 units (out of 3,699 distributed) were examined (including the activity of factor VIII in 66). The average content of Fibrinogen in the tested components was up 444 mg/unit in 2014 and 404 mg/unit in 2015. The average activity of Factor VIII was 117 IU/unit in 2014 and 98 IU/unit in 2015. The table presents the summary of qualitative research. Chart 1 presents the percentage analysis of the amounts of distributed cryoprecipitate. Significant >100% increase in six hospitals was noted. Chart 2 presents quantitative analysis of distributed cryoprecipitate, with a significant increase (>100 units) in four hospitals (three of them are noted in both groups). In total 1,458 units of cryoprecipitate were distributed to these four hospitals, which represents 94% of the total growth.
Summary/Conclusions:
1. In the analyzed period under a significant increase in number of distributed cryoprecipitate to hospitals was observed. Economic factors or insufficient knowledge regarding the recommendations to use cryoprecipitate may be the cause of this situation.
2. Qualitative evaluation of the tested components suggests that cryoprecipitate can be used for hypo-and afibrinogenemia or dysfibrinogenemia as source of fibrinogen due to the very high average content of fibrinogen.
3. Please note that despite producing cryoprecipitate from quarantine fresh frozen plasma it is much safer for patients to use pdCFC which are subject to inactivation of pathogens or to use rCFC.
4. As the observed increase in number of distributed cryoprecipitate was due to four hospitals appropriate training regarding the treatment of bleeding disorders should be considered.

P-188
FOLATE STATUS IN THE CHINA BLOOD DONOR POPULATION
Y Ma
Wuhan Blood Center, Wuhan, China

Background and aims: Folate deficiency have been proved to be related with neural tube defects, cardiovascular disease and selected cancers. Relied on blood centers, many countries have started to test the serum folate levels for their citizens. It was found that sub-optimal status were common in many countries, such as Italy, Germany and other Europe countries, especially for women of childbearing age. And corresponding measures have been effectively implemented for these countries. Unfortunately, little is known about the folate status of Chinese adults. This study evaluated the folate status in a region of middle China, and explore the factors that may affect the folate status B12 levels, such as sex, age, dietary habits, alcohol consumption, smoking habits and physical activity.
Methods and results: Male and female blood donors (n = 113), aged 18–67 years and living in Hubei province in Chine, were enrolled. A brief questionnaire concerning dietary habits, physical activity and smoking were completed; serum folate were measured by an immunoassay on an automated analyzer. The overall geometric mean of serum folate was 14.81 nM (95% CI, 13.58–16.04). When adequate serum folate concentrations were defined as 15 nM, only 38.7% of blood donors showed an adequate level of serum folate (95% CI, 28.6–48.8%) and there is only one woman of childbearing age (19–30 years old) had adequate levels. Significant differences were observed with regard to Age ($P < 0.05$), dietary habits ($P < 0.05$) and alcohol consumption ($P < 0.05$). Folate concentration was higher in middle-aged people (35–50 years) and non-alcohol drinker and in subjects with higher consumption of fruit and vegetable.

Conclusion: This study shows a large proportion of Chinese adults have a low folate status, especially those young/old people or people with excessive drinking or inadequate fruit and vegetable consumption. A public health strategy may be needed for China like other countries, such as supplying grain fortification with folic acid. And blood center should play more active role in this process and remind more people to concern about their own folate status.

P-189

VALIDATION OF STABILITY OF COAGULATION FACTOR VIII IN FRESH FROZEN PLASMA COLLECTED FROM A UNIVERSITY HOSPITAL DONATION CENTRE

ES Sumardi, PL Goh, H Yu Qi, IE Ancheta, SY Lee, PLK Lim, S Lim and LK Tan

National University Hospital, Singapore, Singapore

Background: Fresh frozen plasma (FFP) contains normal amounts of coagulation factors, antithrombin and ADAMTS13. In order to maintain the labile coagulation factors (Factor V and Factor VIII) at its maximum, FFP has to be frozen within 8 h of processing and stored at −18°C or colder, with 12 months' shelf life.

There is no quality control (QC) requirement for FFP stipulated by AABB to test a certain number of units every month to ensure adequate coagulation factors are present.

In Europe, the QC requirements are: all units to be checked for volume (±10% of stated volume); FVIII levels in 10 random units in their first month of storage to be at least 0.7 IU/ml; 75% of units tested must have FVIII levels of more than or equals to 0.7 IU/ml; 1% of all units (at least 4/month) to be checked pre-freezing for residual red blood cells (RBC < 6.0×10^9/l), white blood cells (WBC < 0.1×10^9/l) and platelets (<50×10^9/l).

Thus, the European Directorate for the Quality of Medicines & HealthCare (EDQM) guidelines is used.

Aims: The study aims to evaluate FVIII stability at 0-h post-thawing, and whether FFP processed by our donation centre within 8 h post-collection fulfill EDQM guidelines.

Methods: About 200–250 ml FFP was thawed using Bloodline WPFD-WSCFD 3/6 Plasma Thawer (KW Apparecchi Scientifici, Italy) at 37°C. FVIII:C levels at 0-h post-thawing were measured with STA®-DEFICIENT VIII Assay by STAGO STAR-Evolution machine (Diagnostica Stago, France) utilizing 1-stage clotting assay methodology. FFP collected and frozen within 8 h post-collection, were stored in freezer drawers at −40°C in Panasonic MDF-U5412 Biomedical Freezer (Panasonic Healthcare Co., Ltd., Japan).

Full Blood Count (FBC) was performed on an aliquot using SYSMEX XE-5000 Automated Hematology System (Sysmex, U.S.A) for residual cell count determination pre-freezing.

Results: In the first part of the study, 7 out of 10 FFP (processed following the current freezing method) had FVIII levels of <0.7 IU/ml at 0 h post-thawing. These units were frozen in batches of 6–14 units and stacked in freezer drawers (Figure 1).

Subsequently, to improve product quality, another 10 FFP were placed flat on freezer metal platforms in batches of 6 units or lesser. Seven out of 10 FFP had FVIII levels of more than or equals to 0.7 IU/ml at 0-h post-thawing.

All units displayed acceptable counts of RBCs & platelets. With the exception of two units, leukocyte counts remained within requirements.

Conclusions: The failure to attain FVIII levels of more than or equals to 0.7 IU/ml for the first part of the study was due to small sample size. It is presumed that with a larger sample size, 75% of units tested would achieve FVIII levels of more than or equals to 0.7 IU/ml. Reducing the large batch size, and placing FFP on freezer metal plate, lead to an improved FVIII level recovery. The usage of blast freezers maintained at −65°C would also increase product quality and improve freezing efficiency.

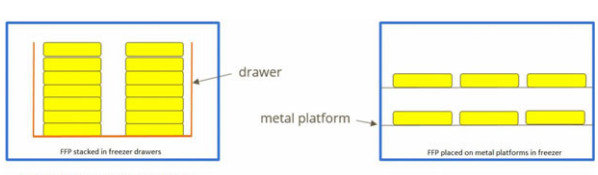


Figure 1. Comparison of different freezing methods

P-190

ARE THE CELL SEPARATORS SPECTRA OPTIA AND AMICUS EQUALLY SUITABLE FOR COLLECTION OF MNCS AND THEIR SUBSETS

GC Leitner[1], M Horvath[1], M Kurz[1] and G Fritsch[2]

[1]*Medical university, Vienna, Austria* [2]*St Anns Children Hospital/CCRI, Vienna, Austria*

Background: The use of mononuclear cells (MNCs) as a therapy is well established and new therapeutic applications continue to emerge. Dependent on the intended use different MNC subsets are favoured, CD34+ cells for stem cell collections CD14+ and CD33+ cells for the generation of dendritic cells. CD3+ cells for donor lymphocyte infusions (DLI). Alpha beta (αβ) and gamma delta (γδ) T-cell populations are CD3 subsets and are natural killer (NK) cells. They include cytotoxic, interferon (IFN)-gamma-producing lymphocytes that actively respond to viral infections; γδ T cells also have well-established protective roles in cancer, activated γδ T cells exhibit potent anti-tumour activity. Thus collection of CD3 subsets may be substantial for future immunotherapeutic approaches for treatment of different types of cancer. In this investigation we analysed the collection properties concerning MNCs and subsets of Amicus (Fresenius) and Spectra Optia (Terumo BCT).

Aim: Our aim was to identify the best suitable device for specific demands.

Donors and methods: Twelve healthy volunteers (3 females and nine males, median 44 years, 21–56) from our routine multikomponent routine donor pool were recruited for MNC donation on Amicus™ (Fresenius) and Spectra Optia® (Terumo BCT) in a cross over design. They all fulfilled the requirements for blood donation. We processed 6,000 ml of blood within 120 min. The MNC products as well as pre- and post peripheral blood samples (to calculate collection efficiencies) were analyzed for cell counts and MNC subsets by FACS analysis using trucount tubes (BD Biosciences) for multi-color single-platform staining according to the technical manual. The FACSDiVa six software (BD Biosciences) was used for cell acquisition and data evaluation. The following MNC subsets were analyzed: CD3, CD19, CD33, CD3 αβ, CD3 γδ CD4 αβ, CD8 αβ, CD56. Furthermore the amounts of concomitantly collected red blood cells and platelets were measured. For statistical analysis the Wilcoxon signed rank test was applied.

Results: The procedures were well tolerated by the donors. Except a mild citrate reaction in one donor no side effects were observed. We found no differences in pre and post values of the donors although Optia collected significantly more platelets than Amicus (mean 2.29 × 10^11/Optia vs 0.64 × 10^11/Amicus, $P < 0.05$). On the other side the concomitantly collected red cell fraction was significantly higher in Amicus than in Optia (9.35 ml/Amicus vs 1.66 ml/Optia, $P < 0.05$). The number and purity of collected MNCs and subsets was comparablel in both devices (81.8%/Amicus vs 84.6%/Optia).

Conclusion: Both devices are equally applicable for MNC collection in general and for all tested subsets. How far the higher platelet numbers in Optia and the higher RBC volume in Amicus, respectively, may negatively influence *in vitro* expansion studies needs further investigation.

Plasma Products

P-191

POSSIBILITIES IN SEPARATION OF HUMAN PLASMA DERIVED PRODUCTS

K Mousavi Hosseini, M Shahabi, MA Jalili and M Fallah Tafti

Blood Transfusion Research Center, High Institute for Research and Education, Tehran, Iran

Background: By the beginning of new century, about 500,000 kg of human serum albumin (HAS) and 40,000 kg of intravenous immunoglobulin (IVIG) is being produced per year. For production of such amounts of albumin and IVIG, more than 20 million liters of starting material which is human plasma is needed. If we notice to more than $7 billion annually production of these human plasma products, it can be concluded that implementation of new technology in production of such biological drugs is a necessity.

Aims: Preparation of biological drugs such as albumin and immunoglobulin from human plasma is the main purpose of plasma fractionation.

Methods: Different studies on developing procedures for the preparation of 'human plasma derived biological drugs' were carried out. One of the methods for separation of proteins from human plasma is salting out. For this purpose ammonium sulphate

as a salt can be added at different concentrations to human plasma solution for precipitation of protein. Addition of ammonium sulphate should be repeated with different concentrations in order to obtain specific protein after each addition of salt. Lactate of 2-ethoxy-6,9-diamino-acridine is another compound which can be used to decrease solubility of proteins in human plasma, which insoluble protein can be seprated by centrifugation. The other technique for plasma derived protein separation is fractionation of plasma by polyethylene glycol. In our study, by this method, intermediate sources for preparation of fibrinogen, α_1-antitrypsin, albumin, IgG, IgA, and IgM were achieved. Affinity and ion exchange chromatography is suitable for separation of labile protein such as coagulation factors. These techniques allowed us to prepare Plasminogen and coagulation factors IX, VII, and immunoglobulin from human plasma.

Results: The most important biological drugs which can be prepared from human plasma are albumin, Immunoglobulin G, coagulation factor VIII, and coagulation factor IX. There are some other compounds which can be separated from blood but with less importance in patient's treatment, due to fewer numbers of patients with specific deficiency of that compound such as α_1-antitrypsin, antitrombin III, ceruloplasmin, plasminogen. The amount which is needed for these compounds is much less than albumin and IgG. For this reason, a method with higher capacity of preparation and possibility for its implementation in industrial scale is the most important factor.

Summary/Conclusions: Development of a process for separation of biological drugs such as albumin and IgG was first suggested by Edwin Cohn from Harvard University. His project was 'plasma fractionation', in order to prepare products such as albumin and IgG for treatment of soldiers suffering from shock and burns, which they need blood volume expander. Nowadays, still most of the modern human plasma fractionation industries are based on Cohn's method.

P-192

DIFFERENCE IN THE LEVELS OF PLASMA PROTEINS IN SINGLE FRESH FROZEN PLASMA AMONG CHINESE POPULATION

L Zhu, P Di, S Wang and D Wang

Chinese PLA General Hospital, Beijing, China

Background: Currently, clinical application mainly is single FFP derived from individual blood donors and there are great differences in plasma proteins between single FFP due to varieties of individual physiology and separated process for blood components. However, there was little scientific data to point out the difference. It is necessary to define the range of this difference to provide evidence to develop relative advanced strategies.

Aims: To investigate and analysis the difference of plasma proteins in single FFP between blood groups, sexes and ages among Chinese population, especially in Beijing area, a possible scientific data will be provided to establish or upgrade relative reference values.

Methods: The people who came to our hospital to donate blood came from all over the country. In our center, one hundred and twenty units of single FFP with 60 units of group A, 25 units of group B, 15 units of group AB, 20 units of group O were thawed and assayed immediately. All data of single FFP were collected and divided into sex groups (male or female) and age groups (18~30, 31~45, 46~60). Plasma proteins were evaluated using coagulometric or biochemical assays including total protein (TP), albumin (Alb), Fibrinogen (Fg), Factor V (FV), Factor VIII (FVIII), Anti-III (AT-III), protein C (PC). SPSS 16.0 using ANOVA with Turkey's post-test was used to analysis data. Significant was set at $P < 0.05$ in all cases.

Results: The levels of each plasma protein showed significant differences with wide ranges in single FFP prepared from Chinese population. And the frequency distributions of all plasma proteins presented normal distributions. The protein level with maximum fluctuation was FVIII from 0.33 to 1.36 IU/ml, the minimum was TP from 45.8 to 69.8 g/l and Alb from 31.9 to 45.6 g/l in a more stable level. Values for other plasma proteins were also clearly ranged. The mean value with 56.07 g/l for TP was above the China Guidelines of 50 g/l, but that with 0.62 IU/ml for FVIII was slightly blew the China Guidelines of 0.7 IU/ml. There were significant differences in the level of plasma proteins among single FFP from Chinese donors between blood groups with upward trends such as TP, Alb, Fg, FVfor group O compared with other blood groups, and downwards trends such as FVIII and AT-III for group O compared with others ($P < 0.05$). There were also significant differences between ages, such as FVfor the age group from 18 to 30 was significantly lower than other blood groups ($P < 0.05$), but no difference between sexes.

Summary/Conclusions: Although single FFP was still applied for clinical transfusion, it had a great difference between single FFP not only in Chinese population but also in other countries. Large scale analysis contributed to demonstrate or establish relative reference values that can conduct and predict plasma transfusion more effectively. Moreover, it also afford scientific base to study other strategies for blood donors or technologies for precise transfusion of plasma.

P-193

PURIFICATION OF THERAPEUTIC PROTEINS FROM HUMAN PLASMA BASED ON AQUEOUS TWO PHASE SYSTEM FRACTIONATION

M Vargas[1], A Segura[1], M Herrera[1], M Villalta[1], A Sánchez[1], T Burnouf[2] and G León[1]

[1]*Instituto Clodomiro Picado, Universidad de Costa Rica, San José, Costa Rica*

[2]*College of Oral Medicine, Taipei Medical University, Taipei, Taiwan*

Background: The current high demand for human plasma-derived products at global levels, particularly IVIg (intravenous immunoglobulin), has generated the need to develop new methods for the recovery of plasma-derived proteins that contribute to solve the demand for these products. In order to establish a domestic facility for processing increasing volumes of local recovered plasma that is not being utilized for direct clinical applications, Instituto Clodomiro Picado in Costa Rica, has developed a fractionation process for human plasma based on an aqueous two-phase system (ATPS).

Aims: This work encompasses the design and evaluation of a methodology that employs a polymer-salt ATPS as a method of primary recovery of proteins from plasma, mainly IgG and albumin, coupled to other purification steps for obtaining therapeutic formulations. These formulations must meet quality requirements according to international standards. In order to determine the potential of ATPS as an alternative to conventional methods for plasma fractionation, we evaluated the partition of plasma proteins in ATPS, the feasibility of coupling ATPS fractions to other polishing steps, the scalability and productivity of the methodology, and the quality of ATPS-obtained final formulations, specifically an IVIg formulation.

Methods: The biphasic and protein partition behavior in PEG (polyethylene glycol) 3,350-potassium phosphate ATPS formed directly in plasma was studied. For this, binodal curves and rheological characteristics of the phases were determined, as well as the partition of proteins under different ATPS conditions. Further identification of plasma proteins in the phases was performed using proteomics. IgG-rich fraction obtained from human plasma fractionation in ATPS was purified by caprylic acid precipitation and anion exchange chromatography to obtain IVIg. The method designed was tested in scales from 1 to 10 l of plasma. The resulting formulations were studied according to their physicochemical and immunochemical characteristics, efficacy and pre-clinical safety.

Results: It was determined that IgG and albumin-enriched fractions are obtained from the fractionation of human plasma in a PEG 3,350-phosphate system. Optimum partition of both proteins, with a yield of 80%, correspond to an ATPS formed with 6–10% w/v PEG 3,350, 12–15% w/v phosphates, in the presence of 15% w/v NaCl, at pH 6. Under these conditions, immunoglobulins were recovered in the upper phase as a precipitate, and albumin in the bottom phase. Other proteins of therapeutic interest were identified in the phases by proteomic analysis. In addition, the density and viscosity difference between the two phases and the interfacial tension were established, which explain the two-phase formation and separation.

Moreover, coupling of caprylic acid precipitation and ion exchange chromatography to ATPS, resulted in an IgG preparation that met international physicochemical and purity requirements characterized by 100% gamma-proteins, low residual IgA, and undetectable IgM. The IgG subclass distribution was not substantially affected by the process. Analyses of Thrombin Generation Assay and amidolytic activities revealed an undetectable *in vitro* thrombogenic risk and the absence of proteolytic enzymes in the final product. Efficacy of the formulation was demonstrated in a murine model based on its protective activity against tetanus toxin.

Conclusions: It was concluded that the designed methodology, based on the fractionation of plasma in ATPS, represents an alternative to conventional methods of plasma fractionation, since it is a simple and scalable method for obtaining functional therapeutic proteins formulations, particularly IVIg, with high levels of purity, yield and quality.

P-194

DIRECT COMPARISON OF SINGLE DONOR FRESH FROZEN PLASMA UNITS WITH POOLED, SOLVENT/DETERGENT TREATED PLASMA (OCTAPLASLG®) IN THROMBIN GENERATION ASSAY AND ROTATIONAL THROMBOELASTOMETRY

A Heger[1], S Janisch[2], K Pock[1] and J Römisch[1]

[1]Octapharma PPGmbH, Vienna, Austria [2]Octapharma, Vienna, Austria

Background: Thrombin generation assay (TGA) and rotational thromboelastometry (ROTEM) have become universal tools for the direct analysis and monitoring of the haemostatic system in the clinical practice and research laboratories. Nevertheless, standardization of the test set-up is still missing.
Aims: The aim of this study was to investigate the role and function of different triggers and plasma proteins in both TGA and ROTEM analysis. Direct comparison of single donor fresh frozen plasma (FFP) and pooled, solvent/detergent (S/D) treated plasma, octaplasLG® should be performed.
Methods: Single-donor FFP units (n = 32) and octaplasLG® batches (n = 16) from Octapharma PPGmbH (Vienna, Austria) and Octapharma AG (Stockholm, Sweden) were investigated. TGA was performed using two fluorogenic assays (Technothrombin TGA from Technoclone GmbH and CAT assay from Stago) and different triggers. Similarly, ROTEM was performed after supplementing plasma with tissue factor and phospholipids. Plasma units before and after spiking with purified protein S, antibodies or depleted plasma were tested.
Results: Thrombin generation capacities were highly influenced by the different triggers used, showing faster thrombin generation in both plasma groups at higher tissue factor concentrations. Spiking studies of protein S depleted plasma with human purified protein S, FFP or octaplasLG® showed decreased peak thrombin concentration by increasing the plasma protein S concentration up to physiological ranges. Parallel studies with ROTEM confirmed the correlation between protein S levels and the clotting times. Although mean thrombin concentration were higher in octaplasLG®, minimum-maximum ranges in FFP units overlapped with the single levels in octaplasLG® batches, as demonstrated in both assays at all tissue factor concentrations.
Summary/Conclusion: Direct comparison of different plasma groups by TGA and ROTEM analysis require clearly defined and standardized test set-ups. Due to higher batch-to-batch variations in the FFP group, the haemostatic potentials of single donor FFP and octaplasLG® are overlapping.

P-195

IMPLEMENTATION EXPERIENCE OF POOLED WHOLE BLOOD DERIVED AMOTOSALEN TREATED PLASMA

T Bocquet and P Bierling

EFS, Rungis Cedex, France

Background: In France, frozen plasma (FFP and FP24) are issued from quarantine or amotosalen/UVA process (INTERCEPT Blood System -IBS). In 2015, the regional French Blood Etablishment of Paris area (EFS IDF) has delivred 89,000 units. The French Regulatory Agency (ANSM) authorized pooled whole blood (WB) amotosalen UVA plasma from July 2015. Then EFS IdF prepared amotosalen pooled whole blood in its two sites of Rungis and Pontoise. Quarantine plasma were also available at this time.
Aims: We report our experience in the implementation of pooled WB plasma treated by amotosalen (IBS, Cerus, Netherlands). The operational aspects as well as the compliance to product standards and variability between early (<8 h) and late (12–19 h) treatment are evaluated.
Methods: Whole blood is collected in top and bottom blood bags DGR7567B (Fenwal, USA) or NPT6288LA (Macopharma, France). After centrifugation (3,947 tr/min, 26 min) WB is separated on OPTIPRESS (Fenwal), then red cells and plasma are filtered separately. The plasma obtained is compliant with the French requirements ($<1 \times 10^4$ WBC/l). Five plasmas are labelled, associated by ABO group and sterile connected to a PLASMIX kit (Grifols, Spain). A plasma pool with a minimum volume of 1.3 l is obtained, then splitted in two sub-pools, each with the maximum volume permitted of 650 ml and treated. Each sub-pool is integrally transferred into an illumination bag with an addition of amotosalen. Sub-pools are exposed to UVA (6 J/cm^2) for about 8 min in a INT100 device prior being gravity flown through a Compound Adsorption Device and split into three storage containers. The six plasmas units are frozen post treatment within 19 h. Quality Control (QC) measurements are performed to verify compliance to product requirements.

	Early process (<8h) Avg +/- SD	Late process(12-19h) Avg +/- SD	Compliance requirements	
FVIIIC (IU/ml) per unit	0.66 +/-0.13	0.61 +/-0.11	≥ 0.5	
Fibrinogen (g/l) per unit	2.30 +/- 0.22	2.27 +/- 0.23	≥ 2	
Number tested	52	39		
	Early process (<8h) NC n=	Late process (12-19h) NC n=	Total Non compliant	% Non compliant
FVIIIC (IU/ml) per unit	3	4	7	8%
Fibrinogen (g/l)	3	3	6	6%
FVIII + fibrinogen	1	0	1	1%
Total Non Compliant n=	7	7	14	15%
Number tested	52 (55% of group O)	39 (48% of group O)		
% Non Compliant	13.5%	17.9%		

Table 1

Results: IBS plasma production reached 1,600 units/month using two illuminators in Rungis site and one illuminator in Pontoise site and one technician(s) by production site. The 19 h window for IBS allows batch production: early in the morning and at the end of afternoon. Primary pooling step takes around 25 min to select, label and pool the five plasmas. Plasma in excess left in the PLASMIX kit is 127.5 ± 41.2 ml, and plasma loss for IBS is 25.7 ± 4.4 ml. Compliance to national coagulation factors QC requirements is reported in Table 1: both types of treatment meet the requirements, no significant difference for the number of non-compliant products (Fishers test: P = 0.572). FVIII and Fibrinogen losses during treatment were respectively of 21.7% ± 7.6 (n = 27) and 8.2% ± 4.8 (n = 23).
Summary/Conclusions: The production of pooled WB derived Amotosalen treated plasma was done without major difficulty. Nevertheless, the production flow needs to be adapted: the FIFO method is not respected for treatment of all WB units because we had to prioritize the adequate ABO group plasmas. No significant difference in non compliant results between early and late treatment was found. This technique meets the French quality requirements for pathogen attenuated therapeutic plasma, and allows to obtain six units from five donors. In comparison with plasma from apheresis, this can reduce the cost per unit obtained. To simplify the production, pooling frozen plasma would allow to schedule the production, to work by batch and to select needed plasma ABO groups.

P-196

EXTENDING THE POST-THAW SHELF-LIFE OF CRYOPRECIPITATE

K Winter, RA Hyland, S Tan, RG Webb, AM Davis, PM Dennington and DC Marks

Australian Red Cross Blood Service, Sydney, Australia

Background: In Australia, cryoprecipitate currently has a post-thaw shelf-life of 6 h, as per the Council of Europe guidelines. This leads to wastage of many units that are thawed in hospitals but not transfused. Wastage could potentially be reduced if the post-thaw shelf-life of cryoprecipitate is extended to 24 h, or possibly even longer.
Aims: The aim of this study was to evaluate the *in vitro* quality of thawed cryoprecipitate after 24 and 48 h of storage at ambient temperature (20–24°C), with a view to extending the post-thaw shelf-life beyond the current expiry of 6 h.
Methods: Forty cryoprecipitate units (20 whole blood-derived and 20 apheresis-derived) were thawed in a 37°C water bath then stored at ambient temperature (22 ± 2°C). Each unit was sampled immediately after thawing (baseline), and then at 6, 24 and 48 h. Within each group there were 10 group O and 10 non-group O cryoprecipitate units. Samples were tested for coagulation factors (FVIII, FXIII and fibrinogen) and von Willebrand Factor (vWF) antigen using an automated coagulation analyser, for vWF activity using an aggregometry assay measuring ristocetin co-factor, and for fibronectin, ADAMTS-13 and total protein concentrations by ELISA. Statistical comparisons were performed using a one-way ANOVA; a P-value <0.05 was considered significant.
Results: The mean volume of the whole blood- and apheresis-derived cryoprecipitate units was 35.0 ± 1.6 and 60.0 ± 2.3 ml respectively. The mean total protein concentration of the whole blood- and apheresis-derived cryoprecipitate units was not significantly different (64.6 ± 5.7 and 63.1 ± 5.0 g/L respectively). All cryoprecipitate units met the Council of Europe specifications for FVIIIc ≥ 70 IU, fibrinogen ≥ 140 mg and vWF activtiy >100 IU, and remained above these limits even at 48 h post-thaw. As expected, FVIII levels decreased during storage at ambient

temperature, from 161 ± 60 IU/unit at baseline to 123 ± 46 IU/unit after 48 h in the whole blood-derived cryoprecipitate and from 286 ± 71 to 241 ± 73 IU/unit in the apheresis-derived units, although neither decrease was significant (P = 0.108 and P = 0.187). At 48 h post-thaw, fibrinogen content was maintained at 416 ± 93 and 676 ± 159 mg/unit, and vWF antigen levels were maintained at 266 ± 70 and 478 ± 120 IU/unit respectively for whole blood- and apheresis-derived units. There were no significant differences in levels of FXIII, vWF activity, fibronectin or ADAMTS-13 after extended storage.

Summary/Conclusions: Whole blood- and apheresis-derived cryoprecipitate components stored at ambient temperature for 48 h meet the Council of Europe specifications for FVIIIc, fibrinogen and vWF. However storage at ambient temperature increases the risk of bacterial growth, and further strategies to mitigate this risk are required prior to extending the shelf-life beyond the current time period.

P-197

COMPARATIVE STUDY ON POTENTIAL OF REGULAR PRP-GEL DRESSING IN ACCELERATION OF WOUND HEALING IN PATIENTS WHO UNDERWENT PILONIDAL SINUS SURGERY: A RANDOMIZED, CONTROLLED, PARALLEL CLINICAL TRIAL

S Mohammadi, A Malek Mohammadi, M Nikbakht, S Nassiri and A Ghavamzadeh

TUMS, Tehran, Iran

Background: One of the most important surgical methods in treatment of pilonidal sinus disease is wide excision with healing by secondary intention followed by a critical complication; wound healing issue. The aim of this study was to investigate the possible effect of platelet rich plasma (PRP) Gel on accelerating wound healing in these patients.

Methods: In this longitudinal, randomized, controlled, parallel clinical trial, patients were randomly divided into two groups (controls and treatment arm) after meeting both inclusion and exclusion criteria. After the surgery, control group were treated by classic wound dressing while the case group was treated with PRP Gel in classic wound dressing platform. The patients were then evaluated for duration of antibiotics consumption, pain and time of returning to normal daily activities. Also both groups were assessed for angiogenesis (by detecting CD34+ on cell surface using immunohistochemical assay), collagen sedimentation (masson's trichrome staining) using pre-complete healing wound biopsy. All the statistical analyses were performed using SPPS 20.

Results: From a total of 116 patients selected according to inclusion criteria, 110 individuals were enrolled and considering the exclusion criteria, they were divided into the two groups of 55 cases. According to the results, patients treated with PRP gel went through a significantly faster healing process (P-value = 0.03) and returned to their routine life activities (P-value <0.0001) while experiencing less pain (P-value <0.0001) and shorter anti-biotic consumption duration (P-value <0.0001). Also the angiogenesis was much higher in treatment arm in comparison to controls (P-value <0.0002). All of PRP-gel treated cases showed collagen sedimentation in their histological evaluations while the controls did not.

Conclusion: Considering the results, authors of this study suggest PRP gel treatment for post operation wound dressing of pilonidal sinus disease for those patients underwent wide excision with healing by secondary intention.

Pathogen Inactivation

P-198

RESIDUAL AMOTOSALEN LEVELS AFTER PATHOGEN INACTIVATION USING THE INTERCEPT BLOOD SYSTEM FOR PLATELETS IN 100% PLASMA

K Ling, T Wilson, H Evans, B Donnelly and A Erickson

Cerus Corporation, Concord, United States of America

Background: The INTERCEPT[TRADEMARK] Blood System (IBS) for Platelets can be used for the preparation of pathogen and leukocyte inactivated platelet components (PC) in 100% plasma. INTERCEPT photochemical treatment (PCT) uses 150 μM amotosalen and 3.0 Joules/cm^2 UVA, followed by incubation with a Compound Adsorption Device (CAD) to reduce the level of residual amotosalen and free photoproducts. Although amotosalen has demonstrated large safety margins in non-clinical studies, a 2.0 μM specification for residual amotosalen has been established to provide maximal safety margins for IBS products based on toxicological evaluation and clinical studies. Approved CAD duration for platelet units in 100% plasma treated with the INTERCEPT large volume platelet processing set (INT22) and the dual-storage set (INT25), which use identical CADs and amotosalen doses, is 16 to 24 h. A shorter minimum duration for the CAD incubation is desirable to improve the flexibility of blood center operations and to make platelet products available for earlier release to hospital transfusion services.

Table 1. Amotosalen Micromolar Concentrations After CAD Incubation (n=6)

CAD incubation (h)	12	16	22-24
N	13	22	15
Average ± SD	0.6 ± 0.2	0.5 ± 0.4	0.3 ± 0.1
Range	0.4 – 1.1	0.3 – 1.8	0.2 – 0.4

Aims: Studies were conducted to evaluate the time required for reduction of amotosalen concentration by CAD in apheresis platelets suspended and treated in 100% plasma. Treatments were done using either the INTERCEPT large volume platelet processing set (INT22) or the dual-storage set (INT25).

Methods: Thirty-four apheresis PCs (3.8–8.3 × 10^{11} platelets per unit in 300–426 ml) were collected in ACD plasma and treated with the INTERCEPT process before the end of the day after donation. Following PCT, Test platelets were incubated in the CAD container on a flatbed platelet agitator with continuous 60-rpm agitation. Samples were removed from each CAD container after 12, 16, and 22–24 h of agitated storage, or some combination of those intervals.

Results: After 12 h of exposure to the CAD, the average amotosalen concentration was <2.0 μM in all experimental units.

Conclusions: This result supports a minimum CAD duration of 12 h for platelet units in 100% plasma treated in the large volume set, or in the dual-storage set.

P-199

This abstract has been withdrawn.

P-200

ANALYSIS OF DONOR PLATELET CONCENTRATE TRANSFUSIONS EFFICIENCY WITH SHELF LIFE FROM 1 TO 5 DAYS, TREATED WITH INTERCEPT BLOOD SYSTEM PLATELET AND GAMMA-IRRADIATED PLATELETS

MH Azimova, MY Drokov, VV Troitckaya, AV Kuzmina, AV Bulgakov and TV Gaponova

National Research Center for Hematology, Moscow, Russian Federation

Introduction: Acceptable shelf life of platelet concentrate is up to 5 days. The use of INTERCEPT Blood System Platelet pathogen reduction technology allows to extend shelf life to 7 days. At the same time, in some countries platelets shelf life is no more than 3 days. The clinical efficacy question of donor platelet transfusion depending on previous storage time remains relevant.

Aims: To perform analysis of platelet concentrate (PC) transfusions efficacy, prepared in 100% plasma depending on storage time (from 1 to 5 days), and on additional treatment (amotosalen + ultraviolet A light and gamma – irradiation with 25 Gy).

Materials and methods: Leucoreduced PCs contained 5 × 10^{11} cells in 100% donor plasma. Part of the platelet concentrate underwent pathogen reduction with INTERCEPT Blood System Platelet (amotosalen + ultraviolet A light (PRPC; n = 205)), the other part underwent gamma - irradiation with 25 Gy (PC25 Gy; n = 107). 106 patients were included in the study (m - 58; f - 48) in the ages from 17 to 83 years old (M = 36). All of them were on treatment at FSBI HSC with diagnoses: aplastic anemia (AA) (n = 28), acute leukemia (AL) (n = 60), limphoproliferative disorders (LPD) (n = 18). Efficacy of PC transfusions was assessed with taking their storage time into account: 1st, 2nd, 3rd, 4th and 5th day. Efficacy criteria were: corrected platelet count increment (CCI) after 1 h and 24 h, maximum amplitude on TEG (MA) and hemorrhagic syndrome (HS) regression.

Results: 312 PC transfusions have been analyzed, out of which: PRPC - 205 transfusions, PC25 Gy - 107. Out of PCs with storage time of 1 day (n = 87) only PC25 Gy were transfused; 2 days storage (n = 113, out of which: PRPC - 96

Storage days	Day 1 (n=87)	Day 2 (n=113)	Day 3 (n=63)	Day 4 (n=41)	Day 5 (n=12)
PLT to ($x10^9$/L)	15,6 ± 1,7	14,8 ± 1,2	14,9 ± 1,2	16,7 ± 1,6	16,2 ± 4,8
CCI/1	18,1 ± 1,5 [2,3]	16,2 ± 0,9 [1]	12,0 ± 1,0 [1,2]	13,2 ± 1,3 [3]	11,7 ± 2,6
CCI/24	12,6 ± 1,2 [2,3]	10,4 ± 1,0	8,0 ± 0,8 [2]	8,5 ± 1,4 [3]	15,6 ± 5,9

Table 1
1- CCI/1 between days 2 and 3 (p = 0,007);
2- CCI/1; CCI/24 between days 1 and 3 (p= 0,001; p=0,004);
3 – CCI/1, CCI/24 between days 1 and 4 (p = 0,027; p=0,033).

Table 1. Efficacy analysis of donor platelet concentrate transfusions with shelf life from 1 to 5 days

transfusions, PC25 Gy - 17); 3 days storage (n = 63, out of which: PRPC - 53 transfusions, PC25 Gy - 10); 4 days storage (n = 41, out of which: PRPC - 38, PC25 Gy - 3 transfusions); 5 days storage (n = 12, out of which: PRPC - 10; PC25 Gy - 2 transfusions). The part of preventive transfusions was 53%, curative: 47%. There were no significant differences in the amount of platelets in patients before to the transfusion (Table 1). As you can see from table #1, significant differences in CCI\1 and CCI\24 were found between days of storage of PC.

Conclusions: No significant differences were found in efficacy of transfusions with various storage time between PRPC and PC25 Gy. General patterns, obtained in this study have shown that the storage time influences quantitative growth of platelets in patients after transfusion, but doesn't influence MA and HS regression.

P-201

QUALITY CONTROL OF PLATELET CONCENTRATED: EVALUATION OF ADAM R- WBC CELL COUNTING SYSTEM

GG Di Lemma, R D' Ambrosio, MG Di Girolamo, M Danzi, G De Caprio, PW Pica, A Stabile and S Misso

S.C. Servizio Trasfusionale ASL Caserta, Aversa, Italy

Background: The GVHD (Graft vs host disease) represents one of the most important and serious transfusion complications due to presence of residues white blood cells (rWBC) of blood components transfused. Furthermore, r-WBC are viral pathogens carriers, as CMV and WNV, resulting a serious risk of infection with lethal consequences, especially in immunocompromised and multiple transfusions patients.

Aim: The aim of our study was to evaluate r-WBC in platelet concentrates before and after pathogen inactivation.

Methods: From June and December 2015 were produced 450 platelet concentrates with TACSI® (Terumo Automated Centrifuge & Separator Integration System for Platelets), of these 150 have been inactivated with the INTERCEPT Blood System[TRADEMARK] (Cerus Corporation). The inactivation with amotosalene and UV light causes irreparable changes of nucleic acids, resulting in inactivation of a broad range of pathogens and inhibition of immune response WBC mediated. WBC count was determined with ADAM r-WBC system (Macopharma). This device counts leukocytes number using fluorescent propidium iodide that colors the DNA in the nucleus of the target cells.

Results: rWBC mean±ds in platelet concentrates vs inactivated platelet was 0,54 ± 0,06/μL vs 0,08 ± 0,01/μL (P = 0,03).

Conclusions: This method may prove useful for the quality assurance and control of WBC-depleted blood products.Inactivation of pathogens and accurate determination of r-WBC in blood components represents an important goal for transfusion safety.

P-202

IN VITRO CELL QUALITY OF REVEOS-DERIVED POOLED PLATELETS IN PLASMA AFTER TREATMENT WITH THE MIRASOL PATHOGEN REDUCTION TECHNOLOGY SYSTEM

ME Shipps, KM Colagrosso, CR Kruk and LM Rouse

Terumo BCT, Lakewood, United States of America

Background: The Mirasol Pathogen Reduction Technology (PRT) System for Platelets combines riboflavin (vitamin B2) and UV energy to inactivate pathogens in platelet products. Platelet concentrates (PCs) in Platelet Additive Solution (PAS) treated

Parameter	8-24 hours post collection processing-Mean Value (N=9)	Day of collection processing, overnight hold IPU-Mean Value (N=5)
Lactate Production Rate mmol/10^{12} platelets/hr	0.071 ± 0.04	0.070 ± 0.02
Glucose Consumption Rate mmol/10^{12} platelets/hr	0.036 ± 0.01	0.029 ± 0.00
$pH_{22°C}$	7.16 ± 0.14	7.21 ± 0.03
Swirl	2.3 ± 1.00	2.2 ± 1.10

Table 1. pH 22°C on Day 5 of storage

8-24 hours post collection processing (N=9)	Day of collection processing, overnight hold IPU (N=5)
7.27	7.23
7.22	7.25
7.04	7.20
6.89	7.18
7.04	7.21
7.27	
7.26	
7.19	
7.22	

Table 2. Platelet cell quality parameters on day 5 of storage at 22°C

with the Mirasol PRT System have been well characterized in numerous studies. However, no studies have yet to explore the cell quality of platelet concentrates derived from pooled Reveos 3 Component (3C) Individual Platelet Units (IPU's) that have been treated with the Mirasol PRT System.

Aim: The objective of this study was to evaluate *in vitro* platelet cell quality data from Mirasol-treated platelets in PAS that were derived from Reveos-processed whole blood (WB).

Methods: WB units were collected and processed in one of two ways. For the first group, WB units were rested for 8–24 h following collection at room temperature without agitation. Units were then processed on day 1 with Reveos to produce IPUs per manufacturer's instructions. For the second group, WB units were processed on the day of collection and held overnight as IPUs. For both conditions, IPUs were pooled and leukoreduced per manufacturer's instructions and all pooled PCs were rested for a minimum of 2 h prior to being treated with the Mirasol System according to a defined protocol. Several *in vitro* cell quality parameters including swirl, blood gas analysis, glycolytic metabolism, cell count, and platelet activation were measured on the day of treatment and on Day 1, 2/4, and 5 of storage.

Results: A total of N = 14 Reveos-derived, Mirasol-treated pooled platelet concentrates in PAS and were included in this study. All N = 14 units (N = 9 WB processed 8–24 h post collection, and N = 5 WB processed day of collection and held overnight as IPU) met the Council of Europe (CoE) Guidelines (18^{th} edition) for $pH_{22°C}$ > 6.4 on day 5 of storage (Table 1). Additionally, all other cell quality parameters indicated platelet quality was maintained throughout the 5-day storage period (Table 2).

Conclusion: Mirasol-treated platelets in PAS derived from Reveos-processed WB held overnight, as well as those processed on the day of collection and held overnight as IPUs exhibit acceptable *in vitro* cell quality and fulfill the CoE pH requirement. The results of this study demonstrate that the application of Mirasol-treatment to Reveos-processed platelet pools in PAS produces a platelet product with acceptable *in vitro* platelet cell quality characteristics.

P-203

This abstract has been withdrawn.

P-204

PATHOGEN REDUCTION OF PLATELETS BY INTERCEPT BLOOD SYSTEM: *IN VITRO* FUNCTIONAL AND SURVIVAL PARAMETERS

MH Azimova, AV Karyakin, ED Skotselyas, GN Kryukova, SS Nechaevskaya, YO Davydova, NM Kapranov, IV Galtseva, MY Drokov and TV Gaponova

National Research Center for Hematology, Moscow, Russian Federation

Introduction: For increasing safety of transfusion donor's platelets different technologies of pathogen reduction are used and the acceptable shelf life varies from 3 to 7 days.

Aims: In this study biochemical parameters and markers of platelets activation were measured during storage of platelets concentrates(PCs) in different media after treatment with INTERCEPT Blood System technologies (IBSt).

Materials and methods: PCs were pooled in 100% plasma (PCs Pl, n = 25) or in mixture 30% plasma + 70% SSP$^+$ (PCs SSP$^+$, n = 25). Each PC was split to obtain identical PCs that were control (CPCs) or treated with IBSt (PRPCs). *In vitro* parameters (pH, Glucose, L-Lactic acid, Citric acid concentration and spontaneous expression of CD62p antigen and ADP-depended specific bindinganti-PAC-1 antibodies) were measured on the day of collection, 3, 5 and 7 days of storage at 22–24°C.

Results: Starting pH CPCs Pl was 7,6 and CPCs SSP$^+$ - 7,4. Both types of control samples had pH increasing on 3rdday, but CPCs SSP$^+$ maintained pH 7,4 for all 7 days while pH of CPCs Pl decreased to 7,1on 7th day. In all samples PR PCs pH decrease in first 3 days, then PR PCs Pl showed decrease in pH to 6,6 but PR PC SSP$^+$ maintained pH7,0.

The glucose utilization (GU) was the same in all PCs Pl samples (CPCs Pl 41,36 mmol and PR PCs Pl- 38,80 mmol). In CPCs SSP$^+$ the GU was slower (23,84 mmol), but in PR PCs SSP$^+$ it increased to 33, 29 mmol and the main it's part (26,79 mmol) was utilized in the first 3 days.

The L-lactic acid production was 21,38 mmol in CPCs Pl, 18,83 mmol in PR PCs Pl, 13,71 mmol in CPCsSSP$^+$ and 18,05 mmol in PR PCs SSP$^+$, in agreement with glucose utilization, but the rate of lactate accumulation was higher after treatment on IBS for the first 5 days in plasma suspended samples (2,71 mmol/day in CPCs Pl and 3,85 mmol/day in PR PCs Pl) and for first 3 days in SSP$^+$ suspended (1,44 mmol/day in CPCsSSP$^+$ and 2,98 mmol/day in PRPCsSSP$^+$.

In all PC samples Citric acid concentration did not change significantly and was 38,6 mmol/l in CPCs Pl, 32,0 mmol/l in PRPCsPl, 29,9 mmol/l inCPCs SSP$^+$ and 31,1 mmol/l in PR PCs SSP$^+$.

In the process of storage, a significant increase of spontaneous activation in all PCs types, and decrease of ADP-activated platelets share in PR PCs Pl, CPCs Pl, CPCs SSP+ was observed. When compared between CPCs Pl and CPCs SSP+, differences in activation were found (share of CD62P+ platelets) on days 1, 5 and 7 accordingly:17,8%±3,4vs 28,7%±2,2(P = 0,009); 46,9%±2,3 vs 55,9%±2,2 (P = 0,008); 61,4%±2,5vs 68,4%±1,5 (P = 0,029). On 7th day of storage, the share of ADP-activated platelets in PR PCs SSP+ is higher than in PR PCs Pl(8,0 ± 2,2vs 18,8 ± 2,8; P = 0,009).

Conclusions: PCs after pathogen reduction (PD) by IBS have more stable biochemical parameters in SSP$^+$. PD did not impact platelets activity. On day 7, ADP induced activity was maintained better by the platelets, prepared in SSP+, but spontaneous activation in SSP+ was higher in all days.

P-205

INACTIVATION OF PLASMODIUM FALCIPARUM WITH AMOTOSALEN/UVA IN PLATELETS CONCENTRATES SUSPENDED IN 100% PLASMA

A Laughhunn[1], P Grellier[2], C Sow[2] and A Stassinopoulos[3]

[1]Cerus Corporation, Concord, United States of America [2]Museum National d'Histoire Naturelle, Team BAMEE, Paris, France [3]Cerus Corporation, Global Scientific Affairs, Concord, California, United States of America

Background: A photochemical treatment process utilizing amotosalen and low energy ultraviolet A (UVA) light, developed to inactivate pathogens and residual leukocytes in plasma and platelet components (PC), is the first pathogen reduction system approved by the FDA in the US. Initially, the system was approved for PC manufactured in 65% platelets additive solution (PAS-3) and now it includes PC suspended in 100% plasma. The objective of this study was to evaluate the inactivation of *Plasmodium falciparum (Pf),* in support of that approval extension. Pf is a species of intracellular *Plasmodium* parasite that specifically infects red blood cells (RBCs) during an obligate asexual blood stage causing malaria.

***Plasmodium falciparum* Inactivation in Platelets Suspended in 100% Plasma**

Replicate	Log Titers ($TCID_{50}$/mL)			Log Reduction[a]	Log Reduction per mL[b]
	Pre-UVA[a]	Pre-UVA per mL[b]	Post UVA		
1	7.5	6.0	≤0.0	>7.5	>6.0
2	7.7	6.2	≤0.0	>7.7	>6.2
3	8.5	7.0	≤0.0	>8.5	>7.0
4	8.0	6.5	≤0.0	>8.0	>6.5
Mean ±SD[c]	7.9 ± 0.4	6.5 ± 0.4	≤0.0 ± 0.0	>7.9 ± 0.4	>6.5 ± 0.4

a. Pre-UVA and Log reduction based on ID50 results per flask at 4 flasks per dilution
b. Pre-UVA per mL and Log reduction per mL based on ID50 results per 28.8 mL/flask.

Methods: For each experiment, a single apheresis PC unit in 100% plasma at ~ 365 ml was inoculated with ring-stage *Pf*-infected human red blood cells (iRBC) to a final concentration of ~10^6 iRBC/ml. An 80 ml control sample was removed prior to addition of amotosalen and illumination of the PC unit. After the control was removed, amotosalen was added, the unit illuminated and a post-treatment sample was withdrawn. For the control and test samples, the iRBCs were isolated by Ficoll separation, resuspended and serially diluted, to 10^{-8} for the control and to 10^{-3} for the test, in flasks containing medium with 5% fresh RBCs. The diluted samples were used to inoculate flasks in quadruplicate and monitored for parasitemia by counting iRBC in blood smears and flow cytometry. Cultures were terminated after they reached >1% parasitemia. Log reduction was calculated as the difference between the mean titer in pre-UVA samples and the mean titer in the post-UVA samples.

Results: The levels of inactivation of *P. falciparum* achieved in platelets collected in 100% plasma (N = 4) is shown in the table below.

Conclusions: Photochemical treatment with amotosalen and UVA of platelet components collected in 100% plasma inactivated >7.9 log, or >6.5 log/ml of *P. falciparum.* These results are comparable with the ≥6 log inactivation achieved in platelet components suspended in 65% PAS.

P-206

INACTIVATION OF BOVINE VIRAL DIARRHEA VIRUS (BVDV) WITH AMOTOSALEN/UVA IN PLATELET CONCENTRATES PREPARED IN PAS USING TRIPLE STORAGE (TS) CONTAINERS

F Santa Maria[1], A Laughhunn[1], R Lenhoff[1] and A Stassinopoulos[2]

[1]Cerus Corporation, Microbiology Department, Concord, California, United States of America [2]Cerus Corporation, Global Scientific Affairs, Concord, California, United States of America

Background: A photochemical treatment process utilizing amotosalen and low energy ultraviolet A (UVA) light, was developed to inactivate pathogens and residual leukocytes in platelet components (PC). It was CE marked for PC in 2002, and was approved in the US in 2014. Approximately 1.5 million kits have been sold for the preparation of inactivated components since their approval and the kits comprise small volume (SV; 255–325 ml), Double dose (DS; 300–420 ml) and large volume kits (LV; 300–420 ml) designed to cover the range between 2.5 and 8.0 × 10^{11} doses. A new triple storage (TS) kit designed to expand the dose range to 12.0 × 10^{11} and the maximum volume to 650 ml, generating either 2 or 3 doses of Pathogen inactivated PC, is being validated for pathogen inactivation (PI) and function. The objective of this study was to evaluate the PI of Bovine Viral Diarrhea virus (BVDV) in TS containers. BVDV is a small, enveloped, positive-sense, single-stranded RNA virus that is a member of the *Pestivirus* genus within the family *Flaviviridae* and serves as a model virus for hepatitis C virus, as they have similar structural organization.

Methods: For each experiment, a platelet pool was prepared in 47% plasma/53% PASIII with a final volume of ~650 ml and a dose of 10–12 × 10^{11} platelets. These conditions, which result in a final concentration of amotosalen at the lower limit of the range (135 μM; nominal ≈150 μM) and the highest range of plasma % represent a worst-case scenario for pathogen inactivation. The platelet unit was inoculated with approximately 7 log of BVDV at a 1:100 dilution and control samples were

BVDV Inactivation in Platelets Suspended in 47% Plasma/53% PASIII in Triple Storage Containers

Replicate	Log Titers		Log Reduction[b]
	Pre-UVA	Post- UVA[a]	
1	4.24	< -1.0	> 5.24
2	4.72	< -1.0	> 5.72
3	4.65	< -1.0	> 5.65
4	4.98	< -1.0	> 5.98
Mean±SD[c]	4.6±0.3	<-1.0±0.0	> 5.6±0.3

[a] When no plaques were detected (0 pfu), the titer was defined as <1 pfu in the volume plated and a theoretical 1 pfu/volume plated was used to calculate the log reduction. Negative logs are a result of >1 mL test volumes.
[b] Log reduction is calculated as Log (pre-UVA titer ÷ post-UVA tier) where titers are expressed as 10^x pfu/mL. Log reductions were determined from numbers that were not rounded.
[c] Means and standard deviations (SD) were determined from numbers that were not rounded.

taken after the addition of amotosalen prior to illumination. Each PC unit was illuminated and a post-treatment sample was collected. Control and test samples were serially diluted and inoculated onto monolayers of Bovine Turbinate (BT) cells to determine pre- and post-UVA viral titers. Log reduction was calculated as the difference between the mean titers in pre-UVA samples and post-UVA samples.
Results: Robust levels of inactivation of BVDV were achieved in platelets collected in PAS and processed in TS Containers (N = 4) (**Table**).
Conclusions: Photochemical treatment with amotosalen and UVA of platelet components collected in 47% plasma/53%PASIII in Triple Storage Containers inactivated >5.6 log, of BVDV. These results are comparable with >6.0 log, inactivation achieved in PC prepared in PAS under nominal conditions.
The TS is not approved for use.

Table 1: Antibiotic susceptibility profiles

Antibiotics Tested (Kirby-Bauer Antibiotic Disc Diffusion Test)	Strain 1	Strain 2
Ampicillin	R	R
Amoxicillin/Clavulanic Acid	R	S
Piperacillin/ Tazobactam	R	S
Cefuroxime	R	I
Ceftazidime	R	S
Ceftriaxone	R	S
Imipenem	R	S
Ciprofloxacin	R	S
Gentamicin	R	S
Tobramycin	R	S
Sulfamethoxazole/Trimethoprim	R	S

R= Resistant, I= Intermediate, S= Susceptible

Table 2: Inactivation Results in Platelets suspended in PAS3

Bacterial Strains	Control log_{10} Titer	Test log_{10} Titer	Log_{10} Reduction
K. pneumoniae_1 Resistant strain	6.6 ± 0.3	≤-0.8 ± 0.2	≥ 7.4 ± 0.3
K. pneumoniae_2 Susceptible strain	6.5 ± 0.3	-0.8 ± 0.2	7.3 ± 0.3

P-207

ROBUST INACTIVATION OF KLEBSIELLA PNEUMONIAE OCCURS IRRESPECTIVE OF GENETICS AND ANTIBIOTIC SUSCEPTIBILITY FOR PLATELET COMPONENTS SUSPENDED IN PAS-3 USING THE INTERCEPT™ BLOOD SYSTEM FOR PLATELETS

N Patel[1], K Goldbeck[1], R Lenhoff[1] and A Stassinopoulos[2]
[1]*Microbiology Department, Cerus Corporation, Concord, United States of America*
[2]*Global Scientific Affairs, Cerus Corporation, Concord, California, United States of America*

Background: The INTERCEPT[TRADEMARK] Blood System for platelets is intended for the *ex vivo* preparation of pathogen inactivated apheresis platelet components (PC) in order to reduce the risk of transfusion-transmitted infection (TTI), including sepsis, and to potentially reduce the risk of transfusion-associated graft vs host disease (TA-GVHD). The INTERCEPT[TRADEMARK] system has been shown to inactivate a broad spectrum of enveloped and non-enveloped viruses, Gram-positive and Gram-negative bacteria, spirochetes, parasites and leukocytes. The mechanism of action is through the generation of adducts in nucleic acids that inhibit replication, translation and transcription. Based on the MOA, we hypothesize that the inactivation of bacteria will not depend on antibiotic susceptibility profile, determined by genomic variations.
Aims: This study compared the inactivation of two genetically different *Klebsiella pneumoniae* strains isolated from human donors, with different susceptibility profiles for at least ten different antibiotics, in apheresis PC suspended in PAS-3.
Methods: Inactivation studies were conducted using the small volume INTERCEPT [TRADEMARK] kit, treated with ~ 150 μM amotosalen and illuminated with UVA light (3 J/cm^2). Two different *K. pneumoniae* strains were selected based on different antibiotic susceptibility profiles to multiple antibiotics (**Table 1**). 16S DNA analysis and membrane analysis confirmed the *Klebsiella pneumoniae* at strain and species level, while antibiotic susceptibility test showed phenotypic differences between the strains. Bacterial stocks were grown to high titers (9–10 log_{10}/ml) under optimal growth conditions and were diluted 1:100 in PCs. Control samples were removed prior to illumination and bacterial viability was determined by plate culture on rich agar media side by side with different dilutions of Test samples, obtained after treatment. Inactivation was calculated by comparison of Test and Control titers (**Table 2**).
Results: See Table 1 and Table 2
Conclusions: Inactivation of *K. Pneumoniae* by the INTERCEPT[TRADEMARK] Blood System for platelets is robust and insensitive to antibiotic susceptibility.

P-208

PRESERVED *IN VITRO* METABOLIC AND FUNCTIONAL CHARACTERISTICS OF DOUBLE DOSE APHERESIS PLATELET CONCENTRATES PHOTOCHEMICALLY TREATED WITH AMOTOSALEN AND UVA LIGHT

P Sandgren, H Lernbäck and B Diedrich
Karolinska University Hospital, Stockholm, Sweden

Background: The INTERCEPT™ Blood System (Cerus Europe BV) uses a photochemical treatment (PCT) with amotosalen and UVA light to prevent pathogens and leukocytes from replicating and functioning. A novel process allows for the production of two pathogen-inactivated therapeutic platelet (PLT) units from one double dose (DD) platelet concentrate (PC). However, only limited data is available on the *in vitro* quality of INTERCEPT-treated double dose (DD) apheresis platelet units.
Aims: The objective of this study was to evaluate potential *in vitro* effects of the INTERCEPT treatment on apheresis DD units as compared to untreated (control) DD units. Functional, phenotypic and mitochondrial properties of such platelets stored for up to 7 days were analyzed.
Methods: Leukodepleted apheresis DD-PLTs of approximately 400 ml with a target PLT content above 5.2 × 10^{11} in 55–60% SSP+ (MacoPharma) and 40–45% plasma were collected (Day 0) from healthy blood donors on the Trima Accel® Automated Blood Collection System (TerumoBCT). All DD-units were then divided into two separate units and stored overnight on a flatbed agitator (Helmer) at 22 ± 2°C. 8 DD apheresis PLT units were PI treated (day 1) with INTERCEPT and 8 untreated DD PLT units served as controls. Treated PLT were exposed to 3 J/cm^2 UVA light in the presence of 150 μM amotosalen. The units were then transferred into the CAD (Compound Adsorption Device) container and stored 15 h under agitation. The INTERCEPT-treated DD-apheresis units were then split into two equal single dose units and stored for 7 days. Functional, phenotypic and mitochondrial properties of platelets were analyzed at days 2, 5 and 7.
Results: No significant differences (repeated measure ANOVA including post hoc test Bonferroni's adjustment) were observed in PLT count, content and LDH. As to be expected, significant differences were found for a number of parameters upon storage including, pH ($P < 0.01$), lactate concentration ($P < 0.05$) bicarbonate ($P < 0.01$) and pCO_2 ($P < 0.01$). Similarly, differences were observed in the expression of CD62P ($P < 0.01$ on day 2 and day 5) and CD42b ($P < 0.05$ on day 7). However, the anaerobic glycolytic rate, ADP and collagen induced PAC-1 expression,

	Untreated (control) platelet units			INTERCEPT DD apheresis platelet units		
Variables	DAY 2	DAY 5	DAY 7	DAY 2	DAY 5	DAY 7
Platelet count ($\times10^9$/l)	1415 ± 217	1398 ± 179	1403 ± 177	1441 ± 196	1430 ± 206	1416 ± 210
Platelet content ($\times10^9$/Unit)	283 ± 44	280 ± 37	281 ± 37	267 ± 30	265 ± 32	262 ± 32
MPV (fl)	8.2 ± 1.1	8.0 ± 0.9	8.1 ± 1.0	7.9 ± 0.4	8.2 ± 0.4	8.2 ± 0.4
LDH activity (%)	4.0 ± 2.5	4.28 ± 1.07	5.21 ± 1.02	3.9 ± 1.8	5.5 ± 0.9	4.54 ± 0.84
pH (22°)	7.470 ± 0.016	7.508 ± 0.084	7.490 ± 0.117	7.212 ± 0.027[‡]	7.327 ± 0.035[‡]	7.313 ± 0.059[‡]
Glucose (mmol/L)	6.8 ± 0.6	4.7 ± 1.1	3.0 ± 1.6	5.5 ± 1.1	3.3 ± 1.4	1.3 ± 1.3
Lactate (mmol/L)	3.5 ± 0.5	7.5 ± 1.6	10.7 ± 2.2	6.5 ± 1.2	10.2 ± 1.6	13.5 ± 1.8
Bicarbonate (mmol/L)	8.1 ± 0.7	7.7 ± 1.4	6.9 ± 2.0	5.9 ± 0.7[‡]	5.2 ± 0.9[‡]	4.7 ± 0.9[‡]
ATP (nmol/10^{11} Pl.Ts)	7.20 ± 0.52	7.15 ± 0.60	7.16 ± 0.40	7.65 ± 0.52	7.65 ± 0.91	7.15 ± 0.38
CD62P (%)	15 ± 07	18.36 ± 2.71	22.96 ± 3.58	21.11 ± 3.53[‡]	26.39 ± 3.35[‡]	27.53 ± 2.69

Table 1. *in vitro* analysis of DD apheresis PLTs (n = 8) stored for 7 days in SSP+ with or without INTERCEPT-treatment. Mean ± standard deviation (SD).

ATP levels and the mitochondrial membrane potential (MMP) remained consistent between test and control platelets. No aggregates were observed in any of the tested units and swirling was maintained at the highest level in all groups at all times. Results for a selection of tested parameters are presented in Table 1.

Summary/Conclusions: Our data demonstrate that photochemical pathogen inactivation of DD-apheresis platelet concentrates with the INTERCEPT Blood System had only minor influence on the PLT *in vitro* quality over the 7 day of storage. PI processing of DD platelets reduces disposable and labor costs compared with single dose treatment.

P-209

IN VITRO PROPERTIES OF WASHED PLATELETS PREPARED WITH AUTOMATED CELL PROCESSOR DURING 72-H STORAGE

J Hirayama, A Iwama, M Chatani, H Onodera, Y Kaneko, M Shiba, T Nagai, M Satake and K Tadokoro

Japanese Red Cross Central Blood Institute, Tokyo, Japan

Background: Washed platelet concentrate (WPC) is useful for preventing transfusion-related adverse reactions. WPC is prepared manually in general, but automated preparation is desirable to minimize variation in the WPC quality and enhance WPC production. It is known that WPC can be prepared with an automated cell processor (ACP), which is already being used to wash red blood cells at blood centers in Japan, but there is no function to mix medical solutions for the preparation of additive solution (AS). To increase the convenience, software of the ACP was recently improved so that the mixing of medical solutions could be performed automatically.

Aim: The aim of this study was to clarify whether or not the *in vitro* properties of WPC prepared with the improved ACP were well-preserved during 72-h storage.

Methods: One-day-old platelet concentrate (2.57×10^{11}), BICANATE (1000 ml×2, Otsuka Pharm. Fac.), ACD-A (300 ml, Kawasumi Lab. Inc.), and a platelet disposable set (238J-00, Haemonetics Corp.) were set up in an automated cell processor (ACP215, Haemonetics Corp.), which was installed with software (215J-B.5) to mix medical solutions for AS and prepare WPC. After that, ACP operation was performed according to the manufacturer's protocol. BRS-A additive solution was automatically prepared by mixing BICANATE and ACD-A at 20:1 in ACP. Prepared WPC was stored on a flatbed shaker for 72 h.

Results: The platelet count ($\times10^{11}$) and volume (ml) of prepared WPC were 2.26 ± 0.13 and 216 ± 1, respectively (n = 10). The plasma protein removal (%) and platelet recovery (%) on WPC preparation were 99.0 ± 0.2 and 87.6 ± 3.4, respectively (n = 10). pH values were maintained above 6.8 during 72-h storage. MPV values 24, 48, and 72 h after washing were almost equal to the pre-wash value, although the values temporarily increased immediately after washing. The CD62P value was increased by 16% after washing, but the change in the values from 24 to 72 h after washing was small. The HSR value was decreased by 10% after washing, but the values 24, 48, and 72 h after washing were higher than the pre-wash value. Aggregation values were maintained above 90% during 72-h storage. Glucose values were decreased in a time-dependent manner, but the value was not zero at 72 h after washing. The swirling of platelets was observed in WPC during 72-h storage.

Conclusions: The quality of WPC prepared with the improved ACP was well-preserved during the 72-h storage. WPC prepared as described in this study was approved on March 2016; therefore, it will be commercially available as a novel blood product in the near future in Japan.

P-210

COMPARISON OF *IN VITRO* PLATELET FUNCTION OF INTERCEPT™ TREATED APHERESIS PLATELET COMPONENTS (PC) PREPARED WITH TWO PAS-E SOLUTIONS (SSP+ AND T-PAS+)

B Donnelly, K Ling, M Von Goetz, H Evans, A Kong, T Wilson, T Diaz, D Hanson, N Mufti and T David

Cerus Corporation, Concord, United States of America

Background: The INTERCEPT[TRADEMARK] Blood System was developed to reduce the risk of transfusion-transmitted infections (TTI) by inactivating pathogens in blood components. This system utilizes amotosalen (S-59) and low energy ultraviolet A (UVA) light to crosslink nucleic acids and prevent replication of pathogens and leukocytes. The INTERCEPT[TRADEMARK] system is an approved device for treatment of PCs in 100% plasma, 32–47% plasma/53–68% PAS-C (InterSol[TRADEMARK], Fenwal) and 32–47% plasma/53–68% PAS-E (SSP+[TRADEMARK], Macopharma). Recently, two "generic"versions of the PAS-E solution were made commercially available in the EU: Grifols PAS-IIIM and Terumo BCT T-PAS+. These solutions are identical to SSP+ with respect to formulation and have similar sterilization/storage conditions.

Table: *In Vitro* Platelet Function Results on Day 7, Mean ±SD (Range)

Assay (Units)	T-PAS+ Test (n=6)	SSP+ Control (n=6)	p Value (paired t-test)
Amotosalen Pre-UVA (μM)	143.1 ±5.6 (135.9 – 151.9)	145.7 ±4.3 (140.8 – 150.5)	Not applicable
Amotosalen Post-UVA (μM)	31.6 ±4.2 (26.0 – 37.0)	33.4 ±5.2 (26.2 – 40.1)	Not applicable
Amotosalen Post CAD 16 hrs (μM)	<0.13	<0.13	Not applicable
Platelet recovery (%) post-processing Day 2	89 ±6 (79 – 97)	91 ±3 (86 – 93)	0.53
Volume recovery (%) post-processing on Day 2	91 ±1 (90 – 93)	93 ±2 (91 – 96)	0.21
Platelet count ($\times10^3$/μL)	1040 ±61 (940 – 1092)	1011 ±63 (917 – 1073)	0.02[a]
Platelet dose ($\times10^{11}$ per unit)	2.7 ±0.1 (2.5 – 2.9)	2.6 ±0.1 (2.4 – 2.8)	0.10
Unit volume (mL)	259.9 ±10.5 (241.1 – 268.9)	261.1 ±4.8 (252.8 – 265.0)	0.73
pH (22°C)	7.2 ±0.1 (7.1 – 7.3)	7.2 ±0.1 (7.1 – 7.3)	1.00
pCO_2 (mm Hg)	20.9 ±2.3 (17.9 – 22.9)	20.6 ±2.4 (17.4 – 23.0)	0.16
pO_2 (mm Hg)	100.3 ±10.2 (87.8 – 117.8)	98.2 ±12.2 (88.2 – 121.7)	0.37
HCO_3 act (mM)	4.7 ±0.8 (3.6 – 5.5)	4.6 ±0.9 (3.4 – 5.8)	0.64
Morphology score (max 400)	272 ±14 (253 – 295)	265 ±8 (253 – 277)	0.08
Extent of Shape Change (%)	24.6 ±1.6 (22.9 – 27.2)	25.3 ±1.6 (22.2 – 26.7)	0.38
Hypotonic Shock Response (%)	57.1 ±6.5 (50.2 – 68.0)	58.8 ±6.8 (52.0 – 69.7)	0.27
Supernatant glucose (mM)	1.3 ±0.9 (0.3 – 2.7)	1.2 ±0.9 (0.0 – 2.5)	0.34
Supernatant lactate (mM)	9.7 ±1.6 (7.1 – 10.8)	9.6 ±1.7 (7.5- 11.1)	0.66
ATP (nmol/ 10^8 platelets)	4.5 ±0.7 (3.6 – 5.4)	4.5 ±0.8 (3.5 – 5.6)	1.00
CD62P (% expression)	61.2 ±2.5 (59.2 – 65.9)	59.3 ±6.7 (48.7 – 67.1)	0.44
Supernatant LDH (IU/L)	101 ±12 (86 – 119)	107 ±9 (100 – 120)	0.05
sCD62P (ng/mL)	89.9 ± 14.5 (77.1 - 111.9)	89.4 ± 17.1 (63.2 - 110.3)	0.96
RANTES (ng/mL)	258.0 ± 37.3 (219.2 - 307.7)	260.2 ± 87.3 (136.8 - 378.4)	0.94
sCD40-L (ng/mL)	11.9 ± 2.1 (9.34 - 14.9)	12.3 ± 3.6 (8.9 - 19.1)	0.71

a. Statistically significant difference (p<0.05), students paired t-test
b. Statistically significant difference (p≤0.01), students paired t-test

Aim: The objective of this study was to compare *in vitro* platelet function (PF) of paired INTERCEPT PCs prepared with either SSP+ or a representative "generic"PAS-E solution (T-PAS+).
Method: PCs in 100% apheresis plasma were pooled, concentrated by centrifugation, equally divided and suspended in either 35% plasma/65% SSP+ (Control) or 35% plasma/65% T-PAS+ (Test) to generate a paired experimental replicate. Each replicate consisted of two identical PCs containing 2.9 to 3.4 $\times$ 10^{11} platelets in 297 to 321 ml of 35% plasma and 65% of either T-PAS+ or SSP+. The PCs were treated using the INTERCEPT[TRADEMARK] Platelet SV Processing Set (INT21), stored at 22°C under agitation and tested for PF on day 7. Function assays included platelet dose, volume, pH, blood gases, morphology score, extent of shape change (ESC), hypotonic shock response (HSR), CD62P expression, ATP, glucose, lactate, LDH and cytokine release (RANTES and sCD40-L). Six replicates were performed.
Results: Following treatment, amotosalen photo-conversion was found to be within specifications in SSP+ and T-PAS+ platelets and essentially equivalent. In addition, similar platelet and volume losses were observed between Tests and Controls (**Table**). On Day 7, all 12 INTERCEPT[TRADEMARK] PCs met Council of Europe transfusion criteria for pH and dose ($pH_{22°C} \geq 6.4$, dose $\geq 2 \times 10^{11}$ platelets/unit). Test units showed a minimal but statistically significant increase in mean platelet count. No statistically significant differences were observed in any of the other parameters tested. INTERCEPT PCs suspended in T-PAS+ and SSP+ showed comparable metabolic activity (pH, blood gases, glucose, lactate, ATP and HSR) and conserved platelet integrity (morphology score and LDH activity). Hemostatic indicators (CD62P and ESC) were similar between Test and Control PCs and there were no significant differences in cytokine release (RANTES and sCD40L) and inflammation markers (sCD62P) between INTERCEPT PCs in SSP+ and T-PAS+ (**Table**).
Conclusions: This *in vitro* study demonstrated that PCs suspended in either T-PAS+ or SSP+ and treated with the INTERCEPT[TRADEMARK] System resulted in equivalent *in vitro* function and cytokine/inflammation secretion profiles up to 7 days of storage. These results support the use of all PAS-E solutions with the same chemical compositions, such as Grifols PAS-IIIM and T-PAS, for the manufacturing of INTERCEPT platelets in PAS.

P-211

FIBINROLYTIC SENSITIVITY OF RECONSITUTED WHOLE BLOOD TO SOLVENT/DETERGENT POOLED PLASMA (SD-PLASMA)

NH Saadah[1], PF van der Meer[2], D de Korte[2], IJ Bontekoe[2], HM Brinkman[3], HH Korsten[2], RA Middelburg[4], JG van der Bom[5] and MR Schipperus[6]

[1]Leiden University Medical Center, Leiden, The Netherlands [2]Sanquin Product and Process Development, Amsterdam, The Netherlands [3]Sanquin Department of Plasma Proteins, Amsterdam, The Netherlands [4]Department of Clinical Epidemiology; LUMC, Leiden, The Netherlands [5]Sanquin CCTR, Leiden, The Netherlands [6]Haga Teaching Hospital, The Hague, The Netherlands

Introduction: Hyperfibrinolysis following transfusion with Solvent/Detergent-treated pooled Plasma (SD-plasma) has been observed in heavily transfused patients.
Study Aim: We aimed to compare the fibrinolytic potential of whole blood reconstituted with SD-plasma vs quarantined fresh frozen plasma (Q-FFP).
Study Methods: 64 whole blood samples were reconstituted from red cell concentrates, platelet concentrates, and various mixtures of Q-FFP and/or SD-plasma to simulate gradual replacement of a patient's plasma fraction with SD-plasma. Hematocrit and platelet count were varied. Thromboelastography (TEG) analysis was used to measure fibrinolytic potential. Tissue plasminogen activator (tPA) concentration was adjusted following tPA calibration runs each day. Four samples representing 0%, 50%, 75%, and 100% SDP in the plasma compartment were run simultaneously with time to 50% clot lysis ($CLT_{50\%}$) for each sample normalized by the $CLT_{50\%}$ of that run's whole blood sample reconstituted with 100% Q-FFP. This yielded relative time to 50% clot lysis ($rCLT_{50\%}$) for each sample. Regression methods were applied to quantify the effects of SD-plasma, hematocrit, and platelet count on relative 50% clot lysis time, maximum amplitude (MA), and initial clotting time (R-time). Sensitivity analyses were performed to ensure the effects of tPA concentration and normalization did not significantly affect relative clot lysis times.
Results: The relative change in $CLT_{50\%}$ as the plasma compartment transitions from Q-FFP to SD-plasma was −0.52 [95%CI:-0.60;-0.45]. Platelets were a significant effect modifier with each 100 $\times$ 10^9 platelets/L increase in platelet count causing a change in relative $CLT_{50\%}$ of −0.06 [95%CI:-0.10;-0.01]), while hematocrit was not associated with relative $CLT_{50\%}$. MA and R-time were not significantly affected by SD-plasma dilution. A sub-analysis of runs with equivalent tPA concentrations yielded a similar relative change in CLT50% as the plasma compartment transitions to SDP (-0.50 [95% CI −0.59;-0.40]), as well as a similar platelet effect (change in relative $CLT_{50\%}$ for each 100 $\times$ 10^9 platelets/L increase in platelet count −0.06[95%CI:-0.11;0.04]). A sub-analysis of runs for which $CLT_{100\%}$ was defined likewise yielded a similar relative change in $CLT_{100\%}$ for the SD-plasma effect (-0.53 [95%CI:-0.64;-0.42]), though the effect of platelets on $CLT_{100\%}$ was here not statistically significant (change in relative $CLT_{100\%}$ for each 100 $\times$ 10^9 platelets/L increase in platelet count −0.04[95%CI:-0.11;0.04]).
Conclusion: SD-plasma is associated with shorter clot lysis times in whole blood. Higher platelet counts may increase the effect of SDP on relative clot lysis time, while hematocrit is not associated with relative clot lysis time. Though the laboratory-derived measures cannot be directly extrapolated to the clinical setting, clinicians should be aware of this effect.

P-212

IN VITRO EVALUATION OF WHOLE BLOOD-DERIVED THERAFLEX MB-PLASMA FOR REGISTRATION IN FRANCE

B Belcour[1], A Dupuis[1], C Geschier[1], V Parentin[1], M Morel[1], C Gachet[1] and S Begue[2]

[1]Etablissement Français du Sang - ALCA, Nancy, France [2]Etablissement Français du Sang - Siege, Saint-Denis, France

Background: The THERAFLEX MB-Plasma process (TMP; Macopharma) uses the addition of methylene blue (MB) and illumination to inactivate pathogens in therapeutic plasma. The treated plasma sourced from aphaeresis was previously on the list of registered blood products in France. A new THERAFLEX MB-Plasma procedure using a new MB source from a different supplier and the Macotronic B2 illumination device treating plasma from whole blood was investigated for registration in France.
Aims: To generate the necessary phase I data according to the French requirements for the registration of a new labile blood product.
Methods: Plasma was derived from 30 units of whole blood and treated with TMP using a new MB pill and the MacoTronic B2 illumination device according to the manufacturer's instructions. Samples were drawn before illumination (T1) and after storage at −25°C for 14 days (T2) and 6 months (T3). Plasma proteins, plasma factors, coagulation tests, fibrinolysis and activation markers were measured. Moreover, fibrinogen and factor VIII were also checked from whole blood (T0) and after 1 year (T4). Residual MB was also determined by HPLC. Thrombin generation tests were done at each time point.
Results: Determined values are summarized in the table.

Sampling	At donation	Before treatment	Storage		
			14 days	6 months	12 months
Quick time (%)	-	98 ± 8.3	93 ± 8.9	94 ± 7.6	
aPTT (ratio)	-	1.10 ± 0.1	1.22 ± 0.1	1.12 ± 0.1	
Fibrinogen (g/L)	2.71 ± 0.46	2.69 ± 0.44	2.53 ± 0.37	2.51 ± 0.38	2.58 ± 0.39
F II (%)	-	99 ± 11	98 ± 10	86 ± 7	
F V (%)	-	105 ± 16	90 ± 16	87 ± 13	
F VII (%)	-	98 ± 16	94 ± 17	83 ± 12	
F VIII (IU/mL)	1.16 ± 0.34	0.97 ± 0.29	0.87 ± 0.27	0.81 ± 0.19	0.82 ± 0.24
F IX (%)	-	108 ± 11	99 ± 10	96 ± 11	
F X (%)	-	96 ± 12	91 ± 11	89 ± 9	
F XI (%)	-	103 ± 12	89 ± 11	77 ± 13	
ADAMTS13 (%)	-	99 ± 8	99 ± 9	86 ± 14	
vWF antigen (%)	-	103 ± 32	102 ± 32	101 ± 32	
vWF activity (%)	-	102 ± 43	100 ± 44	104 ± 37	
Protein C (%)	-	100 ± 15	98 ± 15	101 ± 15	
Protein S (%)	-	80 ± 15	78 ± 15	77 ± 14	
Antithrombin III (%)	-	103 ± 8	103 ± 10	105 ± 10	
Plasminogen (%)	-	98 ± 10	98 ± 10	99 ± 11	
α-2 antiplasmin (%)	-	108 ± 8	108 ± 9	108 ± 9	
TAT (µg/L)	-	3.34 ± 1.3	3.07 ± 1.2	2.63 ± 1.1	3.70 ± 1.9
C3a (ng/mL)	-	69 ± 35	366 ± 157	188 ± 81	
C5a (ng/mL)	-	7.3 ± 4.0	11.6 ± 4.7	9.4 ± 4.8	

Sampling	At donation	Before treatment	Storage		
			14 days	6 months	12 months
Lag time (min)	5.3 ± 1.1	5.3 ± 1.0	5.0 ± 0.9	5.1 ± 0.9	3.8 ± 0.7
Time to peak (min)	11.8 ± 1.8	11.0 ± 1.8	10.1 ± 1.4	10.0 ± 1.6	8.2 ± 1.4
Endogenous thrombin potential (nM x min)	1156.4 ± 377.3	1326.6 ± 422.8	1268.1 ± 344.0	1352.0 ± 270.6	1331.0 ± 236.0
Velocity (nM/min)	19.3 ± 14.9	32.9 ± 25.7	32.7 ± 15.6	37.7 ± 16.6	41.4 ± 17.4
Peak (nM)	107.9 ± 54.0	155.0 ± 78.0	154.9 ± 53.9	170.9 ± 50.6	171.2 ± 44.5

Briefly, moderate reductions due to the treatment and storage for 6 months were observed for FII (13.1%), FV (17.1%), FVII (15.3%), FIX (11.1%), FX (7.3%), and ADAMTS13 (13.1%). Protein C, protein S and antithrombin III are reduced neither by treatment nor storage. The overall average loss of fibrinogen was 0.13 g/L (4.8% of initial content) after 1 year of storage. Average activity of FVIII after treatment after 14 days, 6 months and 1 year of storage was 0.87 IU/ml, 0.81 IU/ml and 0.82 IU/ml (70.7% vs at donation), respectively. Lag time and time to peak were significantly reduced after 1 year compared to 6 months but all other thrombin generation parameters did not significantly change during storage.

Summary/Conclusions: As for any other pathogen inactivation treatment, activities of some hemostasis factors are moderately impaired by the THERAFLEX MB-Plasma process. Nevertheless, all measured factors show activities within the normal range. The maintained balance of all the factors and the normal hemostasis, as showed by the thrombin generation test, demonstrate the ability of the MB-Plasma to fully meet the French regulatory requirements and clinical indications.

P-213

EVALUATION OF WHOLE BLOOD-DERIVED PLASMA QUALITY TREATED WITH THE THERAFLEX PATHOGEN INACTIVATION SYSTEM FOR REGISTRATION IN FRANCE

S Begue[1], C Naegelen[2], N Marpaux[2], D Dernis[3], S Boivin[3], C Geschier[4], B Belcour[4], A Dupuis[4], S Bois[5], S Requiem[5] and M Colombat[6]

[1]EFS Rhone Alpes Auvergne, La Plaine Saint Denis, France [2]EFS Bourgogne Franche Comté, Besançon, France [3]EFS Nord de France, Lille, France [4]EFS Alcase Lorraine Champagne, Nancy, France [5]EFS Bretagne, Rennes, France [6]EFS Aquitaine Limousin, Bordeaux, France

Background: The THERAFLEX MB-Plasma process (TMP; Macopharma) uses the addition of methylene blue (MB) and illumination to inactivate pathogens in therapeutic plasma. The treated plasma sourced from aphaeresis was previously on the list of registered blood products in France. A new THERAFLEX MB-Plasma procedure using a new MB source from a different supplier and the Macotronic B2 illumination device treating plasma from whole blood was investigated for registration in France.

Aims: To generate the necessary phase II data (multicenter trial), for the registration of a new labile blood product, according to the French specifications for pathogen inactivation (PI) of plasma quality based on Fibrinogen and Factor VIII measurements.

Methods: The THERAFLEX MB-Plasma treatment was performed on whole blood-derived plasma by the Etablissement Français du Sang -EFS- (French National Blood Service) the next day from collection, and the freezing process was carried out in less than 20 h from collection. Quality control was performed before PI and freezing (T_0) and after freezing at ≤25°C of temperature and storage for a period between 2 and 14 days (T_2). Fibrinogen and Factor VIII analyses were carried out by each EFS evaluator: Nord de France (Lille), Bretagne (Rennes) Alsace Lorraine Champagne Ardennes (Nancy), Bourgogne Franche-Comté (Besançon) and Aquitaine Limousin (Bordeaux).

Results: A number of 512 units were analyzed. Time-to-freezing (mean ± SD) was 12:11 ± 00:56 hh:mm, Between T_0 and T_2, fibrinogen recovery was 92.2 ± 6.3%, with a rate ≥ 2.0 g/L limit of 82%. Factor VIII recovery was 87.8 ± 7.9%, with a rate ≥ 0.5 UI/ml limit of 91.0% Results are summarized in Table 1.

Summary/conclusions: Both, Fibrinogen and Factor VIII levels, measured from each one of the EFS centers, after pathogen inactivation with the THERAFLEX MB-Plasma system, freezing and thawing, demonstrate the ability of the MB-Plasma to fully meet the French regulatory requirements and clinical indications.

All Blood Groups	Before treatment (T_0)		After treatment (T_2)	
	Fibrinogen (g/L)	Factor VIII (UI/mL)	Fibrinogen (g/L)	Factor VIII (UI/mL)
Mean	2.66	0.89	2.44	0.78
SD	0.52	0.28	0.47	0.25
Median	2.60	0.84	2.41	0.74
Minimum	1.64	0.26	1.50	0.25
Maximum	4.64	2.16	4.16	2.07
Total Units	512	512	512	512
Total Units NC	41	17	92	46
% NC	8.0%	3.3%	18.0%	9.0%

NC: non conform to French regulatory requirement

Table 1

P-214

THROMBIN GENERATION TEST OF POOLED WHOLE BLOOD DERIVED PLASMA AFTER PHOTOCHEMICAL TREATMENT AND STORAGE

C Ravanat[1], A Dupuis[1], N Marpaux[2], C Naegelen[2], G Mourey[2], H Isola[1], M Laforet[1], P Morel[2] and C Gachet[1]

[1]Etablissement Français du Sang Alsace-Lorraine-Champagne-Ardenne, Strasbourg, France [2]Etablissement Français du Sang Bourgogne-Franche Comté, Besançon, France

Background: Thrombin generation test (TGT) allows the measurement of active thrombin concentration, in real time, and not only the time necessary to generate the first traces of thrombin like in the usual coagulation tests. TGT integrates the overall phases of the hemostatic system.

Aims: The INTERCEPT Blood System (IBS) using amotosalen and UV inactivates a large spectrum of microbial pathogens and leukocytes in therapeutic plasma. Our objective was to evaluate the hemostatic quality of IBS plasma, derived from whole blood (WB), using TGT.

Methods: Five leukodepleted plasma units from WB of the same ABO group are pooled and divided into two sub-units of 650 ml which are each pathogen inactivated. Two sites prepared 40 pools (10 O/ 30 non-O) resulting in 2 × 3 units of 200 ml subsequently frozen within 19 h from collection. The plasma quality was evaluated before treatment (T_1) and after 2 weeks (T_2), 6 months (T_3) and 12 months (T_4) of storage below −25°C. Tests were carried out exploring fibrinogen, coagulation FV, VII, VIII, clotting time measurements and TGT at 1 and 20 pM tissue factor (TF).

Results: Following treatment, we observe a moderate reduction of Factors I, V, VII, VIII by respectively 14%, 9%, 24%, 32% and an increase of PT and aPTT by respectively 18% and 11%. However, thrombin generation potential of plasma is adequately maintained with none of the TGT parameters markedly changed with treatment for the concentration of 20 pM TF. The kinetics of TGT is slightly affected at the suboptimal concentration of 1 pM TF without influence on the total amount of thrombin generated. TGT parameters are not significantly affected by storage at −25°C.

Summary/Conclusions: The thrombin generation capacity of IBS and frozen plasma derived from WB is preserved demonstrating its normal hemostatic potential. TGT could be a useful tool for investigating the hemostatic quality of therapeutic plasma preparations.

Mean ± SD (N=40)	T_1/T_2	T_2/T_4
TGT 1 pM TF		
Lag time (min)	4.82 ± 0.56 / 4.69 ± 0.60[a]	4.79 ± 0.63 / 4.79 ± 0.59
Peak (nM FIIa)	310.6 ± 40.2 / 237.0 ± 42.2[a]	234.1 ± 36.8[b] / 236.5 ± 39.3[c]
ETP (nM FIIa x min)	1543.6 ± 117.1 / 1527.8 ± 122.8	1557.0 ± 182.7 / 1509.5 ± 118.6[c]
TGT 20 pM TF		
Lag time (min)	1.43 ± 0.27 / 1.39 ± 0.21[a]	1.43 ± 0.23 / 1.46 ± 0.26
Peak (nM FIIa)	356.0 ± 27.9 / 356.3 ± 28.5	351.5 ± 25.7 / 346.5 ± 29.0[c]
ETP (nM FIIa x min)	1581.1 ± 153.7 / 1662.6 ± 127.3[a]	1636.0 ± 143.7[b] / 1608.6 ± 141.4

T_1 - reference value in pooled plasma before treatment
T_2 – after treatment and 2 weeks of frozen storage
T_3 - after 6 months
T_4 – after 12 months
Two-sided paired t-Test (alpha 0.05)
a, b or c indicate a significant difference ($p<0.05$) when comparing respectively T_2-T_1, T_3-T_1 or T_4-T_1

Table 1. Quality indicators of INTERCEPT treated Whole Blood derived plasma (n = 40)

P-215

This abstract has been withdrawn.

P-216

EFFECTIVE INACTIVATION OF T-CELLS WITH S-303/GSH IN HUMAN RBC AS ASSESSED BY FLUORESCENT LIMITING DILUTION ASSAYS (LDA)

G Castro, J Green and A Stassinopoulos

Cerus Corporation, Global Scientific Affairs, Concord, California, United States of America

Background: Transfusion Associated Graft vs Host Disease (TA GVHD) is associated with major morbidity and mortality and is caused by viable contaminating T-cells present in transfused blood products. Gamma irradiation (GIRR) with 2500 cGy is used to prevent TA GVHD when these products are transfused in high-risk patients. For platelet components and plasma, an alternative methodology to GIRR is the photochemical treatment of PC with amotosalen/UVA (PCT; INTERCEPT™ Blood systems for Platelets and Plasma). PCT has been used as a replacement for GIRR in Europe for more than 10 years and was recently accepted as an alternative to GIRR by AABB. An alternative methodology to prevent TA GVHD after transfusion of RBC may be the treatment with S-303 and GSH (S-303/GSH; INTERCEPT pathogen reduction system for RBC). In this study, we evaluated the capacity of S-303/GSH to inactivate T-cells in human RBC using an LDA design featuring a fluorescent nucleotide analog incorporation assay (Click-iT®).

Methods: Inactivation of T cells by GIRR in PBMC cells isolated form Control RBC, were compared with inactivation of T cells in PBMCs isolated from RBC treated with S-303/GSH (0.2 mM/20 mM) using incubation at RT for 18–24 h, followed by centrifugation and exchange of the supernatant with AS-5. Human leukocytes were isolated from non-leukofiltered RBC in SAGM ($1–2 \times 10^6$ PBMC/ml) through a modified Ficoll procedure using Leucosep™. After isolation, PBMC were incubated (10^6/ml) for a period of 6–12 h in the presence of Allostimulator cells (5×10^5cells derived from 10 donors, treated with 7500 cGy) and growth stimulating factors (PHA and IL2) under standard culture conditions. Proliferation was assessed by the thymidine analog Ethinyl DeoxyUridine (EdU) incorporation into the cells, over a period of 36–44 h. Labeling of the DNA-incorporated EdU in proliferating cells was achieved through a Click-iT® reaction resulting in the fluorescent labeling of residues with an Alexa 488 fluorophore. Measurement of the labeled cell subpopulation of T-cells was achieved through flow analysis of the sample (ATTUNE NxT Flow cytometer; 0.5 ml) and identification of viable cells based on size and fluorescence intensity.

The T-cells that were fluorescently labeled were enumerated after the application of a gate, designed for that population and the specific fluorescent dye.

Results: The analysis showed a strong signal ($1–8 \times 10^4$ cells) associated with the analysis of live untreated cells that could only be observed when allostimulator cells, EdU and fluorescent labeling had been used; while minimal counts were obtained when one of the reagents was missing. No signal (1–7 cells) was observed for the samples derived from S-303/GSH treated RBC, indicating that cells were unable to proliferate. It is expected that if the incubation of the cells is extended for up to 14 days, a larger dynamic range will be achieved. The results obtained with this methodology are consistent with the inactivation of T-cells for the previous generation of the S-303/GSH system (0.2 mM/ 2 mM GSH) obtained through a radioactive thymidine incorporation assay (>5.1 $\log_{10}$).

Conclusions: Treatment of T-cells with S-303/GSH (INTERCEPT Blood System for RBC) results in T-cell inactivation (~4 $\log_{10}$) vs Control. GSH

The INTERCEPT Blood System for Red Blood Cells is not approved for use.

P-217

COMPATIBILITY OF THE INTERCEPT™ BLOOD SYSTEM FOR RED BLOOD CELLS (RBCS) WITH INPUT RBC VOLUMES OF 220–360 ML

MA Schott, D Hanson, B Warbington, G Villegas and A Erickson

Cerus Corporation, Concord, United States of America

Background: The INTERCEPT[TRADEMARK] Blood System for red blood cells (RBCs) uses the small molecule amustaline to crosslink nucleic acids, preventing replication of contaminating pathogens and residual leukocytes. Glutathione (GSH) is included to quench non-specific reactions.

Aims: This study assessed the quality of INTERCEPT RBCs stored for 42 days prepared using SAG-M RBC input volumes of 220 ml (low, L), 280 ml (nominal, N), or 360 ml (high, H). The acceptance criteria were those described for conventional leukocyte-depleted RBCs in additive solution as defined by the Council of Europe (EDQM, 18th Ed.)

Table 1 In vitro function of INTERCEPT RBCs over 42 days of storage (mean ±SD; n=6)

	Day 2			Day 35			Day 42		
	L	N	H	L	N	H	L	N	H
Hb per unit (g)	41 ±2[a]	52 ±3	69 ±2	not measured			not measured		
Hematocrit (%)	59.8 ±1.00	63.6 ±0.72	67.3 ±0.9	58.8 ±0.5	61.1 ±1.3	65.9 ±1.0	62.6 ±1.0	67 ±1.9	71.3 ±1.5
Hemolysis (%)	0.03 +0.01	0.03 +0.01	0.03 +0.01	0.3 +0.1	0.3 +0.1	0.2 +0.1	0.3 +0.1	0.3 +0.1	0.3 +0.1
pH at 37°C	6.72 ±0.02	6.70 ±0.02	6.70 ±0.02	6.34 ±0.02	6.34 ±0.03	6.34 ±0.03	6.29 ±0.03	6.30 ±0.04	6.30 ±0.03
Total ATP (μM/g Hb)	7.4 ±0.6	7.5 ±0.4	7.4 ±0.3	4.8 ±0.4	4.7 ±0.3	4.8 ±0.2	4.2 ±0.2	4.4 ±0.2	4.4 ±0.4
Extracellular Lactate (mM)[b]	2.08 ±0.20	2.20 ±0.17	2.13 ±0.06	6.34 ±0.49	6.74 ±0.35	6.72 ±0.80	6.47 ±0.30	6.44 ±0.70	6.21 ±0.46
Extracellular Glucose (mM)[b]	11.35 ±0.43	9.82 ±0.42	8.46 ±0.46	8.91 ±0.42	7.81 ±0.64	6.01 ±0.34	7.70 ±0.48	6.25 ±0.39	4.84 ±0.17
Extracellular Na^+ (mM)[b]	58.9 ±1.6	52.7 ±1.1	46.7 ±1.0	46.1 ±0.6	41.5 ±2.4	34.0 ±1.9	41.7 ±1.5	34.3 ±2.0	27.9 ±1.5
Extracellular K^+ (mM)[b]	0.234 ±0.03	0.28 ±0.03	0.32 ±0.04	16.67 ±0.93	18.33 ±0.78	18.65 ±0.30	16.80 ±0.88	17.45 ±1.20	17.32 ±1.14

[a] Pre INTERCEPT Hb = 38 g
[b] Corrected for hematocrit

Methods: Eighteen CPD whole blood collections were shipped overnight at room temperature (RT), leukocyte-depleted with EU approved filtration devices, centrifuged and the RBCs suspended in SAG-M. Three ABO matched RBC components were pooled and split to produce one of each L, N and H volume input components for each of 6 replicates.

On day (D) 1, all units were treated with the INTERCEPT process; GSH and RBCs were added to the processing solution followed by amustaline addition (20 mM GSH/0.2 mM Amustaline, based on 280 ml RBC input). After an 18 h RT hold, units were centrifuged; the treatment solution was expressed and replaced with SAG-M. RBCs were stored at 4 ± 2°C for 6 weeks, and sampled for testing on D2, D14, D35, and D42 (**Table 1**).

Results: On D2, INTERCEPT RBCs had Hct within the required range of 50–70%. After INTERCEPT treatment the Hb content was >40 g for 17 of 18 units (54 ± 12 g). Hb content for one L component was 38 g; the input Hb content for this component was also <40 g (38 g). Hemolysis was <0.8% on D35 and 42 for all INTERCEPT RBCs. Over 42 days of storage hemolysis, K^+ and lactate increased, whereas pH, ATP, Na+, and glucose decreased (**Table**).

Conclusions: The INTERCEPT Blood System for RBCs is compatible with SAG-M RBC inputs of 220 to 360 ml. INTERCEPT treated RBCs met the EDQM guidelines (18^{th} ed) for leukocyte-depleted RBCs in additive solution with respect to Hct, Hb content, and hemolysis. All measured *in vitro* parameters of INTERCEPT RBCs indicate suitability for clinical transfusion.

INTERCEPT Blood system for red blood cells is not approved for use.

P-218

QUALIFICATION OF THE INTERCEPT BLOOD SYSTEM FOR RED BLOOD CELLS AT EGE UNIVERSITY HOSPITAL BLOOD BANK

Y Aydinok[1], C Sonar[1], AS Kucukaslan[1], M Demir[1], OH Sonmezocak[1], DY Kucukaslan[1] and K Waldhaus[2]

[1]Ege University Hospital Blood Bank, Izmir, Turkey [2]Cerus Corporation, Concord, California, United States of America

Background: The INTERCEPT[TRADEMARK] Blood System for Red Blood Cells (RBC) inactivates pathogens and leukocytes in RBC components for transfusion using amustaline to crosslink nucleic acids, preventing replication of contaminating pathogens and leukocytes. A Phase 3 clinical investigation in patients with thalassemia major requiring chronic RBC transfusion support in progress in Europe, has been expanded to include additional study sites. Ege University Hospital is providing INTERCEPT treated RBCs for study of the transfusion safety and efficacy of INTERCEPT RBCs compared to conventional RBCs.

Aims: This study was designed to qualify Ege University Hospital Blood Bank to process RBC components using the INTERCEPT process for a Phase 3 clinical investigation.

Methods: On the day (D) of collection, D0, leukocyte-depleted SAG-M RBCs were prepared from CPD whole blood and stored at 4 ± 2°C. Test units were treated on D1 with the INTERCEPT process. Test RBCs (248 ± 20 ml, 226–286 ml) were added to processing solution containing glutathione (GSH) followed by amustaline addition (final concentrations of 20 mM GSH/0.2 mM amustaline, based on 280 ml RBC input). After 18–24 h hold at 20–25°C, RBCs were centrifuged and the treatment solution was expressed and replaced with SAG-M. RBCs were stored at 4 ± 2°C for 35 days and were sampled on D2, D14 and D35 for analysis of *in vitro* parameters (**Table 1**).

Table 1. INTERCEPT RBC in-vitro function (mean ±SD, n=12)			
Parameter	**Day 2**	**Day 14**	**Day 35**
Hematocrit (Hct, %)	59.1±2.2	59.6±2.6	60.8±1.6
Hemolysis (%)	0.24±0.08	0.35±0.14	0.45±0.18
pH (37°C)	6.77±0.03	6.54±0.03	6.33±0.04
Total ATP (μmol/g Hb)	6.2±0.8	5.1±0.9	3.4±0.5
Extracellular potassium (K+, mM)	1.8±0.3	24.0±2.9	40.3±3.1
Extracellular sodium (Na+, mM)	145.2±1.4	124.1±5.2	113.1±5.3
Extracellular glucose (mM)	28.8±1.2	22.7±1.7	18.2±2.0
Extracellular lactate (mM)	5.6±0.7	12.9±1.6	20.0±2.7

Results: All units met the acceptance criteria for site qualification. The volume post treatment was 226–295 ml, with a loss of 2 ± 1 g of Hb attributed to the INTERCEPT process (n = 12). All units had Hb values of ≥40 g ranging from 41 to 56 g. The final Hct was 56–63%, within the 50–70% criterion. After 35 days of storage, hemolysis was 0.21–0.79%, meeting the acceptance criteria of ≤0.8%. ATP values exceeded >2 μmol/g Hb, the level of ATP associated with effective RBC viability, throughout storage (**Table 1**).

Conclusions: The INTERCEPT Blood System for RBC technology was successfully validated in Ege Blood Bank. This study demonstrated that all INTERCEPT RBC units met the EDQM guidelines (18th Ed.) for leukocyte depleted RBCs in additive solution with respect to Hct, Hb content and hemolysis at end of storage. All measured *in vitro* parameters of INTERCEPT treated RBCs indicate suitability for transfusion. INTERCEPT Blood System for Red Blood Cells is not approved for use.

Transfusion Transmitted Infections (TTI): Screening Strategies for TTI

P-219

NUCLEIC ACID TESTING IMPLEMENTATION IN JAKARTA BLOOD TRANSFUSION SERVICE TO REDUCE TRANSFUSION TRANSMITTED INFECTION

NK Ritchie, N Nurdiyati, U Bachrussani and S Salim

Jakarta Blood Transfusion Service, DKI Jakarta, Indonesia

Background: Nucleic acid testing (NAT) is a molecular technique for blood screening donation to reduce the risk of Transfusion Transmitted Infection (TTI) to the patients, thus providing an additional layer of blood safety. In 2005 Soedarmono reported that Indonesian central blood transfusion service found there was 0.64% sero negative was NAT reactive. In 2007 NAT from PROCLEIX® ULTRIO® Plus Assay was introduced to Jakarta blood transfusion service. Regarding the high cost of the NAT testing, only private hospital were committed to use blood screened by NAT for their patients. Therefore in five years, NAT utilization in Indonesia was slightly increased from 0.74% in 2007 to 1.52% in 2011.

Aim: The aim of this study is to show that Indonesian government supports are crucial to improve blood safety and reduce the TTI by using NAT in Jakarta Blood Transfusion Service.

Method:: NAT testing was conducted in Jakarta blood transfusion service from 2007 until 2015. In this descriptive study, there were 9926 blood samples screened by NAT and serology test in 2007–2011. In 2012–2015 there were 501,148 blood samples screened by NAT, serology and discriminatory assay for reactive result confirmation in NAT.

Result: The risk of TTI was quite high in multi transfusion patients. When we found some thalassemia patients had infection from blood transfusion. The pediatricians and hematologists realized that there should be an additional screening method applied in BTS to increase the safety of blood and to reduce the TTI. They did some hearings to the Indonesian Ministry of Health (MoH). In Jakarta, the sero negative but NAT reactive 0.56% in 2007–2011.

Eventually at the end of 2011, NAT testing for thalassemia patients, pregnant woman, neonates and other multi transfusion patients such as hemophilia, cancer and chronic kidney failure were allocated by Indonesian Ministry of Health (MoH). In 2012 there were 30,536 from 303,630 donations (10.1% of blood donations) in Jakarta were tested by NAT, and increasing in 2013, there were 52,783 from 321,925 donations (16.4%).

Not just the MoH, Jakarta Blood Transfusion Service also approached the Jakarta Regional Government. Through sosialization about the risk of the TTI to the patients and its impact to sosio-economic and health budgeting in Jakarta province, we got NAT reagents in 2014 from Jakarta Regional Government. There were 26.95% of blood donations (87,913 from 326,228 donations) were screened by NAT method in 2014.

In 2014, the discriminatory results of NAT reactive were 53.63% Hepatitis B, 12.62% Hepatitis C, 9.78% HIV and 22.08% non-reactive or non-discriminating which mostly anti-HBc positive. These data suggests that the risk of Hepatitis B infection is very high among the donors and could be transferred to patients if no proper regulation for screening TTI in blood donors set up in Jakarta province.

At the end, in 2015 Jakarta Regional Government announce that all blood donors in Jakarta should be tested using NAT and serology test for patient safety transfusion and the government will subsidize the charge of NAT testing.

Conclusion: The government supports are crucial to improve blood safety and reduce the TTI by using NAT in Jakarta. This is shown by the increasing amount of total blood screened by NAT from 0.74% in 2007 to become 26.95% in 2014 and 100% in 2015.

P-220

NAT SCREENING AND THE CHALLENGE OF VOLUNTARY BLOOD DONORS IN EGYPTIAN NATIONAL BLOOD TRANSFUSION SERVICES

D Ekram Shabaan

Egyptian National Blood Transfusion Services, Cairo, Egypt

Background: The Egyptian National Blood Transfusion Services consists of the National Blood Transfusion Center (NBTC), the headquarters of the services, and 27 Regional Blood Transfusion Centers (RBTC) spreading over Egyptian governorates.

A well-established donor program is in place in the National Services following the National Donor Selection Criteria, in addition to a quality assured serology screening program for all donations against Transfusion Transmitted Infections (TTIs). The National Testing strategy include routine screening for HBsAg, HCV-Ab, HIV Ag-Ab and Syphilis Ab validated ELISA assays.

While 100% of the donations in some RBTCs together with the headquarters, the NBTC, are voluntary donations of which 90% are first time donors, donations in some RBTCs within the services remain with accountable percentage of family replacement.

Routine NAT screening was applied to all donations in the NBTC since 2008. Later it was implemented in more than one RBTC within the services. The RBTC in Tanta being one of these, applied NAT screening to all donations since October 2014.

Aim: To compare the number of NAT yield samples in the NBTC (100% voluntary donations) with number of NAT yield samples in Tanta RBTC where almost 20% of the donations are replacement donations during the year of 2015.

Materials and Methods: ID-NAT screening in NBTC and in Tanta RBTC is performed routinely for all donations using a multiplex assay which target multiple viral nucleic acid simultaneously. NAT yield samples are samples that show initial reactivity in NAT screening while non-reactive by routine serology screening. Following the National Screening Algorithm, a discriminatory test is performed later on to identify the exact viral infection.

Both Centers apply the National Donor Selection Criteria on all blood donors.

A monthly statistical record is performed by the screening lab in each blood center that keeps record of the number of NAT yield samples on monthly basis.

Results: By reviewing the monthly statistical records of the year 2015 for both NBTC (100% voluntary donations) and Tanta RBTC (20%replacement donations):

Total samples that were screened in NBTC from January 2015 till December 2015 were 55676samples. The number of NAT yield samples were 32 samples (0.05% approx.).

Total Number of samples that were screened In Tanta RBTC during the same period were 34552 samples. The number of NAT yield samples were 25 samples (0.1% approx.).

Conclusion: NAT screening is important to enhance blood safety, especially in countries with high prevalence of infection. It adds a layer of safety above a quality assured serology screening using validated assays and following national standards. However, collecting blood from safe donors is of the same importance if not more.The implementation of a secure voluntary donor program is the first line of defense against TTIs. More efforts should be exerted by all governments in developing countries to support implementation of a recruitment and educational program nationwide to help achieve voluntary regular blood donors to add up to blood safety and reduce resources waste.

Further research should be done to determine the exact number of NAT yield samples in family replacement donations compared to Voluntary donations in the same blood center.

P-221

TRANSFUSION OF NAT UNTESTED BLOOD PRODUCTS- DATA FROM A DEVELOPING CONTRY

S. Ali, F. Karim and B Moiz

Aga Khan University Hospital, Karachi, Pakistan

Background: Blood transfusion is a life-saving therapeutic option in many conditions. However, blood transfusion is never safe and is associated with risks of transfusion transmitted infections (TTI). To minimize the risk of TTI, all blood donations are screened for infectious diseases as per W.H.O standard. In emergency situation where blood products are required urgently and screened products are unavailable, it may be necessary to transfuse unscreened or partially screened blood products. Some of the indications where use of such products can be justified include: Critical Laboratory Results (defined as Hb < 7gm/dl and platelets $<20 \times 10^9$/l), active bleeding, emergency surgical/ radiological intervention and sepsis. Irrational transfusion of unscreened blood puts a patient at risk of acquiring an infectious disease which can cause severe long term complications. So it is very important they must only be transfused in dire need when screened products are not available and transfusion is the only life-saving option. In our hospital all products are serologically tested and then all sero-negative samples undergo molecular testing known as Nucleic acid testing (NAT). The rate and indications of dispensing NAT untested blood products in our hospital is unknown.

Aim: The aim of this study is to recognize the indications for transfusing NAT untested blood products and assess whether these are rational or not.

Methods: This was a clinical audit conducted by Blood Bank, Section of Haematology, Department of Pathology and Laboratory Medicine, The Aga Khan University Hospital. The Study duration was from May-July 2015. All the request forms received to Blood bank requesting release of NAT untested blood products were reviewed. Additional information regarding the need and indication was extracted from the online integrated laboratory data and medical record chart of patients. Data was recorded on a predesigned proforma. Statistical package for social sciences version 19 was used for data analysis.

Results: During the study period, 254 requisition slips were received to blood bank for 234 patients. Six hundred and seventy two NAT untested blood products were released during study period. The products released included: 154 packed red cell, 493 random donor platelets and 25 whole blood units. Most of the cases where blood bank was not able to provide screened blood were from Section of Clinical Haematology (n = 61, 24%) followed by General Surgery (n = 46, 18%) and Internal Medicine (n = 40, 16%). The location of patients was Emergency room (n = 58, 23%), Operating room (n = 46, 18%), Wards (n = 45, 18%) and special care units (n = 28, 11%).

In 224 out of 254 (88.2%) requests, the indications for transfusion were rational. The most common indication for the release of NAT untested products was Critical Laboratory results (n = 106, 42%) followed by Surgical/Radiological Intervention (n = 65, 26%) active bleeding (n = 53, 21%) and sepsis (n = 12, 5%). Seven percent (18/254) of the requests were not fulfilling any of the indication and transfusion in these cases was not justified. Majority of these requests (n = 14, 77.8%) were from oncology day care transfusion area.

Conclusion: Majority of the indications for transfusion of NAT untested products were rational (93%). Few cases of irrational transfusion of NAT untested products were identified. The audit identified the areas in the hospital which need re-inforcement on the rational use of NAT untested blood products.

P-222

THE EFFECT OF STORE TEMPERATURE AND TIME OF WHOLE BLOOD SAMPLES BEFORE CENTRIFUGATION ON THE CMV NAT RESULTS

H Bi

Wuhan Blood Center, Wuhan, China

Background: For immunocompromised patients, blood screening for cytomegalovirus (CMV) is very important and necessary. Nucleic acid amplification test (NAT) is potential the best methods for blood screening for CMV, because the sensitivity of NAT is very high and can shorten the window period of serological methods. However, as blood screening for CMV by NAT has not been implement in the routine blood screening programs in China, the quality control of blood screening for CMV by NAT should be studied and established.

Aims: To investigate the effect of store temperature and time of whole blood samples before centrifugation on the CMV NAT results.

Methods: The whole blood samples were collected and divided into high, medium and low virus copies groups. Once the samples were collected, they were stored at 4°C, 25°C and 37°C, for 0 h, 1 h, 6 h, 12 h, 24 h, 48 h and 72 h before their centrifugation, and then were tested by real time fluorescence quantitative PCR.

Results: In the high copies group at 4°C, no significant change was found from 0 h to 72 h. In the high copies groups at 25°C and 37°C, no significant change was found from 0 h to 24 h. In the medium copies group at 4°C, no significant change was found from 0 h to 24 h. In the medium copies groups at 25°C and 37°C, no

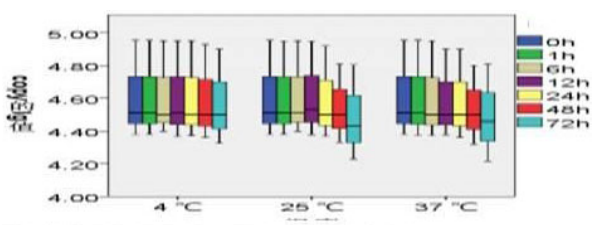


Fig.1 The effect of store temperature and time of whole blood samples before centrifugation on the CMV NAT results in high virus copies group

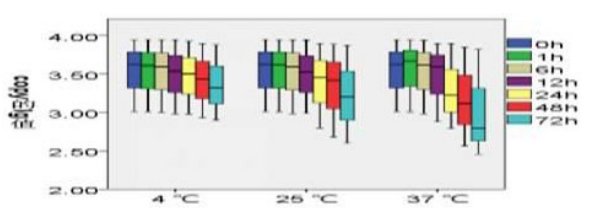


Fig.2 The effect of store temperature and time of whole blood samples before centrifugation on the CMV NAT results in medium virus copies group

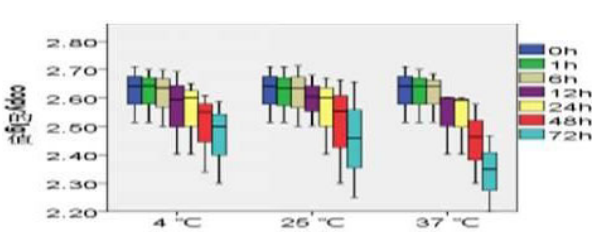


Fig.3 The effect of store temperature and time of whole blood samples before centrifugation on the CMV NAT results in low virus copies group

Table 1. The effect of store temperature and time of whole blood samples before centrifugation on the CMV NAT results ($\bar{x} \pm s$, lg copies/mL)

group	0h	1h	6h	12h	24h	48h	72h
high virus copies group (n=3)							
4℃	4.615 (0.301)	4.615 (0.301)	4.617 (0.293)	4.611 (0.304)	4.610 (0.304)	4.597 (0.296)	4.575 (0.294)
25℃	4.615 (0.301)	4.614 (0.30)	4.621 (0.292)	4.618 (0.295)	4.600 (0.288)	4.547 (0.240)	4.488 (0.291)
37℃	4.615 (0.301)	4.613 (0.304)	4.608 (0.304)	4.592 (0.273)	4.587 (0.280)	4.54 (0.242)	4.496 (0.300)
medium virus copies group(n=3)							
4℃	3.530 (0.478)	3.524 (0.476)	3.517 (0.481)	3.4905 (0.485)	3.470 (0.475)	3.421 (0.484	3.371 (0.494
25℃	3.530 (0.478)	3.529 (0.477)	3.512 (0.489	3.489 (0.480)	3.384 (0.553	3.331 (0.610)	3.225 (0.638)
37℃	3.530 (0.478)	3.545 (0.483)	3.515 (0.487)	3.460 (0.524)	3.304 (0.559)	3.178 (0.645)	3.027 (0.712)
low virus copies group(n=3)							
4℃	2.622 (0.100)	2.619 (0.951)	2.605 (0.996)	2.563 (0.148)	2.551 (0.131)	2.499 (0.141	2.462 (0.147)
25℃	2.622 (0.100)	2.619 (0.98)	2.602 (0.097)	2.596 (0.089)	2.558 (0.139)	2.506 (0.186)	2.455 (0.203)
37℃	2.622 (0.100)	2.618 (0.072)	2.614 (0.072)	2.535 (0.115)	2.532 (0.111)	2.447 (0.140)	2.339 (0.134)

significant change was found from 0 h to 12 h. In the low copies groups at 4°C and 25°C, no significant change were found from 0 h to 12 h. In low copies group at 37°C, no significant change was found from 0 h to 6 h.

Conclusions: In order to ensure the accuracy of the results of CMV NAT, the samples should best be stored at 4°C and centrifuged within 6 h after blood collection, especially under the condition of high temperature.

P-223

EVALUATION OF NUCLEIC ACID TESTING (NAT) FOR THE DETECTION OF HBV, HCV AND HIV FOR BLOOD DONORS

P Le Nhat Minh[1], H Nguyen[2], B Tran[1] and S Nguyen[1]

[1]Cho Ray Hospital, Ho Chi Minh, Vietnam [2]Ho Chi Minh University of Technology, Ho Chi Minh, Vietnam

Background: Blood transfusions are commonly given to patients with injuries, surgeries, bleedings or chronic diseases. To ensure the safety of blood transfusion, many strategies are implemented. To detect Transfusion Transmitted Infections (TTIs) such as Hepatitis B (HBV), Hepatitis C (HCV) and Human Immunodeficiency virus (HIV), serology methods including ELISA and CMIA are used. However, these methods cannot detect virus infections in window period due to low viral loads. Nucleic Acid Testing (NAT) is a technology that allows highly sensitive and specific detection of virus RNA or DNA in samples with low viral load and therefore shortens the window period and ensures safe blood transfusions.

Aim: We evaluated the effects of applying NAT to screen HBV, HCV and HIV for blood donors at Cho Ray Blood Transfusion Center (Cho Ray BTC) of the Cho Ray Hospital in Ho Chi Minh City, Vietnam.

Methods: We analyzed 27,270 samples from blood donations in 2015. First, all samples were screened using the classical serology methods for HBV, HCV and HIV. The positive samples were recorded and identified. Subsequently, the negative samples were tested by NAT using HBV-DNA, HCV-RNA and HIV-RNA.

Results: Out of 27,270 blood samples that were screened by serology methods, 650 were tested positive. The percentages for HBV, HCV and HIV infections were 1.58%, 0.50%, 0.31%, respectively. The remaining 26,620 negative samples were tested by NAT. There were 34 positive samples detected with HBV-DNA, 1 positive sample identified with HIV-RNA and no case was found by HCV-RNA testing. Amongst the 34 HBV-DNA positive cases that were followed up, we found 32 cases with occult HBV infection. The first HIV-RNA detected donor by NAT was identified in the window period.

Conclusion: NAT is a sensitive screening method to detect low viral load and shorten the window period of the virus infection to ensure the safety of blood transfusions.

P-224

DETECTION OF OCCULT HEPATITIS B INFECTION IN BLOOD DONORS BY NUCLEIC ACID AMPLIFICATION TEST (NAT)

S van der Allanki

lobal Hospital, Hyderabad, India

Background: Nucleic acid amplification technology(NAT) has the potential to detect viremia earlier than the current screening methods for blood donors, based on serological assays. Serological screening however may miss infectious donations during the serological window period donations and occult hepatitis B virus infections(OBI). The detection of hepatitis B virus(HBV) in blood donors is achieved by screening for hepatitis B surface antigen(HBsAg), antibodies against hepatitis B core antigen(anti-HBc), followed by NAT for detecting HBV DNA along with human immunodeficiency virus(HIV) RNA and hepatitis C virus(HCV) RNA in a single multiplex assay. OBI is the presence of circulating HBV DNA and anti-HBc in the absence of detectable HBsAg.

Methods: We performed NAT using the cobas Taqscreen MPX test, version 2.0, on the Roche cobas s 201 system. The MPX v2.0 test, is a qualitative multiplex, multi-dye test that enables the simultaneous detection and discrimination of HIV RNA-1 and 2, HCV RNA, HBV DNA and an internal control in a single assay. A total of 19,352 seronegative blood donations were tested in pools of 6 from May 2012 to April 2016. Serological testing was performed for HBsAg, anti-HCV, and anti-HIV by a chemiluminiscence immunoassay(ChLIA), on the Vitros EciQ(Ortho-Clinical Diagnostics, USA). Anti-HBs testing was done for those donations which were anti-HBc seropositive by ChLIA.

Results: We identified 13 NAT-yield donations, which were positive for HBV DNA(1 in 1500 donations). Of the 13 HBV positive donations, 8 donations were positive for anti-HBc and negative for anti-HBS, and the remaining 5 donations were negative for anti-HBc. We did not find any reactivity for HIV or HCV RNA among the blood donors in our cohort.

Conclusion: Among the 13 NAT yield donations, 8 donations were characterized as OBI and the remaining 5 donations were possibly window period cases. However, the window period cases would require confirmation by additional follow up testing. Several recent studies have shown that blood products from donors with occult HBV infection may also result in transfusion-transmitted HBV. Hence, NAT has helped detect potentially infectious HBV donations during the serological window period and prevent the potential risk of transfusion transmitted HBV. As such, NAT is an important and additional layer of blood safety that needs to be added to serological screening routinely.

P-225

AN OBSERVATIONAL STUDY OF CENTRALIZED ID NAT TESTING FOR TTIS USING CONSOLIDATION MODEL TO IMPROVE BLOOD SAFETY IN KARNATAKA, INDIA

A Mathur, S Dontula and L Jagannathan

Rotary TTK Blood Bank, Bangalore Medical Services Trust, Bangalore, India

Background: Blood safety is a challenging task in India; with population of 1.3 billion, India has around 2700 Blood Banks approximately 8 million blood units are collected, blood transfusion service is highly fragmented and poses unique challenges and high prevalence rate of HIV (0.29%) HBV (2–8%) HCV (≈ 2%) in donor population. Every step towards blood safety is important including voluntary blood donation, stringent testing & quality management. NAT shortens window period, thereby offering blood centres much higher sensitivity for detecting viral infections. First ID NAT screening was in India in Delhi in year 2007. Government of Karnataka become the first State Government to implement ID NAT in 2011. Karnataka state has a total of 176 Blood Banks, with a total collection of around 650000 units annually. From January 2014 all units under department of Health & Family Welfare services are tested at NAT Lab established at Bowring & Lady Curzon Hospital, Bangalore and from September 2014, Rotary TTK Blood Bank which is a standalone Regional Blood Transfusion Centre was included in the State Government project.

Aim: Aim of study to demonstrate consolidation of blood transfusion service through a centralised testing centre for NAT manned by experts in transfusion medicine, coupled with an effective transport system that collects samples from 36 centres spread across the State and also to assess safety benefits of implementing IDNAT.

Material & methods: Rotary TTK Blood Bank, collects nearly 40000 units annually from voluntary donors with 30% repeat donations. The donors undergo strict Predonation counselling, donor questionnaire & medical examination. The units collected are tested for HIV, HBV & HCV by Enhanced Chemiluminescence test by Ortho Clinical Diagnostics using VItros 3600. From Sept 2014 all the units are tested

by NAT at central NAT Lab, screened by Procleix® Panther System by Grifols using Procleix® Ultrio Elite Assay kits.
Results: From September 2014 to March 2016, total 50903 samples tested for NAT. Out of 50903 samples, 588 samples (1.15%) were reactive by Chemiluminescence test including 265 for HBV, 188 for HCV & 135 for HIV. Total NAT reactive samples were 254, out of this 11 reactive for HIV-1 (0.02%), 2 reactive for HCV (0.003%), 235 (0.46%) reactive for HBV. There was one HIV and 10 HBV infection cases that were not detected by serology but reactive by NAT. The yield detected is 0.021% or one in 5000. This yield was found lower than other blood centres participated in the project where it was detected one in 300–500 donations.
Conclusion: The ID NAT Consolidation project has helped in preventing window period infections thus eliminating the treatment cost and burden on healthcare.
It has added benefits in blood safety & should be considered along with the basic quality assured blood transfusion system such as volunteer base for blood donation, provision of donor self-deferral, donor notification and quality assured sensitive serological methods.

P-226

HEPATITIS B WINDOW PERIOD INFECTION IN BLOOD DONOR DETECTED BY ID-NAT TESTING: CASE REPORT

N Jurakovic-Loncar, H Safic, I Babic, V Dogic, J Bingulac-Popovic, M Stojic Vidovic, M Miletic Lovric, I Batarilo, M Strauss-Patko and I Jukic

Croatian Institute of Transfusion Medicine, Zagreb, Croatia

Background: Transfusion safety has undergone last two decades significant improvement and many measures have been introduced to achieve near the "zero risk"goal in blood safety. The significant improvement was implementation of nucleic acid testing (NAT) in blood screening worldwide. The main advantages of NAT screening are detection of window period (WP) of infections and identification of occult hepatitis B infections, offering blood centers higher sensitivity in detection of transfusion transmitted infections (TTI). Croatia implemented individual donation NAT testing in March 2013 as a routine blood screening program, concordantly with serological screening. ID-NAT screening was introduced after reorganization of blood transfusion service in whole country and is centralized on one site in CITM.
Aims: We found 2 WP infections during three years after NAT implementation. One WP was HIV-1 infection two years ago and second is WP HBV infection presented in this case report.
Methods: From 2013 to 2016 a total of 573.365 donations from voluntary blood donors in Croatia were collected and screened for HBV, HCV and HIV-1 using ID-NAT, by a multiplex transcription-mediated amplification, TMA test on Procleix Tigris System (Grifols, Spain). Donations that were repeatedly reactive were submitted to additional discriminatory assay to resolve which viral genome was present, and then the confirmatory PCR testing was done. All initial reactive donations were submitted to anti-HBc testing. For HBsAg donations were screened by Abbott Prism HBsAg test (Abbott, USA). HBV-DNA viral load was detected by COBAS AmpliPrep/ COBAS TaqMan HBV test, V2.0 (Roche Diagnostics, Germany). The donors were recalled for follow-up studies and the collection of clinical and epidemiologic data.
Results: By a routine ID-NAT checkup of a 30-years old repeat donor (16 donations) we found positive ID-NAT triplex screening test with negative all serological screening markers (HBsAg (S/CO 0,66), HIV Ag/Ab, anti-HCV i anti-TP. Once the NAT was repeatedly reactive three times, the discriminatory assay was performed and found to be dHBV positive while all HBV serological markers were negative. Viral DNA was confirmed and determined load as 1.11E+2 IU/ml. In the follow up sample taken 14 days after the index donation, we determined the increasing HBV viral load 1.06E+03 IU/ml, and this time also positive HBsAg with S/CO of 4,01 and all other HBV markers (anti-HBs, anti-HBc IgG/IgM, HBeAg and anti-HBe) negative. Donor confirmed risk sexual behavior 1 month before donation.
Summary/conclusion: ID-NAT offered significant early window period closure and prevented a moderate number of residual HBV transmissions previously not detected by HBsAg assays. HBV still remains the most frequent TTI in many countries, as well in Croatia. The infection risk is mainly related to HBsAg negative units collected in WP or during late stage of infection and highly sensitive ID-NAT could detect both. The case report shows successful prevention of the possible HBV transmission by ID-NAT screening.

P-227

VALIDATION OF NON-REACTIVE BLOOD UNITS BY IMMUNOASSAYS USING NUCLEIC ACID TESTING

KA Punzalan[1], E Arcellana-Nuqui[2], RT Yu[1], BM de Vera[1] and IMSC Chamen[1]

[1]*Research Institute for Tropical Medicine, Muntinlupa City, Philippines* [2]*The Medical City, Ortigas, Pasig City, Philippines*

Background: Blood service facilities in the Philippines are limited to the use of serological tests (Chemiluminescence [ChLIA] and Enzyme Immunoassay [EIA]) for blood screening. Due to limited fundings by the government, the addition of nucleic acid testing (NAT) is unfeasible. Lam et. al. in 2010 found that a portion of non-reactive blood units were found to be reactive by NAT. Chigurupati et. al. concluded in their study that NAT could detect HIV, HBV and HCV cases in blood donor samples that were undetected in serological tests. Although constant efforts are exerted to eliminate possible transfusion of infected blood products, a certain level of risk threatens blood safety in the Philippines.
Aims: To determine the capabilities of the blood service facility (BSF) to accurately detect HIV, HBV and HCV by comparing their results with the re-testing done by the TTI-NRL using EIA and NAT; And assess the need to include NAT in the screening algorithm of the BSF in the Philippine setting.
Methods: The Department of Health identified lead blood service facilities (BSF) (*those that perform recruitment, blood collection and testing and supplies blood for a designated region*) to participate in this study and were instructed to send 5% of their total monthly collections of non-reactive samples for a period of six months.
All submitted samples were tested by the TTI-NRL in an automated NAT donor screening system in pools of 6. NAT negative results were reported while those with reactive status were resolved by retesting in pools of 1 to identify the specific reactivity and results were reported. These were further tested using a semi-automated EIA system and negative results were reported while those with reactive status were retested in duplicate using modular method.
Results: Out of 3233 referred samples that were screened non-reactive by the BSF, 63 (1.95%) were found reactive to Hepatitis B by the TTI-NRL, 12 (4.00%) of which were reactive to EIA and NAT, 48 (1.48%) were reactive to NAT only and 3 (0.09%) were found to be reactive to EIA.
Summary/Conclusions: Hepatitis B was the only TTI found to be discrepant in the study. This may be attributed to the possibility of occult blood infections (Seo et. al). Discrepancies in the result of the BSF and the NRL may also imply that quality systems in the BSF must be checked and validated through continuous monitoring of these sites.
Currently, DOH recommended assays for the screening of blood in the Philippines include at least a highly sensitive immunoassay (ChLIA/EIA) that tests for both antigen and antibody for HIV and HCV. The recommendation for HBV is the use of highly sensitive ChLIA/EIA that detects Hepatitis B Surface Antigen (HBsAg). NAT has been scientifically proven to reduce diagnostic window periods, and significantly decrease the residual risk of transfusion infection and based on the results of this study, NAT may be considered for routine blood screening especially in the prevalence of high TTI. However, inclusion of NAT should entail a cost-benefit analysis. In cases of limited resources, inclusion of additional tests for screening may be done (<i*e. inclusion of testing for Hepatitis B Core Antibodies to catch occult blood infections*).

	NAT (+)	NAT (-)	TOTAL
EIA (+)	12 (4.00%)	3 (0.09%)	15 (0.46%)
EIA (-)	48 (1.48%)	3170 (98.50%)	3218 (99.54%)
TOTAL	60 (1.85%)	3173 (98.14)	3233 (100%)

Caption 1: HBV testing results.

P-228

THE ROLE OF DONOR ID-NAT IN ENHANCING BLOOD SAFETY IN THE EGYPTIAN NATIONAL CANCER INSTITUTE

M Fouda[1], R Abou ElFetouh[1] and F Moftah[2]

[1]National Cancer Institute, Cairo University, Cairo, Egypt [2]Dar Elfouad Hospital, Giza, Egypt

Background: Blood components can be a lifesaving treatment in oncology, hematology, surgery and in emergency conditions, which is a major challenge for blood transfusion services to supply safe blood products. Reducing the risk of transfusion-transmitted infections to the lowest possible level, has been the main aim of transfusion medicine practice. Serological screening improved blood safety, but, with longer diagnostic window period than nucleic acid amplification tests (NAT) which narrows the infective window period of HIV, HBV and HCV infections, so, the risk of transfusion transmitted infections (TTIs) is reduced for safer blood transfusion. Nucleic acid testing (NAT) being a highly sensitive and specific molecular technique, is based on amplification of targeted regions of viral ribonucleic acid (RNA) or deoxyribonucleic acid (DNA). It is used for screening donated blood units, reducing the risk of transfusion transmitted infections (TTIs) in the recipients and providing an additional layer of blood safety. NAT also could be used for confirming false positive reaction given by the serological methods especially for donor notification and counseling.

Aim of the Work: The objective was to assess the role of individual donor-NAT (ID-NAT) for human immunodeficiency virus-1 (HIV-1), hepatitis C virus (HCV) and hepatitis B virus (HBV) and its role in reducing transfusion transmitted infections and blood safety at the National Cancer Institute.

Method: Our study was conducted at the National cancer institute Blood Bank, Cairo University from the 1st of July 2011 to the 31st of December 2015. *80840* donated blood units were examined serologically for TTIs (HCV, HBV, HIV), and *77977* (96.5%) blood units tested negative. To increase the safety of these units, ID-NAT was performed using Ultrio Plus assay.

Results: Serological examination showed a higher rate of HCV reactive units (*1877; 2.32%*) than HBV reactive units (693; 0.86%), and 3 unit were HIV reactive (0.0037%). However, ID-NAT yield on EIA negative samples showed a higher rate of positive HBV NAT units (56 units; *0.07%* of negative EIA units), than HCV NAT positive units (23 units; *0.03%* of negative EIA units) and all blood units were HIV-1 RNA negative, with a decrease in the rate of infected donors during the study period due to better donor selection.

Conclusion: Our data clearly indicates the benefit of ID- NAT donor screening for the improvement of transfusion safety; as the implementation of molecular methods for HBV DNA & HCV-RNA detection in the blood center on a routine basis contributed significantly to improving the viral safety of the blood supply. Testing for the three viruses using the ID-NAT prevented the transfusion of 79 of potentially infectious blood units [0.101%] to our immuno- suppressed patients.

Due to its high sensitivity and specificity, NAT efficiently complements serologic testing in detecting pre-seroconversion window period infections, or infections with immunovariant viruses. ID-NAT implementation as a routine donor screening, contributed to safer blood transfusion by reducing the risk of transmitting HCV and HBV infectious units to our immuno-suppressed cancer patients.

Year	ELISA negative units	HCV NAT positive units	HBV NAT positive units	HIV-1 NAT positive units	NAT Yield/No. of units
2011	9 122	8 (0.88%)	10 (0.11%)	Nil	1/507
2012	19 274	5 (0.03%)	16 (0.08%)	Nil	1/918
2013	17 373	4 (0.02%)	14 (0.08%)	Nil	1/965
2014	15 056	3 (0.02%)	12 (0.08%)	Nil	1/1075
2015	17152	3 (0.02%)	4 (0.02%)	Nil	1/2450
Total	77977	23(0.03%)	56(0.07%)	Nil	1/987

Caption 1: ID-NAT Reactive blood units in the National cancer Institute

Year	No of blood donations	HCV reactive units	HBV reactive units	HIV reactive units
2011	9 630	391 (4.06%)	117 (1.21%)	Nil
2012	19 990	523 (2.62%)	193 (0.97%)	Nil
2013	17 902	379 (2.12%)	149 (0.83%)	1(0.0056%)
2014	15 475	290 (1.87%)	128 (0.83%)	Nil
2015	17843	294 (1.65%)	106 (0.59%)	2(0.011%)
Total	80840	1877(2.32%)	693 (0.86%)	3(0.0037%)

Caption 2: EIA Reactive blood units in the National Cancer Institute

P-229

EVALUATION OF PROCLEIX ULTRIO ELITE INDIVIDUAL DONOR NUCLEIC ACID TEST (ID-NAT) ASSAY FOR BLOOD DONOR SCREENING BY AUTOMATED PROCLEIX PANTHER SYSTEM

R Kumar and A Kaur

Dayanand Medical College and Hospital, Ludhiana, India

Background: The early dynamics of viremia and infectious window period of the human immunodeficiency (HIV), hepatitis C and B viruses differ considerably and affect risk of viral transmission by transfusion of blood and plasma products. Introduction of Individual donor nucleic acid testing (ID-NAT) has helped in decreasing window period donations, resulting in increased safety of blood supplies. It combines the advantages of direct and highly sequence-specific detection of viral genomes with an increased analytic sensitivity than that of antigen/antibody/antigen-antibody detection techniques or viral isolation methods.

Aims: The objective of this study is to assess fully Automated Panther System for ID-NAT blood screening and to determine the ID-NAT yield rate among blood donors.

Methods: A total of 39780 donations were tested for HIV 1&2 RNA, HCV RNA and HBV DNA by Procleix Ultrio Elite Assay (Grifols, Emerycille CA USA on Procleix Panther System from Oct 2014 to March 2016 using individual Donor NAT and electrochemiluminescence immunoassay, "ECLIA"(Cobas e, Roche, Germany). All initial ID-NAT reactive samples and serology non-reactive were retested in triplicate and discriminatory assay for HIV - 1 & 2, HCV and HBV were performed.

Results: Among these 748 ID-NAT initial reactive samples, 657 (87.83%) samples were concordant positive with routine serology testing. There were 66 samples (0.16%) identified as ID-NAT initial reactive but serology Non-Reactive. Among these 66 samples, 26 were HBV, 28 were HCV and 12 samples could not be discriminated. There was no case of HIV ID-NAT Reactive but Serology Non-reactive, during this period. Our confirmed ID-NAT yield (NAT repeat reactive and discriminated but serology Non-Reactive) Number is 54 (1 in every 738 samples tested). There were additional 25 (0.06%) samples which were NAT initial reactive but all repeat testing was non- reactive, probably due to very low viral load.

Summary/Conclusions: This study showed that improved ID-NAT testing for HIV - 1 & 2, HBV and HCV can tremendously improve the efficacy of screening by increasing sensitivity. It is superior to serological immunoassays for screening of viral markers for protecting blood recipient from TTIs. It enables the detection of these viruses that were undetected by serological test and thus help in providing safe blood to the patients. In the vast majority of blood unit tested the result of ECLIA and ID-NAT were concordant but in developing country like India with high

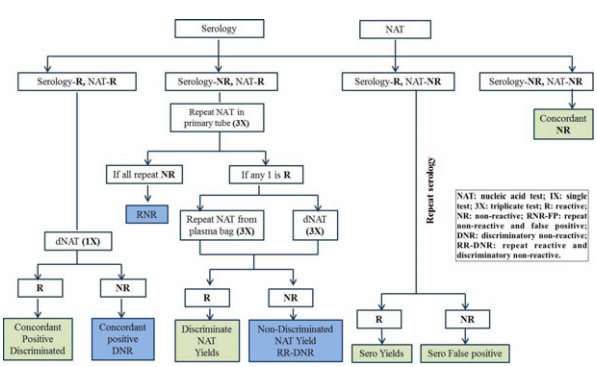


Figure 1. Algorithm used for TTI screening at our centre

	CMIA							
EIA	ANTI HIV		ANTI HCV		HBsAg		ANTI HBc	
	NON REACTIVE	REACTIVE	NON REACTIVE	REACTIVE	NEGATIVE	POSITIVE	NON REACTIVE	REACTIVE
NON REACTIVE/ NEGATIVE	1014 (99.4%)	2 (0.19%)	1008 (98.8%)	3 (0.29%)	996 (97.6%)	0 (0.0%)	906 (88.8%)	2 (0.19%)
REACTIVE/ POSITIVE	1 (0.09%)	3 (0.29%)	1 (0.09%)	8 (0.78%)	2 (0.19%)	22 (2.15%)	4 (0.39%)	108 (10.5%)
KAPPA	0.665		0.798		0.956		0.97	

Caption 1. Details of EIA and CMIA results

seroprevalence of infections, combination of at least two tests would be help us to improve transfusion safety. The introduction of ID-NAT testing in the region would add an extra layer of safety to blood supply by interdicting samples from donor with recent infections.

P-230

FIVE YEARS OF EXPERIENCE WITH ID-NAT AT A TERTIARY CARE CENTRE IN NORTH INDIA: AN INTERDICTORY STEP IN PREVENTING THE TRANSFUSION-TRANSMITTED INFECTIONS

K Chatterjee, P Coshic, R Chaurasia, D Rout, K Dogra and PP Fulzele

All India Institute of Medical Sciences, New Delhi, India

Introduction: Newer principles, improvements in ease of detection lead to better sensitivity, specificity and rapidity of screening tests for effective prevention of transfusion transmissible viral infections (TTVI) in blood donors. The society demands safe blood and as transfusion medicine specialists, it is our sole moral responsibility to provide the community with the same. However, blood safety is a daunting task in India with ~1.25 billion population and high prevalence rate of HIV (0.29%), HBV (2–8%) and HCV (2%) in donor population. Majority of the blood banks in India are using serological techniques for TTVI screening, with a few centers implementing additional Individual Nucleic Acid Amplification Test (ID-NAT) for safer blood supply.

Aim: This study aims at evaluating the impact of ID-NAT as an add-on test on safe blood supply in the current Indian scenario.

Method: A retrospective observational study was conducted to analyse the data of 5 years of add-on ID-NAT (Procleix Ultrio assay, Grifols Diagnostic Solutions Inc., Hong Kong) at Dept. of Transfusion Medicine (Main Blood Bank), AIIMS, New Delhi.

Results: Approximately 1.84% (3,556 of 193,167) units were initially NAT reactive (NAT-IR). A total of 3495 NAT-IR units were further tested, of which 79.25% were discriminated (193 HIV, 421 HCV, 2060 HBV and 96 co-infections). The remaining 13.04% (456) were repeat non-reactive (RNR), 0.71% (25) NAT-IR units were found to have at least 1 repeat reactive result, but dNAT for these were non-reactive (RR-DNR), and 6.98% (244) could not be discriminated (DNR). The overall NAT yield rates were one in 847 and the individual virus-specific NAT yield rates were one in 96,584 for HIV, one in 2683 for HCV, one in 1370 for HBV and one in 14,859 for HBV/HCV co-infections.

Conclusion: ID-NAT screening of all the collected blood donations at our facility over the last 5 years has increased the overall screening sensitivities to check the viral transmissions through transfusion and has interdicted 228 probable TTVIs to 684 (assuming 100% component separation) transfusion recipients in 5 years at our centre. Implementation of NAT along with routine serological tests for screening of the blood donations definitely improves the transfusion safety, the major improvement being the reduction in the risk of viral transmission during the window periods, thus interdicting the transmission that would have been missed by serological screening methods. However, interpretation of NAT reactive results should be adjudged cautiously keeping in mind, the chance occurrence of false-positive reactivity.

P-231

PREVALENCE OF TRANSMISSIBLE INFECTIOUS MARKERS TRANSFUSION AMONG BLOOD DONORS IN MOROCCO

A Laouina[1], R Alami[1], B Adouani[1], A Benahadi[1], S Boulahdid[1], A Tazi Mokha[1], A Mokhtari[2], A Soulaymani[2], A Abouyoub[1], A Ghazi[1], N Bouhlal[1], K Hajjout[1] and M Benajiba[1]

[1]Centre Régional de Transfusion Sanguine, Rabat, Morocco [2]Faculté des Sciences Kénitra, Kénitra, Morocco

Background: Screening for infectious agents is a very important step in the process of biological qualification of blood donations in virology. It contributes to the prevention of transmission of viral diseases following a transfusion of blood or its derivatives in the Moroccan population.

Aims: The aim of this study is to determine the prevalence of infectious markers that are a threat to transfusion safety. Thereafter make a development on current knowledge about the pathogen in our country.

Methods: This study was conducted among 57711 blood donation collected 2015 CRTS Rabat. All these blood donors were examined by a doctor before being selected to donate blood. Screening virus human immunodeficiency (HIV), hepatitis B virus (HBV) and C (HCV) was conducted by enzyme immunoassay based on the principle Elisa and hemagglutination for syphilis.

Results: The prevalence of HIV serological markers, HBS, HCV and syphilis is 0.31%, 0.8%, 0.4%, 1.5%. These results show that there's a tendency to decrease in prevalence in recent years. This is due to several factors, the establishment of a quality assurance system, the choice of the site of collection, donor selection and finally improving the quality of testing and that helped ensure blood safety optimal.

Conclusions: These results are of great importance for blood safety, also the effectiveness of the implementation of the transmission prevention of viral infections by blood transfusion in blood recipients has probably led to this goal.

P-232

COMPARISON OF ENZYME IMMUNO ASSAY AND CHEMILUMINESCENT MICROPARTICLE IMMUNO ASSAY FOR DETECTION OF TRANSFUSION TRANSMITTED VIRUSES

RN Makroo, A Bhatia and UK Thakur

Indraprastha Apollo Hospitals, New Delhi, India

Background: Transfusion transmitted viral infections have been a major concern for transfusion specialists globally for years. Advances in testing technology have effectively minimized the risk of such infections. In India, the Drug and Cosmetics Act, 1940 mandates testing of all donor units for Human Immunodeficiency Virus (HIV), Hepatitis B & C, among others. The technique used for testing is however not standardized and vary from one centre to another. While Enzyme Immunoassay (EIA) based systems have been successfully implemented in donor screening, the Chemiluminescent Microparticle Immunoassay (CMIA) have also gained popularity owing to reduced processing time and random access.

Aims: This study compares the two fully automated systems, one based on ELISA and other on CMIA technology for donor screening.

Materials and methods: A total of 1020 donor samples were tested in parallel using EIA (EVOLIS fully automated system; Biorad) and CMIA (Architect i100SR, Abbott, Wiesbaden, Germany) for anti HIV, HBsAg, anti HCV and anti HBcAb (IgG+IgM). All tests were performed and interpreted in accordance with the manufacturer's instructions. Individual donor Nucleic acid testing was also performed on Tigris fully automated system using Procleix® Ultrio® Assay (Grifols Diagnostics Solution, Formerly Novartis Diagnostics, Emeryville, CA). Kappa (κ) analysis was used to express agreement between test methods; κ-values in the interval 0.6–0.8 were classified as having high agreement, and values in the range of 0.8–1are classified as having very high agreement.

Results: The overall concordance between EIA and CMIA results was 98.5% with Kappa values higher than 0.8 for HBsAg and anti HBcAb and higher between 0.6 and 0.8 for anti HIV and anti HCV. Among the discordant samples, 8 were reactive by EIA alone, while 7 were reactive only with CMIA. The results are detailed in Table 1. ID NAT was negative in all the 15 discrepant samples.

Conclusion: This study compared the results of donor screening tests on two fully automated immunoassay systems. The results of both the assays were comparable. All discrepant results were negative by ID NAT and most likely represent false positivity. These should be interpreted with caution.

P-233

SINGLE CENTRE EXPERIENCE WITH A NEW CHEMILUMINESCENT SYSTEM FOR BLOOD-BORNE INFECTION SCREENING ON HEPATITIS B, HEPATITIS C, HIV 1/2, AND TREPONEMA PALLIDUM SEROLOGICAL MARKERS

C Drexler

Medical University Graz, Graz, Austria

Background: In Austria mandatory serological blood donor screening tests, to be performed on each donation, are HBsAg, anti-HCV, HIV Ab, and total antibodies to Treponema pallidum. In addition screening of neopterin concentrations is performed as means to detect acute infections in a non-specific way, hereby aiming to improve safety of blood transfusions. Besides serological testing, NAT testing for HBV, HCV and HIV-1 is also performed on all donations, in pools of 96 samples when serologically negative.

Aims: Aim of the study was to evaluate the performance of a new fully automated platform, DiaSorin-LIAISON® XL (DiaSorin S.p.A, Vercelli, Italy), in blood donor screening, specifically for hepatitis B surface antigen (HBsAg), hepatitis C antibodies (anti-HCV), HIV p24 antigen, HIV antibodies, and Treponema pallidum antibodies.

Methods: All serum samples from donors collected in Styria between January 4th and April 4th 2016 were assayed for HBsAg, anti-HCV, HIV Ag/Ab, and total antibodies to Treponema pallidum markers by chemiluminescent technology on the LIAISON® XL system (DiaSorin S.p.A.). NAT testing was performed by an external lab.

Results: During the study period more than 13,500 samples were collected, 92% by repeated donors, with a higher proportion of men compared to women (68% vs 32%). In the calculation of specificity all samples showing a reactive result after repetition in duplicate were counted as positive results, regardless of the following confirmation. For Treponema testing, equivocal samples were removed from the calculation, as per manufacturer's instruction for use. Final specificity ranged from 99.77% to 99.99%. Values for each marker, including the 95% of confidence interval are presented in Table 1.

On all reactive samples a confirmation test was performed: One first time donor appeared to actually have hepatitis B, the other samples were negative.

Conclusions: The donors' data, collected during the three months of study, represent a statistically significant sample of the donor population normally pertaining to the transfusion medicine department of Graz.

Data obtained demonstrate that LIAISON® XL assays showed a good level of specificity, that in the context of screening blood donors is desirable in order to prevent unnecessary loss of blood units and donor deferrals. Moreover, LIAISON® XL, being a fully automated system, is also due to its user friendly interface and relatively short hands-on-time easy in use and therefore facilitates the lab work in a blood bank setting.

Marker assayed	Total samples tested	Non reactive results	Repeated reactive results	Specificity	95% Confidence Interval
LIAISON® XL murex HCV Ab	13,550	13,531	19	99.86%	99.78% - 99.92%
LIAISON® XL murex HIV Ab/Ag HT	13,545	13,514	31	99.77%	99.67% - 99.84%
LIAISON® XL murex HBsAg Quant	13,548	13,546	2	99.99%	99.95% - 100%
LIAISON® Treponema Screen	13,519	13,502	17	99.87%	99.80% - 99.92%

Table 1. Final specificity of each marker

P-234

VALIDATION OF ICE- SYPHILIS ELISA METHOD IN UGANDA BLOOD TRANSFUSION SERVICE

G Anyang

Uganda Blood Transfusion Service, Kampala, Uganda

Background: Syphilis is a disease caused by a bacterium *Treponema pallidum.* It's one of the infections transmitted through blood transfusion and its mandatory to be screened for in all the donated blood, therefore it needs a method that is highly sensitive (≥ 99%), specific (≥98%) with high-quality operational characteristics in terms of ease of use on large number of samples with low TAT, technical complexity, inter and intra reader variability. Four assays were used in the study, these included, ICE-Syphilis ELISA (Murex), BIOLINE Syphilis (SD), CMIA-Syphilis (ABBOTT) & TPHA which was taken as the gold standard method.

Aim: To validate ICE Syphilis ELISA kit for use in Uganda Blood Transfusion Service.

Method: A laboratory based descriptive study was conducted. A total of 180 samples of known status using TPHA were used in the study, out of these samples 23, 6, 151 & 0 samples selected were true positives, false positives, True negatives & false negatives respectively. The assays were performed according to the manufactures' instructions from the test inserts.

The test methods included; TPHA, ICE-Syphilis ELISA (Murex), Bio-line Syphilis (SD) & CMIA-Syphilis (Architect).

Results: All the methods evaluated had the same sensitivity and specificity of **100**% and **96.2**% respectively as compared to the manufacturers test performance characteristic for CMIA, ≥**99**% and ≥**99**%, ICE-ELISA (MUREX), 99.95% and 100%, TPHA,100% and 99.5%, BIOLINE (SD),99.5% and 99.3% and the previous studies. CMIA, **100**% and **99.1**%, ICE-ELISA (MUREX), 98.2% and 94.9%, TPHA,88% and 95%, BIOLINE (SD),94.2% and 99.2% for sensitivity and specificity respectively.

Conclusion: In the study it was evidenced that the all screening methods evaluated are equally sensitive and specific despite the performance characteristic, ICE- ELISA method is more convenient in terms of use, volume of work, turnaround times and poses no technical complexity as compared to the other methods. Based on the overall evaluation ICE-ELISA was highly recommended for syphilis screening in a blood transfusion set up.

P-235

ANALYSIS OF TRANFUSION TRANSMISSIBLE INFECTIONS [TTIS] POSITIVE CASES AMONG VOLUNTARY BLOOD DONARS IN A REGIONAL BLOOD CENTER IN SRILANKA BETWEEN 2014 AND 2015

V Ketheeswaran, L Morawaka, TR Pathirana and N Bandara

National Blood Transfusion Services, Colombo, Sri Lanka

Background: sri Lanka has 100% voluntary blood donation system since 2013. transfusion transmitted infection is the major barrier in the blood transfusion services. screening for transfusion-transmissible infections (TTIS) is to exclude blood donations from the risk of transmitting infection from donors to recipients is a critical part of the transfusion service. Effective screening helps to avoid the presence of the most common and dangerous ttis and it reduce the risk of transmission to a very low level. Blood transfusion services should therefore establish efficient systems to ensure that all donated blood is correctly screened for specific ttis and that only non-reactive blood and blood components are released for clinical use. We are having a mandatory system of screening for syphilis (*Treponema pallidum*), HIV-1, HIV-2, hepatitis B, hepatitis C and malaria. Even though we have 100% collection of blood from voluntary non-remunerated blood donors, considerable numbers of donations are tti positive.

Aim: The aim of this study is to analyse the transfusion transmissible infections positive cases among voluntary non-remunerated blood donors.

Method: We reviewed the data of 168 TTIs positive blood donations between 2014 and 2015 from donor declaration forms and post-donation counselling data. The data of those donors analysed according to the age, sex, number of donation, type of infection, history of sexual exposure. Here we considered main four infections such as HIV, syphilis [*Treponema pallidum*], hepatitis b and hepatitis C.

Result: In this study, among the 168 donors there were 79 donors (47%), who are age between 18 and 30, 49 donors (29%) were age between 31 and 40, 37donors (22%) were age between 41 and 50 and 3 (2%) donor was age more than 50 years. among the total number of tti positive donors 114(68%) were first time donors. 54 (32%) were regular donors.

Conclusion: According to the above analysis, age between 18 and 30 years donor populations having more number of ttis positive cases. There are two major reasons for such situation such as 18–30 age group is contributing more to the voluntary blood donation and they are sexually more active age group also. TTI positive cases are more in first time donation when it is compared with regular donation. Because regular voluntary non - remunerated donors are well motivated, educated about risk behaviour and regularly screened. Now it is our responsibility to focus more on the 18–30 age groups first time donors when providing the pre donation education and the pre donation counselling. This will help to reduce the number of tti positive cases in this age group.

P-236

EVALUATION OF THE ELECSYS SYPHILIS ASSAY FOR THE DETECTION OF TREPONEMA PALLIDUM IN ROUTINE SAMPLES FROM THE CHINESE POPULATION COMPARED WITH LOCAL MARKET COMPETITORS

C Tao[1], L Wang[1], X Hao[2], W Xu[3], J Zhang[4], S Pan[5], Z Tao[6], X Li[7], J Lou[8], B Zhang[9], Y Qiu[10], Y Wu[11], Q Ou[12] and X Huang[13]

[1]West China Hospital, Sichuan University, Chengdu, China [2]Xijing Hospital, Fourth Military Medical University, Xi'an, China [3]The First Hospital of Jilin University, Changchun, China [4]Peking University Third Hospital, Beijing, China [5]Jiangsu Province Hospital, Nanjing, China [6]The Second Affiliated Hospital of Zhejiang University School of Medicine, Hangzhou, China [7]The Third People's Hospital of Kunming, Kunming, China [8]Beijing Youan Hospital, Capital Medical University, Beijing, China [9]Shandong Province Hospital, Jinan, China [10]Nanfang Hospital, Southern Medical University, Guangzhou, China [11]Fujian Provincial Hospital, Provincial Clinical College,Fujian Medical University, Fuzhou, China [12]The First Affiliated Hospital of Fujian Medical University, Fuzhou, China [13]The Second Affiliated Hospital of Guangzhou University of Chinese Medicine, Guangzhou, China

Background: The re-emergence of syphilis in recent years has become a serious threat to public health worldwide and also in China during the last two decades. Therefore, control of *Treponema pallidum* transmission and infection is a public health priority. The serologic detection of specific antibodies against *T. pallidum* remains the most reliable method for laboratory diagnosis of syphilis. Currently, the enzyme-linked immunosorbent assay (ELISA) remains one of the most popular methods used to screen for antibodies to T. pallidum in both clinical and blood bank settings in China. However, chemiluminescence-based automated assays offer advantages in terms of their high throughput and their very high sensitivity in early syphilis.

Aims: This multi-center study evaluated the performance of the newly developed, fully automated, Elecsys Syphilis electrochemiluminescence immunoassay and compared it with local market competitor assays routinely used for syphilis diagnosis in a variety of clinical samples from the Chinese population.

Methods: This study involved 13 independent laboratories in China. Besides Elecsys Syphilis assay, the competitor assays assessed were the ARCHITECT Syphilis TP assay, a chemiluminescent microparticle immunoassay (CMIA), the InTec ELISA kit for TP (InTec Products, Xiamen, China), and the KHB ELISA for TP (Shanghai Kehua Bioengineering, Shanghai, China), both of which are two-step DAGS ELISAs. All samples for the assessment of performance were left over samples. The Elecsys Syphilis assay and the competitor assays were tested in four groups of samples, which included random samples from routine diagnostic requests, archived confirmed positive syphilis samples, archived potentially cross-reacting samples and samples considered to be 'borderline' by the routine comparator assay used at the respective study center. All inconsistent and 'borderline' samples were subjected to confirmatory testing with the Mikrogen Syphilis Immunoblot test (Mikrogen recomLine Treponema IgG and IgM, Mikrogen Diagnostik, Martinsried, Germany).

Results: The specificity and sensitivity were assessed in 13,767 samples from routine diagnostic requests; 18 samples with indeterminate results according to the applied confirmation algorithm were excluded from the analysis. The sensitivity of assays was also assessed in 1003 archived confirmed positive syphilis samples. These consisted of 66 samples from patients with primary syphilis, 153 with secondary syphilis, 36 patients with tertiary syphilis, and 301 patients with latent syphilis. The remaining 447 samples were from patients with unknown syphilis stage. The specificity and sensitivity of the Elecsys Syphilis assay were >99.80% (99.81–99.93%) and 100%, respectively, and were superior to the competitor assays in each individual comparison (Table 1). When assessing 'borderline' samples, the Elecsys Syphilis assay gave considerably fewer false-positive results, and zero false-negative results, compared with the competitor assays. The 858 archived potentially cross-reacting samples, of which 188 were confirmed as syphilis positive, included samples from patients with HIV, HBV, HCV, EBV, multiple myeloma, lymphoma, SLE, rheumatoid arthritis, autoimmune disease, tumors, and pregnant women. There were only seven false-positive results and one false-negative in total.

Conclusions: Considering the ease-of-use and automation, high throughput, and high sensitivity and specificity, the Elecsys Syphilis assay represents an excellent choice for the screening of syphilis.

	Elecsys	Architect	InTec ELISA	KHB ELISA
Sensitivity	100%	98.26%		
Specificity	99.81	99.74		
Sensitivity	100%		99.11%	
Specificity	99.93%		99.80%	
Sensitivity	100%			98.56%
Specificity	99.85			99.77%

Caption 1. Specificity and sensitivity of Elecsys Syphilis assay compared with competitor assays

P-237

SCREENING ALGORITHMS FOR SYPHILIS IN DONOR BLOOD

C Politis[1], L Kavallierou[2], M Parara[1], C Richardson[3] and M Asariotou[2]

[1]Hellenic CDC, Athens, Greece [2]Hellenic Coordinating Haemovigilance Centre Hellenic CDC, Athens, Greece [3]Panteion University department of Statistics, Athens, Greece

Introduction: Nowadays, syphilis seems to be a re-emerging disease and a public health problem worldwide. Transfusion-transmitted syphilis is one of the oldest recognized infectious risks of blood transfusion. Serological screening tests are still considered the most useful approach for the diagnosis. Therefore, the traditional approach to the diagnosis of syphilis begins with a non-treponemal assay, either Venereal disease research laboratory (VDRL) or Rapid plasma reagin (RPR). Since these antibodies are not specific for syphilis, reactive non-treponemal tests must be confirmed with a specific, confirmatory assay *such as* the fluorescent treponemal antibody absorption test (*FTA) or* treponema pallidum hemagglutination assay (TPHA/MHATP/TPPA). The reverse algorithm starts with treponemal tests such as Enzyme immunoassays (EIA) and chemiluminescence assay (CLIA) followed by FTA/TPHA assays on positive samples.

Aims: To determinate the prevalence of syphilis among blood donors in Greece. In addition, to strengthen our understanding concerning the use of the appropriate algorithm. The advantages, disadvantages and limitations of current diagnostic algorithms are debated, especially in the context of the diagnosis of blood donors.

Material and Methods: We examined 10,001,526 blood units collected in Greece in the period 1996–2014 for the detection of antibodies to *Treponema pallidum*. The screening assays used for the diagnosis of syphilis according to the traditional algorithm (1996–2005) were nontreponemal tests such as VDRL and RPR test and according to the reverse algorithm (2006–2014) treponemal tests such as EIA and CLIA. All reactive serological samples were confirmed by the use of confirmatory treponemal assays such as FTA ABS or TPHA.**Results** are shown in Table1. Screening for syphilis using the nontreponemal tests (traditional screening) was sensitive, economical and easily implemented, but had high rates of false positive results. The treponemal assay (reverse screening) was rapid, sensitive and more specific but detected a significant higher number of donors with reactive results compared to traditional screening. False positive reactions occurred using both treponemal and non-treponemal tests. In some Blood Services, samples that were reactive by CLIA were then tested by RPR in order to provide a supplementary marker of diagnosis. Most of the positive or discordant results of CLIA/ RPR were then analyzed by FTA or TPHA assays.

Conclusions: This study shows that reverse screening offers a friendly, easy to perform automated screening approach and produces objective true results. As no gold standard methodology exists, more detailed analysis of our data is in progress.

Period	Blood Units	Initially Reactive (%)	Indeterminate (%)	Positive (%)	Negative (%)
1996-2005 Traditional Algorithm	4,972,556	0,003-0,007	0,01	0,008-0,047	0,04-0,05
2006-2014 Reverse Algorithm	5,028,970	0,08- 0,16	0,005	0,007-0,027	0,06-0,07
Total	10,001,526			0,0018	0,005

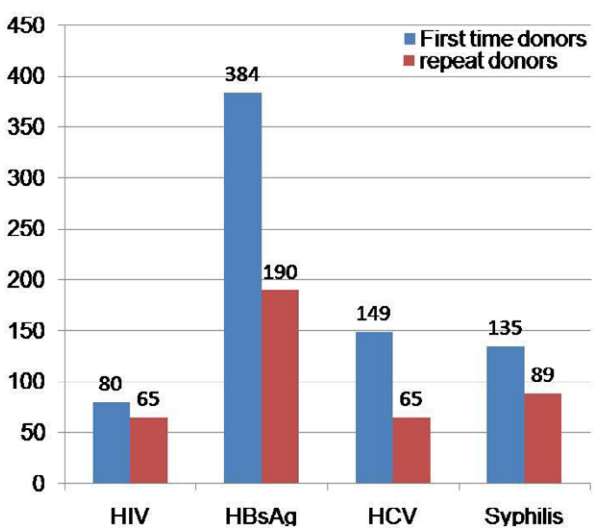
P-238

TRANSFUSION TRANSMITTED INFECTION REACTIVE BETWEEN FIRST TIME AND REPEAT DONORS

S Nachrowi, S Kromodimejo and S Murti

Bekasi county blood transfusion service, Bekasi, Indonesia

Background: Blood transfusion is the part of therapy. Bekasi county blood transfusion service is one of the blood service unit in Indonesia who commits to increase the quality of product, service and management. The cycle of blood service in Bekasi county blood transfusion service consist of donor selection, blood donation, blood component processing, transfusion transmitted infection Testing, blood group confirmation, release product, storage and distribution (patient service). Transfusion transmitted infection Testing is one of the important process to guarantee blood safety.
Aim: We accumulated data to compare the case of transfusion transmitted infection reactive between first time and repeat donor
Method: In Bekasi county blood Transfusion service, transfusion transmitted infection testing perform with EIA method using full automatic elisa processor. Reactive samples will be identify if the absorbance above the cut off value, and non reactive samples if the absorbance below the cut off value. We collected transfusion transmitted infection reactive data in 2014 according to the result of the Test.
Result: Among 33.060 donors in 2014, we found result for all parameters, reactive cases are higher in first time donors. HIV: 80 first time and 65 repeat donors. HbsAg: 384 first time and 190 repeat donors. HCV: 149 first time and 65 repeat donors. Syphilis: 135 first time and 89 repeat donors.
According to the data, we found the result:
HIV reactive: Between first time and repeat donors found the difference 15 people
HBsAg reactive: Between first time and repeat donors found the difference 194 people
HCV reactive: Between first time and repeat donors found the difference 84 people
Syphilis reactive: Between first time and repeat donor found the difference 46 people
Conclusion: We can see in 2014 frequency of transfusion transmitted infection reactive to HIV, HBsAg, HCV and Syphilis were higher in first time donors, it cause by repeat donors more understand about donors requirement and healthy life style. Because of that we have to educate the first time donors about donor requirement before they donate their blood. Donor recruitment must be more selective to get low risk donor, so the frequency of transmitted transfusion infection reactive can be decrease.



P-239

This abstract has been withdrawn.

P-240

SEROPREVELANCE OF HEPATITIS E VIRUS (HEV) IN THE SWISS BLOOD DONORS: BASIS FOR FUTURE STRATEGY FOR PREVENTING HEV TRANSMISSION TO AT RISK INDIVIDUALS

C Niederhauser, N Widmer, M Hotz and P Gowland

Interregional Blood Transfusion SRC, Berne, Switzerland

Introduction: Hepatitis E virus (HEV) the aetiological agent of hepatitis E is a small icosahedral non-enveloped single stranded RNA virus that in recent years has become a growing public health concern in many developed countries. HEV infections though often asymptomatic may cause fulminant or chronic hepatitis E, especially in immunocompromised persons and is thus safety concern for hepatologists and the blood transfusion community. There are 4 known genotypes HEV1–4 which are transmitted to humans via contaminated water and meat, via blood transfusion or vertically from mother to child. In industrialised countries HEV genotype 3 has been shown to be the most widespread virus and the route of infection for this virus suspected to be from infected pork/wild game and meat products. Here we present data on the HEV IgG seroprevalence for the Swiss population which will serve as a basis for determining a strategy for preventing HEV transmission to at risk individuals.
Aim: To determine the HEV seroprevalence in blood donors from different regions within Switzerland and to a data base for establish a strategy for preventing HEV transmission to patients at high risk.
Methods: EDTA Plasma samples were collected from blood donors form different regions of Switzerland. The presence of HEV-specific IgG antibodies was determined using the WANTAI HEV-IgG ELISA (Wantai, Beijing, China). The results were analysed to include different geographical regions, age of donor and sex. The data will serve as a basis for a strategy for future blood donor screening programmes.
Results: On average 19.5% of the 3'609 blood donations collected from regions above and below the Alps were anti-HEV IgG positive. From each region ≥200 samples were included in the analysis and the seroprevalence from these regions ranged considerably between 13% and 34%.
A steadily increase in the HEV seroprevalence of donors with age (~7.7%; 18–22 yrs until ~33.9%; 63–70 yrs) was observed. There was no difference between male and female donors.
Summary/conclusions: At around 20% the current average seroprevalence of HEV in Swiss blood donors is similar to those reported in other middle European countries. This seroprevalence is not constant over the whole of the country but there is a distinct variation, ranging from 13 to 34%, between different regions within Switzerland. Since the seroprevalence increases with the age, new infectious donors must be entering the blood donor pool. These donations pose a threat to high risk donors, such as immunocompromised patients. The data generated in this study were presented to a national working party consisting of hepatologists, blood transfusion and infectious disease specialists that was setup to establish concept for preventing HEV transmission to high risk individuals.

P-241

PREVALENCE OF WEST NILE VIRUS AMONG DANISH BLOOD DONORS

D K Holm[1], S Gjørup Sækmose[2], K Mikkelsen Homburg[2], L Holm Harritshøj[3], H Ullum[3], B Aagaard Jensen[4], M Christiansen[5], UG Liebert[6], J Georgsen[1] and C Erikstrup[7]

[1]Odense University Hospital, Odense C, Denmark [2]Tissue and Transfusion Center, Naestved, Denmark [3]Rigshospitalet, Copenhagen, Denmark [4]Aalborg Universityhospital, Aalborg, Denmark [5]Aarhus Universityhospital, Aarhus, Denmark [6]Institute of Virology, Leipzig University, Leipzig, Germany [7]Aarhus University Hospital, Aarhus, Denmark

Background: West Nile Virus (WNV) is primarily transmitted to humans by infected mosquitos. However, WNV can also be transmitted between humans through infected blood products or solid organs. In Europe, new areas are often added to the map with documented autochtonous transmission of WNV and environmental changes can facilitate a further expansion of the mosquito breeding grounds. In some European countries blood donors living in specific WNV high risk areas are screened by WNV ID NAT, however blood donors often travel increasing the risk of transfusion-transmitted WNV infection also in areas with no WNV transmission. In Denmark, a 4-week deferral of blood donors traveling to WNV risk areas in the period from April 1st to November 1st was implemented to mitigate the risk of transmission to recipients.

Aim: The aim of this study was to estimate the risk of transfusion-transmitted WNV infection in Denmark based on the seroprevalence of WNV, the prevalence of WNV RNA, and a survey of the travel history of Danish blood donors to WNV risk areas.
Methods: Plasma samples from 1228 Danish blood donors collected during February and March 2015 were analyzed for anti-WNV IgG using a WNV Elisa IgG assay (Euroimmun, Germany). To confirm the presence of WNV antibodies, the WNV IgG reactive samples were analyzed using an in-house neutralization test developed at the Institute of Virology, Leipzig University, Germany. Plasma samples from 4114 Danish blood donors were collected in the WNV deferral period in 2013 and 2014 and analyzed for WNV RNA by ID NAT testing using the Procleix WNV assay from Grifols. In addition, the travel history of 11396 blood donors from the Region Zealand of Denmark was surveyed in 2014.
Results: The rate of initially anti-WNV reactive samples was 2.04% (28/1228), but only one sample (0.08%) was confirmed as anti-WNV positive by the neutralization assay. We did not find any WNV RNA positive blood donors in this study. The response rate of the travel history survey was 22.6% (2750/11396) and showed that 31% of the respondents had traveled to regions with a potential risk of WNV infection the preceding year.
Conclusion: Even though many Danish donors travel to regions with a potential risk of WNV infection the seroprevalence of WNV was very low and consequently transmission of WNV to traveling Danish donors is estimatet to be very rare. No WNV reactive donors were found. We conclude that with a 4-week deferral of blood donors who travel to WNV risk regions, the risk of a WNV transfusion transmission in Denmark is extremely limited.

P-242

A QUANTITATIVE IDENTIFICATION OF HSV INFECTED VERO CELLS WITH FLOW CYTOMETRY

L Mousavi, Z Sharifi, R Golestani and E Hajati
Ibto (Iranian blood transfusion organization), Tehran, Iran

Background: For further reduction of transfusion transmitted infections (TTIs), viral inactivation with methylene blue is part of a reducing risk strategy. Virus models and cytopathic effects (CPEs) on cultured cells are used to evaluate viral inactivation. Identification of microscopic cellular changes has lack of sensitivity. Thus improvements to identify cellular changes as viral effect make good tools to better assessing of viral inactivation. HSV is viral model of hepatitis B virus (HBV).
Aims: We aimed to design a method to quantify HSV viral changes on cultured cells using flow cytometry.
Methods: Vero cells at confluency of 80% were incubated with 10 fold serial dilutions of a 7 log HSV titer sample. HSV related CPE on cultured Vero cells assessed in inverted microscope and flow cytometry. Vero cells were incubated with primary human polyclonal Anti HSV antibody and secondary goat anti human FITC conjugate.

Hepatitis E IgG prevalence in Switzerland		
canton	**total # samples**	**% positive samples**
UR	155	**12.9**
AG/SO	226	**13.27**
GE	215	**13.49**
NW/OW	368	**15.22**
LU	172	**16.28**
FR	210	**18.57**
VS	200	**19.5**
Berner JU	265	**19.62**
TG	193	**20.21**
SG/AI/AR	292	**21.58**
VD	402	**22.89**
BS/BL	244	**23.77**
BE	275	**24.73**
TI	344	**33.72**
unknown origin	48	**16.67**
total	**3609**	**19.49**

Results: The CPEs of HSV were identified at the highest titer of 4 and 5 log using inverted microscope and flow cytometry respectively.
Summary/conclusion: Flow cytometry introduced as a sensitive method of detecting HSV that is model of HBV. Flow cytometry can be used in viral inactivation assessments and TTIs.

P-243

REVIEW OF BORDERLINE TEST RESULT FOR VIRAL SEROLOGICAL MARKERS FOR HIV, HCV AND HBV IN BLOOD DONORS

KS Ng, SS Chua, S Lam and WC Tan
Health Sciences Authority, Singaore, Singapore

Background: Viral serological testing for all the three markers: antibodies for Human Immunodeficiency Virus Type 1 and/or 2 (anti-HIV-1/2), antibodies for Hepatitis C Virus (anti-HCV) and Hepatitis B surface antigen (HBsAg) are mandatory screening tests performed for blood donations in Singapore since 1990s. In all three assays, a cut-off value, S/CO, ≥1.00 are considered as initial reactive (IR).To maximize blood testing safety in the early years, Blood Services Group (BSG) has included samples yielding S/CO ratios between 0.70 to 0.89 (classified as HOD) and 0.90 to 0.99 (classified as GZ) to be considered as IR and subjected to the same testing algorithm of IR that includes duplicate testing of the sample and followed by a confirmatory assay on repeatedly reactive (RR) samples. Donations that fall under these categories are discarded.
Aim: Our objective is to determine if donations with initial borderline screening results of HOD and GZ values still have an added value for the detection of HIV, HCV and HBV with current serological tests and concurrent mandatory Nucleic Acid Testing (NAT) in BSG.
Methods: The qualitative viral serological screening tests are performed on the ABBOTT PRISMnEXT system (ABBOTT Laboratories) using chemiluminescent immunoassay. A total of 689,096 donations from 2009 to 2014 were retrospectively evaluated. The number of donations with initial HOD and GZ values and their repeated test results are tabulated for all three serological markers. The confirmatory test results for the donations with RR results were traced.
Results: The number of donations with initial screening result of HOD and GZ was 430 (0.06%) for anti-HIV-1/2; 715 (0.10%) for anti-HCV and 318 (0.05%) for HBsAg. 397/430 (92.33%) of the HOD and GZ results for anti-HIV-1/2 were repeatable reactive (RR); 536/715 (74.97%) of the HOD and GZ results for anti-HCV were RR and 137/318 (43.08%) of the HOD and GZ results for HBsAg were RR. Of these donations with HOD and GZ results for anti-HIV-1/2 and anti-HCV, there were no donations that yield a positive NAT or confirmatory test result for HIV and HCV. However, for the HOD and GZ results of the HBsAg assay, there were 3 donations that were tested reactive for HBV DNA for Nucleic Acid Test (NAT) and anti-HBcore. 2 of these donations were from the HOD group and 1 from the GZ group.
Conclusions: For screening assays with high sensitivity and specificity such as the ABBOTT PRISM assays, setting borderline test results (HOD and GZ) in the testing algorithm do not further enhance blood testing safety on the detection of HIV and HCV. Conversely, borderline test result setting for the HBsAg assay may assist in detection of HBV, especially in the absence of NAT screening. This study shows no added value in blood testing safety with the inclusion of borderline test results in BSG's test algorithm for the three viral serological markers, especially with the concurrent mandatory NAT screening all individual donations. The removal of borderline test results from the test algorithm will lead to additional 0.04% of blood available for use annually.

P-244

THE RELEVANCE OF TESTING FOR ANTIBODIES TO HBV IN THE BLOOD SERVICE

EN Ignatova[1], TA Tupoleva[1], EN Ovchinnikova[1], TYu Romanova[1], NG Yaroslavtseva[1], FP Filatov[2], TV Gaponova[1] and VG Savchenko[1]
[1]National Research Center for Hematology, Moscow, Russian Federation [2]Mechnikov Research Institute for Vaccines and Sera, Moscow, Russian Federation

Background: Analysis of literature data suggests that testing for HBV DNA and HBsAg are not fully secure the viral safety of blood transfusions. To increase the security one needs to identify the other HBV markers (such as anti-HBc) too. The using anti-HBc positive blood components with anti-HBs (>200mME/ml) is

acceptable in several countries. Patients with the blood system diseases are at high risk of HBV infection, and the transfusion is the leading factor.

Aims: Evaluation of the blood donor testing for anti-HBc in terms of viral safety of blood transfusion.

Methods: ELISA, RT PCR (donors - pools of six HBsAg-negative samples, patients - ID).

Donors divided into two groups: the first - voluntary non-remunerated and paid blood donors (13.081), the second - voluntary non-remunerated blood donors par excellence (5.011). Donor samples of the first group were analyzed for HBsAg and HBV DNA. Patients with a hematological malignancy (364) were recipients of the blood components from the first group of donors. The blood samples of donors of the second group and patients were analyzed for HBsAg, HBV DNA, anti-HBc, anti-HBc IgM, anti-HBs, anti-HBe and HBeAg.

Results: HBV markers were identified in 36 donors of the first group: HBsAg - 34, HBV DNA - 2. In the 2nd group 242 donor samples showed positive results: 5 - HBsAg, 1 - HBV DNA, 240 - anti-HBc, 22 - anti-HBc IgM. In 45/240 anti-HBc positive donors no one had anti-HBs marker, whereas anti-HBc IgM were found in 5 out of these 45. In 124 donors out of 240 anti-HBs were detected (titer >200mME/ml) and 13 of these 124 donors were anti-HBc IgM positive.

HBV markers were found in the group of patients: HBsAg - 26, HBV DNA - 30, HBeAg - 4, anti-HBc IgM - 23, anti-HBc - 114, anti-HBe - 63, anti-HBs - 195. Markers of active HBV infection (HBV DNA, HBsAg, anti-HBc IgM, HBeAg) were found in 46 samples. 137 patients were anti-HBs positive only, 112 patients had no HBV laboratory markers.

The percentage of patients with HBV markers treated with transfusion therapy was significantly higher than one without HBV markers, 58% and 37%, respectively ($P < 0.05$). A proportion of patients in these groups with high transfusion load was statistically distinguishable: 20% and 9%, respectively ($P < 0.05$).

Conclusions: Screening the blood samples of the second group of donors for HBsAg and HBV DNA showed only 0.12% of positive samples, and screening the blood samples of the first group for these markers showed 0.28% positive samples, indicating a higher percentage of infected donors of this group. Additional testing of donor blood samples for anti-HBc revealed 4.79% of infected persons, of which 0.44% had the active form of the infection (the presence of anti-HBc IgM), that have presumed the likelihood of incomplete identification of potentially infected blood components the first group donors due to non-use of tests for anti-HB-core. This assumption is confirmed by the high incidence of HBV markers (69.23%), including the active form of infection (12.64%), in recipients of multiple blood transfusions.

Among anti-HBc positive blood donor samples the markers of active HBV infection occurred with similar frequency - both in anti-HBs-negative samples (11.1%) and with the high antibody titer (10.5%), indicating the potential risk of the HBV transfusion transmission even at a high concentration of anti-HBs in the donor blood.

P-245

RESULTS OF SCREENING HTLV-I/II AND CMV-SPECIFIC ANTIBODIES IN SERA OF BLOOD DONORS IN NATIONAL CENTER FOR TRANSFUSION MEDICINE, MONGOLIA

T Bayasgalan[1], E Namjil[1] and M Sonomjamts[2]

[1]National Center for Transfusion Medicine, Ulaanbaatar, Mongolia [2]Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia

Background: People who are Human T-lymphotropic virus (HTLV-I/II) infected, there are usually no clinical symptoms. But HTLV-I may also cause Adult T-cell leukemia or Lymphoma, and some inflammations (Uveitis, Arthritis, Myositis, Alveolitis and Dermatitis etc.).

Most people who are infected with Cytomegalovirus (CMV) have no signs or symptoms, but newborns and people with weakened immune systems are more at risk of developing signs and symptoms than are healthy adults. CMV remains in the body throughout a lifetime. Infected people may spread infection.

We considered screening for HTLV-I/II and CMV-specific antibodies is important to setup the hemovigilance and to ensure the safety of blood products in Mongolian Blood service.

Goal: To determine transfusion transmitted HTLV-I/II and CMV-specific antibodies in sera of blood donors.

Materials and Methods: In this study included subjects, age range of 17–60 years (total of 716) blood donors who were served by the National Center for Transfusion Medicine in first quarter of 2015. Screening for HTLV-I/II-specific IgG and CMV-specific IgG, CMV-specific IgM determined using DIALAB (Austria) kit for ELISA (Enzyme linked immunosorbent assay).

Results: Results of study not determined HTLV-I/II-specific antigen positive case in sera of 716 donors. 99.2% (710) out of blood donors were determined CMV-specific IgG antibodies, 0.1% (1) was CMV-specific IgM antibodies determined positive in sera. 5 of all 6 individuals, who CMV-specific IgG antibodies negative, are included in blood type B(III). CMV-specific IgG antibodies positive individuals into 3 age groups (17–25, 26–40, 41–60) arranged and compared. ($P = 0.65$) The difference of the age groups was not statistically significant. CMV-specific IgM antibodies positive (1) individual was in the age group 26–40 years, female and type of regular donor.

Conclusion: We can conclude from results of the determination of HTLV-I/II-specific IgG in all cases negative, shows there was not a risk of transfusion transmitted infection. And we can conclude from results of the determination of CMV-specific IgM positive 1 case, shows possible that re-infected or reactivated latent infection. There need more efficient and more specific testing methods, nucleic acid amplification technology and CMV-specific IgG avidity test to determine the risks of transfusion transmitted CMV infection.

CMV-specific antibodies and age groups

		17-25	26-40	41-60	Total
IgG	Positive	99% (292)	99.6% (243)	98.9% (175)	99.2% (710)
	Negative	1% (3)	0.4% (1)	1.1% (2)	0.8% (6)
	Total	100% (295)	100% (244)	100% (177)	100% (716)
IgM	Positive	-	0.4% (1)	-	0.1% (1)
	Negative	100% (295)	99.6% (243)	100% (177)	99.9% (715)
	Total	100% (295)	100% (244)	100% (177)	100% (716)

Caption 1. CMV-specific antibodies and age groups

CMV-specific antibodies and blood types

		Blood types				Total
		O(I)	A(II)	B(III)	AB(IV)	
IgG	Positive	100% (272)	100% (182)	97.6% (201)	98.2% (55)	99.2% (710)
	Negative	-	-	2.4% (5)	1.8% (1)	0.8% (6)
	Total	100% (272)	100% (182)	100% (206)	100% (56)	100% (716)
IgM	Positive	-	-	0.5% (1)	-	0.1% (1)
	Negative	100% (272)	100% (182)	100% (206)	100% (56)	99.9% (715)
	Total	100% (272)	100% (182)	100% (206)	100% (56)	100% (716)

Caption 2 CMV-specific antibodies and blood types

P-246

REACTIVE BLOOD DONORS AND THEIR DEMOGRAPHIC CHARACTERISTICS FROM A DEVELOPING COUNTRY

M Borhany

National Institute of Blood Disease an BMT, Karachi, Pakistan

Background: Blood transfusion is an integral and life-saving procedure of modern medicine. Blood donation saves the lives of millions of people worldwide, however, transfusion-transmitted infections (TTIs) often threatens the safety of patients requiring blood transfusion which imposes serious challenges to the medical providers for the availability of blood with safety and affordability.

Aim: The aim of the study was to determine the prevalence of transfusion-transmitted infections in blood donors.

Methods: A prospective cohort study was conducted at our institute. Donors were required to fill a detailed questionnaire and were screened for HBV, HCV, HIV, syphilis and malaria.

Results: Of the total 16602 blood donors, 16557 were males and 45 females, with the mean age28.6 ± 2. Of all the donations, 973(5.8%) had serological evidence of one infection while 58(0.35%) were those having co-infection with more than one pathogen. The prevalence of HCV, HBV, HIV, syphilis and malaria was found to be 1.84%, 1.7%, 0.04%, 2.1% and 0.07% respectively. When the overall characteristics of donors were evaluated, it was found that businessmen and unmarried donors were at risk to be infected among others. However, when characteristics among infections were individually evaluated, unmarried donors had a better chance to be infected by HBV and syphilis rather than other infections.
Conclusion: A substantial percentage of the blood donor's harbored TTIs. Prevention of spreading of transfusion-transmitted infections and future study to assess the data of the blood donor's population should be the main goal.

P-247

COMPARISON OF ANTI-HBC IGG, ANTI-HBC IGM, AND FERRITIN IN THE VARIOUS BLOOD AND BONE DONOR GROUPS

SH Choi

Seoul National University Bundang Hospital, Seongnam, South-Korea

Background: IgG anti-HBc Ab is found lifelong in persons who have been infected with HBV and is a marker of past or current viral replication. Traditionally, it was regarded as a sensitive indicator of hepatitis B virus (HBV) infection. With the development of molecular test such as virus nucleic acid amplification test, the role of anti-HBc Ab became uncertain.
Aims: In this study, we evaluated the usefulness of anti-HBc Ab in various blood and bone donor. Another inflammation marker, serum ferritin, was also evaluated.
Methods: The anti-HBc IgG, anti-HBc IgM, and serum ferritin levels of the donors who visited Seoul National University Bundang Hospital From March 2010 to December 2015 were measured (whole blood donation: 2947 cases, autologous blood donation: 308 cases, Bone donation: 419 cases, autologous serum eyedrops: 349 cases) using Achitect i2000 (Abbott), Modular Analytics E170 (Roche).
Results: Among the total of 1871 cases of whole blood donations, anti-HBc IgG Nonreactive was 1500 cases (80.2%), Reactive was 372 cases (19.8%). anti-HBc IgM Nonreactive was 1871 cases (99.9%) and Reactive was 0 case (0%), QNS 1 case. Among the total of 308 cases of autologous blood donation, anti-HBc IgG Nonreactive was 134 cases (43.5%) and Reactive was 174 cases (56.5%). anti-HBc IgM Nonreactive was 308 cases (100%), Reactive was 0 case (0%). Among the total of 419 cases of Bone donation, anti-HBc IgG Nonreactive was 211 cases (50.4%), Reactive was 208 cases (49.6%). anti-HBc IgM Nonreactive was 419 cases (100%) and Reactive was 0 case (0%). Among the total of 349 cases of autologous serum eyedrops, anti-HBc IgG Nonreactive was 203 cases (58.2%), Reactive was 146 cases (41.8%). anti-HBc IgM Nonreactive was 348 cases (99.7%) and Reactive was 1 case (0.3%). The ferritin distribution of 1877 donors was as follows. Under 12.00 ng/ml was 59 cases, 12.00–14.99 ng/ml was 26 cases, 15.00–30.99 ng/ml was 187 cases, 31.00- 300.99 ng/ml was 1524 cases, above 300 ng/ml was 80 cases. The ferritin level of the each group was not statistically significant.
Summary/Conclusions: We measured anti-HBc IgG, anti-HBc IgM and ferritin using donor blood in the 4 groups. Only the whole blood donation group showed significant difference in anti-HBc IgG testing. In the whole blood donation group, the result of nonreactive anti-HBc IgG was 30%p higher than other group. However, the ferritin levels were not different among 4 groups.

P-248

ANTIBODIES TO HTLV-I/II IN DONORS' AND HIGH-RISK GROUP PATIENTS' BLOOD SAMPLES

RR Abakarov[1], TA Tupoleva[1], AA Gulyaeva[1], EN Ovchinnikova[1], ON Misko[1], FP Filatov[2], TV Gaponova[1] and VG Savchenko[1]

[1]National Research Center for Hematology, Moscow, Russian Federation [2]Mechnikov Research Institute for Vaccines and Sera, Moscow, Russian Federation

Background: In some countries the screening for antibodies to HTLV-I/II (anti-HTLV-I/II) is mandatory for blood donors: in Japan since 1986, in USA since 1989, in Canada since 1990, in France since 1991, in Netherlands since 1993, in Sweden and Denmark since 1994. In Russia analysis for the anti-HTLV-I/II is mandatory only in the Stem Cell Banks. The prevalence of HTLV-I/II is insufficiently investigated in Russia. The blood system diseases patients are the high risk group for infection of HTLV-I/II, the transfusion is the leading factor.

Sex	Age	Amount	First-time donors	Regular donors
male	18 – 25	309	95(1+)	214
female	18 – 25	182	52	130
male	26 – 30	311	71	240
female	26 – 30	154	50	104
male	31 – 40	354	64	290(1+)
female	31 – 40	161	39	122
male	41 – 50	139	21	118
female	41 – 50	79	12	67
male	> 50	50	5	45
female	> 50	28	4	24

Table. Characteristics of donors screened for anti-HTLV-I/II

Aims: To evaluate the relevance and advisability of the testing of blood donors for anti-HTLV-I/II in Russia.
Methods: The study for anti-HTLV-I/II were performed using the certified CMIA test systems Abbott Company on the device ARCHITECT plus i2000sr.
All materials were from National Research Center for Hematology: donors' samples (2.553), bone marrow samples (20), blood diseases patients' samples (53) including T-cell leukemia/lymphoma, hairy cell leukemia, T-lymphoproliferative diseases, and samples from patients receiving multiple blood transfusions (23) with: acute myeloid leukemia, chronic myeloid leukemia, diffuse large B-cell lymphoma and acute lymphoblastic leukemia.
Results: Two of 2.553donors (~0.1%) were identified anti-HTLV-I/II (*Table*). In one case anti-HTLV-I/II were combined with antibodies to Treponema pallidum. These two donors were male, different age (33 and 18) and one was first-time donor and another - regular.
The samples of patients with blood diseases, and with multiple blood transfusions, and bone marrow donors had no anti-HTLV-I/II.
Conclusions: Antibodies to HTLV-I/II were detected in 0.1% of donors. These antibodies were not found in blood samples of patients with hematologic malignancies and recipients of multiple blood transfusions. Today the preparation of blood components uses leukocyte reduction and viral inactivation procedures, which minimizes the possibility of the HTLV-I/II transfusion transmission. Also due to the non-endemic circulation of these viruses in Russia and based on the results of this study, authors believe that the blood donations screening for anti-HTLV-I/II is not necessary.

P-249

EVALUATION OF THE CONTRIBUTION OF THE VIRAL SERUM MARKER 'ANTI-HBC' TO BLOOD SAFETY IN LEBANON BY POLYMERASE CHAIN REACTION

C Al Haddad[1], P Finianos[2], S Matta[2], E Zgheib[2], R Feghali[3], M Germanos[2] and JC Lahoud[2]

[1]CHU-ND Secours/ USEK, Jbeil, Lebanon [2]Faculty of Medicine and Medical Sciences-Holy Spirit University of Kaslik, Jounieh, Lebanon [3]Rafic Hariri University Hospital (RHUH), Beirut, Lebanon

Background: Lebanon is an averagely endemic area for the Hepatitis B virus. In 2013, the Lebanese Committee of Blood Transfusions formed by the Lebanese Ministry of Public Health introduced mandatory testing of anti-HBc in the screening of blood units.
Aims: The objective of this study is to evaluate, by molecular testing, the prevalence of Hepatitis B in a Lebanese donor population that has a negative HBsAg but a positive anti-HBc. Consequently, our study may provide justification for the practice of discarding blood units with such a serological profile in countries where PCR is not routine in blood unit screening.
Methods: A retrospective study was conducted at the Centre Hospitalier Universitaire-Notre Dame de Secours (CHU-NDS) in Byblos, Lebanon on a total of 10,945 donors that were screened between 2013 and 2015 for HBsAg and anti-HBc on an Architect

(Abbott Diagnostics). Donors that had a negative HBsAg but a positive anti-HBc were selected as study samples. Anti- HBs antibodies were measured in all of these on an Architect (Abbott Diagnostics). A subset of 79 samples was then randomly selected and Hepatitis B viral loads were determined by PCR on a Cobas TaqMan (Roche).
Results: 2.05% of the above mentioned 10,945 donors had the serological profile under study (HBsAg negative and anti-HBc positive). 81.3% of these were found to be anti-HBs positive. Viral HBV DNA was found in 2 of the 79 samples that were tested with PCR at a percentage of 2.53%.
Conclusions: HBsAg alone is insufficient to determine the infectivity of donated blood units and to ensure their safety. Even in the absence of HBsAg, anti-HBc may signify contagiosity and discarding of the blood unit is justified. The systematic screening for anti-HBc decreases the risk of post-transfusional transmission of HBV and its measurement is important in settings and countries, such as Lebanon, where PCR is not routinely available due to economic reasons.

P-250

POTENTIAL RISK FOR TRANSFUSION TRANSMITTED INFECTION IN TRANSFUSION CENTER-STRUMICA, MACEDONIA

SD Stambolieva, T Timova, T Malinova, M Sorova, J Vitlarova and V Gorgovska
Institute for transfusion medicine Skopje, Strumica, Macedonia

Background: Blood transfusion can be a life saving procedure. Transfusion transmitted infection is any infection that is transmissible from blood donor to a patient through blood or blood product. But testing for TTI is the final measure for eliminates unsafe blood. Avoiding family donors, selecting donors through questionnaires and regular doctor's examination and limiting the number of blood transfusions can prevent the transmission.
Aim: To estimate the risk of transfusion transmitted infection in blood donors in Transfusion center Strumica in period of one year: from 01 January 2015 year till 31 December 2015 year.
Methods: Our study is an one year retrospective study with blood banking data from our registers and include all donors who were screened for TTI by using immunological methods-enzyme imunoabsorbent assay ELISA for anti-HIV 1,2, Anti-HCV, HbsAg and Syphilis.
Results: Total number of donated blood units in that period was 1976. 1900 of them (96%) were voluntary blood donors, 73(4%) were family. Males are majority of donor population-1584 (80%), females are 389 (20%). First time donors are 267 (13%), 1709 (87%) are regular donors .In that period of one year, initial reactive blood donors were: HbsAg-35(1,78%), anti-HCV-2(0,1%),Anti-HIV-2 (0,1%) and Syphilis- 1(0,05%). 30 of them were retested (from new sample of blood) and the repeat reactive blood donors were confirmatory tested. Confirmatory test (for HBs Ag) was positive in only 4 blood donors (0,2%). Two of them were first time donors, two were regular donors. Initially reactive blood donors for HCV and anti-HIV were invited by letter for a new sample blood, but they did not answer the invite.
Conclusion: Our study documents low percentage of TTI among our blood donors, high percentage of voluntary donors, low participation of female donors and no difference between TTI in first time and regular donors in the studied 2015 year. The target for transfusion specialists should be provision strict criteria in recruitment and deferral of blood donors, proper testing of every blood units by using recommended standard methods how we can improve safe transfusion.

P-251

SCREENING OF TRANSFUSION TRANSMITTED INFECTION (TTIS) IN MULTI-TRANSFUSED PATIENTS

S Maqsood[1], SO Adil[2], SH Ansari[1] and T Shamsi[1]
[1]National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, Pakistan [2]Omair Sana foundation (OSF), Karachi, Pakistan

Background: Hepatitis B, C and HIV are endemic diseases in Pakistan and carry high morbidity and mortality. Risk of these infections is far above the ground in patients receiving multi-transfusions. However, national level estimates of the prevalence of transfusion transmitted infection (TTIs) in multi-transfused patients are currently not available.
Aim: To determine the frequency and trend of transfusion transmitted infection (TTIs) in multi-transfused patients.
Methods: A cross-sectional study was conducted by Omair Sana foundation (OSF) from June 2015 to September 2015 among multi-transfused patients. HBsAg test was performed on Alere Determine HBs Ag Kit, Anti HCV screening test was performed on HCV TRI-DOT/ immunodot Kit and HIV antibody test was performed on ICT and ELISA methods.
Results: Median age of patients was 8(5–12) years. Male preponderance was found to be higher (56.52%). Out of 605 patients, 48 (7.9%) were anti-HCV positive, 11 (1.8%) were hepatitis B positive and 8 (1.3%) were HIV positive. Statistical significant difference was observed in between age and HBsAg (P-value 0.001) and anti-HCV (P-value 0.008). Moreover, number of blood transfusion per year and hepatitis C (P-value <0.001) and number of blood transfusion per year and HIV (P-value 0.001) were also significant.
Conclusion: The present study has highlighted the growing number of HIV infection in multi-transfused patients. Moreover, hepatitis C remains the most threatening TTI in terms of magnitude in these patients. Increase in age and number of transfusions significantly increases the risk of TTIs in multi-transfused patients.

P-252

DEMOGRAPHIC CHARACTERISTICS OF BLOOD DONORS WITH POSITIVE TTI MARKERS

T Makarovska Bojadzjieva, J Nikolova, E Velkova, M Blagoevska, J Samonikov Tosevska, V Dejanova Ilijevska and I Nikoloska
Institute of transfusion medicine, Skopje, Macedonia

Background: Donor selection and TTI markers testing strategies are crucial for blood safety which is the ultimate goal and responsibility of blood transfusion services.
Aim: To estimate the demographic characteristics of blood donors with positive TTI markers who donated blood in the Institute of Blood Transfusion-Skopje in the period of 2 years (2013–2014).
Methods: We evaluated data of confirmatory testing of 243 (150 HBV and 93 HCV) repeatedly reactive samples screened with EIA-Enzygnost-Siemens and CMIA-Architect ABBOTT system. Confirmatory testing was performed with Vidas HBsAg Ultra and Immunoblot Deciscan HCV Plus assay. Donor data (sex, age, ethnicity and number of donations) were evaluated using the ePROGESA software.
Results: The total number of confirmatory positive (CP), confirmatory negative (CN) and confirmatory indeterminate (CI) results was 149 (61.3%), 84 (34.6%) and 10 (4.1%) respectively. The demographic characteristics of the 149 CP donors were the fallowing: 103 (69%) first time donors, 128 (86%) male vs 21 (14%) female donors, mean age of 34.4 years and 1.3 donations/donor (5.5 donations/donor in CN donors). The summarized results are shown on Table 1.
Conclusion: The obvious gender difference is due to the general gender distribution of our blood donors. There was significant difference between CP and CN male donors ($P < 0.01$) but not significant difference in females ($P > 0.05$) was observed. A significant increase of HCV CP results was linked to the low number of blood donations. The number of first time donors (88.8%) is significantly higher in HCV CP donors in comparison to HCV CN donors ($P < 0.01$) which is not the case for HBV ($P > 0.05$) with only 66.4% of HBV CP being first time donors. Further motivation and education of blood donors especially towards self-exclusion should be implemented in upcoming donor selection strategies.

TTI marker No (%)	Male No (%)	Female No (%)	Mean age (18 to 63)	Mean No donations
HBV CP: 131 (87.3)	111 (84.7)	20 (15.3)	35.6	1.3
HBV CN: 19 (12.6)	13 (68.4)	6 (31.6)	34.0	2.5
HCV CP: 18 (19.3)	17 (94.4)	1 (5.5)	33.2	1.4
HCV CN: 65 (69.8)	53 (81.5)	12 (18.5)	36.0	8.6
HCV CI: 10 (10.7)	6 (60.0)	4 (40.0)	36.2	3.2
Total: 243	200 (82.4)	43 (17.7)	35.6	3.4

Table 1. Confirmatory testing results and demographic characteristics of blood donors

P-253

EPIDEMIOLOGY OF TRANSFUSION TRANSMITTED INFECTION AND BLOOD GROUPS AMONG PATIENTS WITH β-THALASSAEMIA MAJOR IN PAKISTAN: A CROSS-SECTIONAL STUDY

RA Kiani[1], M Anwar[2], U Waheed[3], M Asad[4], S Abbasi[5] and HA Zaheer[3]

[1]PMAS Arid Agriculture University, Rawalpindi, Pakistan [2]Department of Blood Bank, Jinnah Postgraduate Medical Centre, Karachi, Pakistan [3]Department of Blood Bank, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan [4]Department of Biochemistry, PMAS Arid Agriculture University, Rawalpindi, Pakistan [5]Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan

Background: Thalassemia is the most prevalent genetic blood disorder in Pakistan. It is estimated that about 100,000 patients suffering from thalassaemia major are in Pakistan. These patients are dependent on regular blood transfusions to sustain life in addition to expensive chelation therapy and other medical management. Transfusion Transmitted Infections (TTIs) continue to be a major risk in transfusions in many parts of the world. The transfusion dependent β-thalassaemia patients are particularly at risk of acquiring TTIs.

Aims: The current study was undertaken to estimate the prevalence of TTIs in transfusion dependent β-thalassaemia patients. Frequency of ABO and RhD blood groups in β-thalassaemia patients was also assessed.

Material and Methods: A cross-sectional study of 1253 multi-transfused thalassaemia major patients was conducted in five different centers of Islamabad, Rawalpindi and Karachi. The study subjects were screened for HIV, HCV, HBV and blood groups. The screening was performed at two centre; Department of Pathology, SZAB Medical University and Blood Transfusion Services, Jinnah Postgraduate Medical Centre from July 2015 to December 2015.The screening was performed through Chemiluminescent immunoassay (CLIA). The blood groups were performed by forward tube technique and confirmed by reverse technique.

Results: Out of the 1253 multiple blood transfused patients, 317 (25.3%) were infected with TTIs. The seroreactivity for HCV was 273 (21.7%) cases whereas for HBV it was 38 (3.0%) cases. The seroprevalence for HIV was estimated to be 6 (0.5%) cases. HCV was more prevalent (P-value = 0.10) in males while HIV in females (P-value = 0.42), both not significant statistically. Blood group B positive was found most frequent 459 (36.6%) and AB negative was the least frequent with 10 (0.8%) cases. The blood group distribution trend was found as B > O > A > AB. The percentage of RhD positive and RhD negative blood groups were 1130 (90.2%) and 123 (9.8%) respectively.

Conclusion: HCV was the leading TTI in multi-transfused thalassaemia major patients in the study. Detection of HIV in thalassaemia patients is a recent disturbing development in Pakistan with this study also witnessing few cases. Improved regulation of blood banks including use of internationally or nationally approved screening kits would bring down the incidence of TTI in high risk groups like transfusion dependent β-thalassaemia patients and more stringent behavioral and serological pre-transfusion screening of blood for TTIs must be implemented in blood banks.

P-254

STUDY OF PREVALENCE OF TRANSFUSION TRANSMISSIBLE INFECTION AMONG DONOR POPULATION IN SRI LANKA

AA Vithanage

National Blood Centre, Colombo 05, Sri Lanka

Background: National blood transfusion service is the sole provider of blood and blood components to the state sector and nearly all private sector health intuitions in Sri Lanka. It consists of the national blood centre (NBC) which is the central body, 18 cluster centers and 96 hospitals based blood banks. National blood transfusion service handles around 400,000 volunteer donors annually. Currently in sri lanka each donor pack is tested for HIV 1 and 2, HBV, HCV, syphilis and Malaria. Recently (in 2015) we have introduced nucleic acid test (NAT) for part of the donor samples in national blood centre.

Aim: When compared with other countries, Sri Lanka used to have a very low prevalence of TTI especially hiv.a lower prevalence of TTI in the donor population also reduces the discard of blood hence results in improved efficiency and use of resources. So in this study I have tried to analyze the prevalence of TTI in last 5 years.

Method: The data was collected from totally voluntary donors from 2011 to 2015 by statistics unit of nbc. The main types of assays used for blood screening are enzyme linked immunoassay (elisa) and enhanced chemiluminescence immunoassay (ECI) and haemagglutination (HA)/particle agglutination (PA) and rapid assays (determine). Syphilis was screened with cardiolipin test mainly and carbon antigen test. Majority of the samples were done by using elisa and ECI.very small amounts of samples (10%) were done by using particle agglutination assays or rapid assays in small laboratories that have limited resources and perform only a small number of tests daily. HIV confirmation was done by sexual transmitted disease (STD)/acquired immunodeficiency syndrome (AIDS) control program. Initially reactive samples in each centre were followed by repeat testing at NBC. Confirmatory testing method for HIV was western blot and for syphilis was treponema palladium particle agglutination test (TPPA). Prevalence for HBV and HCV were calculated by using repeat reactive results done by ELISA and ECI. There is no further confirmatory test for HBV and HCV presently in Sri Lanka. Data analysis was done by minitab 17 statistical software and $P < 0.05$ considered as statistically significant level.

Year	Donations	HIV		HBV		HCV		Syphilis	
		No	Prevalence	No	Prevalence	No	Prevalence	No	Prevalence
2011	330 200	7	0.02	411	1.24	1033	3.12	122	0.36
2012	351 605	16	0.04	405	1.15	1025	2.91	170	0.48
2013	380 808	16	0.04	237	0.62	953	2.50	180	0.47
2014	380 367	26	0.06	394	1.03	657	1.72	152	0.39
2015	395 500	21	0.05	409	1.03	800	2.02	175	0.44

Results: The prevalence (per 1000 donations) of each disease is as follows.

Conclusion: Throughout the last five years (2011–2015) prevalence of all four markers (HIV, HCV, HBV, and Syphilis) are remaining at a very low level. However prevalence of hiv has shown an increasing trend for particular period ($P < 0.05$). Repeat reactive prevalence of both hbv and hcv has remained at very low steady levels for last five years and there is no evidence of any increasing trends.

P-255

THE PREVALENCE OF HAEMOPARASITES IN DONATED BLOOD AT NAKASERO BLOOD BANK FROM EASTERN UGANDA

S Ssenyonga[1] and R Ayikobua[2]

[1]Laboratory, Kampala, Uganda [2]Blood Transfusion Service, Kampala, Uganda

Introduction: The occurrence of haemoparasites (*Plasmodium* species, *Trypanosoma cruzi, Lieshmania* species, *Toxoplasma gondii, Babessia and Microfilaria*) in donated blood is significant health problem facing humanity. In Uganda blood transfusion services, donor blood is not screened for parasites and this raises concern on the safety of the transfused blood to the recipients.

Aims: This study determined the prevalence of haemoparasites in donated blood at Nakasero Blood Bank from Eastern Uganda.

Methods: Thin and thick blood smears were made from donated blood; smears were stained with Giemsa stain and examined under a microscope.

Results: Of the 166 donated blood samples, 3.6% tested positive for malaria as the most prevalent parasite in donated blood, 1.2% tested positive for microfilariae and none tested positive for both Babessia and Trypanosomes. The distribution of haemoparasites according to gender showed that the females had a high prevalence of 44% compared to Males who had 11% in relation to Plasmodium parasites and 11% for both females and males in relation to microfilariae. The predisposing factor ranged from night movements followed by not sleeping under mosquito nets and bushy areas was the least factor.

Conclusion: Despite the prevalence, transfusion of infected donor blood can be fatal to children especially under five years, pregnant women and Immuno suppressed individuals like in cancer and HIV cases. Mandatory donor blood screening should be implemented in the blood bank set ups, since microscopy has its impediments, methods like pathogen inactivation can be put in place for the blood bank centers.

P-256

EVALUATION STUDY OF ROCHE ELECSYS® ASSAYS IN BLOOD DONOR SCREENING IN INDIA

S Pathak

Max Healthcare Ltd., New Delhi, India

Background: Blood banks in India perform screening tests for HBsAg, anti-HCV and HIV Ag/Ab for all donated blood units. Before the introduction of a new automated testing system, an evaluation of different testing systems is recommended. In the Blood Transfusion Centre of Max HealthCare an evaluation of screening assays on Roche cobas® e411 in comparison with the VITROS® ECi/ECi Immunodiagnostic Systems (Ortho Clinical Diagnostics) was carried out.

Aim: The **aim** of this study was to evaluate the specificity of Roche Elecsys® assays on a fully automated analyzer cobas® e411 on blood donor specimens in parallel with our routine system.

Material and Methods: The specificity was evaluated on 2006 plasma specimens from unselected first time blood donors routinely screened on VITROS® ECi HBsAg, Anti-HIV 1 + 2, and Anti-HCV. The samples were retested on the same or the next day on cobas® e411 (Roche Elecsys® HBsAg II, Roche Elecsys® Anti-HCV II and Elecsys® HIV combi PT).

Discrepant results were further investigated by Nucleic Acid Test (NAT) performed routinely at our blood centre as a part of blood screening. NAT using the cobas® TaqScreen MPX test, version 2.0 and/or other confirmatory methods (Immunoblot, single antigen analysis) were utilized as confirmatory testing.

Results: Based on the results from testing 2006 blood donations, the observed specificity of Roche Elecsys® assays on cobas® e411 and VITROS® ECi are comparable.

Specificities of the Roche Elecsys® HBsAg, HIV and HCV assays were estimated **100%**, **99,95%** and **99,85%** respectively.

Specificities of the VITROS® ECi HBsAg, HIV and HCV assays were estimated **99,90%**, **100%** and **99,60%** respectively.

Confirmatory methods have identified false reactive samples i.e. two in HBsAg and eight in anti-HCV for VITROS®; 3 anti-HCV for Elecsys®. One HIV samples indicated reactive with Elecsys® was found reactive with only one HIV-2 specific antigen on antigen confirmatory test, which was non-reactive on VITROS® 3rd generation HIV assay.

Summary: The observed performance of Roche Elecsys® assays on cobas® e411 is comparable to VITROS® ECi assays in blood donor screening. Minimizing false reactive cases is important to reduce burden of confirmatory assays.

P-257

THE ROLE OF PROFICIENCY TESTING AT MBARARA REGIONAL BLOOD BANK, UGANDA

P Wabuyi and S Ssenyonga

Blood Transfusion Services, Kampala, Uganda

Introduction: Proficiency testing is a way of assessing the ability of laboratories in their competence to perform specific tests and measurements. This further provides an external audit for laboratory's own internal quality control procedures. In blood bank transfusion laboratories, Serological tests are used to screen for major transmissible viruses which is critical in blood bank safety precautions. The actual performance in Sub-Saharan Africa is not well documented as measured by proficiency testing. For this reason, Mbarara Regional Blood Bank in Uganda, participated in research conducted by Anglophone Africa Transfusion Research Group in order to determine the quality of our test results and competence in performing test procedures.

Study Design/Methods: We were among the 44 laboratories which participated in the external quality assessment of Blood transfusion laboratories. We received Blinded test panels of 25 serum samples pedigreed for HIV, HBsAg, HCV and negative status. We tested using our routine donor screening methods which was ELISA under the same conditions.

Results: Of 25 serum samples for HIV, HCV, and HBsAg, quality score was 96%, 100% and 92% respectively. Sensitivity was 83%, 100% and 71% respectively with total of 84% sensitivity. Specificity for all tests was 100%.

Conclusion: Specificity was high in all tested parameters but sensitivity was variable in HIV and HBsAg. This indicates that quality control of these tests is still wanting most especially in Uganda where prevalence of transfusion transmitted viruses is high.

P-258

IMPROVE THE MANAGEMENT OF NONSPECIFIC (O UNCONFIRMED) REACTIVITY OF VIRAL SCREENING ASSAYS FOR BLOOD DONOR TESTING IN NORTH ITALY

L Raffaele[1], M Spreafico[1], I Guarnori[1], B Foglieni[1], A Vignoli[1], A Copeta[1], DD de Tommasi[1], L Pecoroni[1], A Lobbiani[1], D Campisi[2], R Trotti[3], P Isernia[4], C Rinaldini[1], M Marini[1] and P Daniele[1]

[1]ASST, Lecco, Italy [2]ASST Grande Ospedale Metropolitano Niguarda, Milano, Italy
[3]Fondazione IRCCS Ca Granda Ospedale Maggiore Policlinico, Milano, Italy
[4]Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

Background: One year after the reorganization of the blood transfusion network in North Italy and the centralization of processing, qualification and validation activities, within the CLQV a technical discussion panel has been established with the aim of defining a regional standard for the blood investigation. In the screening of blood and hemocomponent units there is the need of a proper evaluation in the choice of the tests for each TTI (HBV-HIV-HCV-LUE), in terms of sensitivity and specificity as well as of accuracy, with the purpose of maximizing the transfusion safety and minimize the number of units discarded due to non-specific positive results.

Methods: A retrospective analysis has been carried out on the blood donors who showed up in 2015 at the 9 CLQV in North Italy. The study has been conducted on 294.003 units: the screening has been executed in chemiluminescence and NAT test. To confirm the initial reactivity rate Immunoblotting, Neutralization, second CLIA line and PCR have been used. Serological tests with result s/co≥1.00 were classified as Initially Reactive (IR); those with result 0,7 ≤ s/co≥0,99 as Grey Zone (GZ) and subject to the same protocol as IR samples. Furthermore, a nominal positivity range for Positive Predictive Value (PPV) specific for each antigen was defined, and all samples with result s/co in this range were classified as GZ-positive.

Results: Out of 1.148 IR samples we found an average percentage of GZ-negative samples of 0,06–0,03–0,07–0,04 for LUE-HBsAg-HCVAb-HIV1-2Ab/Ag respectively. None of the samples was confirmed positive after the follow-up tests, except one left as inconclusive. With the GZ-positive samples we found an average percentage of 0,15-0,11-0,21-0,25 for LUE-HBsAg-HCVAb-HIV1-2Ab/Ag respectively. On average, between 93% and 100% of the samples were not confirmed by the follow-up tests.

Conclusions: As expected in each low-prevalence population, the screening positive predictive value for HIV-HBV-HCV-LUE infections is low, and a significant number of units is discarded with the resulting temporary or definitive suspension of the donor. The results of the study show the need of evaluating the choice of methodologies, especially for HCV and LUE, and of limiting the range of the so-called GZ-negative zone, with the only aim of verifying the test accuracy. Within this investigation, a different approach would have resulted in the recovery of 318 units. By using a second diagnostic line for the GZ-positive samples, in case of non-confirmed IR, it would have been possible to recover about 600 units. In addition, it is proposed to investigate the IRs of LUE test with the two different types of treponemal and nontreponemal test, validating the unit in case of negative result. The adoption of agreed and standardized validation algorithms could allow the recovery of most of the units with non-specific positivity result were classified as GZ-positive.

P-259

POST DONATION COUNSELING AND ITS IMPACT ON COMMUNITY AWARENESS ABOUT TRANSFUSION TRANSSIMETED INFECTIONS (TTI): EXPERIENCE AND ROLE OF DEPARTMENT OF BLOOD BANKS SERVICES (DBBS), SULTANATE OF OMAN

K Al-Harthy, J Hamdalla, T Ashraf and S Alhashami

Central Blood Bank, Muscat, Oman

Background: All donated blood should be screened for markers of TTI to ensure the safety of the blood components for therapeutic purposes. In the case of reactive screening results, confirmatory testing performed to identify infected donors before the donors are notified about their infectivity status. Effective confirmation requires appropriate and well-designed confirmatory testing strategies for each TTI, including the selection of assays and algorithms for the analysis and interpretation of results. As per the current protocol of DBBS, each reactive donor is informed about the abnormal test results, counseled, repeat sampling and referred for further confirmation and management to the concerned specialty. So, the study will evaluate experience and role of DBBS, Sultanate of Oman in post donation counseling.

Aim: To evaluate the response rate of TTI reactive donors after notification of their abnormal test results.

Method: This is an observational retrospective study in the DBBS, Sultanate of Oman over a period of 1 year (2015).The central blood bank (CBB) provides blood and blood components for the patients after mandatory testing for TTI that includes HBV, HCV, HIV, Syphilis and HTLV before labeling it as sero-negative.
The study will review:
The prevalence of TTI among the blood donors at CBB, DBBS, Sultanate of Oman
The response rate of TTI-reactive donors for notification of their abnormal test results
Donors perceived understanding of the necessary information.
Whether the donor followed the advice (return -rate).
In case of reactive donor for any marker, the counselor inform the donor (three phone calls and one SMS massage) about abnormal test result (as per communication, Confidentiality is Maintained) with an advice to report to the blood bank for one - to one counseling (interview) and repeat sampling as well as for referral for further management.
Results: During 2015
Among the 26,173 donors 1848 (7%) were found to be reactive in the screening tests (the data is presented only for HBV, HCV, and Syphilis).
1311 (71%) Anti-HB core + Anti-HBs and/or Anti-HBe (past infection)
200 (10.8%) Isolated core
174 (9.4%) HBs Ag + HBs profile
42 (2.3%) Isolated HBs Ag
85 (4.6%) Anti HCV
36 (1.9%) Syphilis
100% phone calls & SMS massage were sent
1706 (92.3%) donors responded
142 (7.7%) not reachable.
1243 (67.3%) donors counseled
262 PCR done and 83 referral to other health sectors
More than 90% donors stopped donation after counseling
Conclusions: DBBS succeeded to:
1. Increase awareness and education about TTI.
2. Recruitment of voluntary non remunerated safe, healthy blood donors (to maximize blood safety).
3. Reduces the burden on the national health system.
4. Early diagnosis, treatment, delaying and / or preventing progression of disease process.
5. Encouragement to take the appropriate vaccinations (contacts).
Also developing and implementing a post -donation counseling protocol to provide appropriate education, follow up, and referral, to be utilized nation-wide.

P-260

CHANGING FROM TIGRIS TO PANTHER – A LABORATORY PERSPECTIVE

C Pistorius

WPBTS, Pinelands, South Africa

Background: Western Province Blood Transfusion Service (WPBTS) performs testing on each blood donation. Virology testing includes testing for HIV, Hepatitis B (HBV) and Hepatitis C (HCV). In 2005 South Africa introduced individual donor nucleic acid testing (ID-NAT) on the Tigris platform. ID-NAT is performed in conjunction with serological testing. Due to a high prevalence of Hepatitis B (HBV) and HIV mini-pool testing is not practical.
At WPBTS, ID-NAT was initially introduced on the Tigris analysers (Griffols) in 2005, which were changed to the Panther analysers (Griffols) in 2014.
Aim: The change from the Tigris analysers to the Panther analysers involved a number of process changes as these analysers are fundamentally different. How did these process changes impact on WPBTS? Are there identifiable benefits or drawbacks for WPBTS from the changeover to the Panther analysers from the Tigris analysers?
Method: Data was compared from the Tigris and Panther analysers. Data from the Tigris analysers was collected between March 2013 and March 2014. Data from the Panther analysers was collected between March 2014 and March 2015. The data gathered was not limited to instrument performance and included data regarding functionality, timesaving and efficiency.
Results: The results obtained can be summarised in these five different categories:
Assay performance
The Panther analysers are able to detect HIV-1/2 RNA using the Ultrio Elite Assay whereas the Tigris analysers were only able to detect HIV-1.
Greater specificity has been shown on the Panther as compared to data from the Tigris.
Cost
The Tigris analysers require both controls and calibrators for each run. The Panther analysers require calibrators to be run once every 24 h and no controls. By eliminating the need for extra calibrators and controls, costs were reduced.
Capacity
The Tigris analysers have a larger waste capacity than the Panther analysers. Each Tigris analyser has a waste capacity of 1000 tests, whereas each Panther analyser has a waste capacity of 750 tests.
Efficiency
The turnaround time for samples using the Panther analysers has been shortened when compared to the turnaround time using the Tigris analysers.
Flexibility
The Tigris analysers are batch system analysers. The Panther analysers are random access analysers. Random access analysers greatly increase flexibility.
Conclusion: The Panther system has greatly decreased the turnaround time of urgent samples, in addition it allows more instrument and assay flexibility.

P-261

RETROSPECTIVE PILOT STUDY TO DETERMINE IF HIGHER RISK EXISTS IN THE DONOR POPULATION THAT HAS INDICATED THAT THEY HAVE BEEN EXPOSED TO HEPATITIS B VERSUS NORMAL ROUTINE DONOR POPULATION

C Pistorius

WPBTS, Pinelands, South Africa

Background and Objectives: Western Province Blood Transfusion Service (WPBTS) is a blood collection, testing and distribution service situated in the Western Cape of South Africa. The race distribution within the Western Cape is different from that of South Africa as a whole (Table 1).
Donor samples are routinely screened for HIV, hepatitis B and C. The prevalence for hepatitis B in South Africa has been reported as high as 18% in some parts of South Africa. If a donor indicates that he/she has had hepatitis after the age of 13, supplementary testing is performed. Due to the fact that most cases of acute hepatitis are subclinical and only a few cases are diagnosed, a need was identified to determine if a higher risk exist in donors that have indicated that they have been exposed to hepatitis, vs donors that have indicated that they have not been exposed to hepatitis.
Materials and methods: 200 random routine negative donors (cohort A) and 200 routine negative donors who have indicated that they have had hepatitis (cohort B) during 2013–2014, were included in this study. Black donors were not included in this study as insufficient data for this race group was available due to limited number of donors.

	White Cohort A	White Cohort B	Mixed Race Cohort A	Mixed Race Cohort B
Total	100	100	100	100
HBc Only	1	2	3	6
HBs Only	15	13	11	9
HBs & HBC Neg	81	80	75	68
HBc Pos and HBs >100	3	3	10	11
HBc Pos and HBs <100	0	2	1	6
Reinstated	99	96	96	88

Caption 1 Results (table 2)

	South Africa	Western Cape	WPBTS donors
Black	79%	33 %	5%
Mixed Race	9%	49%	34%
White	9%	16%	60%
Indian	3%	1%	1%

Caption 2 Racial Distrubution (Table 1)

Each cohort of 200 donors consisted of 100 mixed race and 100 white donors. Hepatitis B core antibodies (anti-HBc), hepatitis B surface antibodies (anti-HBs) were performed on the Architect (Abbott) *i2000* on all the donors. A comparison was then drawn between the 2 cohorts to determine whether or not greater risk, to the blood supply, exists when a donor indicates that they might have been exposed to hepatitis.
Combined presence of Anti-HBc with Anti-HBs levels lower than 100 mIU/ml, were used as markers for hepatitis B risk and these donors were asked not to donate. Routine negative donors with anti-HBs only, anti-HBs ≥ 100, or no anti-HBs or anti-HBc were reinstated and were able to donate.
Results: Average risk associate with cohort A is 2.5%
Average risk associated with cohort B is 8%
Conclusions: After extrapolating these 2 populations to represent that portion of our donor population, it was found that donors who have indicated that they have had hepatitis after the age of 13 do in fact carry a higher risk to our blood supply. Therefore our current screening approach has been proven to be effective.

P-262

PARALLEL EVALUATION OF ASSAY PERFORMANCE AND INSTRUMENT SUITABILITY TO DETERMINE POSSIBLE REPLACEMENT FOR THE CURRENT BLOOD BANK SCREENING SYSTEM

RT Russel
WPBTS, Pinelands, South Africa

Background and Objectives: To ensure the safety of donated blood, screening for transfusion-transmissible infections is mandatory and requires assays with exceptional sensitivity and specificity. The current study evaluated the performance of serologic assays for hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV), designed for use on their respective automated immunochemistry analyzer.
At Western Province Blood Transfusion Service (WPBTS), the Prism system (Abbott Diagnostics) has been the front-line screening system for over 19 years. The Architect i2000 system (Abbott Diagnostics) was used for confirmation. This evaluation study was to determine if any of the evaluated analyzers would be a suitable replacement for the Prism.
Materials and methods: Routine samples from approximately 5000 repeat and 2000 first-time donors were screened using the relevant immunoassays on the cobas e601 (Roche Diagnostics), Liaison XL (DiaSorin), Architect *i*2000 (Abbott Diagnostics) and Evolis systems Bio-Rad). These were compared with routine screening assays currently employed on the Prism system (Abbott Diagnostics). In parallel, all donor samples were screened for HBV, HCV and HIV-1/2 using a multiplex nucleic acid test (NAT). NAT was performed on the Panther system (Hologic/Grifols). Confirmatory testing was performed using western blots (HIV-Ab), immunoblots (HCV-Ab) and neutralization (HBsAg).
Subjective evaluations were performed by the operators on each analyzer to determine the suitability of operations of each instrument. Six areas were identified and a weighted value (1 to 7) was scored by the operators.
Results: The specificity of all immunoassays was >99.5% in both first-time and repeat donors. The majority of the assays showed comparable seroconversion sensitivity. On seroconversion panel PHV925, the Elecsys Anti-HCV II assay detected HCV infection 19 days earlier than comparator assays.
The Architect scored the highest in the subjective evaluation while the Evolis scored the lowest.
Comment/Conclusion: All assays showed high specificity and sensitivity. Significant differences between new and routine assays were not detectable when screening repeat donors, indicating that preselection bias would not be a barrier to updating the screening system.
To replace the current system, a total automated solution was required, i.e. a standalone instrument would have insufficient capacity. The replacement system would require multiple analyzers and pre-analytics, joined by a tracked system. This would automate many of the current manual processes, create extra capacity in case of instrument downtime and allow for future-proofing. Software of the evaluated systems allowed for multitasking and was far more flexible than the Prism system.
The eventual decision was between Roche (PVT and 2 x c8000 systems) and Abbott (2 x Prisms). WPBTS decided to replace the Prism system with the Roche system.

P-263

DETECTION OF BACTERIAL GROWTH IN BLOOD COMPONENTS: EVALUATION OF THE ENHANCED BACTERIAL DETECTION SYSTEM BY MICROBIOLOGICAL CORRELATION IN AN ONCOLOGY SETTING

V Bhat, RS Kelkar and SK Biswas
ACTREC- Tata Memorial Centre, Navi Mumbai, India

Background: Bacterial contamination of blood & blood products can lead to transfusion related complications. It can occur during blood collection, processing or may be donor derived. The prevalence of bacterial contamination in blood products has been estimated to be 0.04–2% with a greater risk in platelet transfusion. Various bacteria have been implicated including Staphylococcus aureus, coagulase negative staphylococci, Streptococcus spp, Yersinisa enterocolitica, Serratia marcescens, Acinetobacter spp, Pseudomonas spp, enterobacteriaceae & Gram positive bacilli. Microbiological cultures and bacterial detection systems may be used for screening blood products.
Aims: The aim of this study was to determine the prevalence of bacterial contamination in blood products by the enhanced bacterial detection system (eBDS) and correlate this with microbiological cultures in a tertiary care oncology setting.
Methods: Single Donor Platelets (SDP) & Packed Red Blood cells (PRBC) were tested by the Pall eBDS (M/S PALL Life Sciences - Pall Medical, Portsmouth, England - Enhanced bacterial detection system). The samples were inoculated into the test pouch provided. Based on the level of oxygen consumption after 30 h incubation at 35°C, results were interpreted as 'Pass' or 'Fail' by the system. All units were also cultured in commercial Blood culture systems Bactec 9050 (Becton Dickinson, USA) as per standard microbiological procedures.
Results: A total of 2995 blood products (1871 SDPs and 1124 PRBCs) were tested by using the eBDS system. Of these, 20 (0.67%) tested as "Fail"which included 9 (0.48%) SDPs & 11(0.98%) PRBCs. Seven (4 SDPs & 3 PRBCs) of these 20 blood products grew microorganisms on bacterial culture. The isolates included Staphylococcus aureus (2), Acinetobacter baumannii (1), coagulase negative staphylococci (2), Enterobacter cloacae (1), and Bacillus spp. (1).
Conclusion: The eBDS system may be used as a screening method for detection of bacterial contamination in SDPs and PRBCs; however, it is associated with some false positivity as shown by the culture negativity of some of the failed eBDs blood products.

P-264

EFFICIENCY OF TRANSCRIPTION-MEDIATED AMPLIFICATION (TMA) IN DETECTING HEPATITIS E IN BLOOD DONORS FROM REGIONAL CENTRE OF TRANSFUSION MEDICINE AND BLOOD BANK IN WARSAW – A NEW RISK IN TRANSFUSION

J Gdowska[1], E Sulkowska[2], P Grabarczyk[2], R Galik[1], D Piotrowski[1] and M Wasiela[3]
[1]Regionalne Centrum Krwiodawstwa i Krwiolecznictwa, Warszawa, Poland [2]Instytut Hematologii i Transfuzjologii, Warszawa, Poland [3]Zaklad Patofizjologii i Immunologii Klinicznej,Wydzial Wojskowo-Lekarski UM Lódz, Lódz, Poland

Background: Safe blood and safe blood products are important for public-health. Various serological and molecular studies (have shown) that hepatitis E (HEV) is a new growing problem in transfusion-related infection in many countries. Usually, the HEV infection is asymptomatic and therefore risk of transmission of infection with the donation may be high. The epidemiological situation in the Polish population is unknown. We present the preliminary study on HEV RNA prevalence in blood donors from Regional Centre of Transfution Medicine and Blood Bank in Warsaw, Poland (using Procleix HEV Assay on fully automated Procleix Panther System '"Hologic/Grifols").
Aim: Evaluation of HEV prevalence in Polish population on the basis of blood test of donors registered in Regional Centre of Transfution and Blood Bank in Warsaw.
Methods: Plasma samples of blood donors were collected from November 2014 to December 2015. EDTA plasma aliquots were stored at −25°C temperature. 7174 plasma samples were analyzed retrospectively and individually with Procleix HEV Assay (95% limit of detection 7,9 IU/ml). The Procleix HEV Assay is a qualitative *in vitro* NAT test,based on transcription-mediated amplification assay (TMA). Initial TMA reactive samples were retested 6–7 times and were tested for HEV antibody using anti-HEV IgG and anti-HEV IgM Elisa Kit (Wantai Biological Pharmacy Enterprise Ca, Beijing China). Positive-HEV IgG,IgM reaction was confirmed with Recom-Line HEV IgG/IgM Mikrogen Diagnostic,German.
Results: Total of 7174 screening samples was tested (including 6141 sample from first-time donors and 1033 samples from repeat donors) with the Procleix HEV Assay .HEV RNA was detected in 7 donors (7 initially reactive sample). 4 of them (3

first donors and 1repeat donors) were repeat reactive. Prevalence in first- time blood donors was 1 in 2047, for repeat donors was 1 in 1033.
Summary: Preliminary results show that the Procleix HEV assay based on TMA is useful for diagnosis of HEV infection in blood donors. Our study results that HEV infection is common in the Polish population. We presume that further epidemiological and clinical studies are necessary for protection of patients are treated with blood and blood products.

P-265

SIXTEEN MONTH EXPERIENCE WITH THE COBAS® MPX TEST ON THE COBAS® 8800 SYSTEM IN A ROUTINE SCREENING SETTING

M Stolz, P Gowland, S Pfister and C Niederhauser

Interregional Blood Transfusion SRC, Berne, Switzerland

Introduction: In January 2015 the cobas® MPX test using the cobas® 8800 system replaced the Procleix® Ultrio assay on the Procleix® Tigris system. The new platform is used for the routine screening of Swiss blood donors for HIV-1, HIV-2, HCV and HBV in an individual donation format (ID-NAT).
Methods: For every blood donation a dedicated 9 ml EDTA plasma tube is taken for the screening with the cobas® MPX test. The samples are tested in an ID-NAT format according to the instructions in the package insert. Confirmation of repeat reactive samples was performed serologically (Abbott Architect) and with an alternative NAT assay (Abbott Real Time PCR Assay).
Results: A total of 214'756 donations were tested since January 2015. 351 donations (0.16%) were reactive in the initial screen:174 HIV (50%), 107 HCV (30%), 70 HBV (20%). From these, 27 samples (0.013%) were repeat reactive (3 HIV, 8 HCV, 16 HBV) and were confirmed serologically and with the other NAT assay. The other 324 samples were false positive. Five donations remained inconclusive. 10'286 sample results were invalid (4.5%), the majority (7'185 samples, 3.2%) due to invalid runs (139 from a total of 4'475 runs). Of the remaining invalid samples 2'503 (1.1%) had an invalid test result. Most of these (87.7%) were due to an impaired amplification, 10% detected clot and 2.3% various other reasons. 322 samples were invalid in repeat runs (0.26%). The invalid runs were void for the following reasons: 67 invalid run controls (48.2%), 29 hardware failures (21.6%), 17 clotting events (12.2%), 15 reagent dispensing problems (10.8%) and 10 other technical reasons (7.2%).
Conclusion: The performance of the cobas® MPX test compares with the data provided by the manufacturer and was supported by our own validation data. The rate of initial positives observed, 0.16% with the cobas® MPX test, is higher compared to our previous Procleix® Tigris system (0.11%). In our setting the cobas MPX test has a repeat reactivity rate of 1: 13 and a specificity of >99.88%. Most of the initial reactive samples could not be confirmed and are regarded as "false"positive results. The number of invalid samples is similar to that observed with the former NAT system (4.2% Procleix® Tigris vs 4.5% cobas® 8800). The majority of the invalid test results were due to an impaired amplification profile and not caused by inhibition events, since dilution steps remain mostly ineffective. The stability of the cobas® 8800 system (3.2%) has surpassed the performance of the Procleix® TIGRIS system (3.6%), which is impressive for such a complex instrument. Although 75% of the invalid results are due to failed test runs, only 21.6% are actually caused by hardware events. Most of the failed runs are caused by invalid run controls mainly the MPX2 (+) C. Improvements in the test algorithm will reduce the number of initially invalid samples and run controls.

P-266

EVALUATION OF THE ROCHE COBAS® DPX TEST PERFORMED ON THE COBAS® 6800 SYSTEM AND COBAS P 680 INSTRUMENT FOR PARVOVIRUS B19 DNA DETECTION IN BLOOD DONORS

A Kopacz, D Kubicka-Russel, G Liszewski, E Sulkowska, M Letowska and P Grabarczyk

Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Background: The cobas® DPX test (DPX test) for use on the cobas® 6800 System, is an *in vitro* nucleic acid amplification technology (NAT) test for the identification and quantitation of human parvovirus B19 (B19V) DNA and the detection of hepatitis A virus (HAV) RNA in human plasma. Samples can be tested individually or optionally in pools comprised of aliquots of 6–96 individual donations. The instrument dedicated for creating pools for DPX assay is the cobas p 680 instrument. B19V testing in Poland is recommended for plasma for anti-D and anti-HBs production and for donors of red cells used for immunization. In addition, plasma for fractionation has to be tested for B19V with sensitivity that ensures the content of less than 10^4 IU DNA B19V/ml in plasma production pool - as recommended by European Pharmacopoeia.
Aim: To evaluate the cobas® DPX test and to demonstrate its ability to detect HAV and polymorphic forms of B19V.
Material and Methods: The analytical sensitivity of cobas® DPX test was assessed by testing dilutions of the WHO B19V Genotype 1–3 Reference Panels (09/110) and IS WHO RNA HAV (00/562) dilution panel. Six dilutions of each B19 panel were tested ranging from 31.6 IU/ml to 0.1 IU/ml of genotype 1, 2, 3 and six dilutions of RNA HAV panel ranging from 10 to 0.03 IU/ml (24 replicates/dilution). Detection and identification of reactive donations tested in minipools was evaluated by testing 767 negative and 1 reactive donations in 8 pools (7 pools of 96 donations and one of 95 donations).
Results: The 50% and 95% limits of detection (LOD) of the cobas® DPX test for DNA B19V were respectively: genotype 1–0.94 IU/ml (0.63–1.38) and 8.70 IU/ml (4.92–21.69), genotype 2–6.25 IU/ml (4.41–9.00) and 37.80 IU/ml (22.24–93.65), genotype 3–6.92 IU/ml (5.04–9.59) and 29.84 IU/ml (19.04–65.60) and for RNA HAV were: 0.13 IU/ml (0.09–0.18) and 0.82 IU/ml (0.49–1.96) respectively. The cobas® DPX test results from the tested samples in minipools were 100% concordant with the expected results. However, sample contaminated with B19V DNA $>10^9$ IU/ml (which exceeds the test linear range) needed to be diluted 1000 fold in order to obtain valid, reactive result. No false reactive or invalid results were observed during donation screening.
Conclusion: The cobas® DPX test allows to identify donations infected with HAV and genotypes 1–3 of B19V that could result contamination of plasma production pools with HAV and B19V beyond acceptable level ($>10^4$ IU/ml, according to recommendations).

P-267

INTRODUCTION OF THE COBAS 6800/ COBAS 8800 INTO ROUTINE MP-NAT BLOOD DONOR SCREENING AND FIRST EXPERIENCE ON A ROUTINE BASIS

K Hourfar, K Gubbe, B Rüster, K Frank, A Karl, T Tonn, UMayr Wohlfart, H Schrezenmeier, E Seifried and M Schmidt

German Red Cross, Frankfurt, Germany

Background: Mini-pool NAT was introduced into routine blood donor screening in 1997 at the German Red Cross Baden-Württemberg - Hesse and Saxony for HBV, HCV and HIV-1 by an inhouse PCR system. The inhouse method was CE certified in 2006. Due to increased future requirements to CE certification the blood donor services introduced the Roche Cobas NAT system on the Cobas 6800 and Cobas 8800 platform in 2015.
Aim: The aim of this study was the qualification of the Roche Cobas NAT system at the test laboratories in Frankfurt, Ulm and Plauen and the subsequent introduction of the new NAT system into routine in 2015.
Methods: The analytical sensitivity was evaluated by probit analysis with WHO standards for HAV, HBV, HCV, HIV-1, B19, HEV and WNV (PEI standard) tested in serial dilution steps of at least 144 samples in six different concentrations. The diagnostic specificity was tested by negative blood samples in parallel to the test of record.
Results: The analytical sensitivities were 98.6 IU/ml, 170 IU/ml, 1487 IU/ml for HBV, HCV and HIV-1, respectively related to the individual donation within a minipool screening of 96 samples per pool. The new NAT system was introduced in December 2015 in Frankfurt and in January in Plauen and Ulm. Up to now 12,358 pools were screened for the MPX and DPXand for limited sample pools in addition for HEV and WNV. Inhibited pools were observed in 0.44%. The percentage of false positive test results was 0.06%.
Conclusions: The validation data were slightly better than the data from the manufacturer. The system convinced by an easy handling, a reduced hands on time and

MPX + DPX	Frankfurt	Plauen	Ulm	Total	(%)
Pools tested	21010	2948	381	24339	
Inhibitions	88	8	13	109	0,45
unspecific pos	12	3	1	16	0,07

Table 1

an extended walk away time. Results were given as negative or positive for qualitative parameter or with a concentration for B19. Access to amplification curves would be desirable in order to improve the interpretation of reactive test results. The utility channel enables blood donor services the integration of new parameters for emerging pathogens and is a topic for further studies.

P-268

EVALUATION OF THE ROCHE COBAS® MPX TEST PERFORMED ON THE COBAS® 6800 SYSTEM AND COBAS P 680 INSTRUMENT FOR HCV RNA, HIV RNA AND HBV DNA SCREENING IN BLOOD DONORS

A Kopacz, D Kubicka-Russel, G Liszewski, P Zwolinska, E Sulkowska, M Letowska and P Grabarczyk

Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Background: The cobas® MPX test for use with the cobas® 6800 System is a qualitative, real-time PCR test for the detection of HIV-1 Groups M and O RNA, HIV-2 RNA, HCV RNA and HBV DNA in human plasma and serum. The test simultaneously identifies the virus (HIV, HCV and HBV). In addition, two different HIV-1 regions are amplified and detected, dual probes for HCV improves coverage of new virus variants. Optional component to the cobas® 6800 System is the cobas p 680 instrument that creates pools from individual samples.
Aim: To evaluate the cobas® MPX test in individual donations and in plasma pools using the cobas p 680 instrument.
Material and Methods: The analytical sensitivity was assessed by testing six, serial dilutions of the WHO International Standards (IS) in 24 replicates for each dilution. The basic dilutions tested were: 31.6–0.1 IU/ml for HCV IS 06/102, 100–0.32 IU/ml for HIV-1 IS 10/152 and 10–0.03 IU/ml for HBV IS 10/264. For HBV WHO IS 10/264 an extra dilution panel created based on CLSI EP17-A2 norm were tested, each dilution: 3, 16; 1, 78; 1; 0, 72; 0, 516; 0, 316 IU/mL in 20 replicates. Clinical sensitivity was evaluated by testing plasma from Polish donors with confirmed infection: 6 HBV (seropositive/DNA positive), 6 HCV in window phase (WP) and 8 HIV WP samples, neat and diluted 1:6 to simulate minipool testing (MP6). Six replicates of each neat and diluted sample were tested. In addition, 12 replicates of the HIV-2 WHO IS 08/150 (31.6–0.316 IU/ml), and HIV-1 group O isolate Performance Panel (100 and 3,16 copies/ml) were tested. Correct identification of reactive donations was evaluated by testing 473 negative and 7 reactive donations in 82 pools (77 MP6 and 5 short pools).
Results: For basic dilution panels the 95% limits of detection (95% confidential limits) were 3.97 IU/ml (2.25–9.75), 7.12 IU/ml (4.40–15.62) and 31.33 IU/ml (17.84–76.80) for HBV, HCV and HIV-1 respectively. For extra HBV dilution panel ranging 3.16-0.316 IU/mL 95% LOD equaled 1,33 IU/mL (0.89-4.25) that was the nearest to manufacturer information. Analytical sensitivity in Probit analysis calculated on all tested HBV IS dilutions came to 2.05 IU/mL. All the Polish seropositive+/DNA HBV+ genotypes A, D and H cases and NAT yield cases: HCV genotypes 1b, 3a and 4, HIV-1 subtype B genotypes were detected when tested neat and diluted. The viral loads in individual donations ranged from 6.9 to 3.15×10^3 IU/ml, $3{,}3 \times 10^4$ to 1.97×10^6 IU/ml, and 7.05×10^2 to 5.61×10^4 IU/ml for HBV, HCV and HIV respectively. All dilutions of the HIV-1 group O and HIV-2 samples were detected. All infected donations were correctly identified during testing in pools (MP6) and in resolution pools of 1 (ResP1). In the entire evaluation the invalid and false reactive pool rates were 1% and 0.1% respectively.
Conclusions: The cobas® MPX test performed on cobas® 6800 System, including the and cobas p 680 instrument demonstrated high sensitivity, satisfactory performance and increased operational efficiency. Results for individual donations indicate the kind of virus detected, thus there is no need for further discriminatory tests.

P-269

REDUCING TESTING COSTS IN THE VIROLOGY LABORATORY – AN HOLISTIC APPROACH

C Pistorius

WPBTS, Pinelands, South Africa

Background: Western Province Blood Transfusion Service (WPBTS) performs stringent testing on each blood donation. In the Virology laboratory, routine screening includes testing for HIV, hepatitis B (HBV) and hepatitis C (HCV). Individual donor testing (IDT) is used for both the serological (c8000 system - Roche Diagnostics) and nucleic acid testing {(NAT - Panther system (Grifols)}. WPBTS screens about 160,000 donations per annum.
WPBTS is a non-profit, independent organisation and is driven to improve and streamline processes and procedures in order to decrease costs, without compromising the safety of the blood supply.
Aim: Identify areas where costs could be reduced and quantify those reductions.
Methods: Laboratory procedures were reviewed holistically. Five areas were identified for modification to reduce costs. These included the manner in which data was obtained from the analytical instruments, modifying the testing algorithms, changing the way tests were requested from the instruments and the advantages of changing the NAT platform from Tigris to Panther. Costs were re-calculated and compared to costs of the previous methods. Efficiencies were determined.
Results: Costs were reduced in the following areas:
Paperless
The current in-house laboratory Information system (LIS) was modified and enhanced. As a result the Virology laboratory is now paperless on both the Panther and Roche systems, i.e. results are automatically transmitted from the instruments through middleware and then onto the WPBTS network. Results are no longer printed. (saving: R 13,224 per annum).
Serological testing
The serological platform allowed more automation than the previous system. This enabled the testing algorithm to be incorporated into the software. As a result, initial reactive samples were automatically reflexed for a second test. If the sample was repeatedly reactive, no further testing was performed on the screening platform. Previously, initially reactive samples would require duplicate repeat testing. (saving: R 41,710 per annum).
Supplementary testing was introduced onto the front-line serological system. Previously these tests were performed on the second-line instrument and only once a week. By introducing these tests on the Roche system, it negated the need to collect an extra sample, reducing costs and turn-around times (saving: R 786 per annum).
Bi-directional LIS
For the first time, a bi-directional LIS was introduced in the Virology laboratory. This enabled the LIS to request the tests from the analysers, for each donor. Unnecessary testing was avoided as certain donors only required specific tests. (saving: R 73,559 per annum).
NAT Testing
The introduction of the Panther analysers increased efficiencies as they no longer required run controls and calibrations were valid for 24 h. This meant more tests were used on donor samples rather than on controls and calibrations. Panther offers random access and shorter processing times. (saving: R1 393,307 per annum).
Conclusion: The holistic approach to cost reduction resulted in a saving of more than R1 500,000 per annum. WPBTS not only saw a reduction in the operating costs to the company, but has also was rewarded with a more efficient, leaner laboratory.

Hepatitis B (HBV)

P-270

HEPATITIS B VIRUS PROFILE IN FOLLOWED UP THE ID-NAT HBV REACTIVE DONORS

K Chaiwong, S Oota, A Nontapoaraya and U Charoonruangrit

National Blood Center, Thai Red Cross Society, Bangkok, Thailand

Background: National Blood Centre, Thai Red Cross Society began using the individual of nucleic acid test (ID-NAT) on 18 December 2015 and shared ID NAT with mini pool of 6 at the beginning stage. Later on ID NAT was fully implemented since 7 Jan 2016, HBV NAT yield rate was 1: 600 which is higher than MP NAT (1: 3361) in previous period. Then blood and its component were discarded more than 5 times from the past. The reactive donors came back for collect new sample for retest ID NAT and confirm with HBV profile.
Aims: To analyze the results of followed ID-NAT HBV reactive donors since Jan 2016, retest with ID NAT HBV and confirm HBV reactive result with HBV profile (anti-HBc IgG& IgM, anti-HBs, anti-HBe, ALT) before counseling.
Methods: Data of HBV ID NAT and HBV profile in followed donors between 1 JAN - 31 MAR 2016 were collected and analyzed.
Results: According to 303 HBV NAT repeated reactive donations, all donors are followed up and discriminated into two groups which are 244 Non-reactive donors and 59 reactive donors. The results of HBV profile in 244 non-reactive donors were positive for anti-HBc IgG (189/244), anti-HBs (180/244) and anti-HBe (82/244). Whereas, in 59 reactive donors were 54/59, 35/59 and 20/59 respectively. Among all followed

up NAT reactive donor, the majority (30%) of them had anti-HBc IgG, anti-HBs positive and anti-HBe Negative with non-reactive NAT. Followed by 22% had positive result for anti-HBc IgG, anti-HBs and anti-HBe but showed non-reactive for ID NAT. While, 32% of HBV NAT reactive donors showed positive result for anti-HBc IgG, anti-HBs but negative for anti-HBe.

Summary/Conclusions: Total followed up donors showed negative results for HBV profile in 35 (12%) NAT non-reactive and 3 (1%) NAT reactive group. From all followed up donors, 243(80%) donors were positive with anti-HBc IgG and only 4 of 243 had anti-HBc IgM Positive, 215(71%) were positive with anti-HBs and 102 (34%) were positive with anti-HBe. While, 12% of followed up donors had HBV profile negative and NAT non-reactive. Then, these donors should be considered to re-entry for blood donation, or not. The expert committee shall set up for decision making and set up the guideline. Moreover, due to increased HBV ID-NAT yield rate at 1: 600. Every 600 donors with positive HBV DNA, 72 (12%) donors might show negative results for HBV profile and NAT after follow up them.

P-271

EFFECT OF VACCINATION FOR HEPATITIS B ON SAFETY OF BLOOD TRANSFUSION

M Mitsopoulos, F Kourgia, M Vini, A Tzolou, E Xanthi and E Zervou

University of Ioannina, Ioannina, Greece

Background: Hepatitis B remains a global health problem. The mandatory implementation of HBV vaccination programs contributes significantly to reducing transmission and leads to disappearance of the infection. Despite the use of highly sensitive methods for detecting HBV DNA in blood donors, HBV transmission by transfusion remains. Blood donors who have been vaccinated and have developed protective anti-HBs antibodies provide greater safety in transfusion.

Aims: To study the effect of HBV vaccination on blood safety by calculating the incidence of hepatitis B markers from previous infection in first-time blood donors who are originating from the region of Epirus in NW Greece and to compare the results with those of a similar study conducted in the years 1994–1996 (Zervou, Transfusion, 2001) as well as to determine the proportion of blood donors with protective anti-HBs antibodies after vaccination.

Methods: Six hundred and nine (441 male and 168 female) donors aged 18–65 years who were born and lived in the four prefectures of Epirus, tested for HBsAg, anti-HBc and anti-HBs. HBV markers were detected with standard, commercially available EIA (Abbott).

Results: Overall, the incidence of hepatitis B markers after infection had a statistically significant reduction in the last 20 years, particularly from 15.8% reduced to 7.7%. According to the sex the decrease in males was from 16.3% to 8% while in females from 13.8% to 7%. In males currently the largest incidence of hepatitis B markers after infection occurs between the ages of 50–59 years (23.75%) and between 60 and 65 years (18.18%) and in females between the ages of 50–59 years (30%). The presence of protective anti-HBs antibodies at a titer >10 IU/ml occurs in 66.6% of blood donors 18–29 years while the following percentages appear in other age groups: 30–39 years 34.7%, 40–49 years 26.2%, 50–59 16.3% and 60–65 years 13%.

Conclusions: The last twenty years there has been a significant reduction of hepatitis B markers after infection in blood donors of Epirus and this reduction is greater in younger donors that have the highest incidence of protective anti-HBs antibodies after vaccination.

P-272

HEPATITIS B AND SYPHILIS CO-INFECTION ARE THEY COMMON AMONG BLOOD DONORS IN THE REGION OF RABAT?

B Adouani[1], A Aguezzar[2], R Alami[2], A Laouina[1], N Bouhlal[1], G Akkazi[1], A Benahadi[1], S Boulahdid[1], A Mokhtari[3], A Soulaymani[3], A Bouyoub[1], K Hajjout[1] and M Benajiba[1]

[1]CNTSH, Rabat, Morocco [2]IFCS, Rabat, Morocco [3]FSK, Kenitra, Morocco

Introduction: Viral hepatitis B and C is a serious public health problem. The Hepatitis B Virus (HBV) and Hepatitis C (HCV) are two pathogens responsible for the majority of liver cancers (Beasley et al, 1984); WHO, 2015). In addition, these two viruses especially HBV sharing with the bacterium Treponema pallidum causes syphilis, certain modes of transmission (sexual, blood . . . etc.) so favoring of coinfections.

The purpose of this study is to calculate prevalence of the VHB at the blood donors (DDS) infected by the syphilis to the Regional center for blood transfusion (CRTS) of Rabat.

Methods: This is a retrospective analysis of DDS files and information extracted from the database of the informatique operating system (PROGESA) in 2009 and 2013.

Age of donors ranges from 18 to 60 years. The analysis for HBsAg was made by the 3rd generation ELISA and syphilis screening was done by the TPHA test (Treponema pallidum in human serum or plasma by passive haemagglutination).

Results: A total of 44136 and 60325 blood donors was selected respectively between 2009 and 2013 CRTS to Rabat.

The overall seroprevalence in 2009 of HBV, syphilis and coinfection HBV-syphilis was respectively 2.56%, 1.19% and 0.01%. Then in 2013 it was respectively 0.98%, 1.51% and 0.0016%.

We found that the rate of HBV infection was decreased contrary to that of syphilis has been increased. It remains to note that the number of co-infections was very low during the two years, compared to individual infections, hepatitis B and syphilis.

Conclusion: Despite its low rates, co infections in DDS should be analyzed with caution especially in determining the serological status of the donor and therapeutic management.

Référence: Beasley RP, Hwang LY. *Hepatocellular carcinoma and hepatitis B virus:* Semin Liver Dis.1984 May; 4(2):113–21.

Hépatite C: Aide-mémoire N°164 Juillet 2015

P-273

ANTI-HBC PREVALENCE IN THE DONORS POPULATION AT THE IMMUNOHEMATOLOGY AND TRANSFUSION SERVICE (SIT) OF TERNI: ANY ROOM FOR BLOOD DONORS SELECTION IMPROVEMENTS?

A Scaccetti, G Palmieri, D Cappuccini, M Piermarini, M Proietti, S Meschini and A Pagnani

S. Maria Hospital Trust, Terni, Italy

Background: New requirements for quality assurance and safety of blood and blood components has been releases by the Italian Ministry of Health on December 2015. This new law establishes that an individual infected by the Hepatitis B virus (HBV) must be permanently excluded from donation of either whole blood and blood components.

The presence of HBV infection it is assessed by the determination of Hepatitis B surface Antigen (HBsAg) and viral nucleic acid (HBV-DNA), markers of active infection and viral replication respectively. Despite the fact that antibody to Hepatitis B core Antigen (anti-HBc) is the marker of HBV exposure, the new requirements don't foreseen the execution of the anti-HBc testing.

Aims: The aim of this study was to define the prevalence of anti-HBc-positive blood donors and to evaluate the impact of anti-HBc testing execution on donors selection.

Methods: All serum-plasma samples from donors attending the SIT of Terni between February 13nd and April 14th 2016 were assayed for anti-HBc in addition to the mandatory screening assays. HBsAg-negative/anti-HBc-positive donors were assayed for all HBV markers by chemiluminescent technology on the LIAISON® XL system (DiaSorin S.p.A.) and for HBV-DNA using TRI-NAT TMA assay (Procleix Ultrio Elite Panther, Grifols).

For all donors, demographic information (i.e. gender, age, place of birth) and classification into first time or repeated donors were available.

Results: During the study period 312 samples, belonging to the same number of donors, have been collected.

87.1% were repeated donors, with a mean and median age of 44 and 45 years respectively. 95.5% of them were born in Italy, with an higher proportion of men in comparison to women (71.2% vs 28.8%).

6.4% of donors tested were anti-HBc-positive. Of these, 90.0% were Italian with a mean and median age of 42 years, and were mainly repeated donors (95.0%). All anti-HBc-positive donors were negative for both HBsAg and HBV-DNA. 62.5% of them were anti-HBs-positive whereas the remaining 37.5% resulted negative for anti-HBs.

Summary/Conclusions: Donors evaluated during the two months of study represent a statistically significant sample of the donor population pertaining to the SIT of Terni.

Comparing the results obtained in this study with previous investigations conducted by the SIT in the period 2004–2008, an increase of 2.0% of anti-HBc-positive donors can be observed (4.0% vs 6.0%).

Most of them (62.5%) are characterized by a serum-virological pattern due to a condition of immunization post-infection, while the remaining 37.5% are classified as 'anti-HBc alone'. This condition may refers to a situation of convalescence or previous HBV infection. Although the infectious potential of the subject 'anti-HBc alone'

is not clear, it is accepted that they should be regarded as subjects 'potentially infectious".
In the present study 1.9% of the analyzed donors are 'anti-HBc alone'. Since it cannot be excluded that the negative blood units screened for HBsAg and HBV-DNA can transmit HBV infection, it would be desirable to revise the current legislation with the addition of anti-HBc in order to better select the donors.

P-274

THE PREVALENCE OF HEPATITIS B AMONG BLOOD DONORS IN THE LAO PEOPLE'S DEMOCRATIC REPUBLIC

C Souksakhone

Lao Red Cross, Vientiane, Laos

Introduction: Southeast Asia is a high prevalence area of the Hepatitis B virus (HBV). Two reports show that the positivity of Hepatitis B surface antigen (HBsAg) in first time blood donors in Laos is 8.7% and 9.6%.[1,2] Because of this high prevalence of HBV, the Laotian government started a HBV vaccination program for newborns in 2002. Recently Xeuatvongsa et al[3] reported that the prevalence of HBsAg is not high in Laos since both mothers (15–45 years) and infants (5–9 years) taken randomly from selected districts in the whole country show positivities of 2.9% (95% confidence interval: 1.7%-4.2%) and 1.7% (95% confidence interval: 0.8%-2.6%) respectively.
In this report, HBsAg positivity was calculated according to age, gender and number of blood donations among donors at the Lao Red Cross National Blood Transfusion Center in Vientiane, Lao PDR.
Methods: HBsAg positivity was calculated from routine screening test results for all blood donors (total 18,612 donors: 16,168 volunteer blood donors and 2444 family donors) at the NBTC in 2014. HBsAg was screened by commercial ELISA kits, Monolisa HBsAg Ultra (Bio-Rad Laboratories, Inc.).
Results: HBsAg positivity for first time donors and repeat donors (2 and more times) was for males 10.3% and 2.5%, and for females 4.2% and 0.8% respectively. The average positivity of first-time blood donors was 7.4%. Males were more than 2 times higher than females, Young blood donors showed high positivity; especially 17 and 18 year old males with 19.3% rate levels. Overall, positivity decreased with age; young were higher than the elderly.
Discussion: First-time donor positivity in Laos was high (7.4%) but this is dependent on age and gender. Female volunteer donor positivity was low (4.2%) similar to the mother positivity rate reported by Xeuatvongsa et al.[3] The reason for high positivity in young blood donors, especially teenagers, is unknown. Probably it is due to horizontal infection by health problems in their normal social lives and not to mother-child infection. Repeat donor positivity rates were lower (1.7%), because the NBTC introduced a notification system to donors who were found to be HBs antigen positive. Those positive are not accepted as future blood donors. The majority of blood donors are from the younger generations in Laos, however currently this group has the highest HBSAg prevalence. Measures for making blood safer should always be considered.

1. Jutavijittum P, et al. Southeast Asian J Trop Med Public Health 38: 674–679, 2007.
2. Jutavijittum P, et al. Vox Sang 106, 31–37, 2014.
3. Xeuatvongsa A, et al. PLOS ONE 9(2): e88829, 2014.

1A	First time donors							
		Roche Elecsys Anti-HBc II			ABBOTT PRISM Hbcore			
Test site	Donations	IR fpos	RR fpos	Specificity	IR fpos	RR fpos	Specificity	Fisher test
Amsterdam	2000	2	2	99.90	1	0	100	0.50
Hagen	1448	0	0	100	1	1	99.93	1.0
Frankfurt	1534	2	2	99.87	2	2	99.87	1.0
Total	4982	4	4	99,92	4	3	99,94	1.0

1B	First time donors							
		Roche Elecsys Anti-HBc II			ABBOTT Architect Anti-HBc II			
Test site	Donations	IR fpos	RR fpos	Specificity	IR fpos	RR fpos	Specificity	Fisher test
Innsbruck	294	0	0	100	0	0	100	1.0
Stuttgart	309	0	0	100	0	0	100	1.0
Total	603	0	0	100,00	0	0	100,00	1.0

1C	Multiple time donors							
		Roche Elecsys Anti-HBc II			ABBOTT PRISM Hbcore			
Test site	Donations	IR fpos	RR fpos	Specificity	IR fpos	RR fpos	Specificity	Fisher test
Amsterdam	2008	3	3	99.85	0	0	100	0.25
Hagen	1539	1	1	99.94	1	0	100	1.0
Frankfurt	1536	2	2	99.87	0	0	100	0.50
Total	5083	6	6	99,88	1	0	100,00	0.03

1D	Multiple time donors							
		Roche Elecsys Anti-HBc II			ABBOTT Architect Anti-HBc II			
Test site	Donations	IR fpos	RR fpos	Specificity	IR fpos	RR fpos	Specificity	Fisher test
Innsbruck	1720	1	1	99.97	1	1	99.97	0.25
Stuttgart	2773	5	5	99.82	0	0	100	0.06
Total	4493	6	6	99,87	1	1	99,98	0.12

Caption 1 Serum plasma comparison

Table 2	Serum				Plasma				
	Donations	IR fpos	RR fpos	Specificity	Donations	IR fpos	RR fpos	Specificity	F-Test
Roche Elecsys	6057	5	5	99,92	4008	5	5	99,88	0,75
ABBOTT PRISM	6057	4	3	99,95	4008	1	0	100,00	0,28
Roche Elecsys	2014	1	1	99,95	3082	5	5	99,84	0,41
ABBOTT Architect	2014	1	1	99,95	3082	0	0	100,00	0,39

Caption 2. First time donor and repat donor comparison

P-275

HEAD-TO-HEAD-TO-HEAD COMPARISON OF THE ROCHE ELECSYS ANTI-HBC II TEST WITH ABBOTT PRISM HBCORE AND ABBOTT ARCHITECT ANTI-HBC II

M Schmidt[1], E Bakker[2], M Boniek[3], M Koot[2], B Luz[3], A Mühlbacher[4], R Rietz[5], O Roehrig[3], B Rüster[1], H Schennach[4], V Schottstedt[5], A van Weert[2] and E Seifried[1]

[1]German Red Cross, Frankfurt, Germany [2]Sanquin Blood Supply, Amsterdam, The Netherlands [3]Klinikum Stuttgart, Katharinenhospital, Zentralinstitut für Transfusionsmedizin, Stuttgart, Germany [4]Zentralinstitut fuer Bluttransfusion und Immunologie Abteilung der tirolkliniken, Innsbruck, Austria [5]DRK-Blutspendedienst West, Zentrallabor, Hagen, Germany

Background: Blood donor screening is done for transfusion transmitted virus infections in parallel by NAT and serology in many countries. For hepatitis B, two diagnostic window periods occur. The first window period will be reduced to a minimum by HBV NAT and HBsAg. The second window period can be reduced to a minimum by HBV ID-NAT and by the introduction of anti-HBc. This study reports the results from a direct head-to-head comparison of the Elecsys Anti-HBc II, with the respective ABBOTT PRISM/Architect instrument immunoassays in a multicenter blood bank evaluation study.
Aim: The aim of this study was to evaluate the specificity and sensitivity of Roche Elecsys Anti-HBc II with fully automated Roche cobas analyzers on blood donor specimens in parallel with the respective routine system.
Methods: Assay validation was performed in the blood screening laboratories of five blood bank centers in Austria, Germany and The Netherlands, where first-time donor samples and multiple time donor samples (in total 17325 donors) were screened.
Results: Of all screened donor samples, 134 (0.77%) were confirmed to be positive. Anti-HBc confirmed positive donations were detected by the Roche Elecsys assay as well as by the ABBOTT PRISM and the ABBOTT Architect test without any exception. Therefore the diagnostic sensitivity was 100% for all three diagnostic assays.

The specificity was 99.93%, 99,88%, 99,95%, 100%, 99.95% and 100% for Elecsys Anti-HBc II serum samples, Elecsys Anti-HBc II plasma samples, ABBOTT PRISM core serum samples, ABBOTT PRISM HBcore plasma samples, ABBOTT Architect Anti-HBc II serum samples and ABBOTT Architect Anti-HBc II plasma samples, respectively. Pre-screened multiple time donors achieved in a direct head-to-head comparisons between Roche Elecsys and ABBOTT PRISM a slightly reduced diagnostic specificity, whereas no differences were obtained for multiple time donors between Roche Elecsys and ABBOTT Architect. A direct comparison between the assays from Roche and ABBOTT according to Bland and Altman analysis demonstrated equivalent quality.

Conclusions: The new Elecsys immunoassay Anti-HBc II demonstrates a overall comparable sensitivity and specificity to the ABBOTT PRISM or ABBOTT Architect tests. In addition, comparable test results between serum and plasma samples enables blood donor services an improved fully automated screening procedure with only 1–2 sample tubes per donor. The time to clean up the donor population for unspecific test results for 1–2 years that might occur by changing to a new screening system will be compensated by a higher grade of automation with reduced hands on time on sample tubes.

P-276

OCCULT HEPATITIS B VIRUS INFECTION AMONG APHERESIS DONORS IN A REGION OF CENTRAL CHINA

L Zhao, G Li, L Yao, T Xu, B Chen and W Cheng

Wuhan Blood Center, Wuhan, China

Background: Occult hepatitis B infection (OBI) is characterized by hepatitis B virus (HBV) DNA-positive, but HBV surface antigen (HBsAg) -negative. Since May 2015, Wuhan blood center has been testing apheresis donors for HBV nucleic acids and improvements in laboratory testing have reduced the risk of transfusion-transmitted infection. The number of apheresis collections increased significantly year by year, however, data on hepatitis B virus marker rates among these donors continue to be lacking.

Aims: To evaluate the epidemic characteristics, incidence and estimate the risk factors of occult hepatitis B virus infection among apheresis donors in a region of central China.

Methods: Apheresis donors' data from May 2015 to Mar 2016 was retrospectively analyzed. All samples were tested for HBsAg, HBV DNA, and other markers. Nucleic acids testing (NAT) was performed on the Roche cobas s201 platform using pools of 6 serologically negative samples and any pools positive would undergo NAT again individually. HBsAg negative, but HBV DNA positive were further tested for HBV DNA quantitative PCR, antibody to hepatitis B surface antigen (HBsAb), antibody to hepatitis B core antigen (HBcAb), hepatitis B e antigen (HBeAg) and antibody to hepatitis B e (HBeAb).

Results: In the evaluation, 26,374 seronegative donations were screened by NAT and a total of 10 HBV DNA-reactive/HBsAg-negative donors were detected. No HIV RNA -reactive or HCV RNA -reactive sample was detected. Complete serologic screening of the index donations indicated that the majority of these donors had an occult HBV infection and the majority of which were married men and the fixed donors with many whole blood or apheresis donations. Age distribution of the age group 31–55 years old showed a large proportion, who accounted for 80% of reported infections. Most of the HBV DNA cases (80.0%) reached senior high school education. The average HBsAg DNA positive rate was 0.038% (10/26374). Incidence among apheresis donors in this period for HBsAg DNA were 3.79/10000. These estimates were comparable to those among repeat whole blood donors.

Conclusions: The risk of occult hepatitis B virus infection among current apheresis donors exists and the introduction of NAT reduces the residual risk of transfusion.

No.	Age	Gender	HBsAg	HBsAb (IU/L)	HBeAg	HBeAb	HBcAb	HBV DNA	*ct*
01	42	Male	n-re	<test	n-re	reac	reac	<2.0 × 10 IU/ml	36.2
02	50	Male	n-re	22.72	n-re	n-re	reac	<2.0 × 10 IU/ml	35.8
03	31	Male	n-re	355.26	n-re	reac	reac	<2.0 × 10 IU/ml	35.3
04	47	Male	n-re	n-re	n-re	n-re	reac	<2.0 × 10 IU/ml	36.7
05	35	Male	n-re	23.49	n-re	n-re	reac	Target not detected	-
06	36	Male	n-re	246.76	n-re	n-re	reac	<2.0 × 10 IU/ml	37.3
07	36	Male	n-re	256.61	n-re	n-re	reac	Target not detected	-
08	21	Male	n-re	232.38	n-re	n-re	n-re	<2.0 × 10 IU/ml	40.5
09	38	Male	n-re	205.01	n-re	n-re	reac	<2.0 × 10 IU/ml	37.2
10	23	Male	n-re	201.55	n-re	n-re	reac	<2.0 × 10 IU/ml	35.5

n-re: non-reactive; reac: reactive

Caption 1 Serologic screening markers profile of HBV DNA-reactive/HBsAg-negative donors

P-277

PREVALENT KINETICS OF HEPATITIS B VIRUS IN BLOOD DONOR POPULATION DURING PAST 10 YEARS IN SHENZHEN, CHINA

T Li[1], Z Wang[1], J Zeng[2], X Zheng[3], X Xu[2], X Ye[2], L Lu[2], W Zhu[2], B Yang[2], JP Allain[4] and C Li[1]

[1]Southern Medical University, Guangzhou, China [2]Shenzhen Blood Center, Shenzhen, China [3]Baoan Central Blood Station, Shenzhen, China [4]Department of Haematology, University of Cambridge, Cambridge, United Kingdom

Background: Hepatitis B virus (HBV) infection is a major threat to public health. Since neonatal hepatitis B vaccination program at birth was implemented nationwide in China, HBV prevalence declined largely in the general Chinese population. However, current HBV prevalence status in blood donations has not been entirely examined as the vaccinees over 18 years old progressively become the majority population of blood donors.

Aims: In this study, we conducted a comprehensive survey and analysis of HBsAg screening results during the past 10 years (2005 to 2014) in donors stratified according to type, geographical origin, gender, age, and vaccination status.

Methods: Approximately 0.5 million blood donors were screened for HBsAg by rapid tests and enzyme immunoassays, and further for HBV DNA by nucleic acid testing between 2005 and 2014 at Shenzhen blood center.

Results: 569,145 blood donors were screened for HBsAg by rapid tests and enzyme immunoassays (EIA), of which 475,538 blood samples with negative HBsAg were screened for HBV DNA by nucleic acid testing (NAT) between 2005 and 2014 at the Shenzhen blood center. An overall HBsAg prevalence of 2.3% was found in the blood donor population during the past 10 years, (2.86% was in 2005, 1.76% in 2010, and 2.79% in 2014, respectively). HBsAg seroconversion occurred in 0.37% of repeat-donors. When stratified by age, the prevalence of HBsAg was found significantly higher in younger donors age 18–25 years (2.73%) than those in 26–35 years (2.13%), 36–45 year (2.03%) and 46–58 years (1.71%) ($P < 0.0001$), unexpectedly suggesting that young blood donors were at higher risk of chronic HBV infection. In presumed non-vaccinated and vaccinated first-time or repeat donors aged 18–22 born before or after 1992, respectively, HBsAg prevalence was found higher in first-time donors born ≥1992 (3.89%), than <1992 (3.51%, $P = 0.005$). The incidence of HBV infection in the 5 year period examined was significantly lower in repeat donors born ≥1992 (0.27%) than <1992 (0.57%, $P = 0.008$). The yield of HBV DNA+/HBsAg- donors was detected in 1:3,302, including 1:4,486 occult infections and 1:43,231 window period infections.

Conclusions: Young blood donors born after implementation of universal vaccination in China presented higher prevalence of HBsAg but lower incidence of HBsAg seroconversion after age 18. This study suggests a need of HBV vaccine boosting for adolescents at 15–17 years old prior to coming of blood donor age when they reach blood donation age.

P-278

This abstract has been withdrawn.

P-279

IMPROVEMENT OF QUANTIFICATION OF ANTIBODY DETECTION TO HEPATITIS B VIRUS SURFACE ANTIGEN

K Chinbordee, J Saejung, A Srisuddee, C Roekchai, S Phiancharoen, A Tatawatorn, S Khanuntong and P Kupatawintu

Thai Red Cross, Bangkok, Thailand

Background: Hepatitis B Immune Globulin (HBIG) is an immunoglobulin for prophylactic and therapeutic use. A suitable anti-HBs quantitative method has to be accurate and consistent yielding.

Aims: To compare the quantitative method on anti-HBs between two series of calibration curve.
Methods: Two series of calibration curve were obtained by Architect anti-HBs reagent kit REF 7C18-25 for two point calibrators and Architect anti-HBs reagent kit REF 7C18-27 for six point calibrators. Both two quantitative methods generated the logistic curve. Those curves were compared using the second international standard for hepatitis B immunoglobulin which was the detected reference. Then the fitting efficiency of two curves was compared.
Results: The average concentration of anti-HBs was calculated using two calibration curves, two point calibrators and six point calibrators. There were 68.74 ± 8.99 and 107.44 ± 9.53 IU/ml respectively. The determinate coefficients of variation were 13.07% and 8.86% respectively. The anti-HBs concentration were compared with the actual value of the detected reference, 100 IU/ml. The anti-HBs concentration when using six point calibrators showed only 4.44% higher than the actual referent concentration while two point calibrators showed 31.25% differently lower.
Conclusion: Architect anti-HBs reagent kit REF 7C18-27 with six point calibrators was the fitting curve for more practical to estimate the actual concentration. It can be utilized as an optimal quantitative method to detect anti-HBs concentration in HBIG.

P-280

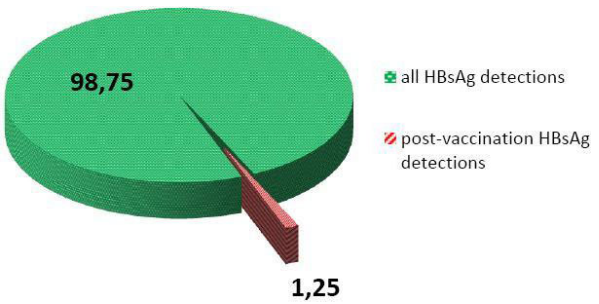
DETECTING THE PRESENCE OF THE SURFACE ANTIGEN OF HEPATITIS B (HBSAG) IN BLOOD DONORS IN THE REGIONAL BLOOD CENTER IN POZNAN IN THE YEARS 2006–2015.

K Piersiala, A Bukowska, K Olbromski and H Skalisz
Blood Center, Poznan, Poland

Background: In Poland hepatitis B is one of the infectious agents which are tested for every donor after each blood donation. In recent years, there has been a significant decrease of detections due to the implementation of vaccination in the 90s. For diagnostic purposes, serological and molecular methods are used. The HBV surface antigen - HBsAg is the marker that is mainly tested in serological stage. The marker is detectable in the blood of people infected with HBV, but also in people that were vaccinated against HBV short time before donating blood as the HBsAg is included in the vaccine. The period of the presence of HBsAg in the blood of vaccinated people has not been precisely defined. Any case of suspected post-vaccinal HBsAg detections requires an in-depth analysis as a positive result for the presence of this marker is the basis for the lifetime deferral from donating blood for medicinal purposes in Poland.

Year	Number of cases
2006	0
2007	1
2008	0
2009	2
2010	1
2011	0
2012	0
2013	1
2014	2
2015	1

Table 1



Graph 1

Aims: The aim of the study was to determine the percentage of post-vaccination HBsAg detections among all cases of detections of this marker in blood donors in the Regional Blood Center in Poznan in the years 2006–2015.
Methods: In the years 2005–2015 donors' blood was investigated for HBsAg using Ortho Clinical Diagnostics, Abbott and BioMerieux tests with high sensitivity and specificity. Each time reactive results were repetitively verified by neutralization tests.
Results: Eight cases of post-vaccinal HBsAg detections in blood donors in Regional Blood Donor Centre were reported in the years 2006–2015, which represents 1.25% of all detections of this marker in the blood centre (Table 1, Graph 1).
The increasing or decreasing trends for this type of detections has been observed.
Summary/Conclusions: The post-vaccination HBsAg detections represent a minor percentage of detections of all markers in blood donors in the Regional Blood Donor Centre in Poznan. However, a special attention must be paid to low repetitively reactive results in the test for the presence of HBsAg which is neutralized in order not to defer from donating blood donors that are not infected but vaccinated against HBV.

P-281

DETECTION OF HBSAG MUTANTS IN THE BLOOD DONOR POPULATION IN ISLAMABAD, PAKISTAN

A Farooq[1], U Waheed[2], R Hafeez[2], M Arshad[3] and HA Zaheer[2]
[1]Department of Biotechnology, IIU and Department of Blood Bank, SZABM University, Islamabad, Pakistan [2]Department of Blood Bank, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan [3]Department of Biotechnology, International Islamic University, Islamabad, Pakistan

Background: Hepatitis B infection continues to be one of the leading health issues in Pakistan, affecting over 6 million people. Hepatitis B infection screening for blood donation is carried out through testing for HBsAg. Poor sensitivity may results due to mutations within and/or outside of the determinants that affect conformational epitope recognition or HBsAg secretion or expression. The existence of HBsAg mutants is well documented in many countries and is a result of selection pressure from immune response (vaccine) or treatment regimes. In Pakistan, most of the blood banks perform rapid or ELISA and CLIA (only limited number of centres) screening to detect HBsAg which may not detect mutant HBsAg. The reagents used in these HBsAg diagnostic assays are directed against epitopes in the "a"determinant. Failure to detect these mutants in blood donors results in transfusion transmitted hepatitis B infection. In Pakistan, HBsAg mutants have not been studied. There is a need for an HBsAg assay to detect mutants in a country where simple, conventional HBsAg assays are used to detect an HBV infection.
Aims: The proposed study compared the routinely used diagnostic tests (ELISA, rapid devices and CLIA) for HBsAg with the Liaison XL CLIA Murex Assay (mutant detection kit) to determine the prevalence of HBV mutants in the Pakistani blood donor population.
Material and Methods: This prospective study was carried out at the Department of Blood Transfusion Services, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, a premier tertiary care hospital of the federal capital. The samples of blood donors from different cities of Pakistan were collected in serum separator tubes, serum separated and sent to the testing site in cold chain. The testing was performed using SD Bioline rapid assay (n = 750), Nanbase C-96 3.0 ELISA (n = 915), and Abbott ARCHITECT® system (n = 900) where the donations were collected. All the blood samples (n = 2565) were re-tested for comparative analysis on the Liaison XL CLIA Murex Assay (DiaSorin). The PCR testing was performed as a gold standard on

all discordant samples. ExiPrep[TRADEMARK] Dx Viral DNA Kit was used to extract viral DNA from serum samples. AccuPower® HBV Quantitative PCR Kit was used to measure the HBV-DNA in serum/plasma. Sensitivity and specificity with 95% confidence intervals (CI) of individual HBsAg assay was determined. Positive predictive value (PPV) and negative predictive value (NPV) (with 95% CI) were also calculated by SPSS software version 18.0 (SPSS Inc., Chicago IL).

Results: The 69/2565 (2.69%) of the samples were positive for antibodies against HBsAg. The sensitivity of SD Bioline, ELISA, Abbott Architect® and Liaison XL CLIA Murex Assay was 62.2%, 71.4%, 90.9% and 98.5% respectively. The specificity of SD Bioline, ELISA, Abbott Architect ® and Liaison XL CLIA Murex Assay was 98.7%, 99.5%, 100% and 100%. The NPV for SD Bioline, ELISA, Abbott Archietect ® and Liaison XL CLIA Murex Assay was 97.9%, 99.1%, 99.7% and 99.96% respectively. The PPV for SD Bioline, ELISA, Abbott Architect ® and Liaison XL CLIA Murex Assay was 75.6%, 83.3%, 100% and 100% respectively.

Conclusion: The failure to detect the HBsAg mutants by routine screening methods may be a major cause of HBV transmission through blood transfusions in Pakistan. LIAISON®XL murex HBsAg assay is a sensitive and specific screening assay for detecting wild type and mutant HBsAg.

P-282

SEROPREVALANCE OF HBSAG AND ANTI-HCV IN BLOOD DONORS OF BURSA-TURKEY, OVER A 18 YEAR PERIOD (1998–2015)

Y Heper[1], SH Bal[2] and LT Kumas[2]

[1]Uludag University Medical Faculty, Bursa, Turkey [2]Uludag University Medical Faculty Hospital Blood Bank, Bursa, Turkey

Background: The seropositivity rates of transfusion transmitted infections in blood donors are related to the prevalence of those infections in the general population. Since 18 years, social consciousness about Hepatitis is rised in Turkey. It can be expected that this will affect our donor population.

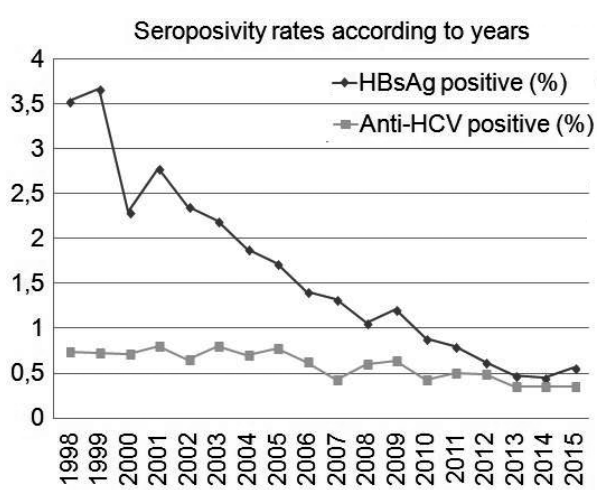
Aims: To investigate if there are any changes in prevalances of Hepatitis B and C markers in our donor population.

Methods: The data of HBsAg and Anti-HCV screenings of 379.025 blood donors who applied to our blood bank in 1998–2015 were evaluated. Screening tests was performed by ELISA (1998-September 2002:Abbot-Axsym, October 2002-February 2008: Vitros-Ortho-Clinical Diagnostics and Abbot-Axsym together, March 2008-January 2013: Abbot-Axsym, since February 2013-today: Abbot- Architect-i1000sr). Repeated reactivity was accepted as positive. Since mid-2011, repeated reactive HBsAg's are confirmed by neutralisation test, and Anti-HCV by HCV-Real-Time-PCR.

Results: Our blood bank is a tertiary hospital blood bank. A very big proportion of our donors are first time, and %95 are replacement donors. Positivity rates in confirmatory tests are 92,8% for HBsAg and 3,9% for Anti-HCV. Because confirmatory tests are used since 2011, results are given in percentages of repeated reactivity in the Table and Graphic.

Year	Blood donors (n)	HBsAg positive* (%)	Anti-HCV positive* (%)
1998	11.201	3,53	0,74
1999	22.020	3,67	0,72
2000	23.832	2,3	0,71
2001	27.466	2,78	0,8
2002	22.169	2,36	0,65
2003	17.090	2,2	0,8
2004	18.810	1,89	0,7
2005	21.285	1,72	0,77
2006	18.528	1,41	0,62
2007	16.351	1,32	0,43
2008	17.390	1,06	0,60
2009	17.651	1,21	0,64
2010	20.635	0,89	0,42
2011	24.112	0,80	0,50
2012	26.397	0,63	0,49
2013	24.359	0,48	0,35
2014	25.203	0,45	0,35
2015	24.526	0,56	0,35
Total	379.025	* repeated reactive	

Caption 2 Seropositivity rates



Caption 1 Seropositivity rates

Summary/Conclusions: The seroprevalance of Anti-HCV is low and didn't change. But there is a statistically significant, steady decline in HBsAg (chi-square test $P < 0.0001$). The decline in the first year can be explained by the massive education of the blood bank staff in our and other blood banks in Turkey and the increased attention in donor selection. Since the beginnings of 2000's, social consciousness about Hepatitis, especially Hepatitis B is rised in Turkey. As a result, many people were screened and also vaccinated. Hepatitis B vaccination has been included in routine childhood vaccines in 1998. Children born after 1998 will begin to reach the age to be blood donors in 2017. Based on this, it can be foreseeable that the decline in Hepatitis B seroposivity in the donor population will continue, perhaps more pronounced. Not changed Hepatitis C seroprevalance (an infection with no vaccine), seem to support the efficacy of Hepatits B vaccination in the general and also donor population.

P-283

PERFORMANCE OF A NEW AUTOMATED ASSAYS FOR HEPATITIS B SURFACE ANTIGEN AND HEPATITIS B CORE

G Schlauder[1], R Makela[1], E Bakker[2], A van Weert[2], J Siregar[1], L Martin[1] and G Williams[1]

[1]Abbott Laboratories, Abbott Park, United States of America [2]Sanquin Diagnostics, Amsterdam, The Netherlands

Background: Blood transfusion safety around the world relies on serologic screening for Hepatitis B surface antigen (HBsAg), as well as serological screening for antibodies to Hepatitis B Core antigen (Anti-HBc), to help prevent transfusion transmitted HBV infection. Sensitive HBsAg assays must be capable of coping with a wide range of mutants while exhibiting an uncompromised specificity. In addition, continued pressures on laboratory operations demand that assays perform on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management.

Aims: To evaluate the overall performance of two new automated prototype chemiluminescence immunoassays for the detection of HBsAg and Anti-HBc on an automated next generation platform.
Methods: The performance of the automated prototype immunoassays for the detection of HBsAg and anti-HBc were evaluated on a next generation automated platform. Precision was assessed over 5 days. Sensitivity was evaluated on 200 known positive samples and 3 commercially available seroconversion panels. HBsAg was also evaluated using 19 mutants and 8 genotypes (A-H). Specificity was evaluated on samples obtained from 2403 first time and repeat donors from the Netherlands and 200 diagnostic specimens from the USA. Results were compared to the Abbott PRISM assay for these markers.
Results: Overall clinical sensitivity was 100% on 200 known positive samples for HBsAg and 100% on 200 known positive samples for Anti-HBc. The HBsAg assay detected 100.00% (19/19) of the mutants while the comparator detected 57.9% (11/19) mutants. All HBsAg genotypes were detected. For the seroconversion panels tested, all positive bleeds detected by the comparator assays were detected by the prototype assays. The overall resolved specificity when compared to PRISM was 100.00% (2403/2403) and 99.88% (2392/2395) for HBsAg and Anti-HBc, respectively. For HBsAg, 4 initial reactive samples were detected. Zero samples were repeat reactive. For Anti-HBc, 11 initial reactive samples were detected and all were repeat reactive. The repeat reactive rate on blood donor samples, excluding confirmed positive samples, was 0.00% (0/2403) for HBsAg and 0.12% (3/2395) for HBc. Resolved specificity when compared to PRISM on 200 diagnostic specimens was 100.00% for both assays. Precision testing over a 5 day period showed percent CVs for positive samples of less than 10%.
Summary/Conclusions: The new automated prototype HBsAg and Anti-HBc assays provided precision and specificity comparable to the current on-market PRISM assays. Anti-HBc sensitivity was also comparable to the on-market assay. However, the HBsAg assay demonstrated a gain in sensitivity over PRISM through the detection of a wider range of mutants.

Hepatitis C (HCV)

P-284

This abstract has been withdrawn.

P-285

PERFORMANCE OF A NEW AUTOMATED ASSAY FOR ANTIBODIES TO HEPATITIS C

G Schlauder[1], R Daruwala[1], A van Weert[2], E Bakker[2], L Fleischmann[1], M Paradowski[1] and G Williams[1]

[1]Abbott Laboratories, Abbott Park, United States of America [2]Sanquin Diagnostics, Amsterdam, The Netherlands

Background: Serological screening for antibodies to Hepatitis C virus (HCV) in conjunction with nucleic acid testing (NAT), are used worldwide to prevent transfusion transmitted HCV infections. While NAT provides improved sensitivity and detection of HCV in the pre-seroconversion window, serological testing provides continued detection of HCV in infected individuals and individuals with resolved infections with no detectable HCV RNA. Blood and plasmapheresis centers require very high throughput anti-HCV assays with high specificity and sensitivity to prevent unnecessary donor deferrals while maintaining the safety of the blood supply. In addition, continued pressures on laboratory operations demand that assays perform on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management.
Aims: To evaluate the overall performance of a prototype chemiluminescent immunoassay for the detection of antibodies to HCV on a new high throughput automated next generation platform.
Methods: The performance of a prototype automated chemiluminescent immunoassay for the detection of antibodies to Hepatitis C was evaluated on a next generation automated platform and compared to an on-market assay. Precision was assessed over 5 days evaluating positive samples. Specificity was evaluated on 5094 blood donor samples and 200 hospital patient samples. Sensitivity was evaluated on 200 known positive samples and 10 commercially available seroconversion panels.
Results: Precision was less than 10% CV for positive samples over 5 days. The overall resolved specificity in a blood donor population was 100.00% (5094/5094) with an initial reactive rate of 0.00%. For hospital patients, the resolved specificity was 98.94%. Overall clinical sensitivity was 100% on 200 known positive samples for anti-HCV. Seroconversion sensitivity was better than the current on-market product as evidenced by prototype assay identifying 3 more bleeds than the on-market assay.
Summary/Conclusions: These results indicated that the new automated prototype anti-HCV assay provided acceptable performance in specificity, sensitivity, and precision. Specificity performance of the new assay was equivalent to a current on-market anti-HCV assay while sensitivity performance indicated improvements over the current on market performance.

P-286

DISCORDANT ANTI-HCV RESULTS CAN INDICATE LOW LEVELS OF HCV RNA IN DONORS BLOOD

N Yaroslavtseva[1], D Tikhomirov[1], T Romanova[1], E Ignatova[1], E Ovchinnikova[1], P Makhnovskiy[2], A Grishechkin[2], O Tikhonova[2], I Nikolaeva[2], T Tupoleva[1] and T Gaponova[1]

[1]National Research Center for Hematology, Moscow, Russian Federation [2]N.F. Gamaleya Federal Resrarch Centre for Epidemiology and Microbilogy, Moscow, Russian Federation

Background: HCV occurs via three types of infection: acute, chronic and occult. During occult HCV infection (OCI) HCV antibodies detection (anti-HCV) can show grey zone or negative results. HCV RNA in OCI may be detected in blood plasma at low level (below 10–15 IU/ml) or in peripheral mononuclear blood cell and hepatocytes. Such low viremia sometimes can't be detected by using routine PCR. OCI often occurs among injection drug users, idiopathic liver dysfunction patients, hemodialysis patients, in asymptomatic HCV-infected patients with sustained virological response after antiviral therapy. Also OCI may occur in asymptomatic anti-HCV-seronegative individuals. The last accidently can become blood donor. Recent data proved cases of reveling of OCI in blood donors in some countries by using ultrasensitive tests.
Aim: Prove viral presence in donor's blood with anti-HCV discordant results.
Methods: 13791 blood donors were screened by chemiluminescent micropartical immunoassay (CMIA) on Architect i2000SR (Abbott) for anti-HCV. Samples with positive or grey zone results (S/CO 0.8–7.7) in CMIA were tested in enzyme immunoassay (EIA) for anti-HCV. EIA negative samples were tested by immunoblot (INNO-LIA HCV Score "Fujirebio") and in addition core-, NS3-, NS4-, NS5-antibodies, core-antigen, HCV RNA.
Results: 38/13791 (0.28%) samples showed positive or grey zone results in CMIA but negative in EIA. HCV RNA was screened by routine PCR (sensitivity 100 IU/ml). All samples were negative. 29 samples were available for immunoblot. 6 of them showed indefinite result (antibodies against C1, E2 or NS3 only). 5 samples were available for core-antigen detection in EIA and sensitive PCR (sensitivity 10–15 IU/ml). All 5 samples were core-antigen negative, 2 samples were RNA-positive. Clinical blood test in 38 samples was taken into account. 16 of 38 showed minimal aberrations mainly by increasing number of lymphocytes (39.9–49.5%) and monocytes (10.4–19.8%).
Summary: Samples with indefinite anti-HCV results may indicate unspecific reactivity or occult HCV. Minimal aberrations in clinical blood test like elevation of lymphocytes and monocytes count can point to occult HCV.

P-287

RISK FACTOR FOR PAST HEPATITIS C INFECTION AMONG CHINESE BLOOD DONORS

CK Lee[1], SC Ng[1], D Chan[2], HK Wong[1] and SS LEE[2]

[1]Hong Kong Red Cross Blood Transfusion Service, Hong Kong, Hong Kong [2]Stanely Ho Centre for Emerging Infectious Disease. the Chinese University of HK, Hong Kong, Hong Kong

Introduction: Hepatitis C (HCV) is an important transfusion transmitted viral infection that has been significantly prevented by stringent pre-donation health screening, coupled with serological and molecular testing of donated blood. Knowingly injection drug use and previous blood transfusion used to be the commonest causes of HCV positivity in blood donors in many countries. Through the application of donor deferral and advances in screening the profile of HCV infection in blood donors might have changed over time. In this study, a prospective survey was

conducted to examine the most likely routes of HCV exposure among Chinese blood donors in Hong Kong.
Materials and Methods: All blood donors screened positive for HCV infections by serological and molecular methods were invited to complete a self-administered questionnaire after informed consent during an interview when counselling and the test results were explained. The responses were reviewed by a medical staff conducting the interview, with further clarifications in case of queries.
Results: Between January 2014 and December 2015, 67 out of 515,219 blood donations (0.013%) were confirmed to be positive for HCV antibody. Forty-six donors were contacted of which only 34 agreed to participate in the study. There were 13 male and 21 females with a mean age of 34 (range: 17–58). A majority (76.4%) were first time blood donors. The main routes of HCV transmission were: previous transfusion (n = 10, 29.4%); surgical exposure outside Hong Kong (n = 6, 17.6%); unclean injection or infusion outside Hong Kong (n = 22, 64.7%); past history of drug abuse (n = 4, 11.8%); and unsafe sex (n = 12, 35.3%). Though medical procedure performed in health care setting constituted a very low risk in acquiring HCV, 33 out of 34 donors reported to have such exposure either over 30 years ago within Hong Kong or lately in cities/countries where the standard of infection control might be suboptimal.
Discussion: The prevalence of past exposure to HCV among young Chinese blood donors in Hong Kong has remained low. Their risk profile suggested that in addition to the usual routes of transmission, a significant proportion might have acquired HCV in other settings such as health care procedures. Their detection and deferral through the pre-donation screening mechanism is important to protect blood safety.

P-288

HBV/HCV CO-INFECTION IS ASSOCIATED WITH A HIGH LEVEL OF HCV SPONTANEOUS CLEARANCE AMONG DRUG USERS AND BLOOD DONORS IN CHINA

YS Fu

Guangzhou Blood Center, Guangzhou, China

Background: Chronic infections with hepatitis virus are very common in China but the factors associated with spontaneous clearance of HCV have not been adequately studied.
Aims: In order to understand the biology of spontaneous clearance of hepatitis C virus (HCV) infection and prevent the sequelae associated with chronic HCV infection.
Methods: We evaluated the spontaneous clearance of HCV among 1918 drug users and 1526 HCV seropositive blood donors in Guangzhou, China. Anti-HCV and HBsAg were detected using two independent enzyme-linked immunoassay (EIA) kits (Kehua Biotech Co. Ltd, Shanghai, China and Abbott HCV EIA 2.0, Abbott Laboratories, North Chicago, Illinois, USA). Anti-HBc, HBeAg, anti-HBs and HBcAg were detected by two commercial EIA kits (Kehua Biotech Co. Ltd, Shanghai, China and Wantai Biotech Co. Ltd, Beijing, China). HCV RNA and HBV DNA were detected using nucleic acid testing (The ProcleixUltrio Assay, Gen-Probe, Novartis, San Diego, CA, USA) according to the manufacturer's instructions. In order to confirm the HCV infection, a recombinant immunoblot assay (RIBA HCV 3.0, MP Biomedical, Singapore) was performed in subjects who were HCV EIA positive but HCV RNA negative ($HCV\ Ab^+/RNA^-$). In order to confirm the HBV infection, Hepatitis B surface antigen quantitative determination was detected by an electrochemiluminescence immunoassay (Roche, Mannheim, Germany) according to the manufacturer's instructions.The IL28B genotype was evaluated by studying the single nucleotide polymorphisms (SNPs). Genotyping of IL28B SNP (rs8099917) was performed by using polymerase chain reaction and direct sequencing. The collected data was analyzed by SPSS software version 19.0.
Results: Among participants who were co-infected with hepatitis B virus (HBV), 41.4% of drug users and 39.5% of blood donors had cleared their HCV infection without antiviral therapy compared to 9.4% of drug users and 16.8% of blood donors who were mono-infected ($P < 0.01$). The proportion of subjects who had cleared their HCV infection was significantly greater in the co-infected subjects whose serum HBV DNA was greater than 2000 IU/ml than those with lower levels. A multiple logistic regression analysis found female gender, IL28B rs8099917 TT genotype, HBV co-infection and blood donors (vs drug users) were associated with increased spontaneous clearance of HCV infection.
Conclusions: Although acute HCV infections are common in China, the incidence of chronic HCV may be reduced among the high prevalence of chronic HBV and IL28B genotypes associated with spontaneous clearance of HCV in Chinese population.

HIV

P-289

ANALYSIS OF HIV INFECTION IN BLOOD DONORS IN THE REGIONAL BLOOD CENTER IN POZNAN IN THE YEARS 2005–2015

A Laba, K Olbromski and H Skalisz

Blood Center in Poznan, Poznan, Poland

Background: The detection of HIV infection is still a serious problem in repeat donors as well as first-time donors. The relatively large number of repeat donors with HIV infection remains still a major concern.
Aim: The aim of the study was the analysis of HIV infection in donors at the Regional Blood Center in Poznań in the years 2005–2015.
Materials / Methods: Donors questionnaires, epidemiological surveys of repeat donors and the data in the 'Blood Bank' computer system were analyzed.
Conclusions: The HIV infection was detected in 43 donors in the years 2005–2015. The largest number of detected infections took place in 2009, and totaled 9 donors. In the group of 43 HIV infected donors 93% were men and 7% were women.
As much as 65% of infected donors were repeat donors which remains a major concern as this group of donors should be the safest and most reliable group of donors.
The analysis of the questionnaires of 43 donors who were infected with HIV showed that none of the donors marked in questionnaire risky behavior, accidental contact with blood or other potential sources of infection prior to the donation.
The analysis of epidemiological questionnaires (i.e. the questionnaires which are filled by the donor himself-herself or with a doctor after receiving the results of virological tests) presented a completely different situation. In 87% of analyzed questionnaires of multiple donors, the same donors marked risky sexual behavior such as: homosexual contact, frequent change of sexual partners, casual sex, or sexual without protection.
In Poland, despite the nationwide campaign regarding HIV and AIDS (among others by National Centre for AIDS) the level of corresponding knowledge is still relatively low in the society. Further education is necessary in particularly targeted at young people. Currently, the aim of the most recent campaign from 2015 from of the National Center for AIDS concerning HIV/AIDS, titled 'Is there anything you have in common? Take an HIV test' is to make a free and anonymous HIV test available for as many people as possible.
Thanks to such campaigns and constant public education we hope to increase the awareness of people who register as blood donors and thereby increase the level of safety of blood.

P-290

FREQUENCY OF HUMAN T-CELL LYMPHOTROPIC VIRUS (HTLV) TYPE 1 IN IRANIAN HIV-1 INFECTED DRUG ABUSERS

L Pirayeshfard and S Zohereh

High Institute for Education and Research in Transfusion Medicine, Tehran, Iran

Background: Co-infection of HIV and HTLV is an increasing danger and a worldwide health problem. One of the ways of transmission of HTLV-1 virus through injecting drug in HIV-positive patients is. The prevalence of HTLV-1 in HIV-1 positive patients between 5 and 10% have been reported.
Aims: In this study, the prevalence and genotypes of HTLV-1 among HIV-positive patients were investigated.
Methods: In this study, 90 samples from HIV-1 positive patients were collected. These samples were analyzed with ELISA kit for the presence of Ag p24 and anti - HIV-1 Ab and then confirmed by western blot. The samples that were HIV-1 positive were examined for the presence of anti-HTLV Ab using ELISA test. For the existence of proviral, after DNA extraction, PCR was done on LTR and TAX genes .Then results were detected by electrophoresis. The PCR products was purified and sequenced.
Results: 13 out of 90 samples were positive (approximately 14.4%) with ELISA methods. Only 5 samples out of 13 samples were confirmed (approximately 5.5%) with PCR and sequencing using both of genes LTR and TAX.
Conclusion: The results of sequencing indicated, all samples were HTLV-1a genotype for both of genes LTR and TAX.

P-291

EVALUATE TRANSFUSION TRANSMITTED INFECTION AMONG NEW AND REGULAR BLOOD DONORS RETROSPECTIVE SIX YEARS STUDY AT CHP

C Ferreira, A Mota, M Sampaio, F Dias, M Amil and J Coutinho
CHP -Oporto, Oporto, Portugal

Background: Transfusion-transmitted infectious diseases (TTID) remain a major concern for blood safety. Serological for Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human Immunodeficiency virus (HIV), Human T-cell Lymphocytotrophic Virus-I and II (HTLV-I/II) and Syphilis (Sy) as well as nucleic acid testing (NAT) are the mandatory screening assays.Aims: To evaluate the prevalence of HBV, HCV, HIV, HTLV and Sy among new and regular blood donors between 2010 and 2015, considering the distribution by gender and age.
Methods: A total of 73554 Blood Donations (BD) where included, from 18446 volunteer and unpaid blood donors. BD were screened by chemiluminsent microparticle immunoassay (for HIV-1/2, HCV, HTLV and HBV.Sy test was performed by ELISA and CLIA. HIV, HBV and HCV single donation NAT: Transcriptase Mediated Amplification (TMA) Procleix® Ultrio PLUS and Procleix® Ultrio Elite [TRADEMARK] Assays. HIV, HCV and HTLV confirmatory test was performed with Innolia®. Results were analysed using SSPS and descriptive statistics were determined in terms of percentages. HBV vaccine it's included in National Vaccination Protocol since 2000.
Results: Of 18446 volunteer and unpaid blood donors, 12216 regular donors (RD) and 6230 first time donors (FTD), 9031(49%) male and 9415(51%) female gave a total of 73554 BD. Due to positive screening tests 488 (0.66%) BD were discarded. Positive screening test for HIV: RD - 49 (0.40%) being five true positive (0.04%); FTD - two(0.03) out of seven confirmed positive 7 (0.11%). Positive screening test for HCV, 64(0.52%) were RD and 19(0.30%) FTD two were true positives, one (0.008%) RD and one (0.016%) FTD. Regarding to HBV, 14 (0.11%) RD and 5(0.08%) FTD had HBsAg positive screening test; five were true positive, one RD (0.008%) and four FTD (0.06%). Isolated positive Anti-HBc:85(0.70%): RD and 19 (0.30%) FTD, all HBV NAT negative. Lookback was performed in all true positive donors for previous donations (frozen sample) and no positive results were found. HTLV positive donors, 9(0.08%) were RD and 4(0.06%) FTD; since 2012 it is only performed in FTD and when clinically relevant and three (0.04%) FTD were HTLV positive. All HTLV positive screening test were negative Innolia® HTLV I/II. Eliminated donations by SY positive occurred in 94(0.77%) RD and 46(0.74%) FTD, and FTD 29(0.24%) were true positive and 28(0.45%) RD. All recipients of previous regular donor's donations which tests became positive were negative for TTI.
Conclusions: According to national hemovigilance data in 2014 in a total of 226882 donors, 19(0.008%) of RD and 7(0.003%) of FTD were HIV confirmed positive. For HCV there were 22 confirmed cases: 1(0.0004%) RD and 21(0.009%) FTD. There were 41 confirmed cases of HBV, 35(0.015%) in FTD and 6(0.003%) RD. One FTD HTLV positive was confirmed. There is no statistical significance between the RD and FTD in our centre, and in the RD and FTD in the general population ($P > 1$). HBV prevalence is expected to diminuend due to the mandatory vaccination programme. The culture of voluntary blood donation is essential to guarantee the availability of safe blood donations. Blood donor information related to behaviour risks, appropriate information and testing are important barriers to assure safer blood components.

P-292

EPIDEMIOLOGY OF HIV INFECTION IN DONOR POPULATION IN TIRANA FROM 2006 TO 2015

Z Abazaj, A Metka and I Seferi
NBTC Tirana, Tirana, Albania

Background: While blood transfusions can be life-saving there are associated risks, particularly the transmission of bloodborne infections. Understanding donor demographics and prevalence of TTI among blood donors is crucial for blood banks in developing strategies to recruit eligible potential donors and to increase blood safety. There is globally an increasing trend for HIV, according to WHO data there are 80% more new HIV cases in 2013 compared to 2004. HIV prevalence among blood donations provides an indication of the relative safety of the blood supply over time.
Aims: To study the prevalence and trends of HIV infection in donor population in Tirana from 2006 to 2015 and to identify the group of donors at risk for this infection.
Methods: The age, sex, and donor type of HIV infected donors were retrospectively analyzed for the time period 2006–2015, for all donations in Tirana. Total number of donations was 154180. Total number of confirmed positive HIV donations was 52 (41 males and 11 females), 24 of them being under 35 years of age and 28 above 35 years of age. The overall prevalence of HIV infected donations resulted 33/100000 donations. The prevalence during years has no significant changes varying from 18/100000 donations in 2008 to 50/100000 in 2006 and 40/100000 in 2015. Related to donor age we found a prevalence of 27/100000 donations in donors less than 35 years old vs 42/100000 in donors above 35 years of age. The prevalence related to donor sex has been found higher in males than in females (37/100000 vs 23/100000). 95% of our donors were first time donors. In family replacement donors was found higher prevalence than in VNRBD donors (35/100000 vs 27/100000).
Summary/Conclusions: There is a high prevalence of HIV infection among our blood donors. This high prevalence is mostly dedicated to the high number of first time donors. Constitution of a group of regular donors is urgently needed in our country. Deferral of at risk donors needs further analysis and improvement. Regular, young VNRBD constitutes basis for long-term sufficiency and quality of blood supply.

P-293

THE HIV PREVALENCE IN THE SOUTH AFRICAN NATIONAL BLOOD SERVICE BLOOD DONORS – WITH SPECIAL REFERENCE TO MEN WHO HAVE SEX WITH MEN

S Ngcobo[1], T Rapodile[1], T Rapodile[1], CF Ingram[1] and T Muthivi[2]
[1]South African National Blood Service, Durban, South Africa [2]South African National Department of Health, Pretoria, South Africa

Background: The South African National Blood Service (SANBS) collects approximately 800,000 donations annually in a country with one of the highest HIV prevalence in the world (18% in the adult population).
The HIV prevalence among men who have sex with men (MSM) in South Africa is estimated as 13%- 49%. In 2014 South Africa lifted deferral on MSM donors instead applying the same risk assessment as for heterosexual donors. The number of donors who were MSM and the HIV prevalence was unknown in SANBS, hence the motivation for the study.
Aim of the study: To assess the HIV prevalence in SANBS MSM and heterosexual blood donors.
Methods: This was a descriptive cross-sectional study. A survey questionnaire was distributed over a 2 month period to all blood donors who presented and were deemed eligible to donate. The Null hypothesis was that there was no difference in the HIV prevalence between the general blood donors (0.22%) and MSM donors.
In addition to the Null Hypothesis assumption the MSM sample size of 146 was determined using:
The donor population size of 479,082 with 226,137 males.
Precision of 5% with 95% confidence interval.
The Fisher's exact test with significance level set at 0.05 was also used to assess if there was any association between HIV statuses and sexuality.
Results: A total of 7344 blood donors were enrolled into the study with the majority being males (n = 4613, 62.92%). The age ranged from 19 to 89 years.
Out of 7312 participants who indicated their sexuality, 120 (1.64%) were homosexual, 7145 (97.72%) were heterosexual and 47(0.64%) were bisexual. Amongst the males 80 were excusively homosexual whereas 29 were men who have sex with men and women (MSMW) giving a total of 109 MSM (1.48%).The HIV prevalence among MSM was 0%. The HIV prevalence amongst 7132 heterosexual donors whose HIV status was verified was 0.39%. HIV prevalence was higher amongst males at 0.47% (n = 20) compared to females at 0.32% (n = 8).
Using Fisher's exact test a P value of 1 was obtained, showing that there was no association between HIV statuses and being either heterosexual or MSM.
Conclusion: The MSM prevalence of 1.48% is consistent with one study which estimated MSM prevalence in the South African general population as approximately 1%. The MSM HIV prevalence of 0% retrospectively justifies the rationale for lifting the deferral on MSM.
One limitation of the study could be that the sample size of 109 MSM might not be a true representation of SANBS MSM population. This smaller sample size (the calculated sample size was 146) could have been due to some reported donor clinic staff not introducing the study to donors citing busy clinics and also to some MSM not declaring their sexuality as in South Africa there is stigma attached to being homosexual.

P-294

PATTERNS IN HIV INCIDENCE IN REPEAT AND FIRST-TIME AND DONORS IN BRAZIL

B Custer[1], E Sabino[2], L Preiss[3], S Keating[1], A Mendrone[4], ME Lopes[5], AB Proietti[6], P Loureiro[7], M Oikawa[8], D Brambilla[3] and MP Busch[1]

[1]Blood Systems Research Institute, San Francisco, United States of America [2]University of Sao Paulo, Sao Paulo, Brazil [3]RTI International, Buenos Aires, Argentina [4]Fundacao Pro-Sangue, Sao Paulo, Brazil [5]Hemorio, Rio de Janeiro, Brazil [6]Hemominas, Belo Horizonte, Brazil [7]Hemope, Recife, Brazil [8]Federal University of ABC, Sao Bernardo, Brazil

Background: Monitoring trends and patterns in HIV incidence in repeat and first-time donors (RD and FTD, respectively) is the most direct measure of changes in the characteristics of the blood donor population that could lead to an increased residual risk of transfusion-transmitted HIV.

Aim: This study assessed HIV incidence over time in both RD and FTD at four large public blood centers in Brazil.

Methods: REDS-II/III donation data from 2007 to 2014 were analyzed. All donor samples were screened by 2 HIV EIAs in parallel. All samples that tested repeat reactive on both HIV EIAs were confirmed by NAT, Western blot, or Limiting Antigen Avidity (LAg) assays. Incidence in RD was calculated using a classical method; for uninfected donors time at risk was from the first to last donation in the interval and for those who became infected time at risk was half of the interval from the first donation to the donation at which the infection was detected. For FTD, LAg results were used to derive incidence using a mean duration of recent infection of 129 days. Uninfected donors contributed 129 days each to the total time at risk, while recently infected donors contributed 64.5 days. For each blood center, results are reported overall and in 4 two-year intervals, and 95% confidence intervals were calculated for the rate in each interval (data not shown). Trends were assessed using Poisson regression.

Results: The four blood centers together collect over 400,000 donations per annum. There were 37 RD and 40 FTD in Sao Paulo, 50 RD and 45 FTD in Recife, 24 RD and 13 FTD in Belo Horizonte, and 13 RD and 7 FTD in Rio with new HIV infections included in the analysis. HIV incidence rates in RD and FTD donors varied between the blood centers and over time (Table). Results for Rio de Janeiro were only available for later intervals. The incidence rate in RD was highest in Recife followed by Belo Horizonte and Sao Paulo, whereas the incidence rate in FTD was highest in Recife followed by Sao Paulo and then Belo Horizonte. High variability was evident in the 2-year intervals for both RD and FTD at each blood center. No clear pattern or trend in HIV incidence was evident. In Sao Paulo, a significant linear trend of increasing incidence in FTD was observed (P = 0.02).

Conclusion: HIV incidence in both RD and FTD varies by region in Brazil. Brazil has a relatively high incidence of HIV in donors, but monitoring incidence rates for evidence of sustained change is challenging. Although not included in the current analysis, differences may be associated with the demographics of the donor population in each blood center.

Table. HIV incidence per 100,000 person-years by interval and blood center

Time period	Fundacao Pro-Sangue, Sao Paulo		Hemope, Recife		Hemominas, Belo Horizonte		Hemorio, Rio de Janeiro	
	RD	FTD*	RD	FTD	RD	FTD	RD	FTD
All years	19.8	34.2	31.7	50.0	26.7	20.4	30.6	N/A
2007-08	24.8	26.6	34.9	34.2	17.5	19.6	N/A	N/A
2009-10	10.4	17.3	37.2	63.1	36.5	36.3	N/A	N/A
2011-12	25.8	33.8	20.8	34.8	17.7	18.6	20.9	0.0
2013-14	18.4	59.8	33.7	65.7	35.4	6.3	43.0	36.1
* Significant linear trend (p=0.02)								

Table

P-295

PERFORMANCE OF A NEW AUTOMATED ASSAY FOR HIV

G Schlauder, D Smith, H Syed, T Biegalski, A Hoffman, K Callear and G Williams

Abbott Laboratories, Abbott Park, United States of America

Background: Blood donations are commonly screened to detect the presence of antibodies (or antibody and antigen) to human immunodeficiency virus Types 1 and 2 (anti-HIV-1/2). Blood centers require very high throughput anti-HIV-1/2 assays with high specificity and sensitivity to prevent unnecessary donor deferrals while maintaining a safe blood supply. In addition, continued pressures on laboratory operations demand that assays perform on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management. In the response for the need for such screening assays, we have evaluated an improved automated prototype assay for the detection of anti-HIV-1/2 antibodies and HIV-1 p24 antigen.

Aims: To evaluate the overall performance of a new prototype chemiluminescence combo immunoassay for the detection of anti-HIV-1/2 antibodies and HIV-1 p24 antigen on a next generation automated platform.

Methods: The performance of the prototype automated chemiluminescence immunoassay for the detection of anti-HIV-1/2 antibodies and HIV-1 p24 antigen was evaluated on a next generation automated platform. Precision was assessed over 5 days evaluating positive samples. Specificity was evaluated on samples obtained from random blood donors. Sensitivity was evaluated using presumed positive samples for HIV-1, HIV-2 and HIV Group O antibodies and HIV-1 p24 antigen. Seroconversion sensitivity was evaluated with 10 commercial seroconversion panels.

Results: Precision was less than 10% CV for positive samples over 5 days. The blood donor specificity was 100% (1000/1000). Sensitivity for HIV-1 antibody positive samples was 100% (n = 107); 100% for HIV group O (n = 46); 100% for HIV-2 (108) and 100% for HIV-1 p24 antigen viral isolates (n = 62). Seroconversion detection was better than the comparator due to the HIV-1 p24 antigen detection capability of the new protoype assay. Eight panels were detected earlier than the comparator assay and 2 panels were detected similarly.

Summary/Conclusions: These results indicate that the new automated prototype HIV Combo assay provided acceptable performance in specificity while providing better sensitivity than the comparator due to the HIV-1 p24 antigen detection.

Bacteria

P-296

FREQUENCY OF BACTERIAL CONTAMINATION IN PLATELET CONCENTRATES IN A TERTIARY LEVEL CARDIAC HOSPITAL IN PAKISTAN

M Noorulamin, A Babur, M Arif, M Hammad, R Hassan, R Faheem and S Salman

Rawalpindi Institute of Cardiology, Rawalpindi, Pakistan

Background: Transmission of transfusion transmissible viral infection like HCV, HBV and HIV has reduced by the implementation of standard operating procedures for the donor enrollment and screening of Transfusion transmissible infections (TTIs) by highly sensitive screening methods. However, Transfusion transmissible bacterial infection especially through platelets Concentrates (PCs) is still one of the main sources of blood borne infections leading to high morbidity and mortality rate particularly in hospitalized patients. Storage temperature for the platelets at 20–25°C provides favorable environment for the bacterial growth. The reported frequency of bacterial contamination varies from ≤0.1% in countries with very high human development like USA to about 10% in low human development countries like Ghana but in Pakistan, this has hardly been ever reported.

Aims: To find our frequency of bacterial contamination in whole blood derived platelets concentrates.

Methods: A cross sectional study was carried out at Department of Pathology & Blood Bank of Rawalpindi Institute of Cardiology (RIC), Pakistan, Which is 272 bedded tertiary level cardiac hospital situated twin cities of Pakistan. A total of 200 whole blood derived PC collected from random blood donors were selected for the study. Ten ml sample from 48 h stored PCs was collected using aseptic techniques and inoculated into Oxoid Signal blood culture bottles. The signal culture bottles were incubated at 35 ± 2°C for 07 days aerobically and inspected daily. Signal culture bottle with positive signals and visual appearance of turbidity were sub-cultured on Blood agar, Chocolate agar and MacConkey agar aerobically at 35 ± 2°C for 48 h. Bacterial growth identification was carried out by standard reference methods.

Results: Out of 200 platelets concentrates, 63 suspected turbid and 02 with positive signal culture device were sub-cultured and identified. *Staphylococcus aureus* was identified in 02 bottles out of 65(3.0%) and the remaining 135 bottles were declared negative for bacterial growth on the basis of negative signal. The overall frequency of bacterial contamination in PCs was found to be 1%.
Conclusion: The frequency of bacterial contamination in PCs found in our study is very high as compared to developed counties and similar to Indian studies (0.48%-1.16%). The storage temperature of PCs provides bacterial friendly environment especially for Gram positive bacteria, so there is need of strict adherence to aseptic measures during blood collection, platelets preparation and their storage, in addition to implementation of standard protocols for the prevention, early detection, and reporting of bacterial contamination in the PCs.

P-297

COMPARATIVE EVALUATION OF 16SRDNA PCR AND BACTERIAL CULTURE RESULTS TO DIAGNOSIS OF BACTERIAL CONTAMINATION IN PLATELETS CONCENTRATE PRODUCED IN TEHRAN REGIONAL BLOOD TRANSFUSION CENTER

M Dehghanian[1], H Avani[2], B Mirzaei[3], M Zadsar[2] and F Shahcheraghi[3]
[1]Iranian Blood Transfusion Organization, Tehran, Iran [2]High Institute for Research and Educational in Transfusion Medicine, Tehran, Iran [3]Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran

Bacterial contamination of blood components constitutes the most frequent infections risk of transfusion. Of all blood products, platelets concentrates (PLT), due to their storage at 20–24°C under agitation, seem to be more prone to be the source of transfusion-transmitted bacterial infection (TTBI). The prevalence of bacterial contamination has been reported and varied considerably in different countries, but the data for bacterial contamination of platelets in Iran are rarely reported.
The aim of this study was to evaluate the prevalence of bacterial contamination of platelets concentrate collected at Tehran Regional Blood Center. In this cross-sectional study, polymerase chain reaction (PCR) amplification assay by 16srDNA method and conventional culture methods were both used, concomitantly.
In this study, initially, two milliliters were randomly taken as sampling from all PLT units from different time points due to storage. By phenotypic technique, all the units were tested for sterility by inoculation of one milliliters of samples into 10 milliliters of thioglycollate broth, followed by the incubation for a maximum of 7 days. Although manual methods, strain were identified based on biochemical properties using analytical profile index 20E (API 20E). By genotypic technique, samples were analyzed by PCR of a highly conserved region of the bacterial universal gene (16s rDNA). Genomic DNA was extracted from each samples using a commercial kit. The 16srDNA sequence were compared with those available in the gene bank database. The resistance pattern from isolated strain were done using standards of the clinical and laboratory standard institute (CLSI). Data were analyzed using fishers exact test with significance test at a P value of <0.05.
Over a period of 6 months (from June through November 2015), a total of 1500 samples were tested during the shelf-life of platelet concentrate (days 0 to 3). One sample was flagged as true positive by both techniques. It was isolated on day 4th of storage time. The responsible organism was identified as Pseudomonas aeruginosa by two techniques. Antibiotic pattern show that the strain was sensitive to the all antibiotics used. By reviewing the donor questionnaire, donor had no symptoms or significant medical history and no evidence of blood stream infection was found.
The performance of this platform demonstrate reduced bacterial contamination rates to comparable to those finding around 10 years ago in Iran. But, finding of the Pseudomonas spp. is suggestive of environmental contamination. The previous studies have shown that improved donor screening, better skin disinfection, removal of initial aliquot of donor blood play an important role in control of bacterial contamination rate. Our finding suggests that more attention should be paid to optimize new method for disinfection techniques and sterilization procedures for preparation places and storage equipment's.

P-298

BACTERIAL CONTAMINATION OF PLATELET POOL

E Valente[1], A Spínola[1], M Sampaio[1], F Dias[1], M Couto[2], S Ponte[2], R Araújo[2], E Machado[2], M Bini Antunes[1], M Amil[1] and J Coutinho[1]
[1]Centro Hospitalar do Porto, Porto, Portugal [2]CHP, Porto, Portugal

Background: Incidence of bacterial contamination in platelet concentrates (PC) varies between 0.08 and 0.70%. Storage temperature increases contamination risk, with residual risk for post-platelet transfusion bacteraemia between 0.05 and 0.07%. Bacterial contamination rate can be reduced by implementing preventive measures related with correct blood donor selection, as well as adequate procedures during blood collection, processing and storage.
Aims: To compare the incidence of platelet pools (PP) bacterial contamination, from 2012 (January 1st) to September 2014- 1rst period, and since then to 2016 (March 31st) - 2nd period, before and after the implementation of quality corrective actions, respectively. And to assess the post-transfusional receptor infection risk.
Methods: In September 2014 deep corrective actions were implemented at the processing facility and training health care professionals. The incidence of contaminated pools between both periods were compared. PP were randomly selected for microbiologic cultures (BacT/ALERTT aero/anae), most of them at the last day of expiry date. The positive results were confirmed by VITEK (BioMerieux). The potential contamination of erythrocyte concentrates (ECs) processed from same whole blood donation as the positive PP was also evaluated. Donor clinical data and notification of adverse reactions in the receptor were reviewed.
Results: Of 4013 PP produced, 904 (22.5%) were submitted to microbiological control (579–14,4% before sept 2014 and 325 – 8,1% after) with 6 positive cultures (3 pooled from five, 2 from four and 1 from three buffycoat) corresponding to 26 whole blood donations. All positive cultures occurred before September 2014 and motivated the implementation of corrective actions. Donor records corresponding to the 26 collections were reviewed and no signs or symptoms of infection during or 1 month after donation were documented. The identified microorganisms were: 4 *Staphylococcus* coagulase negative, 1 *Propionibacterium acnes* and 1 *Alistipes ondordonkii*. Two PP were not transfused due to expired date and 1 due to positive microbiological QC. Three PP were transfused (two *Staphylococcus* coagulase negative and one *Alistipes ondordonkii*): 1 to a 42 years old male patient with acute myeloid leukemia, 1 to a 70 years old female patient with alcoholic cirrhosis and 1 to a 48 years old male with acute myeloid leukemia. Of the 26 blood donations that were pooled into PPs, all 10 ECs (38.5%) tested had negative results. Patients clinical files that received those PPs and ECS showed no evidence of adverse transfusional reaction. Besides, from 2012 to 2016 the hospital haemovigilance system has no report of sepsis associated with transfusion.
Summary: Blood safety is a continuous process that begins with donor selection and goes beyond patient transfusional event. Identified bacterial strains mostly belonged to skin contamination flora. As the minimum incubation period for blood cultures is identical to PP expiring date, transfusion of 3 PP with positive microbiological cultures could not be avoided. However, no reactions occurred after transfusion of any of these components. One explanation could be the fact that most of platelet transfused patients have haemato- oncological diseases and under antibiotic treatment at the moment of transfusion. Implementation of deep corrective actions and training of health care professionals resulted in a significant improvement of quality with no positive cultures detected thereafter and safer transfusion medicine. Microbiological control is performed over legislation requirements but one might question if there is a cost benefit effect of applying this procedure to all PP produced.

P-299

ACETYLSALICYCLIC ACID LIMITS, THOUGH DIFFERENTIALLY, ACTIVATION AND APOPTOSIS OF HUMAN PLATELETS EXPOSED TO VARIOUS *STAPHYLOCOCCUS AUREUS* STRAINS

F Cognasse[1], A Chabert[2], P Damien[2], P Verhoeven[2], F Grattard[2], P Berthelot[2], F Zeni[2], M Darmon[3], MA Eyraud[1], B Pozzetto[4], B Payrastre[5], O Garraud[6] and H Hamzeh-Cognasse[4]
[1]French National Blood System (EFS), St Priest en Jarez, France [2]EA3064—GIMAP, Université de Lyon, Saint-Etienne, France [3]Service Réanimation polyvalente, CHU de St Etienne, Saint-Etienne, France [4]EA3064 - GIMAP, Saint Etienne, France [5]Inserm/UPS UMR 1048 - I2MC, Toulouse, France [6]Institut National de la Transfusion Sanguine (INTS), Paris, France, Paris, France

Background: In addition to their hemostatic functions, platelets can sense pathogens via several receptors, and react differentially depending on the nature of the stimulus, and subsequently adapt the secretory outcome and the inflammatory

response. Platelets are indeed widely considered as active players of inflammatory phenomena. Exacerbated inflammation is a prominent feature of the pathogenesis of several infectious diseases and particularly of the early phase of severe sepsis. *Staphylococcus aureus*, one of the most frequent microorganisms responsible for sepsis, considered as being associated with poor prognosis, proves to induce a drop in the patients' platelet count. Conversely acetylsalicylic acid and statins, that are active on platelet adhesion/aggregation, were shown to decrease the mortality rate in patients with bloodstream infections.

Aims: Therefore, it appeared of particular importance to investigate whether these two molecules (acetylsalicylic acid and fluvastatin) can have a protective effect on platelets exposed to *Staphylococcus aureus*, in order to limit thrombocytopenia and inflammatory response exacerbation.

Methods: Freshly drawn, human platelets were exposed to live clinical and reference strains of *Staphylococcus aureus* in the presence or not of acetylsalicylic acid or fluvastatin. Platelet viability, expression of activation markers, aggregation, and release of soluble CD62P, soluble CD40L, RANTES and GROα and death mechanisms (necrosis and apoptosis) were assessed.

Results: Every tested *Staphylococcus aureus* strains induced platelet activation (increase expression of CD62P and CD63), release of RANTES and GROα none, however, altered platelet aggregation. Staphylococcal strains triggered platelet death with decrease of the mitochondrial membrane potential and increase Annexin V exposure. We next observed that acetylsalicylic acid, but not fluvastatin, limited platelet activation, inflammatory factor release and restored platelet count.

Summary/Conclusions: This study demonstrates that acetylsalicylic acid limits, *in vitro*, some alterations inflicted by clinical strains of *Staphylococcus aureus* on platelets. This offer new paths to prevent bacteremia associated-inflammation.

P-300

RAPID DETECTION OF BACTERIAL CONTAMINANTS IN PLATELETS COMPONENTS: COMPARSION OF TIME TO DETECTION BETWEEN THE BACT/ALERT® VIRTUO AND THE BACT/ALERT® 3D

A Paris[1], P Deol[1], M Adamik[1], J Viray[1], M Dunne[1], K Aplin[2], J Allen[2] and C McDonald[2]

[1]*BioMerieux, Chemin de l'Orme, France* [2]*NHS Blood and Transplant, London, United Kingdom*

Background: The rapid detection of bacterial contamination in platelet units is key to reducing the risk of infection in transfusion of blood components. The BacT/ALERT® (BTA) VIRTUO[TRADEMARK] Microbial Detection System is the next generation of BacT/ALERT® instrumentation providing automation of processes, and an improved user interface. Most importantly, VIRTUO[TRADEMARK] uses a new algorithm designed to significantly reduce the time to detection (TTD) of microorganisms when used with BacT/ALERT® BPA (aerobic) and BPN (anaerobic) bottles for the quality control testing of platelet preparations.

Aim: Validation of VIRTUO[TRADEMARK] for use with BPA and BPN bottles when testing pooled Leukocyte Reduced Whole Blood Derived Buffy Coat Platelets (buffy coats) and Leukocyte Reduced Apheresis Platelets* (LRAP) preparations.

Methods: Pooled buffy coats (plasma only, and plasma plus platelet additive solution (PAS)) and LRAP (without PAS) were seeded with low levels (<1–31 cfu/ml) of organisms, commonly associated with platelet contamination, and inoculated into BPA and BPN bottles. Buffy coats platelet work was performed by NHS Blood and Transplant while LRAP platelets work was performed by bioMerieux. 400 bottles seeded with buffy coats and 11 organisms were loaded into a BacT/ALERT 3D (BTA3D) and a VIRTUO and incubated until declared positive by the instruments or for up to 7 days. For LRAP, 144 and 48 bottles seeded with 13 organisms were loaded into a VIRTUO and BTA 3D respectively. BPA and BPN bottles inoculated with unseeded buffy coats and LRAP platelets were incubated as negative controls. Seeded bottle data were used to evaluate the differences in the overall detection rates between instruments. A minimum of 200 bottles in each instrument (platelets only, no organism) were tested to evaluate differences in the overall negative agreement rates (detection of false positives) between instruments and to serve as sterility controls for the platelet preparations.

Results: Overall, both systems (VIRTUO and BTA 3D) detected 400/400 (100%) of the bottles seeded with buffy coats regardless of bottle type or platelet preparation type. Similarly, all (192) bottles tested for LRAP for both systems were 100% detected. There was no difference in negative agreement rates between systems ($P > 0.05$). There was no difference in detection rates or negative agreement rates between systems overall, or by bottle type or platelet type. VIRTUO was faster than BTA3D, overall, in detecting microorganisms with a difference in the means of 2.067 h ($P < 0.001$). average of 19.7% or 2.75 h ($P < 0.001$) for LRAP platelets.

Conclusions: There was no difference in detection rates between systems by bottle type or platelet type. VIRTUO was faster in TTD for both pooled buffy coat and LRAP platelets preparations.

* *VIRTUO is not commercially available for testing LRAP pending completion of validation studies.*

P-301

DIAGNOSIS OF SUB ACUTE BACTERIAL ENDOCARDITIS FOLLOWING BACTERIOLOGIC SCREENING OF APHERESIS PLATELET DONATIONS: BEWARE OF DENTAL WORK IN DONORS

A Shokoohi[1], S Pearce[1], A Williams[2], R Morgan[1] and S Field[1]

[1]*Welsh Blood Service, Pontyclun, United Kingdom* [2]*Aneurin Bevan University Health Board, Newport, United Kingdom*

Background: Platelets are stored at room temperature and therefore provide a potentially hospitable environment for the growth of many bacterial organisms. Although rare, these contaminated products are source of a huge clinical concern in transfusion medicine. The Welsh Blood Service (WBS) was amongst the first national blood services to establish universal bacterial screening of platelet components as a measure to minimise the risk of septic transfusion reactions. In this setting follow up of confirmed contaminated donations is essential.

Aim: We present two recent cases of bacteriologically contaminated apheresis platelet donations that culture organisms of the *Streptococcus viridans* group. Both of these cases had some dental work prior to their donations and in one case further follow up confirmed persistent bacteraemia and subsequent diagnosis of subacute bacterial endocarditis in the donor.

Methods: All pooled and apheresis platelets collected by WBS are sampled for bacteriologic screening on Day 1 and day 4 after collection and analysed through BacT/ALERT® system. Reactive cases are cultured in aerobic and anaerobic medium for confirmation and identifications (BBL Crystal). Here we describe two donors with confirmed bacteriologically positive donations. We followed these donors up through their diagnostic journey and treatments.

Results: Case 1: a 53 year old male regular platelet donor who had an uncomplicated donation in July 2015. Bacteriologic screening was reactive and subsequent identification of microorganism confirmed *Sterptococcus parasanguinis (a type of Streptococcus viridians)*. Interview with the donor indicated that following donation he has had some constitutional symptoms like night sweats. He confirmed that he had major dental work around 6 weeks prior to his donation. Further blood culture from the donor confirmed persistent positivity and subsequently he was referred to local district hospital. A trans oesophageal echocardiography (TOE) confirmed calcified bicuspid aortic valve with a mobile vegetation. This along with more positive blood culture (same micro organism) confirmed diagnosis of subacute bacterial endocarditis. He received 4 weeks of intravenous antibiotic to complete clinical recovery.

Case 2: a 54 year old male regular platelet donor who had a routine uncomplicated donation in February 2016 and his donation was flagged reactive on bacterial screening. This was confirmed positive and proved to be *Streptococcus parasanguinis*. An interview with the donor revealed that he had episodes of shivering.. He also confirmed that he had dental work 3 weeks prior to donation as well as having personal habit of aggressive cleansing of his teeth and tongue. He was referred to local cardiology department for further investigation and a TOE and repeat blood culture ruled out bacterial endocarditis. Clinically, he recovered fully following a course oral antibiotics.

Conclusion: Despite many different initiatives to prevent bacterial contaminations of platelets (verifying the donor temperature, pre-donation questionnaire, optimizing skin disinfection and diverting the initial aliquot of blood collected during the procedure), these contaminations still happen. Many of these contaminations are skin flora but microorganisms in mouth, *Streptococcus viridans* group, are clinically important. These two reported cases highlight the need for specific attention to mouth hygiene and recent history of dental work in platelet donors. A structured follow up procedures for confirmed contaminated donations facilitates diagnosis of potentially serious clinical conditions among platelet donors.

P-302

REFORMULATION OF ARCHITECT SYPHILIS TP ASSAY LEADS TO IMPROVED SPECIFITY WHEN TESTING BLOOD DONOR SPECIMENS

M Hoffmann[1], M Anderson[1], S Hershberger[2], L Gu[2], V Salbilla[2], C Koch[1], A Vockel[1], U Spies[1], G Williams[2] and G Schlauder[2]

[1]Abbott GmbH&Co.KG, Wiesbaden, Germany [2]Abbott Diagnostics, Abbott Park, United States of America

Background: According to WHO recommendations, every blood donation should be screened for treponemal antibodies using highly sensitive and specific assays to minimize the risk of Syphilis infection through the route of transfusion. The ARCHITECT Syphilis assay underwent reformulation to further enhance specificity and precision.
Aim: We undertook testing of specificity at large scale throughout all phases of the developmental process to demonstrate specificity with a maximum number of lots before launching the product.
Methods: A total of more than 25,000 donor samples (more than 10,000 serum and plasma) from the United States and Europe were tested using 11 different lots manufactured throughout the developmental phase. All initial reactive samples were further tested with 3 immunoblots (INNO-LIA Syphilis Score, Mikrogen *recom*Line Treponema IgG and/or *recom*Line Treponema IgM) for confirmation. A sample was defined as positive if reactive in at least two of 4 assays, including the previous ARCHITECT Syphilis assay version. Imprecision was determined according to protocol EP5-A2 using the assay specific positive controls and 4 plasma panels.
Results: Resolved overall specificity of the assay ranged 99.93% to 99.95% for different donor populations and the SD to cut-off was at least 15.2. The within-laboratory precision using three different lots was determined to be 3.6%CV for the Positive Control and 2.2–2.8% for serum panel members.
Summary / Conclusions: The reformulation of ARCHITECT Syphilis resulted in improved precision and overall specificity for random blood donors. These data are confirmed by feedback from customers using the reformulated reagents.

P-303

PERFORMANCE OF A NEW AUTOMATED ASSAY FOR SYPHILIS

G Schlauder[1], M Anderson[1], E Bakker[2], A van Weert[2], S Worobec[1] and G Williams[1]

[1]Abbott Laboratories, Abbott Park, United States of America [2]Sanquin Diagnostics, Amsterdam, The Netherlands

Background: Blood donations are commonly screened for Syphilis in order to detect the presence of antibodies to the bacterium *Treponema pallidum*. In addition, continued pressures on laboratory operations demand that assays perform on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management. In response to the need for increased specificity for Syphilis screening assays, an improved automated prototype assay for the detection of antibodies to *T. pallidum* was evaluated.
Aims: To evaluate the overall performance of a new prototype chemiluminescence immunoassay for the detection of antibodies to the bacterium *T. pallidum*, on a next generation automated platform.
Methods: The performance of a prototype automated immunoassay for the detection of antibodies to the bacterium *T. pallidum* was evaluated on a new automated platform. Precision was assessed over 5 days evaluating positive samples. Sensitivity was evaluated on 140 known positive samples. Specificity was evaluated on samples obtained from 1819 blood donors and 200 diagnostic specimens.
Results: Precision was less than 10% CV for positive samples over 5 days. Overall clinical sensitivity was 100% on 140 known Syphilis positive samples. The unresolved specificity was 99.92% (2559/2561). Two initial reactive samples were detected. Both samples were repeat reactive. The repeat reactive rate on blood donor samples, excluding confirmed positive samples, was 0.08% (2/2561). Resolved specificity on 200 diagnostic specimens was 100% (200/200).
Summary/Conclusions: These results indicate that the new automated prototype Syphilis assay provided acceptable performance in precision, specificity and sensitivity. Sensitivity and specificity were comparable to the comparator assay.

Parasites

P-304

ANALYSIS ON EFFECTS OF DEFERRAL DONOR SELECTION BY DRUG UTILIZATION REVIEW

WH Lee[1], DJ Oh[1], BJ Hur[2], CY Kim[1] and MS Lee[1]

[1]Korean Red Cross Blood Services, Wonju, South-Korea [2]Korean Red Cross ICT Support Office, Seoul, South-Korea

Background: Korean Red Cross(KRC) has been categorizing donors who have taken deferral medications as deferred donors since 2007 for blood safety. In 2009, Blood Management Act was revised to allow KRC to receive deferral medications from the Ministry of National Defense and Health Insurance Review & Assessment Service (HIRA). However, this information was given weekly, and, to make it worse, it contained some information of patients who were prescribed deferral medications a few years ago. To solve this problem and strengthen blood safety, KRC began using Drug Utilization Review(DUR), system to share the prescription information in real time, to select donors in April 2014.
Aims: This research is to analyze effects of blood donor selection by comparing the status of deferred donors before and after the introduction of DUR service.
Methods: The status of deferred donors and the supply of blood donated by deferred donors for transfusion are compared 1-year before and after April. 2014 when DUR service began.
Results: Deferral rate in donor selection has increased from 0.05% to 0.24% after the introduction of DUR service.(Table 1)Furthermore, the supply of blood donated by deferred donors for transfusion has far reduced from 314 units to 55 units (Table 2).
Summary/conclusions: The introduction of DUR service has a huge impact on the selection of deferred donors and the prevention of their blood supply as DUR service shares the prescription information in real time.

Table 1. Status of Deferred Donors Due to Deferral Medications

No. of Period	2013. 4. ~ 2014. 3.	2014. 4. ~ 2015. 3.
No. of Donors[1)]	3,279,383	3,397,449
Deferred Donors	1,626	**8,222**
Rate	0.05%	**0.24%**

Time to introduce DUR service

[1)] Donors who try for blood donation. It does not mean that they have all donated blood.

Table 2. Supply Status of Blood Donated by Deferred Donors [2)]

Period	2013. 4. ~ 2014. 3.	2014. 4. ~ 2015. 3.
No. of Supply	314 unit	**55 unit**

Time to introduce DUR service

[2)] 55 units of blood had been supplied before deferred donors notified blood centers of PDI(Post Donation Information).

P-305

PERFORMANCE OF A NEW AUTOMATED ASSAY FOR ANTIBODIES TO *T. CRUZI*

G Schlauder, D Smith, J Bryant, A Piotrowski, M Dellamorte, V Salbilla and G Williams

Abbott Laboratories, Abbott Park, United States of America

Background: The parasite, *Trypanosoma cruzi* (*T. cruzi*), is the cause of Chagas disease which is endemic to the Americas and infects 6–8 million people. In order to prevent transfusion mediated transmission of this parasite, blood collection centers require high throughput anti-*T. cruzi* assays with high specificity and sensitivity. In addition, continued pressures on laboratory operations demand that assays perform

on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management.
Aims: To evaluate the overall performance of a new prototype chemiluminescent immunoassay for the detection of antibodies to *T. cruzi*, on an automated next generation platform.
Methods: The performance of the prototype automated chemiluminescence immunoassay for the detection of antibodies to *T. cruzi* was evaluated on a next generation automated platform and compared to another on-market chemiluminescent immunoassay. Specificity was evaluated on 1194 blood donor samples and 200 hospital/diagnostic patient samples. Precision was determined over 5 days.
Results: The overall resolved specificity in a blood donor population was 99.92% (1193/1194) with an initial reactive rate of 0.08%. Resolved specificity on 200 hospital/diagnostic specimens was 100.00% (200/200). Precision testing over a 5 day period showed percent CVs for positive samples of less than 10%.
Summary/Conclusions: These results indicated the new automated prototype anti-*T. cruzi* assay provided acceptable performance in specificity and precision, which were comparable to the current on-market anti-*T. cruzi* assay.

Newly Emerging Pathogens and other Transfusion Related Pathogens

P-306

EPIDEMIOLOGY AND GENOTYPES OF HEPATITIS E IN JIANGSU PROVINCE BLOOD DONORS, AN ACUTE SPORADIC AREA OF CHINA

H Lin, S Zhu, S Zhu, N Jiang, C Huang and J Sun
Jiangsu Province Blood Center, Nanjing, China

Background: Several studies have determined the hepatitis E virus (HEV) prevalence in parts of China, while no data exists about the HEV sero-prevalence among blood donors in Jiangsu province, east of China, an acute HEV infection sporadic area.
Aim: To investigate the prevalence of HEV-antibodies in blood donors and analyze the genotypes of HEV.
Methods: HEV-specific immunoglobulin M (IgM) and IgG were determined with ELISA assay in 1144 blood donors in Jiangsu Province Blood Center from September to October 2015. HEV RNA of antibodies positive samples were extracted and tested by nest-PCR.
Results: Overall, 20.98% (240/1144) blood samples were reactive for HEV-specific IgG antibodies, while 2.19% (25/1144) for IgM. The presence of anti-HEV IgG was not associated with gender or education level; however, it was fundmentally correlated with age and occupations. Our results indicated that the anti-HEV prevalence increases by age and there was a significant difference between the age groups regarding HEV sero-positivity. The sero-prevalence of HEV IgG in peasant was 23.38%. HEV viremia prevalence was 0.26% (3/1144). One is genotype I and two was genotype IV.
Conclusions: High HEV sero-prevalence was observed among the blood donors in Jiangsu province, China. It appears that the exposure to HEV increases with age. Meanwhile, more people should be examined. Further work will focus on the presence of HEV RNA in HEV-antibodies negative samples.

Anti-HEV IgM	Anti-HEV IgM	Blood donors (N=1144)	Frequency (%)	HEV RNA (N, %)
+	+	20	1.74	2 (10)
+	—	5	0.44	0
—	+	220	19.23	1 (0.45)
—	—	899	78.59	Not detected

Table 1. Prevalence of IgM and IgG of anti-HEV among blood donors.

P-307

HEPATITIS E VIRUS – SHOULD WE RECONSIDER ITS ROLE IN BLOOD DONOR SCREENING?

C Sargento, P Achando, C Ferreira, E Silva and J Tomaz
Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

Background: Hepatitis E virus (HEV) is the major cause of fecal-oral transmissible hepatitis worldwide. It is estimated that 20×10^6 HEV infections arise annually of which 3×10^6 become symptomatic and about 57×10^3 die, with a particular focus on pregnant women. It is usually associated with consumption of contaminated water in poor sanitary conditions. In developed countries it is usually transmitted by infected meat that has not been sufficiently cooked. It is also transmitted by blood components transfusion and organ donation.
Aims: To study three different groups: 1- voluntary blood donors, 2 - professional at risk of contracting HEV infection (slaughter house workers, butchers, pig farmers and veterinarians), 3- patients with clinical suspicion of HEV infection or other viral hepatitis (A, B, C, D).
Methods: Research Antibodies (Ab) HEV IgG/IgM ELISA-Euroimmun -Additional test confirmation antibodies: recomLine IgG/IgM HEV Mikrogen -Nucleic Acids - extraction easyMAG-BioMerieux -Polymerase Chain Reaction (PCR-HEV): Ceeram-Tools samples: –238 voluntary blood donors, 58 high risk workers and 217 patients with clinical suspicion of HEV or other viral hepatitis.
Results: 1-Blood donors, n = 238 97.1%HEV PCR negative, HEV IgG/IgM Ab ELISA/recomLine: Negative. 2.16% HEV PCR negative, HEV IgM Ab ELISA/recomLine: Negative and HEV IgG Ab Elisa/recomLine:Positive 0.43% HEV PCR negative, HEV IgG/IgM Ab ELISA/recomLine positive
2-Professional at risk of contracting HEV infection, n = *58*
72.4% HEV PCR negative, HEV IgG/IgM Ab ELISA/recomLine: Negative
24.1% HEV PCR negative, HEV IgM Ab ELISA/recomLine: Negative and HEV IgG Ab Elisa/recomLine:Positive
3.5% HEV PCR positive, HEV IgG/IgM Ab ELISA/recomLine: negative
3-*Patients with clinical suspicion of hepatitis E infection or other viral hepatitis,* n = *217*
92.12% HEV PCR negative, HEV IgG/IgM Ab ELISA/recomLine: Negative
6.90% HEV PCR negative, HEV IgM Ab ELISA/recomLine: Negative and HEV IgG Ab Elisa/recomLine:Positive
0.98% HEV PCR negative HEV IgG/IgM Ab ELISA/recomLine:positive
Conclusions: Among voluntary blood donors we did not detect active infections but HEV seroprevalence was 2.59%.
In workers at risk of contracting HEV infection, potential candidates to donate blood, we registered 3.5% active infections and 24.1% previous infections.
In patients with HEV clinically suspected infection or other viral hepatitis, we did not find active infections, however we identified 7.88% previous infections.
Blood transfusion departments must be very careful, especially when transfusing pregnant women or immunodeficient patients.
Blood centers should take special attention to donors coming from HEV endemic areas or with increased risk for acquiring this infection.
This is a small, single-centered study, thus, further studies are needed to make these results more consistent. Nonetheless it suggests the need to reconsider blood donor screening in selected candidates.

P-308

MOLECULAR AND SEROLOGICAL MARKERS OF HEPATITIS E VIRUS INFECTION (HEV) IN POLISH BLOOD DONORS

E Sulkowska, D Kubicka-Russel, G Liszewski, A Kopacz, M Letowska and P Grabarczyk
Institute of Haematology and Transfusion Medicine, Warsaw, Poland

Hepatitis E (HEV) is detected worldwide in humans and animals both wild (deer, wild boar) and domestic (pigs). In developed countries the infection scope is limited with no significant impact/symptoms. In Poland the number of studies on HEV epidemiology is still rather limited.
The aim of our study was to investigate the prevalence of HEV infection markers among Polish blood donors.

Material and methods: Blood samples were collected from 4409 first time and 1081 repeat blood donors in 21 Polish Blood Transfusion Centers. Each sample was tested individually for RNA HEV with TMA (Procleix HEV Assay, Grifols; sensitivity 7.89 IU/ml) and 2408 first time donors were additionally examined for anti-HEV using Wantai HEV-IgG ELISA and Wantai-HEV-IgM ELISA (Beijing Wantai Biological). RNA HEV and IgM repeat reactive donations were tested with PCR Real Star® HEV RT-PCR Kit 1.0 (Altona Diagnostics) and the latter were confirmed for initial reactivity with recomLine HEV IgG/IgM (Mikrogen, Diagnostik).
Results: Three (3) donations were found initial reactive in RNA HEV (1/1,830 donation), two (2) however (1/2,745 donation) were repeat reactive and further confirmed reactive in real-time PCR.
On average, HEV-specific antibodies were detected in 44.7% serum samples from first time donors. 1053 of 2408 donations were IgG positive (43.7%), 24 of them were also IgM positive (1.03%). Six(6) donors were only IgM positive (0.2%). 7 of 19 (36.8%) IgM positive samples were confirmed in Western Blot. The IgG frequency differed between geographical regions from 30% in Podlasie to 65% in Wielkopolska. The IgG detection increased with age from 25% in the youngest donors (18–27) to over 65% in older donors (47–58). Both IgG and IgM were more frequently detected in males than in females (47.8% vs 39% and 1.04% vs 0.2%, $P < 0.05$).
Conclusions: We identified 33 donors with acute HEV infection markers. The, marker of past infection (anti-HEV IgG) was detected in almost 45% of donors. Up till now HEV diagnostics in Poland was almost unavailable therefore there is an urgent need to update information on the clinical significance of HEV in Poland as well as to protect blood component recipients against transfusion transmitted HEV.

P-309

SEROPREVALENCE OF HEPATITIS E AMONG CROATIAN BLOOD DONORS

M Miletic Lovric[1], M Stojic Vidovic[1], A Hecimovic[1], I Mihaljevic[1], L Jemersic[2], M Strauss-Patko[1] and I Jukic[1]

[1]Croatian Institute of Transfusion Medicine, Zagreb, Croatia [2]Croatian Veterinary Institute, Zagreb, Croatia

Background: Hepatitis E (HEV) has become the topic in transfusion medicine a few years ago since its occurence is increasing in developed countries. Therefore there is a risk of viral transmission by blood. Blood donors in Croatia are not routinely screened for HEV and there are no data regarding HEV seroprevalence in the general population. We also had no *trace-back* for HEV.
Aims: To estimate the seroprevalence of HEV among voluntary blood donors (VBD) in the Croatian Institute of Transfusion Medicine (CITM).
Methods: In October and November 2014 a total of 1036 serum samples of VBD were collected. All donors had previously completed the medical questionnaire to verify that they fulfilled the criteria for blood donation and had given informed consent. The study was approved by the Ethics Committee of CITM. There were 913 (88,1%) males and 123 (11,9%) females. All of the testing was done according to the producer's instructions. Samples were primarily tested for total HEV antibodies using a commercial enzyme immunoassay, HEV Ab, Dia.Pro Srl, Milan, Italy (performed on Gemini analyzer). All reactive samples were tested for HEV IgG/IgM by comercial immunoassays, HEV IgG/IgM, Dia.Pro Srl, Milan, Italy (performed on Gemini analyzer). All HEV IgM reactive samples were confirmed by a comercial immunoblot assay, *recom*Line HEV IgM, Mikrogen, Neuried, Germany (performed on Dynablot Plus analyzer). IgM-positive samples were further tested for the presence of HEV RNA. Viral RNA extraction (QIAamp viral RNA Mini Kit®, Qiagen, Hilden, Germany) was performed from 140 µl of each sera sample. A real time RT-PCR protocol (Jothikumar et al., 2006) for detecting a highly conserved fragment within ORF3 was carried out. The amplification was done in a Rotor-Gene Q machine (Qiagen, Hilden, Germany) by the use of commercially available kits (Rotor-Gene Probe RT-PCR kit, Qiagen, Hilden, Germany) according to the producer's instructions.
Results: 223/1036 (21,5%) samples were reactive for total HEV antibodies. 210/223 were reactive for HEV IgG and overall anti-HEV IgG seroprevalence was 20,3%. 45/223 samples were anti-HEV IgM reactive, 18/45 (1,7%) were confirmed by immunoblot HEV IgM test and HEV RNA was not detected in any of those 18 samples. There is a significant association between age (less and more then 40) and a higher seroprevalence ($P < 0,001$). The difference between males and females was not significant ($P = 0,807$).
Conclusion: We observed a high HEV IgG seroprevalence of 20,3% among voluntary blood donors in comparison to the results of previous Croatian data (used Anti-hepatitis E virus ELISA test, Euroimun, Lübeck, Germany) for different population groups: alcohol abusers 8,9%, war veterans 8,6%, injecting drug users 6,1% and healthcare professionals 2,7%. Results suggest that most autochthonous HEV infections are either asymptomatic or unrecognized. The HEV seroprevalence studies done in developed countries show significant differences, from 0,3% in Greece to 52,5% in southwest France, depending on different HEV antibodies detection assays "Wenzel et al, The Journal of Infectious Diseases, 2013". So, further study will be conducted that compare 3 different anti-HEV IgG/IgM tests.

P-310

No abstract available.

P-311

FROM A TO ZIKA IN TEN WEEKS – RESPONSE TO AN URGENT PUBLIC HEALTH NEED

LL Pate, ML Heil, G Spier, J Sun, S Dugenny, J Niemiec, N Dyer, P Albrecht, M Hoppler, PA Schlag, SA Galel, R Bertuzis, H Mokahel, J Tai, M Agarwal, L Poor, J Duncan, A Starron, G Heilek and J Carlson

Roche Molecular Systems, Pleasanton, United States of America

Background: On February 16, 2016, U.S. FDA issued recommendations to reduce the risk of transfusion-transmission of Zika Virus (ZIKV). Recommendations included cessation of blood collections in a ZIKV-active area unless donors were screened by a nucleic acid test (NAT) for ZIKV or components were pathogen reduced. The recommendation had an immediate impact on Puerto Rico, requiring discontinuance of blood collection, as no FDA approved NAT assay was available and use of pathogen reduction technology is limited to plasma and platelet products. FDA also contacted blood screening NAT manufacturers to determine the feasibility and timeline for ZIKV NAT tests and provided specific requirements for ZIKV NAT tests to be used under IND. Three assay candidates were designed *in silico* using a Roche proprietary process. Based on the urgent public health need, the decision was made to rapidly develop one candidate for use as an investigational assay.
Aims: To describe design, development and deployment of an investigational assay in ten weeks.
Methods: A Zika project team was assembled with representatives from Research, Development, Medical and Scientific Affairs, Quality, Regulatory, Operations and others. A project manager tracked all key activities, technical and quality system deliverables, and timeline. Daily huddle meetings were conducted to coordinate activities, review progress and address challenges. Interactive communications with FDA provided clear guidance on assay performance and IND requirements. To accelerate the timeline, clinical study preparation was concurrent with assay development and performance verification.
Results: Using a Roche proprietary process, three previously-identified, *in silico*-designed candidates were compared using the **cobas omni** Utility Channel, and the optimal design was rapidly developed into a fully-automated screening test with ready-to-use reagents on the **cobas**® 6800/8800 system. Reagent and software development activities were executed at sites in Europe and the U.S. by pre-staging reagents and samples where they would be needed, yielding two workdays from each calendar day. The verified design was transferred to Operations for GMP manufacturing of the investigational assay for donor screening.
Clinical study preparation included development of the study protocol, donor information and consent forms, Spanish translation of donor materials, institutional review board review, execution of contracts with clinical testing and >10 collection sites, contracting with a laboratory for confirmatory and follow-up testing, creation of Instructions for Use, database and case report forms, equipment installation and qualification, operator training, and collection site staff training. In parallel, reagent kit design, reagent forecasting and manufacturing, supply chain logistics, field service support, resource sustainability planning, and frequent communication with regulatory and public health agencies were conducted.
Summary/Conclusion: On March 30, 2016, FDA granted an IND approval for the **cobas**® Zika test for use on the **cobas**® 6800/8800 systems, six weeks after FDA issued recommendations, and ten weeks after assay design was initiated. Collection of donations in Puerto Rico resumed three days later. The rapid availability of the test was possible due to close coordination between FDA, the test manufacturer, the donor testing laboratory, and blood services in Puerto Rico.

P-312

IDENTIFYING ZIKA RNA POSITIVE SPECIMENS

N Dyer, S Dugenny, M Heil, G Spier, J Sun, H Mann, M Lin, B Wood, E Chuck, R Loeschnigg, K Bick, P Albrecht, M Hoppler, E Smith, C Sigua, C Ngo, EH Fiss, LL Pate and J Niemiec

Roche Molecular Systems, Pleasanton, United States of America

Background: The **cobas**® Zika test for use on the **cobas**® 6800/8800 systems was developed for blood screening under Investigational New Drug (IND). To establish sensitivity of the IND test, the FDA required analysis of Zika virus (ZIKV) positive specimens. Procurement of ZIKV positive specimens was challenging due to high demand, scarcity of ZIKV confirmed specimens, and barriers to shipment of specimens from some Zika endemic countries. A second in-house ZIKV PCR was developed as a tool for screening specimens with unknown status. This second in-house ZIKV PCR specifically uses the **cobas omni** Utility Channel, which is ideal for rapid prototyping and high throughput screening. The **cobas omni** Utility Channel utilizes most of the reagents used for cobas IVD tests, except the assay specific primers and probes, allowing for the flexibility to perform laboratory developed tests on the platform.

Aim: To develop a process to identify and confirm ZIKV positive samples in specimens which had been collected from endemic areas.

Study Design/Methods: An in-house test targeting a gene locus separate from that targeted by the **cobas**® Zika test was developed using the **cobas omni** Utility Channel functionality of the **cobas**® 6800/8800 system. This was used to identify "possible"ZIKV specimens by screening samples collected in Colombia and El Salvador between October 2015 - February 2016. Specimens were originally obtained from chikungunya and dengue endemic areas and found to be negative for those viruses. For screening, due to limited volumes, specimens were diluted at least 1:3.75 and tested singly. Broad Ct acceptance criteria were used to identify "possible"ZIKV positive specimens. Specimens identified as "possible"ZIKV by the in-house test were further tested in 4 replicates using RT-PCR with published CDC oligonucleotide sequences (Lanciotti, Emerg Infect Dis, 2008) and once by the **cobas**® Zika test. Specimens were considered to be confirmed for ZIKV if consistent reactivity (4/4 replicates) was observed using the CDC oligos.

Results/Findings: Of 1296 specimens screened by the in-house assay on the **cobas omni** Utility Channel, 111 were identified as "possible"ZIKV positive. Thirty-seven specimens with sufficient volume were tested using the CDC oligos and the **cobas**® Zika test. Three additional specimens with limited volume were diluted 1:100 and tested. Of these 40 "possible"positives, 23 specimens, including the 3 diluted specimens, were confirmed positive by the CDC oligos and all were reactive with **cobas**® Zika. Seventeen specimens had <4/4 reactive results with the CDC oligos; 15/17 tested positive with the **cobas**® Zika test (Table 1).

Specificity of the **cobas**® Zika test was 100% when tested using 500 ZIKV negative specimens.

Conclusion: The **cobas omni** Utility Channel enabled rapid development of an in-house RT-PCR test and facilitated screening of a large number of specimens. This method successfully identified ZIKV positive specimens for use in validation of the **cobas**® Zika IND test.

Number of Individual Specimens N=40	Number of CDC positive replicates per specimen	ZIKV Result interpretation w/CDC oligos	Number of reactive specimens using cobas® Zika
23	4 of 4 replicates	Confirmed	23/23 reactive
4	3 of 4 replicates	Equivocal	4/4 reactive
1	2 of 4 replicates	Equivocal	1/1 reactive
7	1 of 4 replicates	Equivocal	6/7 reactive
5	0 of 4 replicates	Negative	4/5 reactive

Caption 1 cobas® Zika detection in specimens with different levels of CDC reactivity

P-313

EVALUATION OF THE PREVALENCE OF ANTI-HEV IGM AND IGG ANTIBODIES IN 3 GROUPS: A GROUP OF HEALTHY PEOPLE, A GROUP OF PROFESSIONAL FORESTERS AND A GROUP OF HIV-INFECTED PEOPLE

A Bukowska, K Piersiala, H Olbromski and H Skalisz

Blood Center in Poznan, Poznan, Poland

Background: Hepatitis E (HIV RNA) is a small virus which belongs to the family Hepeviridae and gets transmitted by ingestion, with the short period of viremia. There are both acute symptomatic infections (vomiting, weakness, jaundice) and asymptomatic ones. The chronic infections were detected mainly in people who are immunosuppressed. In people with reduced immunity (eg. HIV) HEV infection can cause inflammation of the liver and lead to the cirrhosis. In developing countries, the most common cause of infection is the consumption of contaminated water and in developed countries the infection is linked to travels. In the acute phase of the infection, the virus can be transmitted by transfusion, although the epidemiological data that would allow the evaluation of the impact of HEV on the safety of blood transfusions, is still being collected all over Europe.

Aims: The aim of the study was a retrospective examination of a random group of healthy people, a group of professional foresters and HIV-infected people for the presence of anti-HEV IgG, IgM antibodies.

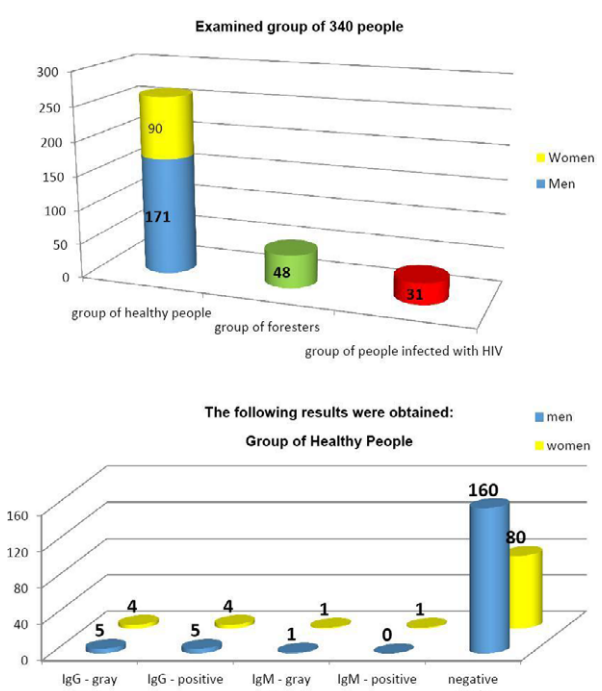
Methods: The study was performed using the EUROIMMUN test by Medizinische Labordiagnostika AG, Luebeck, Germany. The ELISA-type assay (IgM and IgG) is based on the recombinant target antigens of hepatitis E, genotypes 1 and 3.

The following interpretation of the results was adopted:

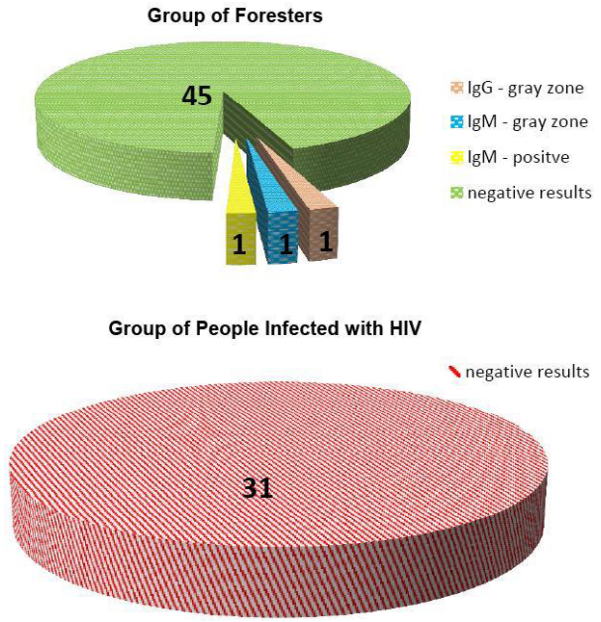
IgM - Ratio <0,8 negative
Ratio≥0,8 < 1,1 borderline
Ratio≥1,1 positive
IgG - < 1,6 IU/ml negative
≥1,6 < 2,2 IU/ml borderline
≥ 2,2 IU/ml positive

It is quite probable that the evaluation performed using various tests will show some differences and there may occur several discrepancies in the results of epidemiological data concerning HEV in Poland. Therefore, it would be advisable to conduct further research.

Results: The study involved a group of 340 people:



Graph 1



Graph 2

- 261 people representing a random group of healthy people (171 men and 90 women aged 18–50 years)
- 48 foresters - professional group
- 31 HIV infected people

Graph 1

Graph 2

Summary/conclusions: The HEV does not seem to be a major problem in the three groups: people infected with HIV, healthy people and the group of professional foresters. Summing up, to conduct reliable evaluation of the risk of HEV infection in Poland, further studies are needed including simultaneous testing of HEV RNA.

P-314

PREVALENCE OF TRANSFUSION TRANSMISSIBLE INFECTIONS IN BLOOD DONORS IN A TERTIARY LEVEL CARDIAC HOSPITAL IN PAKISTAN: A 02 YEAR EXPERIENCE

M Noorulamin[1], A Babur[1], S Salman[1], R Faheem[1], U Waheed[2] and A Hassan[2]

[1]Rawalpindi Institute of Cardiology, Rawalpindi, Pakistan [2]Safe Blood transfusion program, Islamabad, Pakistan

Background: The Safe Blood Transfusion Pakistan (SBTP) and World Health Organization (WHO) suggest that the screening of blood donors for the main transfusion-transmissible infections (TTIs): human immunodeficiency infection (HIV), hepatitis B Virus (HBV), hepatitis C virus (HCV), syphilis and malaria are compulsory. TTIs is one of the major public health concern. TTIs sre more common in replacement donors as compared to the voluntary blood donors.

Aims: The study goals were to observe the prevalence of these TTIs especially syphilis and HIV amongs apparent healthy blood donors at the tertiary level cardiac center.

Methods: A retrospective cross sectional study was conducted at Department of Pathology & Blood Bank of Rawalpindi institute of cardiology, a tertiary level cardiac hospital in Pakistan.Blood units collected from physically healthy blood donors during January 2014 to December 2015 were screened for anti HIV 1 and 2, anti HCV and HBsAg by enhanced chemiluminescence assay on VITROS® ECiQ immunodiagnostics system and Syphilis and Malaria Parasite by Immunochromatographic Technique (ICT).

Results: Of the 6053 consenting blood donors (98.7% replacement donors), the infected donors with HCV, HBV, HIV, Syphilis and Malaria were 1.71% (n = 104), 1.32% (n = 80), 0.16% (n = 10), 1.47% (n = 89) and 0.0% respectively. In 2014,

Year	Total Donation	Reactive HCV	Reactive HBV	Reactive Syphilis	Reactive HIV
2014	3312	67	41	42	04
2015	2741	37	39	47	06
Total	6053	104 (1.71%)	80 (1.32%)	89 (1.47%)	10 (0.16%)

seroprevalence of HCV, Syphilis and HIV were 2.02% (n = 67), 1.26% (n = 42) and 0.12% (n = 4) respectively, while in 2015, the frequency of infected donors with HCV, Syphilis and HIV were 1.35% (n = 37), 1.71% (n = 47) and 0.21% (n = 06) respectively (1). The annual rates indicated decreasing trends in case of HCV infection, but in case of Syphilis and HIV, there was a linear increase. There were no positive donor suffering with Malaria reported because of donor History form was properly filled by blood bank staff.

Summary/Conclusion: Our study raises genuine concerns with respect to the Syphilis and HIV pervasiveness in our country. We observed higher-than-anticipated syphilis and HIV seroprevalence rates in apparent healthy donors, than the quoted in earlier studies.Previously the HIV & Syphilis prevalence rate in our society was minimum but as shown in our study the prevalence rate is rising, the transmission of these diseases could be avoided by screening of the blood for 05 major TTIs by most sensitive assays and by promoting culture of voluntary non remunerated blood donation.

P-315

PERFORMANCE OF A NEW AUTOMATED ASSAY FOR HTLV I/II

G Schlauder, M Anderson, X Qiu, L Gu, L Jiang, S Lou and G Williams

Abbott Laboratories, Abbott Park, United States of America

Background: Blood donations are commonly screened to detect the presence of antibodies to human T-lymphotropic virus Type I and/or human T-lymphotropic virus Type II (anti-HTLV I/HTLV II). Blood centers require very high throughput anti-HTLV I/HTLV II assays with high specificity and sensitivity to prevent unnecessary donor deferrals while maintaining a safe blood supply. In addition, continued pressures on laboratory operations demand that assays perform on platforms capable of increased walk away time and enhanced automation in areas of reagent management, retest options, and commodity/waste management. In the response for the need for increased specificity for such screening assays, we have evaluated an improved automated prototype assay for the detection of antibodies to HTLV I and HTLV II.

Aims: To evaluate the overall performance of a new prototype chemiluminescence immunoassay for the detection of antibodies to HTLV I and HTLV II, on next generation automated platform.

Methods: The performance of the prototype automated chemiluminescence immunoassay for the detection of antibodies to HTLVI and HTLV II was evaluated on a next generation automated platform. Precision was assessed evaluating a number of known positive samples. Specificity was evaluated on samples obtained from 1543 blood donors. Sensitivity was evaluated using dilution panels comprised of three HTLV-I and two HTLV-II positive samples.

Results: Precision was less than 5.0% for samples with values within the range of 2.00 to 6.20 S/CO. The overall resolved specificity was 100.00% (1543/1543). Multiple lots of the HTLV I/II assay showed similar dilutional sensitivity profiles.

Summary/Conclusions: These results indicate that the new automated prototype HTLV I/II assay provided acceptable performance in specificity, sensitivity, and precision.

Immune Haematology: Red Cell Immunology: Serology

P-316

IMPORTANCE OF THE DU TEST IN BLOOD TRANSFUSION

A Verma, D Tiwari, P Negi, J Kumar, M Khan, S Singh and A Kumar
Max Super Speciality Hospital, Ghaziabad, India

Background: The rhesus D (RH D) antigen is expressed on the surface or red blood cells (RBCS). The RH antigens are highly immunogenic and the individuals who do not express the d antigen on RBCS generate antibodies (anti-D) upon blood transfusion with D antigen on transfused RBCS causing a hemolytic transfusion reaction. For this reason, current practice requires RBCS of first time blood donors to be tested for D antigen using two potent agglutinating anti-D reagents and a sensitive automated method. Subsequent donations need to be confined with only a single potent anti-D. The term DU is now redundant and has been replaced by the term weak D, which defines any D phenotype where the expression of D antigen is quantitatively weaker than normal. As red cells expressing qualitatively different D antigens may also give weak reactions with some anti-D antigens this whole area of blood grouping has been a source of great confusion over many years.
Aim: The main aim of this retrospective study was to highlight the importance of DU testing.
Methods: A retrospective study was conducted from January 2015 to March 2016. In total 11,294 blood grouping samples including 5182 donors and 6112 patients were tested for Du in the galileo device using potent antibodies D1 and D2 for RHD typing. Any blood donor sample that was typed RH D negative by the slide or rapid tube method was tested further by an indirect antiglobulin technique. If both tests were negative the donor sample was considered RH negative. If the donor sample tests came positive in any phase of RH D testing the sample was considered RH positive.
Results: Out of 5182 blood donor samples 4944 were positive and 238 were negative. DU test was performed on all the 238 negative samples using antiglobulin technique. Out of 238 samples 236(99.2%) were DU negative and 2(0.8%) were DU positive. Out of 6112 patient samples 5628 were positive and 484 were negative. DU test was performed on all the 484 negative patient blood samples using antiglobulin technique. Out of 484 samples 471(97.3%) were DU negative and 13(2.7%) were du positive.
Conclusions: Study showed if DU test was not performed on donor samples 0.84% of negative samples could have been missed and wrongly marked as RH D negative. Policies regarding D typing procedures and selection of blood components for transfusion should be based on the patient population, risk of immunization and limited supply of d negative blood components. Anti-D is clinically significant antibody and preventing immunization in females of child bearing potential is important to avoid the complication of hemolytic disease of newborn. The complications of anti-D in other patients are less serious and therefore decisions to transfuse rh positive or rh negative blood should be considered individually.

P-317

CLINICAL IMPORTANCE OF D VARIANTS IN PATIENT- CASE REPORT

M Krga Milanovic, N Milosavljevic Knezevic, N Bujandric and R Jovanovic
Blood Transfusion Institute Vojvodina, Novi Sad, Serbia

Background: The significance of the Rh blood group is related to the fact that the Rh antigens are highly immunogenic. D antigen is the most immunogenic antigen in the complex Rh blood group. Routine blood typing for RhD status in blood donors and transfusion recipients has reduced the incidence of hemolytic transfusion reactions caused by anti-D. About 85% of Caucasians are Rh D-positive. Variations of the D phenotype arise when epitopes are only weakly expressed ('weak D phenotype') or when some are missing ('partial D phenotype').
Aims: To present a patient with a serological weak D phenotype who formed anti-D and who have not been identified as having the 'partial D' phenotype by molecular testing.
Case Report: A 37-year-old woman was admitted in the Department of Otorhinolaryngology because of cancer of nasopharyngis, in December 2015. She had a history of two children (13 and 6 years), no previous spontaneous abortions, never received transfusions of blood or blood products. Blood sample was serological tested in The Department of Clinical Transfusion and showed the following results: AB type, weak D phenotype (tube method, anti-A and anti-B CE-Immundiagnostika GmbH, anti-A,B Monoclonal Lorne IgM, anti-D Duoclone Monoclonal Lorne (Blended IgM + IgG) and Anti-D Monoclonal Lorne IgM). Direct Coombs was negative (Liss/Coombs ID Card, BioRad). During hospitalization anemia in patient was treated with two units of red blood cells (RBCs), AB RhD positive. Pre-transfusion testing, which consisted of an AHG (Coombs phase) cross-match, was negative. Three months later the patient was again hospitalized for an operation. The blood grouping retested by automatic technique (QWALYS® 3, Erythrocytes Magnetized Technology). RhD type is not interpreted. Crossmatching with AB RhD positive RBCs became positive. Anti-D and anti-E antibodies were identified (ID-DiaPanel BioRad, LISS/Coombs ID-Card). RHD and RHCE genotyping were obtained by PCR SSP showed test result DCcee, with corresponding phenotype CcDee. PCR RHD and RHCE genotyping was done by an assay based on exons 1, 10 and 5 and could not confirm partial D.
Summary/Conclusios: People who have been identified as having the 'partial D' phenotype should not receive Rh D-positive blood. However in practice people with partial D are difficult to identify. Individuals with the weak D phenotype can receive Rh D-positive blood but it is important to consider that certain molecular types of weak D are capable of making anti-D.

P-318

ANALYSIS OF ABO AND RH BLOOD GROUPS AMONG BLOOD DONORS TESTED AT NATIONAL BLOOD CENTRE, SRI LANKA

I Karunasena
National Hospital of Sri Lanka, Colombo 10, Sri Lanka

Background: ABO and RH blood groups are the most commonly utilized grouping systems in blood transfusion. The incidence of abo and rh groups varies very markedly in different part of the world and in different races. The knowledge of prevalence of different blood group antigens in any given population is always helpful in managing blood transfusion system of a country, blood stock management, cases of alloimmunization, predicting disease prevalence etc.
Aims: To detect the frequency of abo and RH blood groups of blood donors tested at National Blood Centre, Sri Lanka.
Methods: This retrospective study was carried out at National Blood Centre, Sri Lanka during period of one year. ABO and RH grouping results of donors who donated blood or blood components to National Blood Centre, in-house or mobile collection campaigns, were considered. Frequency of A, B, Ab, O blood groups, RH antigen frequency (D, C, E, C and E) and probable RH phenotypes were analyzed in relation to ethnicity and sex.
Results: A total data of 2172 were collected, of which 1706 (75%) were males and 466 (25%) were females. Out of total, 2125 (97.89%) were Sinhalese, 25 (1.15%) were Tamils and the rest, 22 (1.10%) were Muslims. Majority were within the age group of 31–40. The detected ABO blood group frequencies were as follows: o (46.41%.), b (26.75%), a (21.13%) and ab (5.71%). Group o was the most common and group AB was the least common blood group in both RH D positive and D negative donors, in both sexes and in all three races. Percentages of Rh D positive and negative donors were relatively equal in both sex categories. The order of abo blood groups in relation to frequency in different ethnic groups were as follows. In sinhalese – O > B > A > AB, in tamils - O=A > B > AB and in muslims – O > B > A=AB. Among RH antigens, the highest frequency was seen in rh e (99.27%) antigen and RH E antigen (15.17%) was the least. The frequencies of RH antigens in different ABO groups were relatively similar. Rh c antigen percentage in muslims (73%) was higher than other ethnic groups. Other rh antigen frequencies in different ethnic groups show minimal variations. Probable RH-genotypes present in the decreasing order of frequencies were R1R1, R1R, R1R2, RR, R2R, R0R, R'R, R1RZ and R2RZ. R1R1 and R1R were in the commonest RH genotypes detected in both males and females.
Summary/Conclusions: Group O was the most common and group AB was the least common blood group. 95% of the study population is positive for the RH antigen D. R1R1 and R1R were in the commonest RH genotypes. It is necessary to conduct similar well designed studies in all provinces of Sri Lanka in order to determine the blood group frequencies in the population of each part of the country including all races.

P-319

COMPARISON OF THREE AUTOMATED BLOOD TYPING SYSTEMS FOR ABO/D TYPING IN ABO SUBGROUP

BK Jung[1], JY Kim[1], GY Oh[2], JS Lee[2] and CS Lim[1]

[1]Korea University Guro hospital, Seoul, South-Korea [2]EONE Laboratories, Incheon, South-Korea

Background: Blood grouping systems perform basic blood processing tests that include ABO grouping and subgrouping.

Aims: Three fully automated blood typing system, Qwalys-3 (DIAGAST, Loos Cedex, France), BioRad IH-1000 (Bio-Rad Laboratories, Hercules, CA, USA) and Ortho Auto-Vue Innova (Ortho, Raritan, NJ, USA), were compared with those obtained by standard manual methods for ABO subtyping.

Methods: The specimens were collected from Jan 2016 to Apr 2016 in the blood bank laboratory of Korea University Guro hospital. Twenty three cases of suspected ABO subtype were enrolled, and the patterns of agglutination score were analyzed after ABO/D typing by three automated blood typing systems and manual tube method.

Results: When less than one agglutination grade is considered as same reaction intensity, the concordance rates with manual reference method were 34.8% (8/23) for Qwalys-3, 26.1% (6/23) for BioRad IH-1000, and 65.2% (15/23) for Ortho Auto-Vue Innova, respectively. The Qwalys-3 showed relatively high grade reaction with anti-B in comparison with other two automated systems. The 3 automated blood typing system could detect mixed field reaction: 5.9% (1/17) for Qwalys-3, 64.7% (11/17) for BioRad IH-1000, and 23.5% (4/17) for Ortho AutoVue Innova.

Conclusions: Here, we demonstrated the concordance rates of 3 automated blood typing systems in comparison with manual reference method. Ortho AutoVue Innova showed better concordance, however, this degree was not enough for this system to be used as a sole modality for ABO subtyping. We have to consider automated blood typing system could miss exact ABO subgrouping.

TABLE 1 Rh ANTIGENS n673

	NUMBER	%
D	642	95.4
C	385	57.2
c	499	74.1
E	89	30.2
e	667	99.1

TABLE 2 Rh PHENOTYPING n=673

D POSITIVE SAMPLESn=400

Phenotype	number	%
CCDee	172	43.0
ccDEE	5	1.2
CcDee	137	34.2
ccDEe	18	4.5
ccDee	5	1.2
CcDeE	63	15.7

D NEGATIVE SAMPLESn=273

Phenotype	number	%
Ccdee	11	15.0
CCdee	3	0.7
ccdEe	2	1.1
ccdee	157	94.1

P-320

A PILOT STUDY FOR RH PHENOTYPING

M Pedapally and S Ranganathan

Apollo hospital, Hyderabad, India

Background: Rh antigenic system is complex and out of 50 antigens that have been discovered D,C,E,c,e antigens and their corresponding antibodies are of clinical interest in transfusion and obstetrics .A pilot study was undertaken to identify Rh antigens and their phenotypes which would help provide antigen negative blood to patients to prevent alloimmunisation to Rh antigens.

Aims: 1. To evaluate a blood group equipment using the SPRCA principle for ABO grouping and Rh typing; 2. Prevalence of 'weak D' in a population; 3. Pilot study to do Rh phenotyping of both Rh D positive and Rh D negative samples.

Methods: 12854 samples which included 9934 patient samples and 2920 blood donor samples were processed on the NEO (Immcuor,USA) which is microplate based using the Solid Phase Red Cell Adherence (SPRCA) as the principle.This automated blood grouping equipment does the cell and the serum grouping and reports the blood group if there is no discrepancy and the agglutination reaction is >+3. Reactions <+3 are flagged by the machine as NTD(No Test Determined).Rh typing for the samples were done on the same equipment using two antisera D1(IgM) and D2 (IgM + IgG).If both D1 and D2 are positive,samples are given as Rh D positive and if both are negative,Rh D is given as negative. If the results are discrepant between D1 and D2,results are not interpreted by the equipment and are flagged off as NTD. All RhD negative samples and Rh discrepant samples were processed by column agglutination technology gel cards,(Biorad,Switzerland) for weak D testing in the Indirect Antiglobulin phase .Samples showing >+2 were labelled as Rh D+ and samples showing reactions <+2 were labelled as RhD weak positive.Rh phenotyping was done manually for 673 samples which included 400 RhD positive and 273 RhD negative samples by using serological technique for the presence of C,E,c,e antigens using the respective antisera (Tulip,India). Postive reaction indicates the presence of antigen. Manufactures instructions were followed for all the assays.

Results: 1. No serum and cell discrepancies were noted for ABO grouping for 12854 samples.The time taken for blood grouping was approximately 20 min

2. Out of 12854 samples 12264 were RhD+ (95.4%) and 590(4.6%) were Rh D negative .15 samples were categorised as weak D (0.1%)

3. Rh antigens in our pilot study of 673 samples is depicted in Table 1.

4. Rh phenotyping for C,c,D,E,e is depicted in Table 2.

Conclusions: The Neo machine working on the SPRCA principle is easy to operate and gives accurate results .0.1% of our samples are weak D positive which were. The most common Rh antigen in our study is "e"found in 99%,followed by D antigen in 95.4%,E antigen was found in the least number of individuals in 30%.The most common Rh phenotype in RhD positive individuals is CCDee found in 43% and in RhD negative individuals is ccdee found in 94%.This is a pilot study done to study the Rh phenotype which can help provide antigen negative blood to patients to prevent Rh alloimmunisation.

P-321

INCIDENCE AND FREQUENCY OF VARIOUS RBC ANTIBODIES AND THE EXPERIENCE OF PROVIDING ANTIGEN NEGATIVE TRANSFUSION SUPPORT FOR PATIENTS AT A TERTIARY CARE SUPER SPECIALITY HOSPITAL IN INDIA

M Gupta, M Rane, N Mehta and R Sawant

Kokilaben Dhirubhai Ambani Hospital, Mumbai, India

Background: The presence of RBC auto and /or allo-antibodies can pose a serious challenge in terms of finding appropriate antigen negative transfusion support for such patients. Currently available literature focuses on alloimmunization in multi transfused patients, whereas fewer studies have focused on RBC alloimmunization in general patients.

Aim: To assess the frequency and type of unexpected RBC antibodies in a general patient population and analyze the transfusion support and its effectiveness in these patients.

Methods: A retrospective cross sectional study of the data of all patients admitted in our hospital for whom a blood transfusion was requested during the study period of 27 months was analyzed for various demographic, laboratory and clinical variables. Pearsons correlation ant t test (statistically significant at $P < 0.05$) were used for stastical analysis of the data. All patients were screened for atypical RBC antibodies using 3 cell screening panel. In case of a positive antibody screen, antibody identification was performed using a 11 cell identification panel. When the alloantibody was identified, corresponding antigen negative blood was chosen for transfusion. All immunohematology testing was performed using column agglutination technology.

Results: Amongst the 11711 patients screened for atypical antibodies, 73(0.41%) had alloantibodies and 11 (0.06%) had autoantibodies. Of the 84 patients with atypical antibodies, 66 (78.6%) where female and 18 (21.4%) where male. Investigating

the alloantibody specificities detected, antibodies against the antigen from the following blood group systems were detected Rh −54.5%, MNS −21.8% and Lewis-13.6%. Anti-D (32.8), Anti-M (16.4%) and Anti -E (10.9%) were the most prevalent RBC alloantibodies detected. Multiple alloantibodies were seen in 2 (2.7%) cases and autoantibodies co-existing without an alloantibody in 7(9.5%) cases. 541 RBC units were crossmatched for these 84 patients, of which 309 (57.1%) units were negative for the corresponding antigen and were found to be crossmatch compatible. 183 (59.2%) of the above compatible units were actually transfused to 36 (42.8%) patients. The mean increase in Hb per unit RBC transfused was 0.9 g/dl (range 0.5–1.4 g/dl). No serious adverse transfusion reactions were observed.

Conclusion: RBC alloimmunization rate (0.41%) in general patients treated in our hospital is lower compared to other reported rates in our country. Provision of RBC phenotype matched (Rh and Kell) blood for patients likely to get multiple transfusions right from the beginning many reduced alloimmunization rates further.

DONORS BLOOD GROUP RESULTS									
BL.GROUP	A-	A+	AB-	AB+	B-	B+	O-	O+	TOTAL
DISTRIBUTION	367	4944	169	2098	503	7757	521	6823	23182
%	1.58	21.33	0.73	9.05	2.17	33.46	2.25	29.43	

PATIENTS BLOOD GROUP RESULTS									
BL.GROUP	A-	A+	AB-	AB+	B-	B+	O-	O+	TOTAL
DISTRIBUTION	97	1206	36	491	109	1878	100	1689	5606
%	1.73	21.51	0.64	8.76	1.94	33.50	1.78	30.13	

Caption 1 Blood Grouping Results

DONORS PHENOTYPING RESULTS												
PHENOTYPING	D-	D+	C-	C+	c-	c+	E-	E+	e-	e+	K-	K+
DISTRIBUTION	1559	21621	3126	20052	9901	13272	18629	4543	284	22884	22683	488
%	1.12	15.55	2.25	14.42	7.12	9.55	13.40	3.27	0.20	16.46	16.31	0.35

PATIENT PHENOTYPING RESULTS												
PHENOTYPING	D-	D+	C-	C+	c-	c+	E-	E+	e-	e+	K-	K+
DISTRIBUTION	344	5264	788	4822	2219	3398	4469	1147	83	5524	5499	116
%	1.02	15.63	2.34	14.32	6.59	10.09	13.27	3.41	0.25	16.40	16.33	0.34

Caption 2 Phenotyping Results

P-322

THE PREVALENCE OF RED CELL ANTIBODY IN HEALTHY DONORS

RN Makroo, A Bhatia, S Chauhan, M Chowdhry and P Karna

Indraprastha Apollo Hospitals, New Delhi, India

Background: Irregular antibodies against red cell antigens may be present in healthy donors. Alloantibodies may be detected in donors who have either been transfused previously or female donors with previous obstetric events. These antibodies can occasionally cause severe transfusion reaction if a large amount of plasma or whole blood is transfused, as in massive transfusions and in pediatric patients.

Aims: The present study aims to assess the prevalence of red cell antibodies in healthy blood donors at a tertiary care hospital based blood bank in India.

Materials and Methods: A total of 82153 donor samples were screened for irregular red cell antibodies between January 2012 & December 2015 at the department of Transfusion Medicine, Indraprastha Apollo Hospitals, New Delhi. Antibody screening was performed by solid phase method using Immucor Capture -R ready screen (Pooled cells) on fully automated immunohematology analyzer Galileo Neo (Immucor Inc.Norcross,GA). Positive tests were further confirmed using Capture-R Ready Screen (4 cell panel). Advanced investigations to identify the antibody/ies were performed on confirmed positive samples. Antibody Identification was done using various cell panels (IMMUCOR Capture R- Ready-ID, PANOCELL-10, PANOCELL-10, FICIN TREATED). Advanced technique like adsorption, elution etc. were performed as per requirement.

Result: Screening with pooled cells and 4 cell panel was positive in 227 donors (0.27%), 150 of these donors had autoantibodies, 1 had autoantibodies with underlying alloantibody Anti-Jka (0.001%), 76 had alloantibodies (0.09%) alone in their plasma. Anti-M was the most common antibody (43 donors) identified; followed by anti-D (21 donors). Anti-N was detected in 4; anti-Jka in 3 & anti-P1, Anti-C & Ant-E in two donors each followed by Anti-Leb in 1 donor.

Conclusion: Antibodies against red cells can be present in healthy donors detection of which is important in providing safe blood to patient. The prevalence of RBC antibody in healthy donors in this study was found to be 0.27%, while prevalence of alloantibodies was 0.09%. The majority of alloantibodies were anti-M (0.05%) and anti D (0.02%).

P-323

This abstract has been withdrawn.

P-324

RH – KELL PHENOTYPING FOR SAFER BLOOD TRANSFUSIONS – DO WE REALLY NEED IN RESOURCE LIMITED COUNTRIES

A Agrawal

Fortis Escorts Heart Ist., New Delhi, India

Background: Alloimmunisation to red cell antigens is the challenge in currentTransfusion practices. The need for implementing routine Rh-Kell phenotyping for all donors and patient screening is being realised all over the world to prevent the risk of alloimmunisation as far as possible. Fortis Escorts Heart Institute in India is a leading hospital in India, specialized in cardiology and other specialities has high input of pediatric and adult patients from all over the world. With a high number of cardiac surgeries, use of blood components is in accordance. Our patients may need repeated transfusions time, massive transfusions and at times undergo redo surgery having multiple transfusion episodes. So a high risk of alloimmunisation and antibody development exists.

To eliminate the risk of alloimmunisation and ensure safer transfusions along the patient's lifetime, our hospital is among the first few centre in India to introduce Rh-kell phenotyping for all patients and blood donors wef.2012.

Material and Method: From March 2012 to Dec 2015, all the patients anticipating blood transfusion and all donors subjected to Rh-kell phenotyping and 3 cell panel antibody screening.

Tested 28788 samples- 23182 donors & 5606 patients(adult and pediatric) on fully automated immunohematology analyzer- QWALYS 3 (Diagast, France)using Erythrocyte Magnetized Technology®.

Data of the donor units are saved in the Microsoft Excel sheet with the donation & Unit details.

Patient phenotyping and antibody screening (IgM + IgG) is done at the time of admission.

For transfusion, ABO & phenotypically matched units are issued after an Immediate Spin cross match.

Result: Tables.

Conclusion: Introduction of Rh-Kell phenotyping of the donors and the patients has helped us in many ways:

- In identifying the phenotype of our population
- In providing antigen specific red cells to our patients thus ensuring another safety grid by avoiding alloimmunisation
- In reducing the stress & time in obtaining compatible blood component for the patients with existing alloantibodies.

With regards to the high frequency of immunogenic antigens of the Rh-Kell systems, pre-transfusion antibody screening on patients' samples and Rh-Kell phenotyping on donors are essential and needs to be implemented.

References: Gajjar M, Patel T, Bhatnagar N, Patel K, Shah M, Prajapati A. Partial phenotyping in voluntary blood donors of Gujarat State. Asian J Transfus Sci [serial online] 2016 [cited 2016 Apr 12];10:67–70.

AABB Technical Manual

Pineda AA, Chao GJ & Tazwell HF. Red cell antibodies: Clinical & Laboratory relevance in multitransfused patients In: Sirchia G & Zanella A (eds): Thalassemai Today. Milano, 1987, pp 57–61.

Dogra A, Sidhu M, Kapoor R, Kumar D. Study of red blood cell alloimmunization in multitransfused thalassemic children of Jammu region. Asian J Transfus Sci [serial online] 2015 [cited 2016 Apr 12];9:78–81.

P-325

RARE ERYTHROCYTE PHENOTYPES AMONG BLOOD DONORS OF REPUBLIKA SRPSKA

G Guzijan, B Jukic, V Cejic, S Mitrovic, B Đukelic Selak, B Kovacevic and D Udovcic

Institute for Transfusion Medicine of Republika Srpska, Banja Luka, Bosnia-Herzegovina

Introduction: Creating a registry and bloodbank for donors with rare blood groups, in order to provide transfusion therapy for the largest number of politransfused patients is one of the greatest challenges in immunohematology.

Aim: Determining the frequency of rare erythrocyte phenotypes among regular blood donors on the territory of Banja Luka and Republika Srpska. Determining the presence of donors with rare blood groups within the tested blood group systems. Based on the results, the goal is to create a database in the information system, as a base for creating a national registry of blood donors with rare blood groups.

Material and Methods: 384 blood donors were screened in the population of regular blood donors at the Institute for Transfusion Medicine of Republika Srpska in Banja Luka, males and females, the age of 18 through 55, with the blood groups A and O, RhD-positive and RhD-negative. The examinees belong to the category of regular blood donors. In this study 7% of the total number of blood donors on this territory were included. The distribution of clinically relevant erythrocyte antigens C, c, E, e, C^w, K, k, Kp^a, Kp^b, M, N, S, s, Fy^a, Fy^b, Jk^a, Jk^b,Lu^a, Lu^b, as well as the antigens P_1, Le^a andLe^b was tested. To determine the blood groups and erythrocyte antigen typing, the methods used were the test tube method, the gel method and the automatic microplate method (Techno BIORAD), by using different test serums (BIORAD USA, Biognost Zagreb, Novaclone Canada, SanguinHoland, Immucor USA, Diagast France).

Results: In the analysis of Rh phenotypes in the tested sample of 384 blood donors the phenotype Ccddee was found in 1.6% (6 blood donors) of the examinees, of which 3 groups are blood group O and 3 groups are blood group A, whereof two donors have the phenotype Le(a-b-), while one of them also has the phenotype Kp (a+b+) and the phenotype NN, two donors the phenotype Lu(a+b+). The Rh phenotype ccDEE was found in 2.2% (8 blood donors) of the examinees, whereof 4 belong to the blood type A and 4 to the blood type O. Of those, one donor also has the phenotypes NN, SS and Fy(a+b-). The Rh phenotype ccddEe was found in 0.8% (3 blood donors) of the examinees, of which 2 are blood groups A, and 1 belongs to the blood group O and one donor has the phenotypes NN and Fy(a+b-). Moreover, the two phenotypes with the same combination of the other antigens has been found in one individual with the Rh phenotype ccddee. The Rh phenotype ccDee was detected in 2.2% (9 blood donors) of the examinees, where 3 individuals belong to the blood group A and 6 individuals belong to the blood group O. Thereof, one donor has the phenotype SS, while four blood donors have the phenotype NN.

Conclusion: Based on the results, a database was formed in the information system, to serve as a base for creating a national registry of blood donors with rare blood groups for patients with alloimmunization and with clinically relevant anti-erythrocyte antigens, who need the blood of the appropriate phenotypes in the Institute for Transfusion Medicine of Republika Srpska.

P-326

A STUDY OF UNEXPECTED ANTIBODY PREVALANCE IN DISTRICT GENERAL HOSPITAL HAMBANTOTA

M Adikarama, MB Ranasinghe and NK Shilpeswarage

Blood Bank, Hambantota, Sri Lanka

Background: Blood Bank District General Hospital (DGH) - Hambantota is functioning as a cluster center for the Hambantota District of the Southern Province of Sri Lanka, with an annual red cell transfusions around 4000. The hospital gets above 60000 patients per year and has bed strength of 635. There is also a high prevalence of Sickle and Thalassemia patients in the area. In Sri Lanka Antibody identification is done at Immuno-Haematology Reference Laboratory at National Blood Center (NBC) - Colombo. When an alloantibody is detected in the screening procedure antibody identification should be done in order to find out clinical significance of that antibody and to provide compatible blood to the patient.

Aims: To find out the prevalence of alloantibody production of patients presented to DGH Hambantota.

Methods: A retrospective study was done using antibody positive cases for a period of 12 months from March 2015 to March 2016 at blood bank DGH Hambantota. Antibody identification was done by 2–3 cell panels and identification performed by manual indirect agglutination test (IAT) method at NBC.

Results: During the said time period a total of 25 antibody screening positive cases were reported. 22 were inward patients while 3 were from clinics. Out of 25; 20 were female patients and only 5 were male. Out of 25 screening positive cases antibodies were identified in 21 cases while 4 was reported as no antibodies. Of the 21 antibody positive cases, 17 were due to alloantibodies, 2 likely due to exogenous sources (administration of Anti-D) while 2 cases had unclear history to categorize. 3 patients had developed multiple antibodies and all three had a haematological pathology requiring regular blood transfusions.

Identified Antibody	Number of Cases	Percentage
Lewis (2 Le^a, 2 Le^b, 2 Le^a+Le^b)	06	24%
Anti-D	05	20%
Auto Antibodies	03	12%
Anti-c	02	08%
Anti-E	02	08%
Anti-c & Anti-E	01	04%
Anti-C, Jk^b, Di^a	01	04%
Anti-M	01	04%

Table – 01

Lewis antibodies were the commonest with a total of 6 cases (2 Le^a, 2 Le^b, 2 Le^a+-Le^b), 5 Anti-D, 3 Auto-antibodies, 2 Anti-c, 1 Anti-E, 2 Anti-E&c, 1 Anti-M and 1 case with Anti-C, Jk^b, Di^a. The type of antibodies identified are shown in Table 1.

From the study group 57.1% had clinically significant antibodies and 81% of them had a history of previous blood transfusion or sensitization event. Of the screening positive females majority (65%) were pregnant or had pregnancy related complication. ABO blood group was analyzed in the study group, with the normal prevalence of ABO grouping in Sri Lankan population using chi square; Chi Square value was 0.1905 and P value was 0.979112 which was not significant at $P < 0.05$.

Conclusion: Alloantibody formation is more common in female population especially in pregnancy. The commonest antibodies in antenatal women were Lewis & anti-D. This is comparable with a study; prevalence of red cell alloantibodies in antenatal women in Sri Lanka done by Kohombange CG. Patients with haematological conditions requiring regular blood transfusions are more prone to develop clinically significant antibodies. Similar results showed in a study; prevalence of red cell alloantibodies in antenatal women in Sri Lanka done by Rupasena IP. Therefore it is important to provide phenotypically matched blood for these patients in order to prevent alloantibody formation.

P-327

SELECTING COMPATIBLE RED CELL UNIT FOR A PATIENT WITH PAROXYSMAL COLD HAEMOGLOBINURIA WHO DEVELOPED SEVERE HAEMOLYSIS FOLLOWING TRANSFUSIONS

SMGS Manchanayake

National Blood Centre, Colombo, Sri Lanka

Background: Paroxysmal cold haemoglobinuria (PCH) is a form of hemolytic anaemia mediated by a biphasic IgG autoantibody, with the specificity of P antigen and it triggers complement-mediated intravascular hemolysis. Though most cases are acute, self-limited conditions, severe and rapidly progressive anaemia can occur in some requiring transfusions. This is a case of PCH presented with severe anaemia and aggravating haemolysis with transfusions.

Case Report: A 2 year old previously healthy girl was admitted with one day history of fever, loose stools and passing dark colour urine. She was very pale and icteric with mild hepatomegaly. Investigations revealed Hb of 5.5 g/dl. Blood smear showed red cell agglutination with polychromatics and erythrophagocytosis. Laboratory findings revealed the intravascular haemolysis. PCH was suspected and confirmed with positive Donath-Landsteiner (D-L) test. Since next day Hb was 3.5 g/dl, three units of blood were ordered. Blood group was A Rh D positive. DAT was positive with C3d specificity and antibody screening was negative. Group specific cross match compatible blood units were transfused using a blood warmer. Precautions were taken to avoid exposure to cold. Transfusions did not lead to a satisfactory rise in the Hb level. Patient passed dark colour urine within several hours following each transfusion and laboratory findings confirmed the haemoglobinuria. Routine transfusion reaction investigations were performed and results were compatible with pre-transfusion findings.

Since the possibility of allo-antibody was excluded, haemolysis of transfused erythrocytes must probably triggered by the Donath Landsteiner antibody of the patient. As facilities are not available for selecting P antigen negative donor units in our centre due to lack of anti-sera or molecular methods for typing, selecting a compatible unit for this patient was a challenge.
Aim: To describe a procedure for selecting a compatible blood unit for a patient with PCH under limited facilities.
Method: 50% red cell suspensions were made from randomly selected 10 units of group A Rh D positive blood units. To each labeled test tube, one volume of 50% red cell suspension and nine volumes of 37^{O} C separated patient's serum (containing D-L antibody) were added. As negative control, serum of a normal person was added to 50% suspensions of cells prepared from mixture of three group specific blood units. Each tube was incubated at 4°C for one hour and then at 37°C for 30 min as performing routine D-L test. Following incubation, supernatant was inspected after centrifugation for the presence of haemolysis.
Results: In nine out of ten tubes, haemolysis was detected in compared to negative control. Haemolysis was not detected in the supernatant of one tube confirming the absence of probably P antigen in red cells tested. Results were confirmed with repeat testing. The selected unit was also negative for the P1 antigen.
Summary/Conclusions: This method can be applied for selecting a compatible blood unit for patients with PCH similar to our case, in a laboratory with limited facilities and with a minimum cost. A future study is needed to find out the prevalence of P antigen in Sri Lankan population.

P-328

RBC ANTIBODIES IN PATIENTS WITH HAEMATOLOGICAL DISORDERS

E Velkova, T Makarovska Bojadzjieva, M Blagoevska, J Samonikov Tosevska, I Nikoloska and V Dejanova Ilijevska

Institute of Transfusion Medicine, Skopje, Macedonia

One of the grates challenges in immunohematology testing is to determine the specificity of irregular RBC antibodies when the presence of both auto and alloantibodies is suspected and blood transfusion is needed.
Aim: The aim of this study is to evaluate the rate of RBC antibody identification in patients with positive antihuman globulin test (AHG).
Method: We evaluated antibody identification data from 450 patients with AIHA and hematological malignancy. The patients were screened positive for irregular RBC antibodies mainly during the compatibility testing in the period from 2013 until April 2016. The antibody screening and identification were performed using AHG method in gel (Bio-Rad ID-System). Antibody elution was performed with acid elution kit (DiaCidel from Bio-Rad). Blood samples were taken in EDTA.
Results: In 68 (15%) of the patients only DAT was positive. In the rest of the 382 (85%) patients DAT was positive in combination with IAT. In 242 (64%) of the DAT/IAT positive patients the antibody identification process did not reveal specific antibodies in the plasma. In the rest of the 140 (36%) patients the specificity of the antibodies was as follows: anti-E (27%), -K (21%), -c (13%),-C (4%), -e (5%), Jka (6%), Fya (3.5%), -Jkb and -Fyb (1.4%), -M (11%), -P and -N (0.7%). None of the identified antibodies was detected in the eluate from the corresponding RBC. Multiple antibodies were identified in 14 pacients (anti-E+Jka, anti-K+Fya, anti-E+c being the most frequent combinations). RBC from 143 patients were subjected to elution. Only in 3 eluates specific antibodies were identified (1 anti-c, 1 anti-e and 1 anti-C). Patients were antigen positive for the corresponding autoantibody and recent transfusion was excluded.
Conclusion: It is essential to exclude or to confirm the presence of clinically significant RBC antibodies underlying nonspecific irregular antibodies in all cases in which auto and alloantibodies are suspected in order to provide compatible blood for transfusion.

P-329

THE PREVALENCE OF RED CELL BLOOD GROUP ANTIGENS AMONG EGYPTIAN POPULATION IN COMPARISON WITH OTHER ETHNIC GROUPS

R Abou Elfetouh[1], F Moftah[2], T Metwally[3] and M Fouda[1]

[1]*National Cancer Institute, Cairo, Egypt* [2]*Dar Elfouad Hospital, Cairo, Egypt*
[3]*National Blood Transfusion Services, Cairo, Egypt*

Introduction: Blood group antigens are inherited macromolecules on the outer surface of the red blood cells; some of these antigens are present on the cell surface of various tissues, as they are not red cell specific.

The prevalence of Rh phenotypes among Egyptians in comparison with other ethnic groups – Table 1

	Egyptians	Cote d'Ivoire	Indians	Whites [Caucasians]	Blacks	Iran	Asians
D	84.68%	92.93%	93.4%	85%	92%	90.2%	99.0%
C	65.64%	21.97%	84.8%	68%	27%	75.9%	93.0%
E	19.7%	13.82%	17.9%	29%	22%	29.5%	39.0%
c	78.78%	99.85%	52.8%	80%	98%	73.9%	47.0%
e	97.92%	99.85%	98.3%	98%	98%	97.9%	96.0%

The prevalence of other blood groups phenotypes among Egyptians in comparison with other ethnic groups – Table 2

	Egyptians	Caucasians	Blacks	Asians	Indians
K	8.23%	9%	2%	Rare	3.5%
k	99%	99.8%	100%	100%	99.97%
Fya	26.66%	66%	10%	99%	87.4%
Fyb	48.87%	83%	23%	18.5%	57.7%
Jka	83.88%	77%	92%	72%	81.4%
Jkb	58.75%	74%	49%	76%	67.6%
M	78.99%	78%	74%	79.7%	88.8%
N	40.1%	72%	75%	67.4%	65.4%
S	56.77%	55%	31%	8.7%	54.8%
s	86.1%	89%	93.0%	100%	88.7%
Lea	26.26%	22%	23%	------	-----
Leb	46.49%	72%	55%	------	-----

The [ISBT] identifies almost 344 red cell surface antigenic determinants classified to: *36 blood group Systems, Collections (200 series), 700 Series* and *901 Series*.
Besides the antibodies of ABO and Rh, antibodies to other clinically significant antigens e.g. Kell, Kidd, Duffy, MNSs, P, Lewis, and Lutheran are known to cause HTR, HDFN, or shortened survival of transfused red cells.
Certain blood types may affect susceptibility to malarial infections, as the Duffy antigen, which presumably as a result of natural selection, is less common in ethnic groups from areas with a high incidence of malaria.
A correlation between Duffy blood group phenotype and risk of breast cancer was also suggested.
Aim of the study: Is to detect the prevalence of red cell blood group antigens among Egyptian population and to compare it with that of other ethnic groups.
Having the knowledge of the prevalence of the implicated antigens in the local population is much more helpful to manage blood transfusion for chronic patients.
The information obtained can be used to develop new, and refine existing molecular techniques and to establish the correlation between blood group genotype and phenotype and to determine the occurrence of particular genotypes in different populations.
Methods and Techniques: The classical method used for testing of the blood group antigens was haemagglutination; Colum Agglutination Technique using Mono-specific anti sera (Biorad) was the serological technique used to test the red cells of our blood donors. It is the gold standard method to detect the presence or absence of blood group antigens on RBCs. This technique is simple, inexpensive, and when done correctly, has a specificity and sensitivity that is appropriate for the clinical care of the vast majority of patients.
Results: A total number of 3219 regular blood donors in NBTC and the blood bank of the National Cancer Institute was subjected to extended Rh system phenotyping (D, C, E, c and e) and to phenotyping for other blood group systems.
The prevalence of phenotypes associated with the blood group antigens among Egyptians in comparison with other ethnic groups is shown in tables 1, 2
Summary and Conclusions: The distribution of the blood group antigens among different races shows different findings.
This has clinical significance in transfusion medicine and in hemolytic disease of fetus and newborn [HDFN].

It is essential to provide antigen-negative blood to patients with medical conditions, who often require regular blood transfusion and who may have developed multiple antibodies. The prevalence of different blood group antigens in any given population is helpful in managing cases of allo-immunization in multiply transfused patients.

P-330

DIRECT BLOOD GROUP TYPING USING MDMULTICARD LATERAL FLOW TECHNIQUE WITH ANTIBODIES FROM POLYCLONAL SOURCE

M Binda

Medion Grifols Diagnostics, Duedingen, Switzerland

Background: A lateral flow assay for simultaneous typing of ABO, RhD, Rhesus phenotype and K with stable end-point and without a centrifugation step is in routine use since several years (MDmulticard) (Geisen, Vox Sang, 2006). For the typing of all these parameters IgM class antibodies from monoclonal source have been used. Recently, the typing of Fya, Fyb, Jka, Jkb, S, s, P1 and k with the same technique was reported. In this study, the successful use of monoclonal antibodies of the IgG class has been demonstrated (Caesar, Transfusion, 2015).

Aims: The aim of this study was to evaluate the feasibility of using polyclonal antibodies for direct blood group typing in a lateral flow assay. As model system, Kpa and Kpb, two antigens of the Kell blood group system, have been chosen.

Methods: Fresh blood was obtained from individuals previously determined serologically for Kpa and Kpb with established CE certified techniques (Anti-Kpa and Anti-Kpb, Medion Grifols Diagnostics, Duedingen, Switzerland).

The credit-card sized lateral flow test device consists of a membrane, which is equipped in a cassette housing. Two equidistant detection areas with parallel lines of antibody reagents against up to ten different blood group specificities are left and right of a central application zone. In this study, affinity purified polyclonal antibodies against Kpa and Kpb antigens were applied on the membrane below the left detection area.

For blood group typing, 100 µl of diluted whole blood or erythrocyte sediment are pipetted to the central application zone, followed by 300 µl of a rinsing solution. Results may be interpreted after 5 min. Positive results clearly impose as distinct red bands, whereas negative results lack the respective bands.

Results: By using affinity purified polyclonal Anti-Kpa and Anti-Kpb in the lateral flow technique, the phenotypes Kpa+b-, Kpa+b+ and Kpa-b+ could be reliably detected. Accelerated stability testing indicated stabilities that are comparable to monoclonal antibody based tests in the same format.

Summary/Conclusions: For the first time, the successful use of polyclonal antibodies for direct blood group typing in lateral flow technique is described.

MDmulticard lateral flow technique was presented earlier with unique features, e.g. simultaneous multiparameter testing without the need of centrifugation and results within 5 min. The detection of antigens in this technique was, however, limited to the use of monoclonal antibodies as source. In this study, we demonstrate the feasibility to directly detect with MDmulticard antigens for which no monoclonal antibodies are available, e.g. Kpa and Kpb. This may be the base to detect any blood group antigen with lateral flow technique in a direct 5 min assay. Further studies are needed to confirm the findings of this feasibility study.

P-331

INCIDENCE OF RHESUS ANTIGEN IN ABU DHABI BLOOD DONOR POULATION

N Oumeziane and E Al Zaabi

Seha / skmc, Abu dhabi, United Arab Emirates

Introduction/Background: The Rh (Rhesus) blood group system is one of the most polymorphic and immunogenic; it is the most important after the ABO in transfusion medicine; it is a complex blood group system that is currently comprised of more than 50 different antigenic specificities.

Aim: The aim of this study is to evaluate the frequency of Rh-phenotype in Abu Dhabi blood donor population and to compare it with other data from similar studies within the region.

Method: The red cell antigen typing has been introduced in Abu Dhabi blood bank in January 2014 for all blood donors to help better serve patients with special transfusion requirements.

The present retrospective study was carried out during a period of one year, from January to December 2014. A total of 20648 blood donors were considered medically fit and accepted for blood donation during the study period. The Blood donors tested were from both sexes and of age between 18 and 65 years including all nationalities.

Table 1: Frequency of RH1-RH5 antigens

Rh antigens	Number of donors (Percentage)
D	18769 (90.9%)
C	16198 (78.5%)
E	4495 (21.8%)
c	13604 (65.9%)
e	20316 (98%)

Table 2: Percentages of phenotypes and predicted Rhesus genotypes

Phenotypes	Predicted genotypes	Study population	Indian population (Makroo, Raj 2014)	Kalba Study UAE Population (Jenan Y Taha)
DCe	R1R1	33.7%	40.9%	28.1%
DcE	R2R2	1.5%	.78 %	1.8%
DCce	R1r	30.6%	30.9 %	30.9%
DCcEe	R1R2	13%	14.3%	11.5%
ce	rr	8.2%	4.76 %	7.4%
DcEe	R2r	6.6%	3.69%	6.7%
Dce	R0r	5%	1.15%	10.9% (R0R0)*
Cce	r'r	0.66%	2.32%	1.2%
DCEe	R1RZ	0.36%	0.32%	NA
cEe	r"r	0.17%	0.05%	NA

* Dce reported by Jenan Y Taha R0R0 as predicted genotype

The blood donors have been typed for Rh antigens (D, C, c, E, e) using a gel method.

Results: In this study, the predominant donors belonged to age group between 18 and 35 years (61%). Male donors (88.7%) were more than female donors (11.3%).

Rhesus group D positive is more prevalent at 90.9% while Rhesus negative population is at 9.1%. The most frequently occurring antigen was found to be e followed by D, C, c and E as depicted in Table 1.

The most frequent phenotypes were by decreasing order: DCCee 33.7% followed by DCcee 30.6%, DCcEe 13%, dccee 8.2%, DccEe 6.6%1, Dccee 5%, DccEE 1.5%, dCcee 0.66%, DCCEe 0.36% and dCCEe 0.17%.

The predicted Rh genotypes present in decreasing order of frequency as follows: R1R1 6957 (33.7%), R1r 6320 (30.6%), R1R2 2682(13%), rr 1691(8.2%), R2r 1369 (6.6%), R0r 1038 (5%), R2R2 314 (1.5%), r'r 136 (0.66%), R1RZ 75 (0.36%), r"r 35 (0.17%).

Conclusion: It was concluded through our study that the most frequent antigen amongst five major antigens of Rh system was **e** while the least common was antigen E, the most common phenotype was DCCee and the most frequent probable genotype was DCe/DCe (R1R1) while in Rh negative samples it was dce/dce (r r).

Among the D negative blood donors 90% were ccee (r r), 7.2%, Ccee (r 'r) and 1.9% were ccEe (r"r).

The results obtained in this study are similar to the results obtained in kalba study, Jenan Y Taha Bahrain Medical Bulletin, on UAE nationals except for the genotype R1r which comes in the first position for UAE nationals. Table 2.

P-332

RED BLOOD CELLS PHENOTYPING OF BLOOD DONORS IN ISLAMABAD, PAKISTAN

F Jabin[1], S Ahmed[2], K Haroon[3], U Waheed[3], Y Zaman[3], M Arshad[4], A Arshad[5] and HA Zaheer[3]

[1]PMAS Arid Agriculture University, Islamabad, Pakistan [2]Husaini Blood Bank, Karachi, Pakistan [3]Department of Blood Bank, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, Pakistan [4]Department of Biotechnology, International Islamic University, Islamabad, Pakistan [5]Department of Zoology, PMAS Arid Agriculture University, Islamabad, Pakistan

Background: At present, 33 blood groups representing more than 300 antigens have been identified globally. The blood transfusion services in resource limited countries including Pakistan only test for ABO and RhD antigens during cross matching. However, the transfusion of ABO-RhD compatible but unknown phenotype blood may

result in alloimmunization especially in multi-transfused patients. The most significant red blood cell alloantibodies encountered are from the Rh, Kell, Duffy and Kidd blood group system. The typing of these minor blood groups is frequently underrated and scarce data is available regarding the frequency of the blood group antigens other than ABO and RhD in Pakistani population.
Aims: Current study investigates the prevalence of ABO, Rh, Duffy, Kell, Kidd and MNSs groups among the population of Islamabad, Pakistan.
Material and Methods: The study was conducted at the Department of Blood Transfusion Services, Shaheed Zulfiqar Ali Bhutto Medical University, Islamabad, after ethical approval from the Ethical Review Board. Blood samples from 625 randomly selected blood donors (volunteer/replacement) were collected for extended antigen typing during a period of six months from August 2015 - February 2016. Direct (forward) blood grouping was performed using commercially available antisera (Bio-Rad Laboratories, DiaMed, Switzerland). All donors voluntarily participated in the study. The statistical analysis was done using SPSS version 20.0. Qualitative or categorical variables were described as frequencies and proportions. Gene frequencies were calculated using the Hardy Weinberg equation where $p + q = 1$ and $p = \{2 \times obs(AA) + obs(Aa)\}/2 \times \{obs(AA) + obs(Aa) + obs(aa)\}$; thus $q = 1 > 'p$.
Results: The study included 625 healthy blood donors, of which 425 were males and 200 were females. The mean age for male donors was 25.36 and 29.0 for female donors. Of the 625 blood donors, 575 were RhD positive (92%) and 50 (8%) were RhD negative and Du negative. The frequency of ABO blood group system was A (24.64%), B (34.72%), AB (9.28%) and O (31.36%). Amongst minor blood group antigens, Kell (K) antigen frequency was 4%, Duffy (Fy^a) 58.24%, Duffy (Fy^b) 39.84%, *Kidd (Jka) 65.28%, Kidd (Jkb) 42.24%,* M 86.88%, N 46.24%, S 49.44%, and s 30.4%. The gene frequency for various blood group systems was, Kell K (0.04), Duffy Fy^a (0.481), Fy^b (0.519), Kidd JK^a (0.537), JK^b (0.462), M (0.699), N (0.301), S (0.444), s (0.556). Most common phenotypes in Duffy system were Fy a + b- (34.56%), Fy a+b+ (27.04%), Fy a-b- (23.04%), Fy a-b+(15.36%). Most common phenotypes in MNSs system were M+N- (53.92%), S+s- (36.96%). M+N+ (32%), S-s- (32%), S-s+ (16%), S+s+ (15.04%), M-N+ (14.08%). Most common phenotypes in Kidd system were JK a+b- (40%), JK a+b+ (27.52%), JK a-b- (18.88%), JK a-b+ (13.6%).
Conclusion: Current study depicts the prevalence of the blood group antigens among Pakistani blood donors to be statistically different from those in the Caucasian, Black and Chinese populations. The information about the minor blood group antigens prevalence can assist in the management and prevention of erythroblastosis foetalis and providing antigen-negative compatible blood to patients with multiple alloantibodies.

P-333

FIRST REPORT OF THE RARE LITTLE P BLOOD TYPE IN PEOPLE OF IRANIAN DESCENT

T Peyrard[1], G Laiguillon[1], A Raneri[1], F Hassany[2], P Peihani[2], S Poupel[1] and M Moghaddam[2]

[1]Institut National de la Transfusion Sanguine (INTS), Paris, France [2]Blood Transfusion Center, Immunohematology Department, Tehran, Iran

Background: Anti-PP1Pk (previously known as anti-Tj^a) is a naturally occurring antibody made by the rare little p individuals. This antibody to a high-frequency antigen is considered highly clinically significant, either in transfusion (risk of severe and acute hemolytic transfusion reaction) or obstetrics (frequent and recurrent early miscarriages). In case of blood transfusion, p negative rare blood must systematically be used, but finding compatible donors represents a real challenge for blood bankers in many countries due to extreme scarcity of this phenotype.
Aims: To investigate antibodies to a high-frequency antigen of undetermined specificity in 9 patients originating from Iran, through an international collaboration
Methods: EDTA blood samples were collected from 9 patients with pan-agglutinating antibodies of undetermined specificity.
The blood samples were referred to the French National Immunohematology Reference Laboratory (IRL) for serological investigations, together with the initial test results and clinical information obtained by the IRL of the Iranian Blood Transfusion Organization.
Results: Five out of the 9 patients were identified as carrying a rare p phenotype, with anti-PP1Pk in their plasma. No underlying common alloantibodies were identified in the 5 p patients; autocontrols and DAT were negative. Three out of those 5 p patients were initially referred to the Tehran IRL by obstetricians/gynecologists specialists, because of spontaneous and multiple miscarriages. The close relatives of the 3 p female patients were also studied and 2 of their brothers were identified as presenting a p rare type as well.
Summary/Conclusions: The plasma from 9 patients from Iran, with antibodies to a high-frequency antigen of unknown specificity, were studied and 5 of them were found to show an exceptional p phenotype, also known as Tj(a-), with a potent anti-PP1Pk (anti-Tj^a). As the rare p phenotype was never detected or reported in Iran before, the antibodies of those 5 patients were considered in the initial investigation performed in Tehran as antibodies against a high-frequency antigen with an undetermined specificity.
The patients and their siblings with a p phenotype were informed about their unique blood group and the precautions they should take in case of hospitalization or pregnancy. The results of the 3 female patients with a history of recurrent miscarriages were sent to their respective obstetrician/gynecologist, with information about a specific clinical follow up in case of further pregnancy.
The rare p phenotype was previously described to be more frequently encountered in Scandinavia (Finland, Sweden), Japan and Northern Africa, with several different molecular backgrounds. We plan to further investigate the molecular basis of our p cases, which might be similar to the one reported in Northern Africa.
Iran has implemented with great enthusiasm a 'National Rare Donor Program' since 2010.This interesting simultaneous finding of 5 individuals with an exceptional p type allows for the opportunity to store their own blood (autologous donation) at −80°C for a possible future use, the better management of possible future pregnancies in the three p female patients, and the possibility to cryopreserve reagent red blood cells with a p type as well as anti-PP1Pk antisera, in order to identify new similar cases in an autonomous way in the IRL of Tehran.

P-334

STUDY ON PREVALENCE AND CHARACTERISTIC OF IRREGULAR ANTIBODIES OF PATIENTS HAVE BLOOD DISORDER DISEASES AT NATIONAL INSTITUTE OF HEMATOLOGY AND BLOOD TRANSFUSION, VIETNAM (2011–2015)

VD Binh, BTM An, HT Nga and NA Tri

National Institute of Hematology and Blood Transfusion, Hanoi, Vietnam

Background: Blood transfusion is an important support treatment method for patients have blood disorder diseases. Patients who were transfused can develop irregular antibodies if patients were not transfused blood group antigen matching units during treatment time. Transfusion reaction can occur in patients have irregular antibodies in next transfusion times. Study on prevalence and characteristic of irregular antibodies to select antigens matching blood units in order to transfuse patients will help transfusions more safely and effectively for patients.
Aims: Study on prevalence and characteristic of irregular antibodies of patients at national institute of hematology and blood transfusion (NIHBT), vietnam from 2011 to 2015. Materials: 9.860 patients who were treated in nihbt from 01/01/2011 to 31/12/2015.
Methods: Cross-sectional descriptive study. Using column agglutination technique and screening cell, panel cell which were made in nihbt to detect irregular antibodies of patients.
Results: The irregular antibodies prevalence was 3,4%; irregular antibodies prevalence in female and male was 4,4%, 2,6% respectively. Irregular antibodies prevalence related to blood transfusion times (no transfusion: 1,6%, less than 5 times: 2,2%, from 5 to 10 times: 3,7%, more than 10 times: 6,0%); there were 212 patients had 1 irregular antibody among 339 patients had irregular antibodies, anti-E and anti-MIA were found maily in this group (31,3% and 23,6% respectively); there were 127 patients had combined irregular antibodies: 2 irregular antibodies: 72 patients (21,2%), 3 irregular antibodies: 41 patients (12,1%), 4 irregular antibodies: 11 patients (3,2%), 5 irregular antibodies: 2 patient (0,6%) and 6 irregular antibodies: 1 patient (0,3%), anti-E combined anti-C was the highest prevalence (9,7%), follow anti-E combined anti-C and anti-MIA (8,3%), anti-E combined anti-MIA were met 6,5%. Anti-E of Rh blood group was met highest prevalence (62,8%) follow anti-MIA of MNS (45,1%) and anti-C of Rh blood group (26,8%), other irregular antibodies were met low prevalence: anti-C: 2,9%, anti-E: 2,9%, anti-D: 0,6%; anti-S: 2,4%, anti-M: 0,3%; anti-JKA: 6,5%, anti-JKB: 3,2%; anti-FYB: 2,9%; anti-P1: 1,5%; anti-LEA: 0,3%, anti-LEB: 0,3%.
Conclusions: Research has shown the picture of irregular antibodies' prevalence and characteristics of patients at nihbt:irregular antibody prevalence was 3,4%. Irregular antibody prevalence related to sex (male: 2,6%, female: 4,4%) and blood transfusion times (no transfusion: 1,6%, more than 10 times: 6,0%). Single irregular antibody was found much more than combined irregular antibodies (62,5% and 37,5%

respectively). Anti-E, anti-C of Rh blood group and anti-MIA of MNS had high prevalence in single antibody group and combined antibodies group. We met 2 patients had 5 irregular antibodies and 1 patient had 6 irregular antibodies: anti-E combined anti-C, anti-MIA, anti-JKB, anti-S and anti-FYB.

P-335

RESEARCH RESULTS OF BLOOD GROUP ANTIGEN MATCHED TRANSFUSION FOR PATIENTS HAD BLOOD DISORDER DISEASE AT NATIONAL INSTITUTE OF HEMATOLOGY AND BLOOD TRANSFUSION (2011–2015)

B An, VD Binh, HT Nga and NA Tri

National Institute of Hematology and Blood Transfusion, Hanoi, Vietnam

Background: Blood transfusion is an important support treatment method for patients have blood disorder diseases. Patients who were multi-transfused can develop irregular antibodies lead to uneffective blood transfusion and left the severe consequences for patients. Selection blood group antigen matching blood units in order to transfuse patients will help transfusions more safely and effectively.
Aims: Study on result of blood group antigen matching transfusion for patients had irregular antibodies at national institute of hematology and blood transfusion (nihbt), vietnam from 2011 to 2015.
Materials: One hundred and ten patients who were treated in nihbt from 01/01/2011 to 30/06/2015 were transfused blood group antigen matching units.
Methods: Cross-sectional descriptive study combine clinical intervention study, 110 patients who had irregular antibodies were transfused antigen matching blood units, assessing result of blood transfusion by some indexes before and after transfusion: red blood cell count, hemoglobin, hematocrit, indirect bilirubin, LDH and number of selected blood units.
Results: 2.024 blood group antigen matching units were selected to transfuse for 110 patients who had irregular antibodies: 790 blood units for 44 patients had anti-E, 408 blood units for 23 patients had anti- MIA, 198 blood units for 11 patients had anti- C and anti-E. . ., special we selected 18 blood units for 1 patient had anti-E, anti-C, anti-mia, anti-JKB and anti-FYB. There was different statistically significant between before and after transfusion about some indexes: average hemoglobin, red blood cell count, HCT, indirect bilirubin and LDH of patients such as hemoglobin before transfusion was 68,4 ± 13,9 g/l and after transfusion was 96,2 ± 8,9 g/l; red blood cell count before transfusion: 3,1 ± 0,9 t/l and after transfusion: 4 ± 0,6 t/l, hct before transfusion: 0,23 ± 0,05 and after transfusion: 0,32 ± 0,04 t/l, indirect bilirubin before transfusion: 38,4 ± 28,6 μmol/l and after transfusion: 37,4 ± 25,4 μmol/l, LDH before transfusion: 1046,9 ± 934,5 ui/l and after transfusion: 969,8 ± 856,2 ui/l. We found out 1,8% patients disappeared some irregular antibodies after blood group antigen matching transfusion: 5 patients disappeared 1 irregular antibody and 1 patient disappeared 2 irregular antibodies.
Conclusions: Blood transfusion with matched blood group antigens was initially effective for patients who had irregular antibodies, patients were transfused more safely and effectively: 2.024 blood group antigen matching units were selected to transfuse 110 patients who had irregular antibodies. Suitable blood units were selected to transfuse patients who had 1 irregular antibodies and patients who had combined irregular antibodies (198 blood units for 11 patients had anti- c and anti-e, 18 blood units for 1 patient had anti-E, anti-C, anti-MIA, anti-JKB, anti-FYB). Average hb, red blood cell count and hct indexes after transfusion also increased statistically significant compare with before transfusion.

P-336

RED CELL ALLOIMMUNISATION IN REGULARLY TRANSFUSED BETA THALASSEMIA PATIENTS IN PAKISTAN

SH Ansari, U Zaidi, M Borhany and D Zahid

National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, Pakistan

Background: In Pakistan routine blood group typing of thalassemia patients identifies ABO and Rh(D) antigens only. Therefore, other antigen incompatibilities between blood donor and blood recipient may cause alloimmunisation.
Aims: The aim of this study was to estimate the frequency of alloimmunisation and to evaluate the risk factors associated with its development in beta ()-thalassemia patients receiving regular blood transfusions.
Methods: In total 164 thalassemia patients were included in this study. An extended red cell antigen panel was performed to detect antibodies. Patients received red cell concentrates, which were matched for aboand Rh(D) antigens. Clinical and laboratory data were collected and analysed to estimate the frequency of alloantibodies and the factors influencing immunisation in patients on regular blood transfusion.
Results: The median age of patients was 6–7 (range: 0–5-25) years. A total of 16 (9.7%) patients developed alloantibodies against red cell antigens.the most frequently occurring alloantibodies was anti-e (3.03%), anti-k (2.4%), anti-e (1.2%) and anti-d (0–6%). Five (3-1%) patients developedmore than one red blood cell (RBC) alloantibody. Age at first transfusion in alloimmunised patients was 1–22 ± 0–87 years. The frequency of blood transfusion in alloimmunised patients was 23 ± 8–81 days and in those without alloimmunisation was 31–8 ± 16 days (P = 0.02). Logistic regression analysis showed no independent risk factor associated with alloimmunisation.
Conclusion: The frequency of transfusion was increased in patients who developed alloantibodies. Typing patients and donors to match for rh and kell antigens would prevent more than 90% of RBC alloantibodies and reduce the frequency of transfusion in thalassemia patients.

P-337

RED CELL ALLOANTIBODIES IN THALASSEMIA PATIENTS' BLOOD REFERRING TO THE REGIONAL BLOOD TRANSFUSION CENTER OF TEHRAN, IRAN

P Ebrahimisadr[1], SM Tabatabaei[1], H Majidiani[2], R Ahmad Monfared[3], A Abbasian[3], Z Sharifi[3] and F Ghaffarifar[2]

[1]High Institue for Education and Research in Transfusion Medicine, Tehran, Iran [2]Department of Parasitology, Faculty of Medical Sciences, Tarbiat Modares Univers, Tehran, Iran [3]High Institute for Research and Education in Transfusion Medicine, Tehran, Iran

Background: Thalassemia is associated with genetically determined reduction in rate of synthesis of one or more types of normal hemoglobin polypeptide chain. Alloimmunization is provoked against unknown types of RBCs which includes one of the important complications in thalassemia patients. The objective of this study was to explore the frequency of red cell alloantibodies among β thalassemia patients referring to the regional blood transfusion center of Tehran.
Material and Method: In this study, we performed antibody screening test (Dia-cell I. II and III) on 184 thalassemia patients. An identification experiment by the Dia panel, comprising of 11 diverse group O RBCs were employed to investigate sera with Dia cell (I or II or III).
Results: In our study, males and females constituted 66 patients (35.87%) and 118 patients (64.13%), respectively, among which 116 subjects (63%) were found to possess Alloimmunization. Furthermore, 68 thalassemia individuals (37%) lack any alloantibody. Among 184 patients with β thalassemia major, anti-K (Kell system), anti-D and anti-E (Rhesus system) were the most abundant found alloantibodies with occurrence rates of 24 (13%), 11(5.98%) and 10 (5.4%), respectively.
Conclusion: Prior to RBC transfusion, regular RBC antigen phenotypes as well as resolving the problem of alloantibody production by receiving compatible blood in terms of Kell and RH subgroups are suggested for all of the transfusion-derived thalassemia cases.

P-338

STUDY OF RED BLOOD CELL ALLOIMMUNIZATION IN MULTITRANSFUSED THALASSEMIC CHILDREN IN YANGON, MYANMAR

T Aung[1] and I Nozaki[2]

[1]Ministry of Health, Yangon, Myanmar (Burma) [2]Japan International Cooperation Agency, Yangon, Myanmar (Burma)

Background: Thalassemia is one of the most common genetic disorders of hemoglobin synthesis worldwide with a particularly high frequency in broad belt existing from the Mediterranean basin to India and Southeast Asia. It was also reported that high incidence of thalassemia in the populations of malaria-endemic remote area of Myanmar. It is one of the most common causes of frequent blood transfusion in the country. Although RBC transfusion is life saving for these patients, it may be associated with some complications including RBC alloimmunization.
Aims: The aim of the study was to investigate the frequency and type of alloimmunization alloantibodies among the multitransfused thalassemic children in Yangon.
Methods: This was a descriptive study involving a hundred of confirmed beta thalassemia major pediatric patients treated at two teaching hospitals in Yangon, and

received more than 10 units of blood transfusion. The patients who have the history of collagen vascular disease and lymphoproliferactive disorder were excluded from the study. After getting informed consent, relevant clinical and laboratory data was collected with reference to age at the start of transfusions, total number of transfusions received and splenectomy status. Antibodies screening and antibody identification were performed for all study participants during the period between January and March 2009.

Results: The median age of the participants at the time of study was 8.0 years (range: 0.8–15.0 years), the median age of the first blood transfusion was 2.0 years (range: 0.2–10.0 years), and the median of the total amount of blood the participants received was 28 units (range: 10–127 units). Of the 100 participants, 39 were male, 31 received splenectomy. The alloantibodies were detected in the five participants, including two cases of anti-E alone and three cases of anti-E and anti-c antibodies. Among these 5 identified cases, the median age of the participants at the time of study was 12.0 years (range: 4.8–14.0 years), the median age of the first blood transfusion was 4.8 years (range: 1.0–10.0 years), and the median of the total amount of blood the participants received was 24 units (range: 14–41 units). All five cases had growth retardation, iron pigmentation, hepatomegaly, and two cases received splemectomy.

Conclusions: Transfusion support to thalassemic patients in a resource limited setting is still recognized as a big challenge. Fragmented transfusion services in the country, lack a component preparation facility or trained personnel or both in the peripheral hospitals, lack of routine screening of minor blood group antigens, and lack of provision of pre- or poststorage leukofiltered blood, were reported hurdles for the optimum management of these patients in the resource limited setting. In order to reduce alloimmunization, a policy for performing extended red cell antigen phenotyping especially Rh D, C, E, c, E should be provided for transfusion to these patients.

P-339

IMPLEMENTATION OF A SICKLE CELL DISEASE PATIENT BLOOD PROGRAM AT THE WESTERN PROVINCE BLOOD TRANSFUSION SERVICE AND THE EFFECT ON THE RARE DONOR PROGRAM

R Soeker and R Valensky

WPBTS, Cape Town, South Africa

Background: During March 2012 the Reference Lab of WPBTS noticed an increase in Sickle Cell Disease (SCD) patients with multiple antibodies to high incidence antigens. The majority of patients were of Central and West African descent.

The number of SCD patient cases increased from 12 in September 2013 to 104 in March 2015. This is an increase of 92 patients in 18 months. Some of these patients presented with multiple antibodies to high incidence antigens, particularly in the Rh system. It was challenging finding blood for these patients as our donor and patient populations are extremely diverse.

Aims: To antigen match SCD and Thalassaemia patients from their first transfusion in order to prevent possible allo-immunisation

To introduce a systematic approach for dealing with these patients in order to reduce time and costs incurred while randomly performing donor antigen typing.

To confirm if patients that typed Fy $^{a-b-}$ and received Fy^{b+} blood had the GATA-1 binding site mutation

To build up a panel of extensively typed antigen negative units thereby increasing our Rare Donor panel and to maintain these donors over time.

Methods: All SCD patients were typed for ABO, D, C, c, E, e, K, Fy^a, Fy^b, Jk^a, Jk^b, S, s utilizing tube technique.

As WPBTS blood donors are routinely typed for ABO, D, Rh (C, c, E, e) and K antigens on the Olympus PK7300, the Reference lab decided that extended antigen typing (Fy^a, Fy^b, Jk^a, Jk^b, S, s) be performed on targeted donor units in order to build up a panel of suitable donors.

Total SCD patients in program	167
Total SCD patients extended antigen typed	167
Total SCD patients genotyped	148

Table 1

Total donors extended antigen typed	1658
Total donors genotyped	325
Donors with rare combinations of antigens identified for SCD patient use	350

Table 2

As this was labour intensive, certain Group O donors had extended phenotyping performed (Fy^a, Fy^b, Jk^a, Jk^b, S, s) since May 2015 utilizing the Diagast PK7300 kit.

Genotyping utilising Grifols ID Core XT was introduced to confirm genotyping of some SCD patients and to verify certain suspected rare donors.

Results to date: (April 2016): Additions to WPBTS Rare Donor panel:

1 U Negative, 2 U variants, 2hr^S neg, 20hr^B neg, 1Js^b neg, 3 Jo^a neg, 10 k neg

Conclusions: All SCD and Thalassaemia patients are provided with blood with as close an antigen match as possible.

Extended antigen typed units are routed to the Blood bank and held for SCD and Thalassaemia patients as needed. No allo-antibody production is evident in any of the patients that receive antigen matched units.

It has been confirmed that all the Fy $^{a-b-}$ SCD patients who received Fy^b positive blood have the GATA-1 binding site mutation.

Genotyping patients using Grifols ID Core XT has reduced patient antibody problems with regard to confirming antigen status when rare cells are not available. Some rare donor phenotypes have also been confirmed and WPBTS Rare Donor panel has increased phenomenally.

P-340

VARIATION IN ANTIBODY DETECTION IN PRE-TRANSFUSION PRACTICE BY DIFFERENT METHODS

S Mangwana[1], N Bedi[1] and V Gangwar[2]

[1]Sri Balaji Action Medical Institute, New Delhi, India [2]Immucor India Pvt Ltd, New Delhi, India

Background: Pre-transfusion testing methods have been introduced and improved progressively for the safe and effective transfusion. After introduction of anti-globulin test in 1945, range of antibody detection methods expanded in the 1960s and 1970s. There are several traditional manual methods available but for the benefit of the patients, these traditional tests are eliminated and replaced with automated technology. In recent years there is significant improvement to detect antibody prior to transfusion and identification of clinically significant antibodies by these methods. In our tertiary-care facility, Solid Phase Red Cell Adherence (SPRCA) technology was implemented (Galileo, fully automated immunohematology analyzer) for the routine pre-transfusion testing with combination of semi-automated testing on Column Agglutination Technology (CAT).

Aims: Considering the recent knowledge about the clinical significance of reactive antibodies and suggestions to perform individual cost-benefit analysis in every institution, it was decided to compare the sensitivity of SPRCA and CAT to detect clinically significant red cell antibodies in pre-transfusion practice and to decide which high-sensitive screening system should be applied.

Methods: In a period of 32 months, 23638 patients were tested for antibody screen & cross matching using CAT (Bio-Rad). In last three months period, between January- March 2016, total 1048 patients were tested for 3 cell antibody screen on SPRCA using Capture RS3 (Galileo Immucor, Norcross GA)and positive samples were further subjected to identification for clinically significant antibodies.

Results: In 29 months period, seven antibody positive results were found out of 22576 patients using CAT while SPRCA detected nine antibody positive results out of 1048 which is statistically highly significant(P value <0.001). Out of seven positive patients by CAT, five were of only auto antibodies; both warm and cold and two patients showed combination of auto and allo-antibodies (one warm antibody with Anti-E, Anti-c and other cold antibody with Anti-M allo-antibody). After implementing SPRCA, nine out of 1048 patients showed positive screen in three months. Six of Nine patients (67%) showed Rh antibodies of which three patients

(33%) showed Anti-D allo-antibodies. Two patients (22%) showed presence of multiple allo-antibodies; one with Anti-E, Anti-Fy^a and Anti-S while other patient had Anti-C and Anti-E allo-antibodies. Other clinically significant allo-antibodies were Anti-c, Anti-P and auto antibodies.
Conclusion: Selection of test in transfusion service should not only be cost effective, it must also have technology that is sensitive & specific. SPRCA was able to detect more number of clinically significant antibodies in pre-transfusion testing than Column Agglutination Technology. Sensitivity is the major advantage of SPRCA compared to CAT. Detection of clinical significant antibodies prior to transfusion is good indicator to provide safe blood transfusion. Both technologies give a huge variation to detect these antibodies. Resources should be better directed that benefit the patient. Testing that result in clinically insignificant discrepant results places additional strain on the resources and may delay transfusion. Blood transfusion services may fail to recognize the impact of change in method or introduction of new method or reagents. Hence, it is important to select and validate effective pre-transfusion test practices.

P-341

SERUM TRANSFERASE TESTING APPLIED IN ASSESSING ABO SUBTYPES

TS Tsay, SL Lin, HC Lin, CW Wu, KT Lin and CM Hung
Kaohsiung Blood Center, TBSF, Kaohsiung City, Taiwan

Background: The A and B alleles of the ABO gene express enzymes with glycosyltransferase activities that differ, adding either N-acetyl galactosamine or galactose to the H antigen, converting it into the A or B antigen respectively.
Aim: The goal of this study was to estimate the suitability of serum transferase in the assessment of ABO subtypes.
Methods: The subtype blood donors in this study have been demonstrated by genetics test, saliva test and RBC adsorption and elution test. We performed serum transferase tests for A subtypes (1 of A_m and 10 of A_{el}), B subtypes (10 of B_{el}), AB subtypes (3 of $A_{el}B$, 1 of AB_{el}, 1 of A_xB and 1 of *cis*-AB) and para-bombay (10 of O_{Hm}, 6 of OA_{Hm}, 9 of OB_{Hm}, and 4 OAB_{Hm}) among 56 blood donors. The reaction products were tested for their ability to inhibit hemagglutination of human erythrocytes.
Results: We found A transferase in the serum of the A_m, A_xB, *cis*-AB, OA_{Hm} and OAB_{Hm} blood donors, and B transferase in the serum of the *cis*-AB, OB_{Hm} and OAB_{Hm} blood donors. After counting the agglutination reaction scores of the serial dilution anti-A, anti-B and anti-AB reagent with the red cell tested by transferase, we found that the amont of A transferases in A_m and A_xB blood donors is quite low.
Conclusion: The presence of the ABO-gene-specific transferases in the ABO subtype blood donor serum can be detected, as to assist blood group determination.

P-342

NEW WORKFLOW EFFICIENCY OF THE FULLY AUTOMATED ERYTRA® ANALYZER IN A BLOOD DONOR CENTER ENVIRONMENT

M Carpintero[1], S Solé[1] and M Serra[2]
[1]Grifols S.A, Sant Cugat del Vallès (Barcelona), Spain [2]Grifols International, Sant Cugat del Vallès (Barcelona), Spain

Background: Automated blood grouping systems for donor center routine should demonstrate high loading capacity and self-organization to provide maximum processing effectiveness. The Erytra® (Diagnostic Grifols) is a fully automated, high-capacity analyzer for blood group determination and pre-transfusion compatibility testing that uses the 8-column gel agglutination technique Grifols DG Gel® cards.
Aim: The main objective of this study was to assess how the Erytra® workflow efficiency could be tailored to different needs in a donor center environment, in terms of turn-around times from process start to end, efficiency, ease of use and hands-on as needed by the laboratory staff.
Study Design/Methods: Fresh whole blood samples in EDTA were collected from donors and tested for the screening of unexpected antibodies on Erytra® with the DG Gel® Coombs card (Diagnostic Grifols) and the one cell panel Sero-Cyte® Pool 0.8% (Medion Grifols Diagnostics). Weak D samples obtained from D negative donors were tested on Erytra® with DG Gel® Coombs card and the new Anti-D IgG Mono-Type® (Medion Grifols Diagnostics), a monoclonal murine/human IgG anti-D (clone ESD1). In case of detecting D variants, they were determined using molecular biology (ID RHD XT,Progenika Biopharma, Grifols). Different possible samples workloads were assessed in a fully equipped Erytra® (reagents and system solutions). The following performance metrics were assessed: time to first result (TTFR), turn-around time (TAT) from first result to last result (cadence, results/h), manual "hands-on"time required and walk-away time. For the ease of use evaluation, the number of steps and timing of the following activities were tracked: sample preparation, sample sort and loading, routine testing, post-run procedures, consumables used, IT/data review and space requirements.
Results/Findings: Results showed that the Erytra® workload management optimizes the loaded sample processing to provide the highest results cadence. Independently of the different sample workload scenario, Erytra® provided a cadence value of 205 results/hour for the unexpected antibody screening test. Moreover, the TAT was 40 min, without being affected by the sample loading frequency. The TTFR for one single sample was 26 min. The multi-drawer Erytra® system and the visual blue alert allowed samples and reagents continuous loading, thus shortening time to start processes and requiring minimum hands-on. In addition, Erytra software allowed accurate and timely notification of cards tracking and reagent status. Being a see-through instrument provided a clear view of all operating processes that were happening at the same time.
Conclusions: High workflow efficiency of Erytra® was evidenced through its increasing and adaptable performance with different sample loadings. Erytra® was particularly valued for its ease of loading and minimum hands-on, which lead to efficiency and time savings. According to the obtained results, the Erytra® is suitable for a blood donor center environment.

P-343

COMPARISON BETWEEN THE PREVALENCE OF COMBINATIONS OF TTIS AND EACH PARAMETER IN VOLUNTARY NON- REMUNERATED BLOOD DONORS

M Youssef and A Gamal
Alex Regional Blood transfusion Center, Alexandria, Egypt

Backgroud: Transfusion transmitted infections (TTIs) is a major challenge to the transfusion services all over the world.
The problem of TTIs is directly proportionate to the prevalence of the infection in the blood donor community. Currently, prevention of TTIs depends on proper pre-donation selection of all voluntary non remunerated blood donors. The pre-donation questionaire is considered the first line of defence against TTIs.
Aims: To know the different prevalence rate of each parameter of TTIs and combinations.
Methods: The study took place in the Alexandria Regional Blood Transfusion Center (Alex RBTC). It was conducted on all voluntary non remunerated blood donors

Parameter	Prevalence
HBV	304(0.4%)
HCV	708(1%)
HIV	59(0.09%)
Syphilis	108(0.16)

Combinations	Prevalence
HBV, HCV	9(0.013%)
HCV, HIV	8(0.012%)
HCV, Syphilis	7(0.011%)
HIV, Syphilis	3(0.004%)
HBV, HIV	2(0.003%)
HBV, Syphilis	1(0.002%)
HBV, HIV, Syphilis	1(0.002%)
HCV, HIV, Syphilis	1(0.002%)

Caption 1. Prevalence of parameters and combinations

during the period from January 2015 to December 2015. Screening was done using EIA HBs Ag, HCV- Ab, HIV Ag-Ab and Syphilis Ab.
Results: Alex RBTC donations were 66234 in total over one year (2015).
The total number of repeatedly reactive and confirmed positive donations for:
Hepatitis B surface antigen: 304 of 66234 (0.4%), hepatitis C virus antibody: 708 of 66234 (1%), human immunodeficiency virus (HIV): 59 of 66234 (0.09%), Syphilis Treponema Pallidum antibody: 108 of 66234 (0.16%).
For combinations:
HBV & HCV: 9 (0.013%), HCV & HIV: 8 (0.012), HCV & Syphilis: 7 (0.011%), HIV & Syphilis: 3 (0.004%), HBV & HIV: 2 (0.003%), HBV & Syphilis: 1 (0.002%), HBV, HIV & Syphilis: 1 (0.002%) and HCV, HIV & Syphilis: 1 (0.002%)
Conclusion: We found that the highest prevalence rate of TTIs is HCV and the lowest one is HIV. For combinations, the highest one is a combination of HBV& HCV and the lowest are combinations of HBV & syphilis, HCV, HIV & syphilis and HBV, HIV & syphilis.

P-344

AUTOMATED ABO ANTIBODY TITRATIONS ON THE GALILEO NEO® PLATFORM: SPECIFIC AND PRECISE ASSAYS TO MEET CLINICAL NEEDS

E Preuss, S Oldekamp, J Muehl, AK Gellerer and SJFE Blincko
Immucor GmbH, Rödermark, Germany

Background: ABO antibody titration is clinically relevant for many different applications such as organ and stem cell transplantations, ABO incompatible pregnancies and blood group O donor characterization in transfusions. However, the consistency of methods employed has not been satisfactory and studies can show as much as 7 doubling dilutions differences in results. Automated titrations on the Galileo NEO® platform, employing quality reagents, have been developed and have been evaluated for their consistency.
Aim: This study describes the comparison of fully automated titrations for IgM and IgG antibodies on the Galileo NEO® to the corresponding manual gel methods. In addition the precision of the automated methods are tested.
Methods: The Galileo NEO® offers fully automated IgG full range assays with dilutions from neat up to 1/4096 and full range IgM assays from neat to 1/128. The Galileo NEO® also offers titer screen assays for the detection of high titer specimens with dilutions from 1/16 to 1/128. All methods are fully automated with the titer result obtained by the instrument without manual intervention. The Galileo NEO® assays do not require dithiothreitol (DTT) treatment to specifically measure human IgG (the detection employs a specific monoclonal to human IgG). IgG Gel methods without DTT by contrast measure a mixture of IgG and IgM according to the manufacturers' pack inserts.
A mixture of donor and patient (pregnancy and transplant) specimens were employed in the following studies. Method comparison: 75 specimens (including those from blood groups A, B and O) were tested by the full set of Galileo NEO® assays and by gel with and without DTT following the AABB manual method.
Repeatability and reproducibility: two specimens were tested on each assay in triplicate, morning and afternoon, on three Galieo NEO®s, on 5 non-consecutive days within a 21 day period. Lot to Lot consistency: ≥2 specimens were tested on each assay with three discrete sets of reagents on one Galileo NEO®. Range vs titer screen comparison: ≥10 specimens were tested on each screen and corresponding range assay in triplicate.
Results: The method comparison studies show that the Galileo NEO® IgM assays give good correlations to their corresponding gel method ($R^2 > 0.76$). The good correlations ($R^2 = 0.78$, 0.81 and 0.71 on A1, A2 and B cells respectively) between the Galileo NEO® IgG and the corresponding gel method with DTT support the contention that the Galileo NEO® assays are specific for IgG.
Repeatability and reproducibility, lot to Lot consistency and range vs titer screen studies showed results with ranges no greater than ±1 doubling dilution from the median results and usually better.
Conclusions: The automated Galileo NEO® ABO IgG titration assays demonstrate better specificity than the gel method and excellent consistency across lots, instruments and time. These assays offer a specific, consistent and complete picture of IgG and IgM titers and automate the currently labor intensive and less precise manual alternatives. These results will need to be confirmed by users in clinical settings.

P-345

PRETRANSFUSION SURVEY TESTING IN THE PHILIPPINES

R Alcantara, A Domasian, C Amaro and R Chua
Philippine Blood Coordinating Council, Quezon City, Philippines

Background: Pretransfusion (compatibility) testing is performed to prevent incompatible red cell transfusions that may cause immune-mediated hemolytic transfusion reactions resulting in serious complications and even death to the recipient. The Philippine Clinical Practice Guidelines for Rational Use of Blood and Blood Products recommends that pre transfusion testing should include ABO and RhD typing, red cell antibody screening and a suitable crossmatching technique. Blood banks in the Philippines use different methods in performing pretransfusion testing.
Aims: The objective of this study is to assess the current testing practices for ABO grouping, RhD typing, red cell antibody detection and crossmatching performed by blood banks in the Philippines.
Methods: A voluntary national survey on pretransfusion testing practices was conducted. Survey forms were distributed to different private and government blood banks in the Philippines. Participants were asked to provide information on the pretransfusion testing done in their blood banks including the method used. Participants were also asked on what pretransfusion testing problems are usually encountered in their blood bank. Completed survey forms were returned to the authors for review and all information was entered into a database for analysis.
Results: A total of 78 survey forms were received from the different regions in the Philippines (25 and 53 from the National Capital Region and the regional provinces respectively). There were 27 government blood banks and 51 private blood banks who participated in the survey. All participants performed ABO and RhD typing with 78% using the tube method. Most of the participants performed both forward and reverse ABO typing (85%) while 13% only performed forward typing. Only 18 (23%) of the participants performed red cell antibody screening, 10 of them were located in the National Capital Region. More than half of the participants (51%) used column agglutination based testing in performing crossmatch followed by the tube method (46%). An incompatible crossmatch was the most common (62%) pretransfusion testing problem encountered by the participants followed by ABO discrepancy (27%).
Summary and Conclusion: Results of the survey provided insights into current practice trends in pretransfusion compatibility testing in the Philippines. Most blood banks currently favor tube method when performing ABO and RhD typing while column agglutination technology is the most common method used for red cell antibody screen and crossmatching. The continued education for improvement of techniques used in pretransfusion testing test should be required because a significant number of blood banks who participated in the survey do not use red cell antibody screening and "forward typing only" is still being performed to determine the ABO type in some blood banks. Individual blood banks should regularly review their choice of test methods to ensure that they comply with standards, represent the recognized best practice available and are cost-effective. This survey also provides an opportunity for hospital blood banks in the Philippines to compare their current practices with those of their peers.

P-346

EVALUATION OF A NEW FULLY AUTOMATED IMMUNOHEMATOLOGY TESTING INSTRUMENT FOR PERFORMANCE

TS Casina, W Malomgre, R Witkowski and A Eckhardt
Ortho Clinical Diagnostics, Raritan, NJ, United States of America

Background: Evaluation of a new immunohematological testing system is necessary to show that the performance of the new instrument demonstrates equivalence from a method-based perspective. Results are generated that are compared to the performance of a predicate method or instrument.
Aim: The ORTHO VISION® Max Analyzer is a new instrument designed to fully automate *in vitro* immunohematological testing of human blood using the ORTHO BioVue® System column agglutination test. A study was conducted to evaluate the performance of the new instrument compared to the ORTHO VISION® Analyzer.
Methods: A variety of direct and indirect agglutination tests were completed the same day on both instruments. Discordant samples were repeated using the site's manual ORTHO BioVue® System workstation. Testing was executed on a total number of 2505 samples to meet required sample criteria. Data from direct agglutination testing and direct and indirect antiglobulin testing were assessed by comparison of interpreted test results to determine percent concordance between the two systems at

the lower 95% confidence bound. The criteria for concordance were ≥ 99.4% for direct agglutinating testing and ≥98.0 for direct and indirect antiglobulin testing.
Results: Acceptable concordance was observed between the two systems for both direct agglutination testing and direct and indirect antiglobulin testing. Direct agglutination testing was performed on 1460 samples with 2795 interpreted results. 2793 results were concordant and 2 discordant. The system comparison demonstrated a concordance of 99.9% agreement with a 99.8% at a one-sided lower bound 95% confidence interval for direct agglutination. Direct and indirect antiglobulin testing was performed on 1045 samples with 1428 interpreted results. 1418 results were concordant and 10 discordant. The concordance demonstrated was 99.3% concordance agreement with a 98.8% at a one-sided lower bound 95% confidence interval for direct and indirect antiglobulin testing.
Conclusions: This evaluation demonstrated that the ORTHO VISION® Max Analyzer system showed equivalent performance vs the predicate system in the intended use environment.

ABO Discrepancy	Causes	Incidence	Total
Reverse Group	A Subgroups	5 (9.8%)	31 (60.8%)
	Age Related (Baby\Elderly)	13 (25.49%)	
	Cold Auto Antibody	3 (5.88%)	
	Alloantibody (Anti-M), High Frequency Antigen	5 (9.8%)	
	Acquired B	1 (1.96%)	
	Weak Reverse Reaction Not Related To Age	4 (7.84%)	
Forward Group	A Subgroups	11 (21.56%)	20 (39.2%)
	B Subgroups	1(1.96%)	
	Transfusion	3 (5.9%)	
	Feto-maternal Hemorrhage	3 (5.9%)	
	Acquired B	1 (1.96%)	
	Not Resolved	1 (1.96%)	

Caption 1. Causes of ABO discrepancies

P-347

RELATIONSHIP OF IMMUNOGLOBULIN SUBCLASSES AND BLOCKING PHENOMENON

E Lee[1], C Jones[2], A Ebbs[3], R Modasia[1], K Hart[1] and T Adewole[1]
[1]NHS Blood & Transplant, London, United Kingdom [2]Peterborough City Hospital, Peterborough, United Kingdom [3]Quuen's Elizabeth Hospital, King Lynn, United Kingdom

The presence of potent maternal antibodies blocking antigens in the newborn causing false-negative typing results is uncommon. The blocking phenomenon has been reported with anti-D, anti-K and anti-Fya and possible for other blood group antibodies. There has been no information in relationship to the immunoglobulin IgG subclasses of these potent antibodies. We reported an interesting observation with different immunoglobulin subclasses that may affect the antigen typing results by monoclonal antibody(ies).
Case Study: A 29-year-old Caucasian female (p2 g2). She grouped as B, R1R2 K- Fy(a-b+) S+s- with anti-Fya+s level (titre 256) detected on her previous pregnancy. Her partner was grouped as O, R1R2 K- Fy(a-) S- s + . Maternal antibody levels at current delivery were 128. Baby born was group O, R1R2 K- Fy(a-b+) S+s+, DAT positive (5 + IgG) and anti-s was eluted. No false negative typing was observed with monoclonal anti-s (P3BER), however results were weaker with murine monoclonal anti-s (MIMA-74) suggested partially blocking. Additional investigation identified maternal anti-Fya was IgG1 and anti-s was IgG3.
Discussion: The level of anti-s is compatible with blocking phenomenon with anti-D, anti-K and anti-Fya (titre 128 to 256) reported previously. In this case no, or minimal, blocking phenomenon was observed. IgG3 has a much larger hinge region than IgG1 and is more flexible, less steric hindrance to GPB, also there are more GPB molecules than FY (6,900) or KEL (4,000 to 18,000) protein that may lead to less saturation of the s antigen sites (4,700 to 88,000). This is an unusual observation for this blocking phenomenon, and further investigation may be required to prove this hypothesis.

P-348

THE ABO BLOOD GROUP DISCREPANCIES AMONG PATIENTS REFERRED TO THE RED CELL REFERENCE LABORATORY (RCRL) AT THE DEPARTMENT OF BLOOD BANKS SERVICES

L Al mashaykhi[1], H Al- Lawati[2], R Al- Muhaidri[2] and Z Al fana Alarimi[2]
[1]OMSB, Muscat, Oman [2]Depratment of Blood Banks services, Muscat, Oman

Background: ABO blood group system is discovered in the beginning of 20th century by Landesteiner and is considered as a corner stone in the transfusion medicine. So, ABO test is done for all samples received at blood banks by forward group which test cell antigen and reverse group which test patient's serum against known A and B cells. The disagreement between the two tests is called ABO discrepancy. In the FDA Annual Summary for 2014, fatalities reported following blood collection and transfusion, ABO Hemolytic Transfusion Reaction (HTR) were reported to account for 13% of fatalities. In annual SHOT report 2014, there were 10 ABO incompatibility transfusion due to clinical error. Therefore, to provide a high quality and safe blood, ABO discrepancy should be resolved before any blood group is assigned to the patient and the blood donor to prevent the transfusion reaction due to ABO Haemolytic Transfusion Reaction.
The Red Cell Reference Laboratory (RCRL) is considered as a reference lab for the blood banks in Oman. It provides the technical support and transfusion advice for all the regional blood banks and resolves the difficult immunohaematology cases.
Aim: To provide information on the causes of ABO discrepancies among the referred patients to the RCRL Between January 2014 to December 2015.
Methods: It was retrospective analysis performed on samples referred with ABO discrepancy between January 2014 and December 2015. Samples referred to RCRL which showed ABO discrepancy between red cell grouping and serum grouping were analyzed as per the classifications of the ABO group discrepancies into forward and reverse group discrepancies. Then further classification of causes of each group is done.
Result: The total number of patients samples referred to the RCRL from January 2014 to December 2015 was 1863, Forty-two patients were found to have ABO discrepancies with incidence of 2.25% among all referred patients. 60.8% of ABO discrepancies causes were due to reverse ABO group discrepancy, and most common cause in this category was age related which give week antibodies on reverse grouping with 25.49% of all causes observed. While, Forward group discrepancy was observed in 39.2% of patients and A subgroup was the common cause seen in this group. Three cases (7.14%) showed both reverse and forward group discrepancy.
Conclusion: The incidence of ABO discrepancy among referred patient to RCRL in the period of the study was found to be 2.25% with the majority (60.8%) of them due to reverse group discrepancy. In order to find compatible blood for recipients, ABO discrepancies must be resolved to prevent ABO Hemolytic Transfusion Reaction.

P-349

COMPARISON OF SENSITIVITY AND SPECIFICITY OF THE BIO-RAD ID CARDS LISS/COOMBS WITH THE GRIFOLS GEL COOMBS CARDS IN A MANUAL APPROACH

H Hustinx, E Münger and S Lejon Crottet
IRB Blood Transfusion SRC Ltd, Berne, Switzerland

Background: Antibody screening and identification is, besides blood grouping, an important part of the patient pre-transfusion process. As the Swiss immunohematology reference laboratory uses the Bio-Rad ID coombs cards it is very important to know if the method used has a good sensitivity and specificity in comparison with similar systems like Grifols.
Aim: The aim of this study was to compare the sensitivity and specificity of the two methods used by most immunohematology laboratories in Switzerland.
Methods: A total of 1001 frozen random samples from a German hospital (anonymous) and 200 samples with known antibodies were investigated. These antibodies were chosen due to their clinical relevance and weak antibody reactivity or due to their unspecific reactivity (antibodies against substances in the stabilisation solution= anti-stabi). These were determined using ID coombs cards (ID/IAT) and in-house test cells and kept at 4°C for 2–4 months. Both sample collections were tested with a set of 3 test cells from Bio-Rad (ID-DiaCell I-III DiaMed, Switzerland) and Grifols (Serascan Diana 3, Medion Grifols Diagnostics AG, Dudingen, Switzerland) using the ID/IAT and Grifols Gel Coombs cards (Gel/IAT).
Results: For 999 of the 1001 random samples no difference between the systems was observed. Two samples showed in each of the systems a reaction that could not be confirmed by the in-house system. In 13 of the 999 samples antibodies were

detected, of which one anti-stabi. In 48 of 200 antibody containing samples differences between Bio-Rad and/or Grifols and our in-house system were observed. Twenty-one of 24 samples with anti-stabi were only detectable with fresh serum, whereas two were detected by Grifols only and one by Bio-Rad only. For the 24 samples showing very weak reacting antibodies, one anti-D, one anti-Lu(a), one anti-M and one anti-Le(a) were not detected by Grifols and one anti-K and one anti-M by Bio-Rad. Five anti-Kp(a) (Bio-Rad) and two anti-Cw (Grifols) could not be detected as the antigens were not present on the screening cells.
Conclusions: Both screening systems showed an equal good performance, with a sensitivity of 100% and a specificity of 99.8% for the 1001 random samples. From the 200 samples with known antibodies, only four very weak reacting antibodies were not detected by Grifols and two by Bio-Rad. However, both systems detected all anti-Fy and anti-Jk antibodies. Both test sets lacked a mandatory antigen according to the Swiss regulations (but not to other regulations): Cw for Grifols and Kp(a) for Bio-Rad. The samples with anti-stabi were sent to our laboratory from all-over Switzerland and in all cases the observed reactions could be confirmed in ID/IAT with our in-house cells. However, the anti-stabi samples could only be detected in two out of 24 samples by Grifols and in one of 24 samples by Bio-Rad. The negative reactions could be due to the sample storage at 4°C and a putative instability of IgM antibodies.
Conflict of interest: The study was supported by DiaMed GmbH, Switzerland.

P-350

ABO-MISMATCHED PLATELET TRANSFUSION: EVALUATION OF A STRATEGY TO REDUCE THE RISK OF HEMOLYSIS

C Bub, EP Bastos, L Visciglia, LF Almeida, AM Sakashita, SMC Lira, MG Aravechia, L Castilho and JM Kutner

Hospital Israelita Albert Einstein, São Paulo, Brazil

Background: ABO-mismatched platelet transfusion is an accepted practice when ABO-identical platelets are unavailable. However, acute hemolytic transfusion reaction (HTR) can be a severe complication after ABO-mismatched platelet transfusion due to the passive transfusion of antibodies from donors possessing unusually high-titers anti-A and/or anti-B. Strategies to reduce the risk of PLT-associated HTRs include volume reduction of PLTs and screening donor plasma for high-titers antibodies by performing anti-A and/or anti-B titration or assays for *in vitro* hemolysis. Non-standardized methods of IgM or IgG isohemagglutinins titration and the lack of agreement of a "critical titer"that will predict *in vivo* hemolysis have made the determination of ABO antibody titers difficult and limited to a few blood banks.
Aims: In order to define a safe strategy to reduce the risk of passive hemolysis in ABO-mismatched transfusion of platelets, we evaluated IgM and IgG ABO antibody titers in group O donors and compared the results with hemolysis grade. We also compared the IgM antibody titers in the donors' plasma samples and in their platelet products.
Methods: A total of 164 group O donor plasmas and their respective PLT products were analysed. Plasmas and PLT products were tested against A1 and B reagent RBCs using an automate gel test system (Wadiana, Grifols, Spain) at dilutions of 1 in 64. IgG titers were determined by treating the plasma samples with 0.01 dithiothreitol (DTT) and tested at dilutions of 1 in 64 in gel. Test for qualitative hemolysin was performed by standard procedure in tube adding a fresh source of complement and graded as complete hemolysis and partial hemolysis. IgM and IgG ABO antibody titers were compared with the result of hemolysins.
Results: Anti-A and anti-B IgM high titers (>64) were significantly associated with partial and total hemolysis ($P < 0{,}001$) while no significant association was found between IgG high titers and hemolysis. Anti-A hemolysin was more prevalent than anti-B hemolysin (60% and 40% respectively). When we compared IgM ABO antibody titers between the plasma sample and the PLT products we verified that plasma samples showed higher titers of ABO antibodies (at least one dilution) than PLT products.
Summary/conclusions: We found a significant correlation between high titers of ABO IgM antibodies and hemolysis in group O donor plasmas and PLT products and showed that PLT products have lower IgM antibody titers than plasma samples. Therefore, the isohemagglutinin titration of IgM antibodies in PLT products using an automated screen method at dilutions of 1 in 64 can be a good approach to be used as prophylactic test to prevent HTR with less impact on the platelet inventory.

P-351

ALLO-ANTIBODIES MASKED BY AUTO-IMMUNE COLD ANTIBODIES IN REGULARLY TRANSFUSED EGYPTIAN PATIENTS (FROM JANUARY 2015 TO JANUARY 2016)

M Saleh

Egyptian National Blood Transfusion Service, Cairo, Egypt

Background: Autoimmune cold antibodies are clinically non-significant but they can interfere with the pre-transfusion tests like (antibodies screening, cross-matching, etc. . ...) by masking the presence of other antibodies that may be clinically significant.
Aim: The aim of the study is to find out:
The prevalence of autoimmune cold antibodies among regularly transfused Egyptian patients.
The prevalence of allo-antibodies masked by autoimmune cold antibodies.
Methods: The study conducted on transfusion dependent patients (500 patients) with: Thalassemia, aplastic anemia, leukemia, renal failure and anemia of chronic diseases; presented to Egyptian NBTC for blood transfusion.
According to National Egyptian Testing Strategy, Patients were subjected to:
Screening for allo-antibodies against group O reagent RBCs (panel of 3 cells) using appropriate IAT at 37°C by CAT.
Antibody identification for positive screening samples against group O reagent RBCs (panel of 11 cells) using appropriate IAT at 37°C by CAT.
Screening at room temperature to detect the presence of cold antibodies.
Screening for allo-antibodies against group O reagent RBCs (panel of 3 cells) using appropriate IAT at strict 37°C with pre-warming of both serum & reagents(in case of positive results of Screening at room temperature).
Antibody identification for positive screening samples to identify the specificity of allo-antibodies masked by autoimmune cold ones against group O reagent RBCs (panel of 11 cells) using appropriate IAT at strict 37°C with pre-warming of both serum & reagents.
Results: A total of 500 patient were examined for the presence of autoimmune cold antibodies & allo antibodies that may be masked by them.
Out of a total of 500 we found that 47 (9.4%) patients had autoimmune cold antibodies and the co-incident prevalence of masked allo-antibodies was as follows:
Autoimmune cold antibodies only:32 out of 47 patients (68%)
Allo-anti D:1 out of 47 patients (2.1%)
Allo-anti C:2 out of 47 patients (4.2%)
Allo-anti E: 3 out of 47 patients (6.4%)
Allo-antic:3 out of 47 patients (6.4%)
Allo-anti K: 2 out of 47 patients (4.2%)
Allo-anti Fya: 1 out of 47 patients (2.1%)
Allo-anti Jka: 2 out of 47 patients (4.2%)
Allo-anti S:1 out of 47 patients (2.1%)
Summary/conclusions: Pre-warming techniques are helpful in differentiation between cold non-significant Abs and warm Abs, for proper pre-transfusion testing of the patients' samples.

P-352

COMPLEX ANTIERYTHROCYTE ALLOIMMUNISATIONS IN PATIENTS – NINE YEARS' EXPERIENCE AT THE BLOOD TRANSFUSION INSTITUTE OF SERBIA

N Mladenovic, L Mijovic, S Jovanovic Srzentic, M Jovicic and M Dukic Novakovic

Blood Transfusion Institute, Belgrade, Serbia

Introduction: Criteria for managing complex antierythrocyte immunisations are applied in patients with 3 or more alloantibodies, 2 or more alloantibodies with autoantibodies and antibodies to high frequency antigens. Recommended strategies in transfusion treatment of those patients include establishment of regional registers of rare blood group donors and banks of rare blood groups. On February 2nd, 2015, project of establishing national typed donors register (NTDR) was initiated at the blood transfusion institute of serbia (BTIS).
Aim: Analysis of complex alloimmunisations from January 1st, 2007 till December 31st, 2015 in patients at the department of pretransfusion testing of the btis, as well as applied transfusion treatment.
Method and Material: Clinically significant antierythrocyte antibodies were identified at +37°C using gel method (anti igg rabbit or anti IGG+c3D ID liss/coombs cards), by commercial test erythrocytes (Bio Rad, grifols and sanguin-pelikloon), papain treated test erythrocytes in combination with corresponding nacl cards (Bio

Rad). Antibody identification at +22°C or +4°C was performed by tube method using "in house" test erythrocytes. Bood groups were determined using sanguin-pelicloon, Bio Rad, and lorne test reagents. Data were collected retrospectively from written protocols and btis computer database.

Results: During the observed period, pretransfusion tests were performed for 214,462 patients. Because of positive screening and/or positive interreactions, identifications of antierythrocyte antibodies were performed in 4676 patients, out of which 93 (0,04% of the total number of tested patients) had complex immunisations: 77 with three antibodies, 14 with four, and 2 with 5 antibodies. Multiple antibodies were most frequent in haematological polytransfused patients, 50 to 60 years old (42%). All patients with complex immunisations had at least one rh system antibody. The most frequent combination with three antibodies was anti-d+c+e in rh negative patients. The most frequent antibodies in complex immunisations were of anti e and anti k specificity. In order to provide adequate blood units, majority of those patients required up to 20 interreactions (in one case over 100). At the same time, blood units were typed (most often up to 20 and for 5 patients over 100). Autologous blod predeposit was provided for 10 patients, and for the treatment of kell null patients use of erythropoietin and iron substitute was recommended. From the date of the register establishment, currently containing 1274 donors, blood for 13 patients was provided thanks to the database.

Conclusion: Establishment and enlargement of NTDR contributed to the increase of typed blood stocks, which facilitated and shortened adequate blood searching procedure needed in patients with complex immunisations. Consumption of reagents and the number of collected samples was reduced. The register enables administration of adequately phenotyped erythrocytes, from the inclusion of transfusion therapy, in order to prevent immunisation in patients expected to undergo multiple blood transfusions. NTDR is the cornerstone for the establishment of the National Rare Blood Group Bank.

P-353

EFFECT OF RH AND K MATCHED RED BLOOD CELL TRANSFUSIONS ON ALLOANTIBODY FORMATION IN PATIENTS WITH HEMATOLOGIC DISEASES

C Bub, RO Cruz, TEM Barata, PBC Freire, M Mota, AT Kondo, AP Yokoyama, MG Aravechia, L Castilho and JM Kutner

Hospital Israelita Albert Einstein, São Paulo, Brazil

Background: Blood transfusions are life sustaining in chronically transfused patients. However, certain complications, such as alloimmunization to red blood cell (RBC) antigens, can create challenges in the management of those patients. Routine phenotyping of blood recipients and the use of phenotype-matched blood units for transfusion has been useful to lower the occurrence of RBC alloantibodies in chronically transfused patients. These protocols range from providing limited phenotype-matched RBCs for only Rh and K antigens to providing extended phenotype-matched RBCs for Rh, K, S, Fy^a, and Jk^a antigens prior to transfusion Nevertheless, extensive phenotyping is expensive, laborious and cannot be performed in certain situations.

Aims: We aimed to assess the effectiveness of Rh and K matched RBC transfusions in reducing alloimmunization in chronic transfused patients with hematologic diseases such as lymphomas, myeodysplastic syndromes and plasma cell dyscrasias.

Methods: Age, sex, number of transfusions and the rate of alloimmunization were determined during the course of 6 years in 159 patients who received Rh and K-matched units and in 249 patients receiving ABO-D-matched RBC transfusions. A control group of 131 chronically transfused patients with non-hematologic diseases receiving only ABO-D-matched RBC transfusions was also included.

Results: Of the 249 patients who received ABO-D-matched units with a median of 21 red cell transfusions, 142 were males and 107 females, median age was 52 years old. Of the 159 patients transfused with Rh and K matched units with a median of 31 red cell transfusions, 103 were males and 56 females, median age was 50 years old. Of the 131 controls with a median of 12 red cell transfusions, 69 were male and 62 were female with a median age of 64 years old. The rate of red cell alloimmunization in the patients receiving Rh and K matched units was 0.6% while the rate of alloimmunization in the patients receiving ABO-D-matched units was 6%. In the control group, the rate of alloimunization was 27%. The majority of antibodies developed by the two groups of patients receiving ABO-D-matched RBC transfusions were against Rh and K antigens. Our results revealed a very low rate of alloimmunization in patients with hematologic diseases receiving Rh and K matched units compared to the patients receiving ABO-D matched units (P = 0.021, OR=0.09, 95% IC=0.70).

Summary/Conclusions: Rh and K matching reduced the alloimmunization risk by 90% in chronically transfused patients with hematologic diseases. This finding should be considered in cost-benefit analysis for implementation of a prophylactic matching strategy in those patient populations.

P-354

DEVELOPMENT OF MIMICKING AUTOANTIBODIES IN ASSOCIATION WITH ALLOIMMUNISATION – CASE REPORT

N Jurakovic-Loncar[1], B Bosnjak[2], Z Kruhonja Galic[1], S Jagnjic[1], M Strauss Patko[1] and I Jukic[1]

[1]Croatian institut of Transfusion Medicine, Zagreb, Croatia [2]Clinical Department of Transfusion Medicine, Clinical Hospital Centre, Osijek, Croatia

Background: The possibility of alloimmunisation by foreign red blood cell (RBC) antigens rise with the number of RBC transfusions. Beside alloantibodies (alloAbs), our immune system can subsequently produce autoantibodies (autoAbs) which seem to be of the same specificities. In the past this was described as Matuhasi-Ogata phenomenon.

Aims: To represent the case of mimicking autoantibodies following transfusion.

Methods: Seventy-five years old woman was refered to our laboratory because of suspicion of developing multiple RBC Abs. She received 8 units of B, RhD negative RBCs from 2010. till 2014., due to repeated ortopedical operations on her right knee. In summer 2015., during the removal of infected endoprothesis, she got 2 units of B, RhD positive RBCs (1^{st} EE, Kk, 2^{nd} Jk^{ab}) because of shortage of B, RhD negative. All of the pretransfusion testing were negative. There were no transfusion reactions and the patient did not refer any disturbances. Following repetitive infections of endoprothesis, she received numerous antibiotics (penicilin, cephalexin, sulfametoxasole, trimethoprim, vancomycin, ciprofloxacin, clindamycin) and developed allergic reactions on each of them. During the preoperative treatment she succesfully received meropenem. For thromboprophylaxis she got enoxaparin and warfarin.

Results: In January 2016., during the last preoperative testing indirect antiglobulin test and crossmatches with 2 units of B negative RBCs were positive. Serum was tested by ID Diapanel (BioRad, Cressier, Switzerland), 0,8% Resolve Panel A and 0,8% Resolve Panel C - both ficin treated and untreated and Resolve Panel A and B (Ortho Clinical Diagnostics, Rariten, USA). For identification we also used phenotyped RBCs of our blood donors. In serum, there were identified anti-D, -E, -Jka, -S and -K (anti-K by micromethod only) and slightly reaction with her own RBCs were obtained. Direct antiglobulin test (DAT) in ID Card BioRad Liss/Coombs was score 1. In eluate, there were anti-D, -E identified by heat elution. The patient RBCs were typed B, Ccdee, K-, Jk(a-b+), S-s+, M+N-, Fy(a+b+). After triple autologous ZZAP adsorption and autologous PEG adsorption, in serum, there were anti-D, -E, -Jka, -S identified. DAT performed on RBCs after the third ZZAP adsorption was negative.There was no evidence of clinical signs of hemolysis (Hgb, Htc, Rtc, bilirubin, haptoglobin and LDH were normal).

Conclusion: As patient's last transfusion was 7 months ago, we concluded there were autoanti-D, -E with mimicking specificity and alloanti-D, -E, -K, -Jka, -S. It is interesting that as long as she got RhD negative RBCs she formed no RBC Abs. After receiving 2 units of RhD positive RBCs she produced multiple autoAbs and aloAbs. It seemed that after longlasting microbial infection, patient's immune system was finally triggered by RhD positive RBCs to start producing different auto- and alloAbs.

P-355

IDENTIFICATION AND FREQUENCY ANALYSIS OF ALLO ANTIBODY INDUCED BY DELAYED SEROLOGIC TRANSFUSION REACTION (DSTR)

TL Cheng, HF Liu, PI You, ZY Hsu, KH Lin and JH Chien

Taichung Tzu Chi Hospital, Taichung, Taiwan

Background: Delayed transfusion reactions may occur days or even months after transfusion. According to AABB guideline, delayed serologic transfusion reaction (DSTR) is defined as blood recipient develops antibodies to RBC antigens between 24 h and 28 days after transfusion. Unlike delayed hemolytic transfusion reactions (DHTR), the DSTR patients that develop alloantibodies may be with or without any transfusion reaction signs and symptoms. However, the routine screening and alloantibody identification are very important for prevention of subsequent

Figure 1. Distribution of DSTR according to gender

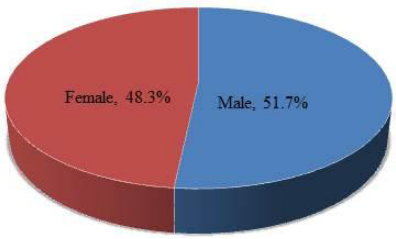


Figure 2. Frequency of DSTR according to age group

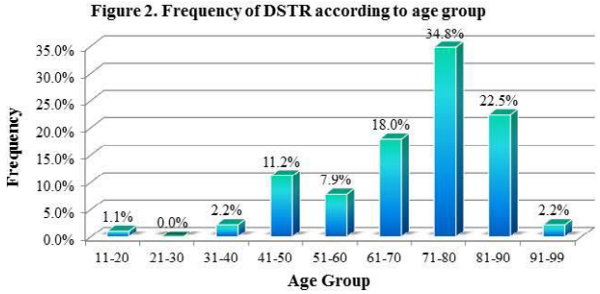


Figure 3. Distribution of blood types of DSTR patient

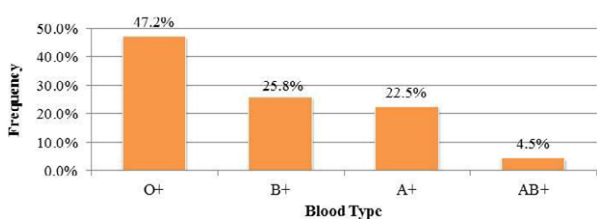


Table 1. Frequency of alloantibody among DSTR

Type of Alloantibody	Patient No. (n=89)	Frequency (%)
Anti-E	20	22.5%
Anti-Mia	17	19.1%
Anti-E,Anti-(c)	8	9.0%
Autoantibody	8	9.0%
Anti-C	4	4.5%
Anti-(c)	2	2.2%
Anti-Dia	2	2.2%
Anti-E & Autoantibody	2	2.2%
Anti-M	2	2.2%
Anti-P1	2	2.2%
Others	22	24.7%
Total	89	100%

transfusion complications. Compatible RBC antigens matched blood components could prevent the potential acute transfusion reaction and reduce alloimmunization.

Aims: The acute or delayed transfusion reaction can be caused by different type of RBC alloantibody. In order to provide the valuable information of alloantibody specificities and prevent the potential transfusion reaction, we conducted this study by a retrospective review on alloantibody of DSTR patients in our hospital.

Methods: All reviewed data of 19561 transfusion patients from 2011 to 2014 were included in this study. Blood samples were screened by manual polybrene method or classical 3 phases method, and further alloantibody identified by using RBC panel cells (Sanquin, Netherlands). Patient transfusion history, age, gender, alloimmunization, and transfusion reaction were retrieved from hospital computerized blood bank records.

Results: Total of 19561 patients received blood transfusion in Taichung Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation. There are total of 244 cases were identified as seroconversion (development of detectable irregular antibodies after transfusion). Among all seroconversion patients, there is 36.5% (89/244) patients developed new alloantibody within 28 days, and 63.5% (155/244) patients develop alloantibody after a longer period, even months or years. Those 155 patients developed longer than 28 days were excluded in this study. Immunization rate in males and female is 51.1% vs 48.9%. The incident rate is significantly greater at the age of 71–80 years old. The most frequent alloantibody is anti-E (22.5%) followed by anti-Mia (19.1%) and Anti E, c (9.0%) and autoantibody (9.0%).

Conclusion: The clinical consequences of alloantibody may cause serious transfusion reaction once patient receives unmatched RBC blood components. To prevent those unwanted complications, most blood banks provide compatible blood as possible. In emergency situations, it is difficult to have antigen matched blood component and may delay the lifesaving. However, to reduce alloimmunization is very important to improve transfusion safety and clinical outcome.

P-356

HOW PERFORMANCE OF PROPER AND RELIABLE PRE-TRANSFUSION TESTS COULD HAVE THE IMPACT ON RED BLOOD CELL ALLOIMMUNIZATION OF RECIPIENTS UNDERGOING ELECTIVE SURGERY

S Moradinasab[1], A Gharehbaghian[2] and A Abdollahi[3]

[1]Iranian Blood Transfusion Organization, Tehran, Iran [2]Department of Hematology and Blood Bank, School of Allied Medical Sciences, Shah, Tehran, Iran [3]Department of Pathology, Imam Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran

Background: Red blood cell alloimmunization is a major complication of transfusion therapy and may cause hemolytic transfusion reactions. Non-ABO antibodies have been one of the main leading cause of transfusion-associated death. Clinically significant alloantibodies also cause morbidity in a form of anemia, hemolytic disease of newborn and decrease in the survival of transfused RBCs. The other problem is autoantibodies formation against RBC antigen after alloimmunization, these alloantibodies make the hemolytic condition more severe, so it is important to study transfusion history of patients who form alloantibody after transfusion and carrying out pre-transfusion tests (ABO group and Rh D type, antibody screening test and identification of unexpected antibodies) for all the patients who were to receive transfusion.

Aim: The aim of this study was to investigate the prevalence and specificity of red blood cell alloantibodies among transfused patients due to surgery in Tehran, Iran.

Methods: A total of 6029 patients (3982 female and 2067 male) who were to undergo elective surgery in Imam Khomeini hospital of Tehran were retrieved for analysis from September 2015 to January 2016. The data collected from Imam Khomeini hospital, the biggest general teaching hospital in Iran that has the most active blood bank with the highest cross-matches ratio (more than 10,000 in a year). Clinical data included sex, age, medical history, transfusion history, pregnancy and abortion history were collected. Ab screening was performed by tube method using the 11 cell identification panel.

Results: Data from 6029 were analyzed and a total of 50 alloantibody found in 31 patients. The incidence of RBC alloimmunization in the patients for whom cross-match has been done was 0.5%. This lower alloimunization rate in comparison to similar studies sheds the light on the necessity of improvement in pre-transfusion screening tests. The most frequent antibodies were anti-D (38%) (19 patients), anti-E (20%) (10 patients) and anti-K (12%) (6 patients).

Conclusions: The most common clinically significant alloantibody in male was anti-K and in female was anti-D. The women shown a higher rate of alloimmunization than men, this is possibly because of more exposure to immunizing events through pregnancy. In this study, pregnancy for women and non-extended matched transfusions for men appeared to be risk factors for alloimmunization. Identification of alloantibodies with pre-transfusion tests have a significant role in reducing the risk of a hemolytic reactions and increase the survival of transfused RBCs and must be done for all the patients who might be candidate to receive blood transfusion.

P-357

ESTIMATING RED BLOOD CELL ALLOANTIBODY PREVALENCE AMONG BLOOD DONORS AND PATIENT POPULATION AT A TERTIARY CARE HOSPITAL IN KERALA, INDIA

N Mohanan and SP Dathan

Jubilee Mission Medical College, Thrissur, India

Background: A thorough knowledge on alloimmunisation among our population is important as it increases the risk of transfusion reaction, extends the time required for crossmatching, may delay treatment. Data on the prevalence and characteristic of alloantibody is hitherto unidentified from Kerala in South India.

Aims and Objectives: To estimate the prevalence of alloantibody among general patient population including antenatal cases and among blood donors approaching Jubilee Mission Medical College, Thrissur, Kerala.

To perform antibody screening among all samples received for pre-transfusion testing at our centre and identify all possible alloantibodies.

Materials and Methods: A prospective observational study was done on all patients who came for pre-transfusion testing and among blood donors from March 2014 to August 2015. Antibody screening was performed using commercial pooled 'O' cells and three-cell panel for donors and patients respectively. An 11-cell panel using Column Agglutination Technology (CAT from Biorad) was used for identification of alloantibodies. Autocontrol and DCT positive cases were excluded from the study.

Results: Among 23074 subjects who were screened for alloantibodies, 10382 belonged to patient population whereas 12692 were blood donors. Among the total patients (including the antenatal cases) alloimmunisation rate was 0.6%. Excluding antenatal cases it was 0.56% which is in line with most studies done across India. Among the 10382 patients screened, 3801 (36.6%) were males and 6581 (63.4%) were females. The prevalence of alloantibodies among males and females were 0.49% and 0.65% respectively, hence females were more prone for alloimmunisation. The most common alloantibody among patients was from the Rhesus system (45.1%) followed by the Lewis system (22.5%). The order of antibody against various blood group systems followed Rh > Lewis > MNS > Kell > Kidd = IH = High Frequency Antigens. Among patients with anti-M alloantibody, 63% showed crossmatch incompatibilities with the first unit cross-matched, hence showing clinical significance. Alloimmunisation rate among antenatal cases was 0.72%. The antibodies identified were anti-D > anti-c > anti-E > antibody against a high frequency antigen in Rh negative and positive mothers. Neonatal management ranged from phototherapy to Exchange transfusions. Among donors, the rate of alloimmunisation was a low 0.08%, naturally occurring antibodies like anti-Lewis^a, anti-Lewis^b and anti-M being more common than the immune antibody anti-E.

Conclusion: The alloantibody prevalence was comparable to other Indian data. Antibodies against Rh group were the most, anti-D being most frequently identified. Contrary to common belief, anti-M alloantibodies were found to have clinically significant transfusion incompatibilities.. The high rate of prevalence of alloantibodies among antenatal cases and unfavourable neonatal outcomes point towards emphasis on proper anti-D immunoprophylaxis and screening even the Rh positive antenatal mothers. Donor population had higher alloimmunisation rate compared to other Indian data. Some antibodies were unidentified could be due to some indigenous antigens hence developing indigenous panels must be considered. As alloimmunization complicates blood transfusion outcomes, routine implementation of antibody screening and identification of alloantibody as a part of pre-transfusion testing must be made aware among clinicians.

P-358

IDENTIFICATION OF A RARE ALLOANTI-GP.HIL (ANTI-MNS20) IN A THAI PATIENT

J Janthaaksorn[1], M Emthip[1], P Kupatawintu[1], O Nathalang[2], S Bejrachandra[1] and U Charoonruangrit[1]

[1]Thai Red Cross Society, Bangkok, Thailand [2]Graduate Program, Faculty of Allied Health Sciences, Thammasat University, Pathumtani, Thailand

Background: Glycophorin (GP) phenotypes and associated antigens (previously the Miltenberger subsystem) is considered as low-incidence antigens in Caucasians, but is somewhat more common among Asians, especially GP.Mur phenotype. Consequently, there are some case reports of delayed hemolytic transfusion reactions (DHTR) or hemolytic disease of the fetus and newborn implicating anti-GP phenotypes. A special requirement of positive MNS7 (Mi^a) antigen should be included in both in house and commercial panel cells for Asian populations.

Aims: To report serological findings of alloanti-GP.Hil in a Thai patient who required blood transfusion.

Case Study and Methods: A 35-year-old male Thai patient with severe anemia required two units of red blood cells (RBCs) had positive antibody screening. The antibody identification result was inconclusive. Thereafter, the blood samples were sent to the Reference Laboratory of the National Blood Centre, Thai Red Cross Society, Bangkok, Thailand for further investigation.

Results: The patient was Group A and Rh(D) positive. Antibody identification using in house panel cells demonstrated a suspicion of antibody to low-incidence of GP phenotypes because two out of three panel cells with MNS7(+) were positive and all were negative with enzyme test. The autocontrol was negative. It was found that two panel cells with positive results were GP.Hil(+); whereas, one panel cell with negative result was GP.Hil(-). The patient RBCs tested with human anti-GP.Hil and found to be negative. Additionally, the patient plasma was also tested with two extra panel cells with GP.Hil(-) and showed negative results. Therefore, two RBCs units of GP.Hil(-) with compatible crossmatch were provided to this patient.

Conclusions: The GP.Hil(-) phenotype was confirmed in a Thai patient who had alloanti-GP.Hil. Interestingly, in a case of unusual antibody identification results suspected antibody to GP phenotypes and associated antigens, additional testing with extra panel cells of those GP phenotypes and specific antisera is recommended to provide safe blood transfusions.

P-359

PRINCIPLES TO IDENTIFY ANTI-DIEGO A AND ANTI-DIEGO B ALLOANTIBODIES IN KOREA

D Shin[1], KH Lee[2], HS Kim[1], YJ Hong[2], SM Hwang[2], JS Choi[3], JS Park[2], KU Park[2], MN Hur[4], YA Lim[5], CS Lim[3], DA Kim[6], JH Song[2], YJ Cho[3] and KS Han[1]

[1]Seoul National University Hospital, Seoul, South-Korea [2]Department of Laboratory Medicine, Seoul National University Bundang Hospital, Seongnam, South-Korea [3]Department of Laboratory Medicine, Korea University College of Medicine, Seoul, South-Korea [4]Department of Laboratory Medicine, Konkuk University School of Medicine, Seoul, South-Korea [5]Department of Laboratory Medicine, Suwon, South-Korea [6]Department of Laboratory Medicine, Hanyang University School of Medicine, Seoul, South-Korea

Background: Di^a antigen is very rare in people of European or African ancestry but has a prevalence of 6.4~14.5% in people of Korean. However, most of the current antibody screening panels used in Korea are from abroad. So the antibody screening panels have no or only one Di^a antigen positive cells, and it makes laboratory professionals difficult to identify anti-Di^a alloantibody. On the other hand, Di^b is a high-prevalence antigen in almost all populations. In Korea, Di^b negative frequency is known as 1.2%. In most antibody screening or identification panels, positivity or negativity of Di^b antigen is usually not described, so it is difficult to identify anti-Di^b alloantibodies. Anti-Di^a and anti-Di^b alloantibodies can cause hemolytic disease of the fetus and newborn (HDFN) and acute or delayed hemolytic transfusion reactions. Considering the clinical significance and prevalence in Korea, detection of anti-Di^a or Di^b alloantibodies is important.

Aims: The purpose of this study is to demonstrate 13 cases with anti-Di^a or anti-Di^b alloantibodies, and to suggest the principles to identify the anti-Di^a and anti-Di^b alloantibodies.

Methods: As a part of Korean Rare Blood Program (KRBP), patients with rare blood groups have been registered and/or accomplished for additional tests. We reviewed and examined 13 cases from 9 institutes, from November 2014 to April 2016. We performed antibody screening, identification, and genotyping for red cell antigens. In cases with antibody screening-positive, which showed agglutination with only 'Di^a positive' cell, we performed additional test to confirm the presence of anti-Di^a alloantibody. We already had 'frozen red cells', from various donors, which had been genotyped before they were frozen. We selected the Di^a antigen positive red cells and the Di^a antigen negative red cells, deglycerolized selected red cells, and performed Indirect Antiglobulin Test (IAT) with these red cells and serum of each cases. For Di^b, IAT was performed by the central laboratory of The Swiss Red Cross.

Results: 5 of 13 cases showed agglutination with only Di^a positive screening panel cell. Additionally we performed IAT with these cases. In IAT, all of 5 cases showed agglutination with Di^a positive cells and no agglutination with Di^a negative cells. So we confirmed that these 5 cases have anti-Di^a alloantibody. 8 of 13 cases showed agglutination with all of screening and identification panel cells, so-called 'panagglutination'. We suspected that the panagglutination may be due to presence of high-prevalence alloantibody in the patients' serum. Results of red cell genotyping for these cases showed negative for Di^b antigen, although Di^b is high-prevalence antigen. So we suggested that there is a possibility of presence of anti-Di^b alloantibody. 2 of 8 cases were confirmed to have anti-Di^b alloantibody by IAT. In the rest

6 cases, the specimens were not submitted to central laboratory for IAT or the quantity of specimens were not sufficient.
Summary/Conclusions: We confirmed 5 cases with anti-Di^a alloantibody and suggested a possibility of anti-Di^b alloantibody in 8 cases. We also demonstrated that genotyping for red cell antigens and RBC freezing/deglycerolizing technology can be useful in antibody identification.

P-360

This abstract has been withdrawn.

P-361

THE PREVENTATIVE MEASURE OF ERYTHROCYTE ALLOIMMUNIZATION IN PATIENTS REQUIRING CHRONIC BLOOD TRANSFUSIONS: RESULT OF A REFERENCE CENTER IN CENTER ANATOLIA, TURKEY

M Yay, B Eser and E Unal
Erciyes Universty, Kayseri, Turkey

Background and Aims: The compatibility tests (CTs) are obligatory tests that must be done before a blood transfusion. In Turkey, the cross matching tests are the only legal obligatory test that had to be performed between these tests. However, cross matching tests are the final stage of CTs including ABO typing, antibody screening and antibody identifications tests. Recent guidelines advise not to perform cross matching tests even in these screening tests are done correctly. In general, the overall positivity rate of antibody screening were reported approximately 0.5–1%. We aimed to share our experience with reduction of erythrocyte alloimmunization in patients requiring chronic blood transfusions.
Materials and Methods: Laboratory and medical records of donors, and patients receiving erythrocyte transfusion from Erciyes University Blood Bank were evaluated, respectively. Historical improvements were noted; and results of different time periods were compared.Results; Routine antibody screening tests were started after 2006. Antibody identification tests were performed in 137565 patients from 2006 to 2013. Gel centrifugation method (DiaMed ID microtyping system) was used for antibody identification. Antibodies were identified and defined in 380 (0.27%) patients. Among these alloimmunized patients 285 (%75) patients have hematological disease, 95 (25%) of them were patients with surgical disease. The 317 (83.4%) of detected antibody was Rh subgroup, and Kell; 63 (16.6%) of them was others (MNS, KIDD, etc). These results showed that the majority of alloimmunazition originated from more antigenic Rh and Kell systems. After 2013 all of our blood donors were routinely screened for ABO + D Reverse typing, and Rh + K (C, c, E, e, K). The antigen typing was done by Neo microplate system, Immucor.
Results: The distribution of 60413 donors in this period, 16756 (27.7%) were negative, 43657 (72.3%) were positive for 'C' antigen; 14449 (23.9%) were negative, 45964 (76.1%) were positive for 'c' antigen; 43072 (71%) were negative, 17341 (2.9%) were positive for 'E' antigen; 1774 (2.9%) were negative, 58639 (97.1%) for 'e' antigen; 56585 (93.6%) were negative, 3828 (6.4%) were positive for 'K' antigen. In addition to these laboratory improvements, for prevention of erythrocyte alloimmunization in patients requiring chronic blood transfusions, blood groups, the result of antibody screening-identification tests, and Rh sub group, Kell identifications of patients and donors were electronically recorded between 2013, and 2015. The computer system of donor and recipient antigen typing does not only serve to find suitable donor from previously recorded data but also allow inviting the known donors to donate blood in the absence of suitable blood product in the stock. By this system we only detected antibodies in patients with surgerical or gynecological problems who have history of previous pregnancy or transfusion story. Furthermore we did not encounter any new alloimmunization in patients with hematological disorders such as thalassemia, sickle cell anemia, and recipients of hematopoietic stem cell transplantation between 2013, and 2015.
Conclusion: Erythrocyte alloimmunization is one of the important complications of transfusion especially for patients requiring chronic blood transfusions. Our experienced showed that the laboratory and software improvement was resulted in no alloimmunization for 5212 units of erythrocyte suspension for 58 patients with thalassemia, sickle cell anemia; whom the historical erythrocyte alloimmunization was found to be 6 in 11254 transfusions in the same group.

P-362

PREVENTION AND MANAGEMENT OF ALLOIMMUNIZATION BY ESTABLISHMENT OF EXTENDED RED CELL PHENOTYPE VOLUNTARY DONORS DATA BASE IN TERTIARY CARE HOSPITAL: A DISTANT VISION

A Bajpayee and A Kaur
AIIMS JODHPUR, Jodhpur, India

Background: The well-known risks associated with repeated transfusions include alloimmunization and increased donor exposure. The use of extended red blood cell (RBC) antigen matching has been well documented in reducing alloimmunization. The knowledge of RBC antigen phenotype frequencies in a local population is helpful in terms of their ethnic distribution, when dealing with patients dependent on chronic transfusion therapy and who have developed multiple alloantibody. Registry of extended red cell antigen phenotyping in repeat voluntary donors can create a credible database of antigen- negative donors for patients having clinically significant irregular antibodies and phenotype match transfusion program in thalassemics and sickle cell disease patients.
Aims: The present study was conducted to create a voluntary donors data base of young donors of known clinically significant minor blood group antigens, to provide extended antigen phenotype match blood to local patient population of thalassemia and sickle cell disease.
To provide antigen negative blood for patients having multiple antibodies in emergency.
Material and Methods: The prospective study was conducted in AIIMS,Jodhpur from Dec'14 to Mar'16. The KAP (knowledge, attitude and practice regarding voluntary blood donation) study was conducted in local population. From the participants of the KAP study prospective regular voluntary donors of group O were identified by ABO grouping and extended antigen phenotyping for RBC antigens was done in these donors for antigens D, C, c, E, e, K, Fy^a, Fy^b, Jk^a, Jk^b, M, N, S & s status by serologic methods using tube method and Gel cards (Diamed, Switzerland).
Results: Out of 1000 participants of KAP study, 200 young (18–25 yrs of age) "Group O"voluntary donors were recruited for RBC antigen phenotyping. The antigen frequency among Rh blood group system was found to be D (92.2%), C (84.2%), c (62.3%), E (23.7%) and e (98.2%). Within the MNS blood group system, antigen frequency was M (89.5%), N (51.7%), S (64%), and s (78.7%) and in the Duffy blood group system, antigen frequency was Fya (87.7%) and Fyb (51.7%). The antigen frequency for Jk^a and Jk^b was 81.6% and 50% respectively. Among 200 donors we listed 18 C neg, 21 Jk^a neg, 12 M neg and 14 Fy^a neg donors and reserved these donors for those patients who need antigen negative units due to alloantibodies. The newly registered thalassemia and sickle cell anemia patients were phenotyped for minor RBC antigens and we made a registry of minor RBC antigen matched donors for each patient. This strategy helps us to bleed these clinically significant minor antigen typed donors on the base of our requirement.
Conclusion: The Apex centers of the country that hold up chronic transfusion therapy in transfusion dependent patients, should maintain extended red cell phenotype voluntary donors data base in a software where antigen negative donors can be found on one click. It could be helpful in cases of emergency and complex cases of multiple alloantibodies. Phenotype matched transfusion in thalassemia and sickle cell anemia can largely reduce the problem of alloimmunisation and incompatible cross-match.

P-363

COMPARATIVE CHARACTERISTICS OF AGGLUTINATION METHOD IN GEL AND SOLID PHASE TECHNOLOGY 'CAPTURE-R' WHEN DEFINING ANTI-ERYTHROCYTIC ANTIBODIES OF THE DONORS

OS Kalmykova, IV Dubinkin, TV Gaponova and VV Zhuravlev
Federal State-Funded Institution National Research Center for Hematology, Moscow, Russian Federation

Background: Defining anti-erythrocytic antibodies is a very important analysis performed *in vitro*, to determine the compatibility in case of erythrocytes transfusions, as well as for culling donor hemocomponents of clinical application. Currently, there is no universal method to define all clinically significant anti-erythrocytic antibodies.
Aims: Comparison of the technology - solid-phase red cell adherence (SPRCA) "Capture-R"and agglutination in the gel cards when defining anti-erythrocytic antibodies of the donors.

Materials and methods: During the period between January and December 2015 12624 blood samples from 6100 donors at the Federal State-Funded Institution National Research Center for Hematology of the Ministry of Healthcare of the Russian Federation were studied. The studies were conducted by means of the analyzer "Galileo Neo"(Immucor Gamma, USA) and gel cards LISS/Coombs Anti-IgG + C3d (Bio-Rad, USA).
The specificity of antibodies was defined on a 14 cells panel using "Capture-R"technology and on 11 cells panel of standard erythrocytes in the gel technology. Besides, we were using erythrocytes of walk-in blood donors O(I) native and treated with bromelain.
Results: In case of SPRCA "Capture-R"out of 6100 donors there were 16 cases of anti-erythrocytic antibodies, while in the gel technology there were 10 cases (all positive in SPRCA): 3-anti-D, 2-anti-Kell, 1-anti-E, 1-anti-C^w, in 3 cases the specificity was not established. With 6 donors we found antibodies only in the solid phase technology and no antibodies were found in the gel cards on the panels of standard erythrocytes. Cold-reactive antibodies with native standard erythrocytes in high titers were not found. The direct Coombs test (direct Coombs) (anti-erythrocytic antibodies to own erythrocytes) in the gel cards was weakly positive (the result from doubtful ± to 2+). The treatment of standard erythrocytes and erythrocytes of walk-in blood donors demonstrated hyper responsiveness of serums of those 6 donors with all samples of erythrocytes in the gel cards (4+), while in case of native erythrocytes the reaction was negative.
Conclusion: The solid phase technology has a higher sensitivity when defining anti-erythrocytic antibodies. The issue of the clinical significance of all anti-erythrocytic antibodies defined by SPRCA is rather debatable.

P-364

ABO ANTIBODY TITER TESTING IN SOLID ORGAN TRANSPLANT PATIENTS: CONVENTIONAL TUBE TECHNIQUE VERSUS COLUMN AGGLUTINATION TECHNOLOGY

M Gupta, RA Shaikh, N Mehta and R Sawant
Kokilaben Dhirubhai Ambani Hospital, Mumbai, India

Background: ABO incompatible renal and liver transplants are done these days with the help of immunosuppression medication and apheresis. Performing ABO antibody titers is critical for determining both, the effectiveness of pre-transplant regimes and when titers are low enough to permit transplant. Patients may be overtreated or undertreated, owing to the inconsistency in performing and monitoring ABO antibody titers.
Aim: To compare the ABO antibody titration test in solid organ transplant patients using the conventional tube technique (CTT) and the column agglutination technology (CAT)
Methods: The isoagglutinin titers were determined in 135 patients (84-O, 43-A and 8-B blood group) using the CTT, including a 30 min room temperature phase and a manual CAT (without DTT), by the same technologist in parallel.The concordance of the titers with both the techniques was compared.
Results: 47.7% of the IgG titers reported by the CTT were between 1:2 and 1:8, 39.2% were between 1:16 and 1:64, 9.6% were between 1:128 and 1:512 and 3% were between 1:1024 and 1:4098. On comparing the IgG antibody titers by CTT with the IgG CAT titers, perfect concordance between CTT AHG and CAT AHG titers was observed in 31.8% cases, IgG CAT titer was greater than the CTT AHG titer in 58.1% cases, whereas IgG CAT titers were less than CTT AHG titers in 9.1% cases. The variability in titers with reference to the technique used shows a significant correlation ($P < 0.05$) with the blood group. The CTT AHG showed a higher titer than IgG CAT in blood group A and B.
Conclusion: The variation in titers was significant with reference to the testing method used as well as the ABO blood group tested. This variability needs to be taken into account in interpretation of the ABO antibody titer, taking the method into consideration, particularly while making a clinical decision like plasmapheresis, etc for antibody removal from the patient.

P-365

NECESSITY OF TUBE METHOD IN RBC ANTIBODY SCREEN: PERSPECTIVE FROM A CASE OF COLD AGGLUTININ DISEASE

Y Li[1], X Luo[1], S Liu[1], I James[2], G Morewood[1], ML Grant[3], K Simmons-Massey[2], S Nikolaidis[1], S Nance[4] and DN Moolten[4]
[1]*Lewis Katz School of Medicine at Temple University, Philadelphia, United States of America* [2]*Temple University Hospital, Philadelphia, United States of America*
[3]*Thomas Jefferson University Hospital, Philadelphia, United States of America*
[4]*American Red Cross Penn-Jersey Region, Philadelphia, United States of America*

Background: Tube testing (TT) and gel testing (TT) are U.S. Food and Drug Administration (FDA) approved methods for red blood cell (RBC) antibody screen (ABS). GT is more sensitive than TT in general but less sensitive for cold antibodies (cAbs). Although most of cAbs are not clinically significant, for patients with cold agglutinin disease (CAD) who may need cardiovascular surgery requiring hypothermic cardioplegia, the detection of cAbs is important.
Aims: To report a CAD case with negative ABS by GT and strongly positive (4+) by TT and to discuss the importance of TT in RBC antibody screen for CAD patients and potential candidates for hypothermic procedures.
Methods: RBC antibody screen was performed with both GT (ORTHO ProVue; Ortho Clinical Diagnostics, Rochester, NY) and TT on this A CAD case. The different results of GT and TT were investigated and discussed.
Results: An 85 year-old woman with a history of rheumatoid arthritis, low-grade B-cell lymphoma, monoclonal IgM κ immunoglobulin, cold agglutinin disease, and severe aortic stenosis was admitted for aortic valve replacement (AVR). Findings upon admission included finger cyanosis and frank hemolysis (Table 1). An EDTA blood specimen was received for blood type and ABS (T&S). The specimen showed no evidence of hemolysis after centrifugation. Per routine, T&S were performed with GT. ABO gel cards at 25°C revealed A+ in the forward typing and O+ in the reverse. ABS was negative by GT (37°C), but was strongly positive (4+) at the immediate-spin phase by TT with a 3+ autocontrol, which was eliminated by rabbit erythrocyte stroma adsorption. Direct antiglobulin test was 2+, negative, and 3+ at polyspecific, IgG, and C3d phases, respectively. The ABO discrepancy was attributed to cAbs. One hour later (at room temperature) the plasma of the specimen became red and the RBC layer formed a large gross clot. Testing at 4°C and 20°C showed anti-I specificity. Thermal amplitude studies showed: titers at 4°C were >2048 with autologous, adult O, A_1 and A_2 RBCs, 512 with I-RBCs; at 20°C 2 with autologous RBCs, 4 adult O RBCs, 8 with A_1 and A_2 RBCs; and 0 at 30°C and 37°C (Table 2). During her stay in the hospital the patient's room was continuously warmed. The operating room and IV fluids were warmed using standard external warming methodologies for the transcatheter AVR. The surgery was uneventful. No significant perioperative hemolysis occurred (Table 1). No blood product was administered. The patient was discharged 2 days after the surgery.
Summary/Conclusions: ABS was negative by GT in this case with CAD, while strongly reactive cAbs were identified by TT during the ABO discrepancy workup. TT should be advocated for ABS in patients with suspected CAD or potential candidates for hypothermic procedures, especially in group O patients in whom cAbs cannot be detected by reverse typing.

Tempreture	37°C		30°C		20°C		4°C	
RBCs Tested	Titer	Score	Titer	Score	Titer	Score	Titer	Score
Autologous RBCs	0	0	0	0	2	23	>2048	NA
Adult O RBCs	0	0	0	0	4	35	>2049	NA
I- RBCs	0	0	0	0	0	0	512	117
A_1 RBCs	0	0	0	0	8	42	>2049	NA
A_2 RBCs	0	0	0	0	8	39	>2050	NA

Note: Partial Hemolysis was observed at 4°C with the first 3 dilutions of the patient's serum when tested with I+ RBCs.

Table: Clinical data

P-366

COMPARATIVE STUDY OF SOLID PHASE & COLUMN AGGLUTINATION TECHNOLOGY FOR IDENTIFICATION OF IRREGULAR ANTIBODIES IN HEALTHY BLOOD DONORS

A Verma[1], A Bazaz[1], K Upadhyay[1] and A Gupta[2]

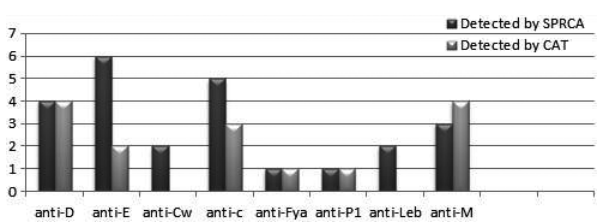
[1]Rotary Blood Bank, New Delhi, India [2]Immucor India Pvt. Ltd., New Delhi, India

Background: A lot of technology and methods are available to detect irregular antibodies in patient or blood donor but they vary to detect its clinically significance and specificity. We studied it on healthy blood donors by two most known automated methods available in india i.e. Solid phase method (immucor gailieo-neo) & column agglutination technique (auto-vue innova).this is a comparative analysis of the antibody types & number of antibodies detected, to determine the effectiveness of solid phase & cat in routine antibody screening for patient & donors.

Aim: To compare the results obtained using the column agglutination technique (CAT) by ortho biovue® system on auto-vue innova (Ortho Clinical Diagnostics, Inc., Raritan, NJ), and the results obtained on solid phase, capture-r® ready screen®, on galileo-neo (immucor, norcross, USA).

Material & Method: In a period of 1 year, total 22742 samples were collected and tested for type and screen. For antibody screening, surgiscreen® cells were used on ortho biovue®system in anti-IGG+c3d beads card, & capture-r® ready screen® plates (coated pooled 'o' cells) were used on immucor's galileo-neo. Samples were equally distributed both on automation for antibody screening. The reagents used in cat were polyspecific coombs serum which detects both IGG and c3d, & monospecific igg in solid phase. Positive screen test were repeated on both automation and sent to the ocd's & immucor's reference laboratory for identification.

Results: 22742 samples were evaluated during the study period. There were 22704 concordant negatives and 11 concordant positives. 26 samples (23 were solid phase positive and cat negative; 3 cat positive and solid phase negative) were discordant. After investigation from reference laboratory the concordant positive antibodies were identified as anti-d (29%), anti-e (15%), anti-c (21%), anti-m (21%), anti-p1 (7%) & anti-fy^a (7%). The specificities of antibodies (not detected by cat) were identified as anti-e (18%), anti-cw (8%), anti-leb (8%), anti-c (8%) & warm auto antibody (58%). Antibody which was detected only by cat identified as anti-m, but later confirmed by sprca extended panels. The sensitivity of solid phase was found to be 94.8% which was substantially higher than that of cat (34.3%) for the detection of clinically significant antibodies in normal serum as well as in weak titer, while the specificity of these two tests was similar. See Table (1.1) Table1.1: antibodies identified by spraca/cat discussion: this comparative study showed that solid phase capture technology detected a larger number of irregular antibodies in compare to cat. As per the specificity of antibody, capture detects mostly clinical significant igg antibodies while cat may detect both igg and igm with a lower sensitivity than capture. But in some cases both systems have limitation to identifying some non-specific antibodies.



P-367

DISCREPANCIES IN ISOAGGLUTININ TITERS AMONG DIFFERENT MICROCOLUMN ASSAYS

H Kim, YJ Hong, KU Park and KS Han

Seoul National University College of Medicine, Seoul, South-Korea

Background: Standardization of red cell antibody titration has long been a problem unresolved in the field of immunohematology. Efforts such as implementation of uniform methods have been described with inspirations and limitations in reducing inter-institutional variances. Since ABO-incompatible solid organ transplantation becomes a routine practice in many countries, further research on factors that may contribute to improve precision in antibody titration is required.

Aims: In this study, we measured the isoagglutinin titers of group A, B, and O pooled plasma using different commercialized microcolumn assays and compared the results.

Methods: Plasma from group A, B, and O individuals was pooled to a volume of 40 ml (group O plasma was diluted with saline at a ratio of 1:4). The plasma was divided into ten aliquots of 4 ml and stored at −30°C until testing. Isoagglutinin titration was performed twice daily for five consecutive days. Anti-A and anti-B titers were measured with 0.8% A_1 and B reagent RBCs (Affirmagen, Ortho-Clinical Diagnostics, High Wycombe, UK) using BioVue Reverse Diluent and Anti-Human Globulin (AHG) Polyspecific microcolumn cassettes (Ortho-Clinical Diagnostics) at room temperature (RT) and the AHG phase, respectively. Comparison tests were also done using ID-card (Bio-Rad, Cressier, Switzerland) microcolumn cassettes. Titers were determined at endpoints of 1+ and ± reactivity, and the overall strength of hemagglutination was calculated by the Marsh scoring system. Antibody titers converted to $\log_2$ and the hemagglutination scores were compared using the Wilcoxson signed rank test. A P value of <0.05 was considered as statistically significant.

Results: The antibody titers at both endpoints of anti-A and anti-B in group O using BioVue were significantly higher compared to the results using ID-card. The difference in mean antibody titers tended to be higher at the AHG phase (1.9–2.3) than RT values (1.2–1.7). Anti-A titers (AHG) and anti-B titers (RT, AHG) in groups B and A were also significantly higher when determined by BioVue than ID-card at both endpoints. The difference in mean antibody titers in groups B and A (0.6–1.2) inclined to be lower than that of group O. Hemagglutination scores of anti-A and anti-B in group O using BioVue were also significantly higher for more than 10 points compared to the results using ID-card.

Conclusions: Isoagglutinin titers using BioVue was higher than that of ID-card, especially in IgG dominant samples such as group O plasma. Antibody titration performance disagreement among different manufacturers should be considered when microcolumn assays are used. Further investigation regarding this issue may be informative in interpreting proficiency test results, comparing inter-institutional desensitization protocols in ABO-incompatible organ transplantation, and for establishing isoagglutinin titration standardization.

P-368

FREQUENCY OF A_2 SUBGROUP IN A AND AB BLOOD GROUPS IN THE POPULATION OF SOUTH BACKA DISTRICT

J Grujic and N Bujandric

Institute of Blood Transfusion of Vojvodina, Novi Sad, Serbia

Background: Blood subgroups are weaker forms of blood group antigen. The polymorphism of the gene encoding the ABO blood group system leads to the presence of a subgroups. A1 and A2 are the most common subgroups of blood group A and differ from each other qualitatively and quantitatively. The frequency of A2 subgroup in the population of South Backa District of the Autonomous Province of Vojvodina is not known.

Aim: To establish the frequency of A2 subgroup in the population of the South Backa District.

Methods: Blood sample was collected in 1 pilot tube immediately after the collection of blood unit. ABO (forward and reverse typing) and Rh typing are performed on all donated blood. Blood typing was performed by standard gel technique. Detailed serological workup was done for all donor units where forward and reverse grouping showed discrepant result in the ABO blood group system. Depending on the presence of agglutination of erythrocyte with lectin from Dolichos biflorus seed, the samples were classified into A1 and A2 subgroups as well as A1B and A2B. Agglutination comes in contact with A1 and is absent with A2 erythrocytes. Frequency of A2 subgroups was established. Statistical significance was analyzed with chi-square test.

Results: A total of 700 blood donor's samples was tested. Among 500 samples of blood group A 99.6% is detemined as A1 and 0.4% as A2. Among 200 samples of AB blood group 92.5% is classified as A1B and 7.5% as A2B. Proportion of A2B subgroup in AB samples was statistically more significant then the presence of A2 in samples of blood group A ($P < 0.0001$).

Conclusion: The serologically determined weak ABO phenotypes require confirmation through genomic analysis for correct determination of ABO blood group status. Correct blood typing of an individual is essential to prevent ABO incompatibility in case of weaker subgroups. The results of the study show a significant proportion of A2B subgroup compared to the A2 subgroup. It is important that 1–8% of A2 and 22–35% of A2B individuals can produce anti-A1 antibodies.

P-369

PREVALENCE OF CLINICALLY SIGNIFICANT ANTIBODIES IN DONATED BLOOD UNITS

B Pekovic, J Grujic, Z Gulan and Z Budakov Obradovic

Institute of Blood Transfusion of Vojvodina, Novi Sad, Serbia

Background: Inside the serum/plasma one can detect the antibodies as a result of the transfusion utilization or pregnancy. Detecting clinically signficant antibodies inside a voluntary blood donor's (VBD) plasma is of crucial importance, as they can be involved in the creation of hemolitic transfusion reaction (HTR) at patients. Their presence can usually be detected at a VBD using an antibody screening with each blood donation.
Aim: To evaluate prevalence of anti-RBC antibodies in donated blood unites at Institute of Blood Transfusion of Vojvodina.
Methods: A screening blood samples of VBDs have been analyzed at the Institute of Blood Transfusion of Vojvodina during 2014. In 2014, the antibody screening was conducted using an automatized method on BioRad IH-1000 (ID-DiaCell POOL, ID card Liss-Coombs, BioRad) and on Diagast Qwalys 3 device (Hemascreen POOL, Diagast). The samples containing the positive screening results have been further tested using the IAT method (ID card, ID-DiaCell I-II BioRad). The samples with the positive IAT have been used to examine the specifity of the antibodies applying the gel identification method (ID-DiaPanel, BioRad).
Results: During 2014, 26499 samples of VBDs were tested out of which 67 (0,25%) had a positive screening result, and the further tests proved that 32 (47,7%) samples contained the specifity of the antibodies: anti-K 7 (21,8%), anti-M 4 (12,7%), anti-c 2 (6,2%), anti-C 2 (6,2%), anti-D 4 (12,5%), anti Le^b 1 (3,1%) and 12 (37,5%) samples containing irregular antibodies of an unknown specifity. 35 blood samples were screening positive on Diagast Qwalys but further tests did not expose presence of the antibodies.
Conclusions: False-positive antibody screening result can be contibuted to usage of two different screening methods especially because in further testing we use reagences of only one manufacturer. By checking Questionnarries of VBDs with positive antibody screening it was determined that most of the donors didn't inform doctors anything about illnesses, operations, receiving blood transfusions or sensibilisation in pregnancies. Sensitive methods for detection of alloantibodies contributes to the higher safety of the transfusion treatment of patients.

P-370

This abstract has been withdrawn.

P-371

PREVALENCE OF RED BLOOD CELL ALLOIMMUNIZATION AMONG PREGNANT WOMEN AWAITING CAESAREAN OPERATION

JV Galhenage

District General Hospital Matara, Matara, Sri Lanka

Background: Red cell alloimmunization can occur by means of fetomaternal haemorrhage in pregnancy and child birth or following blood transfusion. Clinically significant unexpected antibodies in the maternal serum may cause haemolytic disease of the foetus and newborn. Further, it may also interfere with the pre transfusion compatibility tests. Screening for unexpected red cell antibodies in Rh D positive pregnant women is not routinely done in Sri Lanka. Therefore such antibodies are usually detected for the first time during the pre-transfusion compatibility testing of blood for Caesarean operations.
Aims: The aim of the study was to determine the prevalence of unexpected red cell antibodies with their specificities and frequencies among pregnant women to undergo Caesarean section and to evaluate the pattern of interference of such antibodies with the pre transfusion compatibility test.
Methods: A retrospective study was conducted for 12 months from January 2015 to December 2015 in District General Hospital, Matara, one of the main state sector health care institution in the southern part of Sri Lanka, to analyse the data of antibody screening done as a part of pre transfusion compatibility testing of 3390 maternal patients who were to undergo caesarean operation. Antibody screening was done at the same institution and identification was done at National Reference Laboratory, National Blood Centre, Colombo.
Results: Out of the 3390 cases analysed, only 53(1.6%) were found to be positive for clinically significant antibodies. The majority of the cases (42) had a single antibody whereas 11 cases had multiple antibodies identified as one case with anti-D+C and other 10 cases with anti-Le^a +Le^b. Most common antibody was anti-Le^b found in 20 cases (37.7% of total positive samples) followed by anti-Le^a in 10 cases (18.9%), anti-Le^a+Le^b in 10 cases(18.9%) anti-D in 4cases (7.5%) anti-E in 4 cases (7.5%), anti-M in 2 cases(3.8%) and anti-c in 2 cases(3.8%). Least frequently found antibody in the study population was anti D+C combination (1.9%). Out of 53 samples with antibodies, 6 were Rh D Negative in which all had developed anti D except for one case that had found to be with anti- Le^b. Lewis antibodies (Le^a, Le^b and Le^a + Le^b) had been the cause for 75.5% of total number of positivity.
Conclusions: The results of the study showed that most frequently occurring antibodies in this study group were those of the Lewis blood group system that does not clinically interfere with the pregnancy. But it contributed to 75.5% of the positive pre transfusion antibody screening tests that ware done before reservation of blood for Caesarean operation causing delays in the procedure. To resolve the problem, blood units with IAT crossmatch compatibility at 37°C were selected after identification of Lewis antibodies.

P-372

A 43-YEARS OLD PREGNANT WOMAN WITH ANTI-JK3

N Revelli[1], MA Villa[1], C Paccapelo[1], E Magnabosco[1], MC Manera[1], NN Fantini[1], MC Rossi[2], C Leone[2], M Dassi[3] and M Marconi[4]

[1]IRL - Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy [2]ASST Nord Milano, Immunoematologia e Medicina Trasfusionale, Sesto San Giovanni, Italy [3]ASST di Monza, Immunoematologia e Medicina Trasfusionale, Monza, Italy [4]IRL - Fondazione IRCCS Cà Granda Ospedale Policlinico, Milan, Italy

Background: The Kidd blood group system is composed of three antigens, the antigens Jk(a) and Jk(b) associated with a single nucleotide change and Jk3, a high-prevalence antigen absent in JK null individuals. Several alleles, mostly comprising single nucleotide polymorphism have been reported to weaken or silence the expression of the JK protein.
Methods: A sample from a 43-old female, untransfused primigravida at the 16th week gestation, was received from another hospital for investigation of a reactivity with all tested cells in antibody screening. Her group was A, Rh(D)+, ccEe, Kell negative. The antibody screening, performed by indirect antiglobulin test (IAT) in microcolumn method (CAT) with BioVue System (Ortho-Clinical Diagnostics, Raritan, USA), was positive with all cells. The antibody identification, performed in IAT by CAT and by tube method with polyetylene glycol (PeG) and by saline method, was positive with all cells in IAT and negative in saline. The direct antiglobulin test (DAT) was positive due to C3d. An alloantibody to a high-prevalence antigen (HFA) was suspected. We performed an extended phenotype by molecular typing using two kits (Hea BeadChip, Immucor-BioArray Solution, Warren NJ, USA; IDCORE^{XT}, Progenika Biopharma, Grifols, Derio, Spain) and by serology with tube method and with NeoGalileo instrument (Immucor, Rodermark, Germany).
Results: A panel of Jk(a-b-) cells were tested with patient plasma and eluate and they were negative. The positive DAT was due to anti-JK3. During the last control, the titer of anti-Jk3 antibody was 1:8 in IAT by tube method without additive. The red cell typing for Jk^b and s antigens was discrepant between serology and molecular typing. The typing was Jk(a-b-) and s- by serology while Jk(a-b+) and s+ by molecular typing. Therefore, the sample was sent to the Grifols Immunohematology Center (S. Marcos, Texas, USA) for the sequencing study. DNA sequencing showed that the patient was Jk^b homozygous but also heterozygous for c.810A polymorphism in exon 8. Although the c.810A does not encode an amino acid change, JK*B (810A) has been reported as a null allele based on adsorption-elution tests. Given its proximity to the exon-intron boundary, c.810A may have an effect on mRNA splicing. It is possible that in this patient there is a slightly weakened Jk^b antigen, as reported in literature for Jk^a antigen. Moreover, the patient showed variant allele GYPB*s(IVS5 + 5A) which has been reported to encode a s-phenotype. In our Regional Lombardy Bank were not stored compatible frozen units with this rare phenotype, thus the patient was scheduled to the pre-deposit of two autologous red blood cell units in advance of the expected need for mother or baby. The patient delivered at the 38th week of gestation. The newborn's DAT was positive and presented no clinical symptoms of hemolytic disease fetus neonate. No transfusion support was required by either mother or child.
Conclusions: Variants associated with very weak antigen expression are often missed by routine serological typing. This could cause alloimmunization in recipients and thus donor genotyping can increase transfusion safety and reduce potential alloimmunization.

P-373

THE PERSISTENCE AND EVANESCENCE OF RED CELL IRREGULAR ANTIBODIES

L Fan

Shanghai Blood Center, Shanghai, China

Background: Red cell irregular antibodies may become weak and undetectable over the course of time after transfusion-related alloimmunization .They can cause a delayed haemolytic reaction if their past existence is not known before a transfusion. So the research of persistence and evanescence of red cell irregular antibodies is valuable in theory, and can offer valuable data for safe transfusion. Only a few studies have been published on this topic.

Aims: We wanted to find the evanescence rate of irregular antibodies all kinds .this research can offer valuable data for safe transfusion.

Methods: In our research, We collected and detected the blood samples of patients from all of hospitals in Shanghai between 2000 and 2016. All warm-reacting irregular antibodies were included, with the exception of ABO antibodies, cold-reacting antibodies, autoantibodies, antibodies produced in women of childbearing age. If the antibodies were tested again after the first detection, we recorded these antibodies data included the strength of antibodies, the length of follow-up, and calculated the rate of evanescence.

Results: We retrieved the records of 110 antibodies(included -E/Ec, -Ce/C, -D, -Jk^a, -Jk^b, -Fy^b, -Di^a, -Wr^a, -M, -S), which had been tested again after the initial detection, and found that 10% were non-persistent. The average rate of evanescence of Rh blood system antibodies is below the others blood system antibodies.

Conclusions: Red cell irregular antibodies commonly tend to disappear over the course of time, especially when they become weak and undetectable. The increased sensitivity of our current screening tests does not protect us against this risk. To avoid delayed haemolytic reactions,it is necessary to rely on previous records.

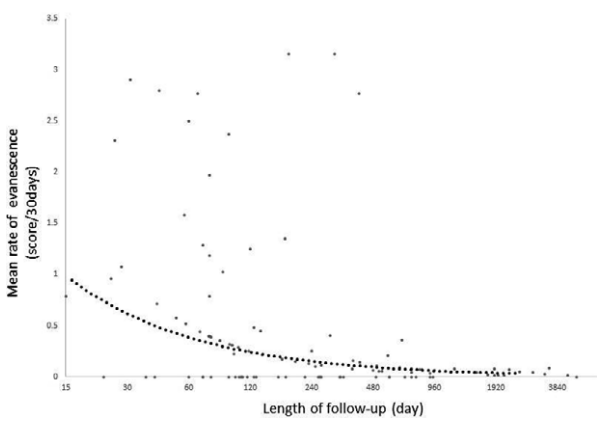


Figure1. Rate of evanescence of antibodies

	Antibody	Num of Sample	Mean of tests	Num of disappearance	Length of follow-up (day)	Mean rate of evanescence (score/30days)
Rh	F/Ec	66	3.03	3	518.5 (23-3483)	0.209
	Ce/C	11	2.54	1	453.3 (43-3049)	0.785
	D	10	3.50	0	1793.4 (70-4298)	0.0136
	Jk^a/Jk^b	11	3.7	6	114.8 (6-408)	1.76
	Fy^b	3	2.67	1	680.7 (37-1885)	0.443
Non-Rh	Di^a	1	14	0	457.0 (457)	0.263
	M	3	3.5	0	71.0 (57-85)	0.966
	S	1	1	0	530.0 (530)	0.056
	Wr^a	4	2.50	0	258.0 (101-397)	0.227
Total	/	110	/	11	/	/
Mean	/	/	3.09	/	569.7 (6-4298)	0.457

Table 1. Summary table of study

P-374

SEVERE IMMUNE HEMOLYTIC ANEMIA ASSOCIATED WITH WARM-REACTING IGM AUTOANTIBODIES: A DIAGNOSTIC (AND THERAPEUTIC) CHALLENGE

M Raos, M Lukic, IL Burnac, F Plenkovic, I Aurer and B Golubic Cepulic

Clinical University Hospital Center Zagreb, Zagreb, Croatia

Background: Autoimmune hemolytic anemia (AIHA) can present with severe intra- and extravascular hemolysis. In warm AIHA (WAIHA), characterised by IgG autoantibodies, hemolysis is tipically extravascular; however, complement-mediated intravascular hemolysis can be seen in "mixed" type AIHA, characterised by both IgG warm autoantibodies and IgM cold autoantibodies reactive up to 30 to 37°C, and in rare WAIHA caused by IgM warm autoantibodies. Diagnostic of IgM WAIHA is very difficult. It can be confused with cold agglutinin syndrome, caused by IgM cold autoantibodies reactive up to 30°C or with "mixed" type AIHA. Although death from WAIHA is considered rare (4–11%), it is particularly high (22%) in IgM WAIHA.

Aims: Our aim is to present a patient with chronic lymphocytic leukaemia (CLL) and severe AIHA caused by warm-reacting IgM autoantibodies with a special focus on difficult serologic diagnostics.

Methods: A 57 years old patient with CLL was admitted to our hospital because of severe anemia (Hb 52 g/l). He was previously diagnosed with CLL Rai stage 4, Binet C, failed to respond to FCR and was receiving bendamustine + rituximab. Patient's red blood cells (RBC) were washed 6 times with 37°C saline. For direct antiglobulin test DAT IgG/IgA/C3c/C3d card (DC-Screening I, Bio-Rad) was used. Eluates were prepared from the patient's RBCs using acid elution kit (DiaCidel, Bio-Rad) and 56°C heat elution. Master dilutions of serum samples were prepared for titration and thermal amplitude analysis at temperatures 37°C, 30°C, 20°C and 4°C. Serum samples were tested with untreated and papain-treated RBCs. RBCs tested were pool of adult RBCs, pool of cord RBCs and autologous RBCs. Serum samples with acidification, after addition of a fresh normal serum as a source of complemet, and serum treated with DDT were also tested with untreated RBCs.

Results: Agglutination of the patient's RBC by autoantibodies was observed, therefore washed RBC were tested. Positive results were obtained with anti-IgG (2+), -IgM (3+), and -C3d (3+). Neither of the eluates directly agglutinated test RBCs, nor reacted in the antiglobulin test. Agglutinin reactive against untreated RBCs at 37°C was detected in patient's serum. It also reacted at 30°C and 20°C and in the antiglobulin test. Agglutinin reacted against all tested papain-treated RBCs at four temperatures, the strongest at 37°C (37°C (4+), 30°C (3+), 20°C (2+) and 4°C (1+)), and also after serum acidification and the addition of fresh normal serum. The latter did not enhance reactions. Titration and thermal amplitude analysis showed that the antibody optimally reacted at 37°C and 30°C. Titer at 4°C was 1:2. The serum sample treated with DTT showed only IgM antibodies.The patient was treated with RBC transfusions, steroids, intravenous Immunoglobulins and plasmpaheresis, but failed to respond and died due to anemia complications after 5 days.

Conclusions: WAIHA associated with IgM warm autoantibodies is very difficult to diagnose. Since hemolysis may be life-threatening and difficult to treat, precise serologic diagnostics is of paramount importance for determining prognosis.

P-375

PERFORMANCE CHARACTERISTICS OF A NOVEL ANTI-MIA MONOCLONAL ANTIBODY-BASED REAGENT IN A CANADIAN POPULATION

J Saldanha[1], T Dolnik[2], X Lin[1], JC Yang[1] and J MacEachern[3]

[1]Immucor Inc., Norcross, United States of America [2]Canadian Blood Services, BC & Yukon, Vancouver, Canada [3]Dominion Biologicals Limited/Immucor, Dartmouth, Canada

Background: The GP.Mur (Mi III) glycophorin of the Miltenberger subsystem, one of the eleven classes of the Miltenberger subsystem, is relatively common in South-east Asia especially along the south-east coast lines of China and Taiwan. Although glycophorins in the Miltenberger subsystem can be phenotypically defined by using class-specific antisera, cross-reactivity of the epitopes can limit precise identification of the glycophorin variant. For example, the currently available antibodies against GP.Mur consist of polyspecific antibodies, such as anti-Mi^a, anti-Mur, anti-MUT, anti-Hil and anti-MINY. In addition, the supply of class-specific antisera is limited making precise identification of the glycophorins in the Miltenberger subsystem difficult. Immucor has developed an IgG mouse monoclonal antibody (GAMA210)

reagent, the NOVACLONE[TRADEMARK] Anti-Mi^a Mouse Monoclonal Typing Reagent (anti-Mi^a typing reagent), for detection of 6 of the 11 glycophorin variants.
Aims: A study was carried out at two centers in Canada to evaluate the performance of the anti-Mi^a typing reagent.
Methods: A manual tube test was used to evaluate the performance of the anti-Mi^a typing reagent and compare the performance with that of a human anti-Mi^a polyclonal antibody. A total of 578 samples were tested, including adult male and female and neonate samples (2%). One of the test sites chosen were selected due to the population demographics. As the Mi^a antigen has a higher incidence in the Asian population, the majority of test samples were tested at Canadian Blood Services, BC & Yukon (over 30% of the population of Vancouver is of Chinese or SE Asian descent) and the remaining samples were tested at a second Canadian site. Samples with discordant results between the two antibody reagents were sent to Bioarray Solutions (Immucor, Warren) for further analysis using molecular methods, including GYP Exon 3 hybrid gene sequence-specific primer (SSP)-PCR, GYP Intron 3 SSP-PCR, GYP.Vw SSP-PCR, nucleotide sequencing and HEA BeadChip analysis.
Results: The Novaclone Anti-Mia typing reagent detected 11 positive samples in 578 samples tested (1.90%), while one sample was missed by the polyclonal reagent (10/578 = 1.73%). The sample with the discordant result was tested by molecular methods. GPY Exon 3 SSP-PCR and GYP Intron 3 SSP-PCR confirmed the absence of glycophorins GP.Mur, GP.Hil, GP.Bun, GP.Hop and GP.HF in the sample. However these primers cannot detect the GYP.Vw allele by SSP-PCR. GYP.Vw SSP-PCR suggested the presence of the GYP.Vw allele in the sample and this was confirmed by sequencing. The HEA BeadChip assay showed that the sample was N (+) and s (+), which are the known common haplotypes with glycophorin Vw in the MNS blood group system.
Summary/Conclusions: The NOVACLONE[TRADEMARK] Anti-Mi^a Mouse Monoclonal IgG Typing Reagent was shown to have a comparable performance to that of a polyclonal antibody reagent. In addition, the reagent was able to detect a Gp. Vw, which was not detected by the polyclonal reagent. The ready availability of the NOVACLONE[TRADEMARK] Anti-Mi^a Mouse Monoclonal Typing Reagent makes it the reagent of choice for typing individuals in populations where GP.Mur is reasonably common.

P-376

IMMUNE CHARACTERISTICS AND EVANESCENCE OF ANTI-MUT AND ANTI-MUR RED CELL ALLOANTIBODIES IDENTIFIED DURING ROUTINE PRE-TRANSFUSION AND ANTENATAL TESTING

VS Nadarajan[1], I Jeyajothi[2], SMN Kyaw[2], MT Htut[2] and MS Shahila[2]
[1]University Malaya, Kuala Lumpur, Malaysia [2]University Malaya Medical Centre, Kuala Lumpur, Malaysia

Background: The MUT and Mur antigens are expressed on glycophorin variants within the MNS blood group systems that result from rearrangements between the glycophorin A and B genes. Among East Asians, the GP.Mur phenotype, which expresses both MUT and Mur, is a common phenotype. Alloantibodies directed against both antigens are one of the most common antibodies identified in this population when appropriate screening cells are used. Limited information is however available on the characteristic differences between anti-MUT and -Mur.
Aims: To determine the differential characteristics of anti-MUT and -Mur with regards to association to transfusion sensitization events and antibody evanescence.
Methods: Samples for pre-transfusion and antenatal testing were subjected to antibody screening using Abtectcell[TRADEMARK] III (CSL Limited, VIC), a three-cell screening panel with one cell engineered to express MUT and Mur epitopes. Antibody identification using a variety of methods and cells were carried out on samples that were positive on antibody screening.
Results: Of 75,597 unique patient samples tested, 229 (0.30%) patients were identified to have anti-MUT, and 158 (0.21%) with anti-Mur. The prevalence of anti-MUT among transfused patients was 0.72% and was significantly different ($P < 0.001$) when compared to 0.26% in non-transfused individuals. No significant difference was observed in prevalence of anti-Mur between transfused (0.33%) and non-transfused (0.21%) patients. Sequential antibody screening results exceeding a period of more than 30 days from first identification, were available for 86 and 47 patients with anti-MUT and -Mur respectively. Anti-Mur was transient in 27 (57%) of the patients with median time to evanescence of 281 days, contrasting with 28 (32%) patients harboring anti-MUT. The median time to evanescence was 1111 days in the anti-MUT group, which was significantly different from anti-Mur ($P = 0.003$).
Conclusions: The prevalence of anti-Mur does not significantly differ between transfused and non-transfused patients, irrespective of gender, suggesting that the antibody has a greater propensity to be naturally occurring and not require a transfusion sensitization event. Anti-Mur is also more likely to be undetectable with time. These characteristics are in contrast to anti-MUT that has greater tendency to occur following a transfusion event and show persistence. The results suggest that the detection of anti-MUT in a patient may be more clinically relevant.

P-377

A PATIENT OF CAUCASIAN ORIGIN WITH ANTI-SC3 AND THE SC-NULL PHENOTYPE DUE TO A NOVEL ERMAP MUTATION

C Henny[1], V Karamatic Crew[2], A McNeill[2], S Lejon Crottet[1], C Niederhauser[1], TR Braschler[3], H Hustinx[1] and N Thornton[2]
[1]Interregional Blood Transfusion SRC, Bern, Switzerland [2]International Blood Group Reference Laboratory, NHS Blood and Transplant, Bristol, United Kingdom [3]Division of Hematology, Luzerner Kantonsspital, Lucerne, Switzerland

Background: Scianna (SC) is a blood group system composed of seven antigens, five of high prevalence (Sc1, Sc3, STAR, SCER, SCAN) and two of low prevalence (Sc2, Rd), all expressed on the human erythrocyte membrane-associated protein (ERMAP) encoded by the *ERMAP* gene located on chromosome 1. Anti-Sc3 produced by patients with the SC_{null} phenotype (SC:-1,-2,-3) has been reported to have caused mild delayed transfusion reactions and mild haemolytic disease of the fetus and newborn. Three molecular backgrounds of the SC_{null} phenotype have been reported: c. 307_308delGA (from Saudi Arabia), c.994C>T (from Pacific Islands) and c.349C>T (from India).
Aim: In this study we present results from serological and molecular investigations of a case of anti-Sc3 with a novel molecular background of the Sc_{null} phenotype.
Case Study and Methods: Samples from a 31 year old pregnant Kosovar female were investigated because of an alloantibody in her plasma which reacted with all cells tested except her own. The patients samples (A, RhD positive) were collected in the 20th (sample A) and 29th (sample B) week of gestation. Serological investigations were performed by IAT (tube and column agglutination). Papain and IAT/trypsin techniques were also utilised. Soluble recombinant Sc blood group proteins (Sc-rBGP) (Imusyn, Germany) were used in neutralization tests. The clinical significance of the antibody was assessed by a monocyte monolayer assay (MMA). Genomic DNA was isolated from whole blood and all the coding exons (3–12) of *ERMAP* were amplified by PCR and analysed by Sanger sequencing. Blood samples from the patient's two brothers were subsequently analysed.
Results: The two serum samples (A and B) reacted by IAT (titre 1:4) with all panel cells tested. An anti-Sc3 was identified and the patient was found to have the SC:-1,-2,-3 phenotype. The antibody was neutralized with Sc-rBGP, thereby confirming Sc specificity. The monocyte index (MI) for sample A was 10% with SC:1,-2,3 cells, which increased to 26.5% for sample B. The samples from the two brothers revealed the following phenotype: brother 1, SC:-1,-2,-3 (O, RhD positive) and brother 2, SC:1,-2,3 (O, RhD positive). Cells from brother 1 were compatible with the patient's serum. *ERMAP* sequencing revealed a novel homozygous 8 bp duplication in exon 5, c.479_486dupTGATCCTG. This duplication introduces a frameshift and premature stop codon at amino acid 163. Brother 1 was found to be homozygous, and brother 2 heterozygous for this novel mutation.
Summary/Conclusions: We report the case of a pregnant woman of Caucasian origin with the rare SC_{null} phenotype and the corresponding anti-Sc3 antibody. The phenotype was shown to be due to the novel mutation c.479_486dupTGATCCTG. One of her brothers was found to have the same phenotype and genotype. During the period from 20 to 29th week of gestation the samples showed an increased MI. As an MI over 5% suggests a clinical relevance for the corresponding antibody, the patient's anti-Sc3 was classified as clinically relevant. Antigen-negative red cells were recommended for transfusion and the patient's compatible brother identified as a possible donor.

Red Cell Immunology: Molecular

P-378

A NOVEL CIS-AB VARIANT ALLELE ARISING FROM A DE NOVO NUCLEOTIDE SUBSTITUTION 796A>C IN THE B GLYCOSYLTRANSFERASE GENE

B He

Nanning Institute of Transfusion Medicine, Nanning, China

Background: The CIS-AB phenotype is very rare, and only nine genotypes that correspond to specific ABO allele changes have been reported. It may lead to ABO discrepancies and a delay in establishing the blood group. Until now, gene sequences of nine cis-*ab* alleles were characterised and none of these cis-*ab* alleles were reported as *de novo* polymorphisms.
Aims: The aim of this study was to investigated the cis-ab phenotypes were identified in a chinese family.
Methods: We performed phenotype investigations by serology studies, analyzed the dna sequence of the abo gene by sequencing of exon 6 and exon 7 after cloning.
Results: A novel cis-ab allele arising from nucleotide substitution 796A>C in the B glycosyltransferase gene were discovered in the blood from the proposita and one of her sons.
Conclusions: The Chinese family described carries a novel cis-ab allele that differs molecularly from all previously reported cis-ab alleles.

P-379

TWO NOVEL MUTATIONS IDENTIFIED IN THE ERYTHROID CELL-SPECIFIC REGULATORY ELEMENT OF THE ABO GENE

Q Chen and JY Xiao

Jiangsu Province Blood Center, Nanjing, China

Background: A3 and B3 phenotypes are characterized by mixed-field reaction with a few large agglutinates among large amounts of free cells when typing with anti-a and anti-b, respectively. The underlying mechanisms of A3 and B3 phenotype were diversified. A regulatory element was identified in the in the first intron of the abo gene with a functional gata-1 erythroid transcription factor binding site. Studies have shown that mutations in the erythroid cell-specific regulatory element (+5.8 kb) can cause variant abo phenotypes.
Aims: To perform serologic and genetic analysis to investigate the molecular mechanism of ABO serologic discrepancy detected in one A3 and one B3 phenotype patients referred to our laboratory.
Methods: Serologic tests were done by the standard serologic protocol. Genomic DNA were extracted. The abo gene upstream CBF/NF-Y enhancer region, promoter, ABO +5.8-kb site, exons and adjacent introns were amplified and sequenced.
Results: A novel single nucleotide substitution close to the gata site (A>T substitution at position c.28 + 5827) in the +5.8-kb site on the a-allele was found in one proband with a3 phenotype. Another novel single nucleotide substitution within the gata motif (c>t substitution at position c.28 + 5885) on the b-allele in the other proband with b3 phenotype. No other mutations were found in the promoter, CBF/NF-Y enhancer region, coding regions or the exon-intron boundaries of the abo gene. The genbank nucleotide database accession numbers for these two novel alleles were kr780754 and ku234272, respectively.
Summary/Conclusions: Our study suggested that the A>T substitution at position c.28 + 5827 and the C>T substitution at position c.28 + 5885 within the gata motif in the regulatory of the abo gene might diminish transcription activity causing the generation of the A3 and B3 phenotype, respectively. To increase the safety of blood transfusion, correctly identify these subgroups to improve the accuracy of blood typing is necessary.

P-380

IDENTIFICATION OF A NOVEL RHD*730C ALLELE ASSOCIATED WITH A PARTIAL D PHENOTYPE

GP Luo[1], YL Ji[1], Z Wang[1], JZ Wen[1], QN Liang[2], L Wei[1], H Luo[1] and YS Fu[1]

[1]Guangzhou Blood Center, Guangzhou, China [2]Guangdong No.2 Provincial People's Hospital, Guangzhou, China

Background: Compared with the great numbers of D variant alleles reported in the Caucasians population, less D variant alleles (including 27 weak D alleles and 14 partial D alleles) are identified in the Chinese population to date. *RHD*weak partial 15* and *RHD*DVI.3* are the most common D variant alleles, accounting for more than 70% of D variants in the Chinese population. In this study, the *RHD* genotype was analyzed in the Chinese donors with D variant phenotype and one novel *RHD* variant allele was identified.
Aims: To investigate the variant alleles of *RHD* in the Chinese population.
Methods: The blood samples with D variants phenotype were collected from the Guangzhou Blood Center. The *RHD* and *RHCE* genotypes were analyzed by the developed Multiplex Ligation-dependent Probe Amplification (MLPA) genotyping assay. Further sequencing of ten exons of *RHD* gene was conducted in the donor with the *RHD* variant allele that could not be defined by the MLPA analysis. The serological typing for D antigen was conducted by using the two monoclonal anti-D reagents (Clone Rum-1 and TH-28/MS-26) and the commercial panel anti-D (D-Screen, Diagast).
Results: One donor with a novel *RHD* variant allele defined by a missense mutation (c.730G>C, p.244Ala>Pro) was identified by sequencing of *RHD* gene. *RHD*730C/RHD*01N.01* (RHD deletion) and ccEE genotypes were determined by the MLPA analysis. RBCs of this donor negatively reacted with two monoclonal IgM anti-D (Clone Rum-1 and TH-28) by the tube method, while weakly reacted (1+) with the IgG anti-D (Clone MS-26) in the Coombs gel card. Besides, for the reaction with the panel anti-D (D-Screen), only weak positive agglutination (1+) was detected with the two monoclonal IgG anti-D (P3*290 for epD5.4 and P3*249 for epD2.1) by the tube IAT method, while D+ control with a strong positive (4+) reaction and D- control with a negative reaction.
Conclusions: The missense mutation (c.730G>C) defined the novel *RHD*730C* allele caused one amino acid change (p.244Ala>Pro) located in the 8th membrane-spanning domain of RhD protein, which indicated to result in a partial D phenotype.

P-381

A NEW MUTATION IN THE PKLR GENE LEADS TO AN INSTABLE PYRUVATE KINASE ENZYME AND SEVERE HAEMOLYTIC ANAEMIA

TRL Klei, S Kia, M Veldthuis, BM Beuger, E Clifford, J Berghuis, F Ait Ichou, R van Bruggen and R van Zwieten

Sanquin, Amsterdam, The Netherlands

Background: Pyruvate kinase (PK) deficiency is one of the most common causes of hereditary non-spherocytic haemolytic anemia and is caused by mutations in the Pyruvate Kinase, Liver and RBC (*PKLR*) gene. PK deficiency disrupts red cell glycolysis which can result in a severe reduction of erythrocyte lifespan. The underlying cause of the deficiency can strongly influence clinical outcome. In the most severe cases, patients suffer from iron overload and are dependent on regular lifesaving transfusions. In this study, genotypic analysis of the *PKLR* gene, as well as protein expression of PKLR was investigated in the blood cells of a 7 year old boy from Iran suffering from life threatening haemolytic anaemia.
Aims: In this study we aimed to unravel the molecular basis for the haemolytic anaemia observed in this young boy.
Methods: The *PKLR* gene of the patient and other members of this family was sequenced. PKLR expression, activity and PKLR mRNA stability were determined. Since the patient is receiving transfusions on a regular basis, PK activity could not be assessed in erythrocytes. Therefore, reticulocytes, which are derived from the patient, were isolated and compared to control reticulocytes for PK expression by Western blot and enzymatic activity. Furthermore, we assessed expression of PKLR and pyruvate kinase muscle isozyme (PKM2), an isoenzyme of PKLR, in reticulocytes and erythroblasts derived from this patient to determine the contribution of PKM2 in glycolysis in erythrocyte precursors.
Results: A homozygous amino acid substitution (G195–>R) in the *PKLR* gene of the patient was found. Reticulocytes isolated from the patient exhibited 20% PK activity compared to controls. We found by Western blot that PKLR expression in both reticulocytes as well as erythroblasts of the patient is absent. The remaining 20% PK activity can be attributed to low levels of PKM2 expression in the reticulocytes of

the patient. PKM2 expression in both reticulocytes as well as erythroblasts was comparable to controls.
Conclusion: The absence of PKLR in both erythroblasts as well as reticulocytes of the patient, results in severely impaired erythrocyte glycolysis which causes the severe haemolytic anaemia observed in this patient. The expression of the isoenzyme PKM2 in reticulocytes and erythroblasts is likely to be responsible for the residual PK activity in these cells and may rescue these cells from apoptosis.

P-382

A NEW KEL MUTATION LEADS TO AN ALTERED EXPRESSION OF KEL2 EPITOPES

A Matteocci, A Moscetti, A Collaretti, K Castagna, L Rogai, F Pirelli and L Pierelli
A.O. S. Camillo - Forlanini, Rome, Italy

Background: Antigens belonging to the Kell system result from variations in *KEL* sequence, a gene coding for a zinc-dependent metallo-protease. *KEL* is highly polymorphic and presents 2 major alleles, *KEL*01* and *KEL*02*, resulting from a C>T substitution in exon 6 (c.578C>T) that determines the presence of Met or Thr at position 193 in KEL1 and KEL2, respectively. Several additional point mutations may cause a weakened (Kmod) or abolished (K0) expression of Kell antigens.
Aims: We describe a new *KEL* mutation that determines an altered or null expression of *KEL*02* in a Caucasian woman.
Methods: Automated serological analyses were performed with microplate methods on NEO instrument (Immucor). KEL1 was determined by the use of K1.1.21.HM.EF clone (Immucor), while KEL2 was typed through a solid phase test (Immucor). KEL1 and KEL2 antigens were also typed on Bio-Rad test tube, and KEL2 was additionally determined in Bio-Rad gel cards. DNA was extracted by using Qiagen columns and analyzed with the HEA BeadChip kit (Immucor-BioArray Solutions) and the KKD Ready Gene SSP kit (Inno-Train). The whole coding sequence of *KEL* was finally analyzed through direct bi-directional sequencing.
Results: Our tests showed an open discrepancy in determining the status of KEL2, depending on the method used: all serology tests resulted negative, while molecular tests such as the HEA BeadChip and SSP analyses typed it KEL2-positive. DNA sequencing also showed a heterozygous condition at c.578C/T, thus confirming the *KEL*01/KEL*02* typing obtained with other molecular methods, but at the same time revealed the presence of a heterozygous polymorphism (1491 + 2t>g) at intron 13 involving the splicing site of exon 13.
Conclusions: As described in a study we have recently published (Matteocci et al, Transfusion 2014), mutations similar to the one we report here are linked to Kmod or K0 phenotype. The intronic mutation 1491 + 2t>g, though, has not been reported previously and still requires further investigations to better clarify what is the mechanisms that underlies its capacity to interfere with the expression of KEL2 epitopes. Absorption/elution studies with antisera from different clones will be necessary to accurately characterize this new variant. Molecular analyses highlight a non-negligible incidence of variant antigens in the Kell system and point out the need to integrate serological tests with genotyping in order to confirm K+/k- results. As reported in the current literature, a diagnostic algorithm that includes both the phenotype and genotype approaches in blood group determination would prevent allo-immunization due to the administration of Kmod units to K+/k- recipients.

P-383

A NOVEL MUTATION 460A>G IN RHCE*02 ENCODES WEAKENED EXPRESSION OF C AND E ANTIGENS

JR Storry, AK Hellberg, E Pesjak and JA Karlsson
Clinical Immunology & Transfusion Medicine, Lund, Sweden

Background: Rh blood group antigens are carried on two similar multipass membrane proteins encoded by *RHD* and *RHCE*. *RHD* encodes D and several partial D-associated low prevalence antigens whereas *RHCE* encodes C, E, c, e as well as many other antigens of high or low prevalence. In addition, mutations/polymorphisms in the genes may affect the level of protein expression, typically demonstrated by the weak D phenotype.
Case Study and Aims: Routine Rh phenotyping on samples from a group A RhD positive 41-year-old female blood donor revealed the following results: $C+^{w}c+E+e+^{w}$. A follow-up sample was requested at the time of her next donation. At this time, her red blood cells (RBCs) typed $C-c+E+e+^{w}$. The aim of this study was to investigate the molecular basis of the *RHCE* gene and to further characterise the C and e antigens serologically.
Materials and Methods: Routine in-house allele-specific PCR of *RHD/RHCE* was performed. Sequence analysis of *RHCE* exons 1–10 was achieved by exon-specific amplification followed by sequencing (ABI BigDye v3.1, Life technologies, Carlsbad, CA, USA); interpretation was made with CodonCode Aligner (CodonCode Co., Centerville, MA, USA). A panel of 6 monoclonal anti-C and 6 anti-e from different reagent manufacturers were tested by standard serological techniques with the donor's RBCs.
Results: Allele-specific PCR revealed an apparent *RHCE*02/03* genotype, consistent with the C+c+E+e+ phenotype. Sequence analysis revealed heterozygosity for a novel mutation in exon 3, 460 A>G, which encodes a change of Arg154Gly. Tests with anti-C revealed weak reactivity only with one reagent, which contained the MS273 clone; while tests with anti-e revealed weak reactivity with one of two anti-e reagents that contained a blend of MS16/MS21/MS63 clones and a 4 + reaction with anti-e containing clone BS260. The other reagents were nonreactive.
Discussion and Conclusions: We describe a novel mutation in an *RHCE*02* allele in a Swedish blood donor that generates both quantitative and qualitative changes in the RhCE protein. Residue 154 is associated with the start of the 5th transmembrane helix and it is likely that a change from arginine to glycine could affect how the protein is inserted into the RBC membrane. The amino acid polymorphisms responsible for C and e antigens are encoded by exon 2 and exon 5, respectively and are thus not directly affected but transmembrane alterations in the similar RhD protein have been shown to cause both quantitative and qualitative changes to RhD. The strong positivity of the RBCs with anti-e clone BS267 and variable reactivity with other anti-e suggests that the e antigen expressed is a partial antigen, i.e. that it expresses some but not all epitopes of e, parallel with the partial D antigens. This raises the question of whether this donor is at risk of producing anti-e if challenged, similar to individuals of the $e+ hr^{B}-$ phenotype.

P-384

A NOVEL NUCLEOTIDE INSERTION IN THE RHCE GENE RESULTING IN SILENCING OF C EXPRESSION

C Paccapelo[1], F Truglio[1], S Scognamiglio[1], A Manazza[1], N Revelli[1], MA Villa[1], M Crotti[2], T Hutchinson[3] and M Marconi[1]
[1]Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico, Milan, Italy [2]ASST di Cremona, Immunoematologia e Medicina Trasfusionale, Cremona, Italy [3]BioArray Solution, Warren, United States of America

Background: The Rh blood group system is the most polymorphic red blood cell system. The Rh antigens are encoded by two highly homologous genes, *RHD* and *RHCE* and a number of *RHCE* alleles which are associated with silencing of Rh antigen expression have been identified. This study describes a novel insertion of one nucleotide in exon 1 of the *RHCE* gene resulting in silencing of C expression.
Aims: The aim of this study was to investigate the cause of the discordancy between the *RHCE* genotype and the RhC phenotype in a female, Caucasian blood donor of Italian origin.
Methods: Rh phenotyping was done by the microcolumn methods (BioVue System, Ortho Clinical Diagnostics, USA and ID-Cards BioRad, Switzerland) and tube methods using commercial, monoclonal reagents (Immucor, Germany and Medion Grifols, Switzerland). Genomic DNA was isolated from whole blood using the QIAamp DNA Blood Kit (QIAGEN, Germany) and the *RHCE* genotype determined using the HEA and RHCE BeadChip™ kits (Immucor-BioArray Solution, Warren NJ, USA) and the PCR-SSP kit (RH-TYPE BAGene, Germany). Since the phenotype and genotype results were discordant, the sample was further characterized by sequencing all ten *RHCE* exons using Sanger sequencing.
Results: As this donor was identified with an uncommon R_0R_0 (ccDee) phenotype, the donor was genotyped. However, both the HEA and RH-TYPE kits predicted a R_1R_0 (CcDee) phenotype, while the RHCE BeadChip™ test did not identify any altered alleles. In addition, the RBCs were non-reactive with multiple, commercial anti-C monoclonals, including MS24 and MS273. Sequence analysis of the *RHCE* gene showed that the individual was C+c+ heterozygous but also heterozygous for one nucleotide insertion at *c.93_94insT* in exon 1, resulting in a frameshift mutation and a premature stop codon at p.(Asp35Stop). The result was an aberrant *RHCE*Ce* sequence resulting in the silencing of C expression. In principle, the mutation should also have resulted in silencing of e but since the individual was e+e+ homozygous this could not be demonstrated. A homologous insertion has been previously described in the *RHD* gene in the same location, resulting in a DEL phenotype.

Conclusions: We describe a novel insertion in exon 1 of the *RHCE* gene resulted in silencing of C expression in an Italian blood donor with an uncommon R_0R_0 (ccDee) phenotype. Although DNA typing for the prediction of blood groups has great value, the genotype is not the phenotype. There are many genetic events that cause apparent discrepant results between hemagglutination and DNA typing.

P-385

BW PHENOTYPE CAUSED BY A NEW MISSENSE MUTATION IN EXON 7 – A FAMILY STUDY IN NORTHERN MORAVIA

M Písacka[1], M Králová[1], J Králová[1], M Korístka[2], Z Cermáková[2], Å Hellberg[3], A Hult[3], JR Storry[3] and ML Olsson[4]

[1]Institute of Haematology and Blood Transfusion, Prague, Czech Republic [2]Blood Centre, University Hospital, Ostrava, Czech Republic [3]Clinical Immunology and Transfusion Medicine, Office of Medical Services, Lund, Sweden [4]Div. Hematology & Transfusion Medicine, Dept of Lab. Medicine, Lund University, Lund, Sweden

Background: Weak variants of the B blood group are detected much more rarely than weak A subgroups in Europe. B subgroups are difficult to classify, and an analogy with A variants is used. The B_w phenotype has been associated with 38 different alleles to date (dbRBC). Here we report a family study showing that a novel single nucleotide mutation in an otherwise normal *ABO*B.01* allele results in weakened B antigen expression and is associated with the phenomenon of allelic enhancement.

Aim: The investigation was prompted by discrepant blood grouping results in a 55-year-old male patient whose red blood cells (RBCs) initially typed as group O but whose plasma gave only a weak reaction with B test RBCs. Initial genotyping results revealed an *ABO*B.01/O.01* genotype consistent with group B. Samples from family members were drawn under informed consent for testing.

Materials and Methods: Blood grouping was performed on gel cards (BioRad; Grifols) by manual tube agglutination tests (Exbio; MTC) and by automatic analyzer in microtitre plates (Galileo, Immucor). Genotyping was done by PCR-SSP (Innotrain; BAG) and on a microarray (BloodChip Reference, Grifols). *ABO* exons 1 to 7 were sequenced using Big Dye Terminator reagents on the ABI 3500DX (v3.1, Applied Biosystems), and analysed using CodonCode Aligner software. RBCs were tested with monoclonal anti-A, anti-B and anti-H by flow cytometry.

Results: See the table for a summary of ABO phenotype and genotype results. B antigen was detected weakly in gel cards on the niece's RBCs only. Sequence analysis showed a novel mutation in ABO exon 7, c.662G>A in the B allele from the proband, his sister and niece, predicting an amino acid change p.Gly221Asp. This residue is completely conserved among the members of the GT6 family of glycosyltransferases and centrally located in the enzyme, 7 amino acids from the DVD motif that coordinates the UDP-galactose donor substrate. Analysis of ABO exons 1–6 on the proband sample did not show any other change. Flow cytometric analysis showed very weak B antigen expression on RBCs from the proband and his sister and notably stronger expression on RBCs from the niece. While the same mutation was present in all three individuals, the niece had also inherited a normal *ABO*A1.01* allele and the increased B antigen expression is consistent with allelic enhancement. Subsequent to the family study, an unrelated person, also of Czech origin with the same mutation (genotype *B.01/O.01*) and identical phenotype was identified in our laboratories.

Summary/Conclusions: This family study revealed a new inherited mutation in the *B* gene predicting an amino acid change near the catalytic site with weakening effect of the B transferase activity. The interesting difference of more emphasized weakening effect when this allele is inherited with *O.01 in trans* compared with being paired with *A1.01* demonstrates the phenomenon of allelic enhancement. This mutation together with observed presence of anti-B in plasma could cause discrepant or in some cases even wrong ABO determination and might be underdetected by routine serological methods, as indicated by the coincidental finding in an unrelated individual.

Supported by Ministry of Health, Czech Republic - conceptual development of research organization Institute of Haematology and Blood Transfusion IHBT CZ00023736.

Sample ID	Initial *ABO* genotype	Predicted ABO phenotype	Observed ABO group	Flow cytometry
Proband	*ABO*B.01/O.01.01*	B	O weak anti-B	B_w
Mother	*ABO*O.01.01/O.01.01*	O	O	O
Sister	*ABO*B.01/O.01.01*	B	O weak anti-B	B_w
Niece	*ABO*A1.01/B.01*	AB	DG-Gel: AB_w Galileo: A weak anti-B	AB_w
Nephew	*ABO*A1.01/O.01.01*	A	A	A

P-386

NEXT GENERATION SEQUENCING (NGS) IN RED BLOOD CELL MEMBRANE DISORDERS

OJHM Verhagen[1], M Veldthuis[2], R Dee[2], M de Boer[1], K van Leeuwen[1], E van der Schoot[1] and R van Zwieten[2]

[1]Sanquin Research, Amsterdam, The Netherlands [2]Sanquin Diagnostic Services, Amsterdam, The Netherlands

Background: Red Blood cell (RBC) membrane disorders like spherocytosis, elliptocytosis, poikilocytosis and stomatocytosis are classified according to their morphological appearance. The underlying defects have been found in the genes that encode the proteins of the RBC membrane. Current moluculair diagnosis involves Sanger DNA sequencing. However, this technology is laborious and expensive, and it therefore only allows for the analysis of a limited number of genes. Instead, Next Generation Sequencing (NGS) technologies, which allow for large scale targeted sequencing of many genes at the same time, have rapidly developed. The resulting NGS workflow, reliability of the generated sequencing data, data-analysis, and cost-effectiveness have reached such a level of improvement that it is absolutely necessary to implement it into molecular diagnostics.

Aim: To develop a NGS-based diagnostic test for RBC membrane disorders.

Method: The NGS method was based on the Ion AmpliSeq Targeted Sequencing Technology from Ion Torrent (ThermoFisher Scientific). A custom-made primer (AmpliSeq-) panel was designed to cover all exons, flanking intronic regions and untranslated regions (UTR) from 10 genes involved in RBC membrane disorders: SPTA1, SPTB, ANK1, SLC4A1, EPB41, EPB42, RHAG, PIEZO1, SEC23B and UGT1A1 with the Ion AmpliSeq Designer Software. Libraries from DNA isolated from 36 patients with suspicion for RBC membrane disorders were robotically prepared and sequenced on the Ion S5 sequencer. Run statistics were performed in Torrent Suite Software and variant calling was performed in Ion Reporter in combination with resources like HGMD, ClinVar and Pubmed. An algorithm was developed to filter potential disease making mutations among the called variants.

Results: The designed AmpliSeq panel covering the 10 genes of interest results in a panel with 327 amplicons (amplicon length 68 bp-336 bp, mean 288 bp). The coverage of of the submitted sequences is 99.05% (88.9 Kbase) and include all known disease making (DM) mutations listed in Human Gene Mutation Database (HGMD) for these genes. The mean targeted mapped reads per sample is 317524 (range 152–615k). Coverage analysis shows that 320 out of 327 amplicons (98%) have a coverage of at least 20 times. In 14 patients 1 HGMD DM variant for Spherocytosis was found (11x SLC4A1 and 3x SPTB). In 3 patients a HGMD DM variant for Elliptocytosis (twice a SPTA1 variant, once a EPB41 variant) was found. In 1 patient a HGMD DM variant for Congenital Dyserythropoitic Anemia type II (CDAII) in Sec23B was present. In 1 patient a potential HGMD DM variant in SEC23B was found for Anaemia (CDAII). In 1 patient 2 HGMD DM variants in PIEZO1 were found for Spherocytosis. In 10 patients unpublished mutations were found, which have to be confirmed by proteomics and/or functional tests. In 6 patients no suspected variants were found. All 13 mutations known from previous Sanger sequencing were recognized.

Conclusion: We have developed a NGS-based diagnostic workflow to identify known disease causing mutation in 10 genes related RBC membrane abnormalities. Moreover, by this workflow we also detect novel mutations that might cause or contribute to the severity of disease.

P-387

No abstract available.

P-388

PARTIAL D AND DEL (K409K) VARIANT AMONG RHESUS NEGATIVE BLOOD DONORS AND THEIR CLINICAL IMPLICATIONS

S Ahmed[1], R Hassan[2], CY Ang[2], S Ponnusamay[2], K Muthaya[3], S Md Noor[3] and R Rejab[3]

[1]School of Medical Sciences/ Universiti Sains Malaysia, Kubang Kerian /Kota Baru, Malaysia [2]Department of Haematology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia [3]Department of Transfusion Medicine, Hospital Permaisuri Bainun, Ipoh, Perak, Malaysia

Background: In transfusion medicine laboratories, Rhesus blood group is determined by serological assays based on the detection of RhD antigen on red blood cells using specific anti-D antibody. However in some instances especially those labelled as serologically Rhesus negative may not be true negatives. As a consequence, blood transfusions of some of these Rhesus variants may cause alloimmunization.
Aims: This study was carried out to detect the rhesus variants (partial D VII and DEL (K409K) among rhesus negative blood donors.
Materials and Methods: Eighty seven (87) rhesus negative blood donors were recruited for this study. Whole blood was collected from each of the donors. The D antigen status was detected using a monoclonal anti-D preparation (Diamed, Switzerland). DNA was extracted from peripheral blood followed by polymerase chain reaction using sequence specific primers (SSCP) for genotyping using the BAGene RH-TYPE (BAG Health Care, Lich, Germany).
Results: Partial DV1 and DEL (K409K; 1227G>A) were detected at in 5.9% and 12.9%, respectively, in the blood samples of the serologically typed RhD negative blood donors. All blood donors with DEL (K409K) were genotyped as RhCcee.
Conclusions: The rhesus variants under study appear to be common in RHD negative blood. Anti-D alloimmunization could be a concern if blood carrying these variants were to be transfused to apparently Rhesus negative recipients. Hence, a Rhesus D negative with RhCcee genotype donor should be further screened for DEL variants for more effective and less eventful transfusion practice.

P-389

GENOTYPING OF SEROLOGICAL WEAK D, D-NEGATIVE AND PERSONS WITH ANTI-D IN BLOOD TRANSFUSION INSTITUTE OF SERBIA-FIRST RESULTS

S Jovanovic Srzentic[1], T Dovc Drnovsek[2], P Rozman[2], M Dukic Novakovic[1], Z Andric[1], N Jankovic Pavlovic[1], S Kosovic[1] and I Djilas[1]

[1]Blood Transfusion Institute, Belgrade, Serbia [2]Blood Transfusion Centre, Ljubljana, Slovenia

Background: Blood donors with some types of weak D and DEL may be determined as D-negative by serology and are potential risk factor for immunization of D-negative recipient. Determination of D status using molecular methods may overcome the serological limits.
Aims: To present the first results of *D gene* analysis in BTIS and the use of proper algorithm to determine D status in blood donors and pregnant women.
Methods: The criteria for the selection of blood samples for *D* genotyping were: a) blood donors with direct agglutination of ≤2 +, performed by tube method using IgM/IgG anti-D test sera (Sanguin, Immunodiagnostic, Bio-Rad, BTIS-performed 'in house') and by gel method (DiaClon ABO/Rh for Donors, monoclonal anti-D, Bio-Rad), and at the same time the agglutination of ≤3 +, using Veryfication of D ^{weak} by IAT and ID/cards (Bio-Rad); b) D-negative donors positive for C and/or E; c) the presence of anti-D in plasma of D-positive (4 +) samples. Anti-D was detected by gel method using NaCl and Liss/Coombs ID/cards by ID-DaCell IP-IIP-IIIP and ID DiaCell I-II-III screening test red cells. The antibody specificity was determined using ID-DiaPanel P and ID-DiaPanel (Bio-Rad). Eighteen samples fit to criteria a), twenty two to criteria b) and two to criteria c).
Blood of 42 investigated persons were collected in EDTA. DNA was isolated manually, using GeneJET Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific Inc) and tested by PCR-SSP method using RBC-CDE, RBC-D weak/variant and RBC-vERYfy kits (Inno-Train). PCR products were detected by fluorometric detection, using FluoVista apparatus (Inno-Train). Because the genotyping method was in the phase of the implementation into the routine diagnostics in BTIS, the first results were confirmed in Blood Transfusion Centre of Slovenia, with routinely used RBC-Ready Gene CDE and RBC-Ready Gene D weak kits (Inno-Train) and agarose gel detection.
Results: Using molecular approach, 17 (94.4%) out of 18 serologically weak D samples were confirmed as D weak types: 10 (58.8%) as weak D type 1/1.1, 3 (17.6%) as weak D type 3, 3 (17.6%) as weak D type 14 and 1 (5.8%) as weak D type 15. One (5.65) sample (5.6%) had normal *D* gene. The absence of *D* gene was confirmed in 20 (90.9%) out of 22 samples of serologically D-negative, C and/or E-positive blood donors, 1 sample (4.5%) was weak D type 11 and 1 (4.5%) sample, with C+c-E-e+ phenotype, had D variant which cannot be determined by kits we used. The same undetermined result was obtained at donor with anti-D and normal serological expression of D (4 +). A pregnant woman with anti-D and normal expression of D (4 +) was determined as DNB variant.
Conclusion: The D blood group molecular testing was successfully implemented in BTIS. The molecular testing also confirmed the chosen algorithm for detection of serologically weak D types at blood donors and point out the necessity to investigate the frequency of all *D* alleles in Serbia in order to improve D testing and to establish national transfusion strategy for donor and prenatal testing and its clinical relevance.

P-390

THE SEROLOGICAL AND GENETIC CHARACTERISTICS OF WEAK D TYPE 72 IN THE CHINESE DONORS

YL Ji[1], Z Wang[1], JZ Wen[1], QN Liang[2], L Wei[1], H Luo[1], GP Luo[1] and YS Fu[1]

[1]Guangzhou Blood Center, Guangzhou, China [2]Guangdong No.2 Provincial People's Hospital, Guangzhou, China

Background: The *RHD*weak D type 72* allele (*RHD*01W.72*) was only identified in one Chinese individual from Shanghai in 2006, lacking of the serological data and just with the accession number of Genbank (EF103573). The variant allele was defined by the specific mutation (c.1212C>A) in the exon 9 of *RHD* gene resulting in the amino acid change (p.404Asp>Glu) located in the cytoplasmic C terminal domain of RhD polypeptide. In this study, five Chinese donors carried the *RHD*weak D type 72* allele were identified in the Guangzhou Blood Center and its serological and genetic characteristics were analyzed.
Aims: To obtain the serological and genetic characteristics of weak D type 72 in the Chinese donors.
Methods: The blood samples from sixty-eight donors with D variants phenotype were collected in the Guangzhou Blood Center. The *RHD* genotypes were analyzed by the developed Multiplex Ligation-dependent Probe Amplification (MLPA) assay. For the donors with D variants phenotype but without aberrant *RHD* variant alleles identified by the MLPA analysis, the ten exons of *RHD* gene were further sequenced. The detailed serological typing for D antigen was conducted by using the panel anti-D (D-Screen, Diagest) and another seven kinds of monoclonal anti-D available (Clone LHM 169/81 for epD 6.3, IV-I-60 5C8 for epD 2, HIRO-16 for epD 3, IV-I-65 LOS1 for epD 6/7, LHM 76/58 for epD 8.1, IV-I-64 8D8 for epD 9.1, IV-I-63 LOS2).
Results: Five donors (5/68, 7.4%) carried the *RHD*weak D type 72* allele were identified among the 68 D variant donors through the MLPA analysis and the sequencing of *RHD* gene. Four of them with a *RHD*weak D type 72/ RHD*01N.01 (RHD deletion)* and Ccee genotypes and one of them with a *RHD*weak D type 72/ RHD*DVI.3* and CCeee genotypes were determined. The blood sample was available in one donor (Du-48) with the *RHD*weak D type 72/ RHD*01N.01* genotype for the detailed serological typing of D antigen. In the primary typing for D antigen, RBCs of Du-48 were weakly positive reacted with the monoclonal anti-D (Clone Rum-1 and TH-28) by the tube method, strong positive (4 +) with the anti-D (Clone MS-26) in the Coombs gel card. In addition, RBCs of Du-48 reacted with all nine monoclonal anti-D of D-Screen with the agglutination strength of 1 + ~2 + and also positively reacted with other seven kinds of monoclonal anti-D with the agglutination strength of w+~1 + by the tube method for IgM anti-D and the tube IAT method for IgG anti-D, while D+ control with strong positive (4 +) reaction and D-control with negative reaction.
Conclusions: Compared with the rare distribution of weak D type 72, it is more common in the D variant individuals from Guangzhou located in the southern China. Whether the D epitopes of weak D type 72 is complete on the surface of RBCs and the recipient could not produce anti-D are still needed to investigate in the future.

P-391

ANTI D ALLOIMMUNIZATION IN RHD+ SICKLE CELL DISEASE PATIENT

Z Al fana Alarimi, R Almuhaidri, H Al lawati and M Al Belushi

Department of Blood Banks Services, Muscat, Oman

Background: Alloimmunization is of particular concern in patients with Sickle Cell Disease (SCD) patients receiving regular blood transfusion. The main problem with alloimmunization is the risk of delayed haemolytic transfusion reaction (DHTR). Many studies have demonstrated the effectiveness of phenotypically matched Red Blood Cell (RBC) transfusion in reducing the rate of alloimmunization in SCD patients. Tahhan and coworkers, showed that none of the 40 SCD patients who received antigen matched transfusion for the Rh, K, S, Fy(a), and Fy(b) developed antibodies while 34.8% of the 46 patients who received antigen matched and non-antigen matched transfusions developed significant alloantibodies. However, Recent studies demonstrated that the risk of RBC alloimmunization still occur despite giving Rh phenotypically matched RBC which was attributed to the inheritance of variant RHD and RHCE genes.

Aims: We report a case of SCD who was found to have anti D despite being serologically strong RhD+. Full sequencing of the RhD for the patient revealed that the patient harbour a partial D allele DAU-3 which harbour two mutations V279 & T379M.

Method: A 28-year-old women with SCD admitted with history of multiple transfusion and is known to have multiple antibodies with anti E, anti C and anti Kell antibodies. She was admitted with SCD crises and required blood transfusion. The blood bank investigations showed that the blood group is O Rh D positive and confirmed the presence of the already known antibodies with anti C, anti E and Anti Kell specificities. However, there are additional reactions observed suspicious of the presence of anti D detected by enzyme method only. The Direct Anti globulin Test (DAT) is negative.

Given the above findings, the transfusion recommendation was to give antigen negative for D, C, E and Kell and cross match compatible.

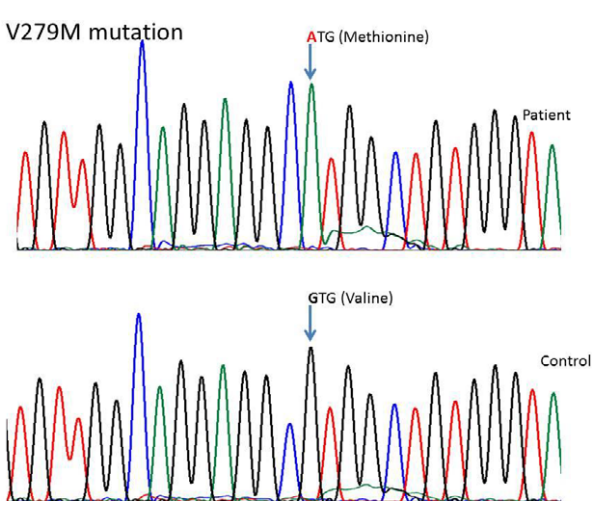
Six months later, the patient sample was send again as she required transfusion and the antibody identification test confirmed the presence of anti D in addition to the already identified antibodies with anti C, anti E and anti Kell specificities.

The anti D used for the Rh D typing demonstrated that the patient 's sample reacted strongly (3+– 4+) Using Ortho Clinical Diagnostics. The previous patient's D typing demonstrated the same findings using different Rh D typing reagents.

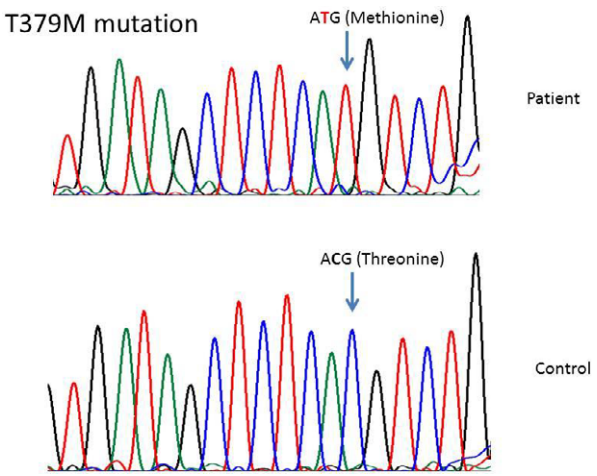
Given the patient's findings, genotyping was performed to explain the paradoxical findings of anti D in serologically normal Rh D positive patient.

Result: Analysis of RHD gene and Sequencing of the *RHD* exons revealed that the patient *harbor DAU-3 RHD allele* (V279M,T379M) which have been described in African ethnic origin.

Summary/Conclusion: Anti-D immunization in patients with SCD harboring partial D alleles is a problem despite giving phenotypically matched RBC transfusion as current serological D typing may not detect some partial D variants harbouring some of the DAU alleles. Molecular testing should be considered for patients with SCD on chronic transfusion who develop anti D even when the Rh D typing reacts strongly with the D Typing reagents as some partial Rh D variant may give normal reaction with Rh D typing reagents.



Caption 1. Rh D mutation 1



Caption 2 Rh D mutation 2

P-392

MOLECULAR RHD SCREENING FOR DETECTION OF DEL PHENOTYPE IN D-NEGATIVE KOREAN BLOOD DONORS

SA Lee[1], TE Kim[1], LS Shin[1], AH Lim[1],CH Jung[1], YJ Park[1], S Chun[2], SY Cha[2], D Cho[2], DJ Oh[1] and SJ Park[1]

[1]Korean Red Cross, Seoul, South-Korea [2]Samsung Medical Center, Seoul, South-Korea

Background: Serological RhD blood group tests have limitation for the detection of variants. In Korea, cases of anti-D immunization by transfusion of D-negative red blood cells (RBCs), which were eventually confirmed Asia type DEL variants, have been reported.

Aims: We present our experience to set a practical *RHD* genotyping algorithm and supply the D-negative blood based on the RHD genotype results.

Methods: Phase I study: From December 2014 to September 2015, a total of 240 serologically confirmed D-negative club members were tested using polymerase chain reaction sequence-specific primers (PCR-SSP) for *RHD* promoter, exon 4, exon 7, and exon 10. Samples with no detectable alleles were interpreted as total *RHD* deletion, and those with only promoter and exon 10 were interpreted as *RHD-CE-D* hybrid. Samples with the complete *RHD* allele were further analyzed by sequencing of exon 9 for c.1227G>A and c.1222T>A variations. Full exon sequencing was performed for undetermined samples.

Phase II study: D-negative blood donors who agreed to participate in this study at six blood centers accounts for about 65% of D-negative blood donations were tested by *RHD* genotyping algorithm from December 2015 to February 2016. If participants donated blood that contains RBC products, the issue of RBC units were hold until the genotyping results were confirmed to prevent to transfuse the DEL RBCs to apparent D-negative patients. We monitored the demand and supply of D-negative RBCs.

Results: In phase I study, of 240 D-negative Korean individuals, 71.3% completely lacked *RHD*, 10.0% were *RHD-CE-D* hybrid, and 17.8% had c.1227G>A mutations, which comparable to previously reported Korean frequencies. In phase II study, 231 blood donors were enrolled this study, 70.1% of them donated RBCs. Of 231 D-negative blood donors, 70.1% had *RHD* total deletion, 6.5% had *RHD-CE-D* hybrid, 22.5% had c.1227G>A, and 0.4% had c.1222T>C. One case with unknown variation (c.1154–1G>C) was detected. Mean turnaround time was 6.5 days. Only one RBC unit with Rh C negativity was distributed before confirming the genotyping result because of emergent need, which were ultimately confirmed as apparent D-negative.

Summary/Conclusion: Molecular *RHD* screening provides safer RBC units to prevent anti-D alloimmunization. A practical *RHD* genotyping strategy is applicable to D-negative Korean donors.

P-393

PERFORMANCE EVALUATION OF A GENOTYPING ASSAY FOR THE DETECTION OF HIGH-PREVALENCE RHD NEGATIVE AND WEAK D TYPES IN A COHORT OF RHD VARIANTS SAMPLES

A Molano[1], M López[1], D Tejedor[1], K Billingsley[2], M Kalvelage[2] and F Puente[3]

[1]Progenika Biopharma, A Grifols Company, Derio, Spain [2]Scientific Support Services, LifeShare Blood Centers, Shreveport, United States of America [3]Servicio Inmunohematologia Banco de Sangre y Tejidos de Aragón, Zaragoza, Spain

Background: It is well established that the higher-prevalence RHD weak D 1, 2 and 3 phenotypes are not at risk for forming allo-anti-D, whereas a few weak D and all partial D and negative phenotypes are. Most importantly, routine serologic D typing does not distinguish among weak D subtypes or partial D phenotypes, and consequently *RHD* genotyping is recommended, especially in patients ("Flegel, Pathol Lab Med, 2014", "Sandler, Transfusion, 2015", "Haspel, Transfusion, 2015").

Aims: The objective of this study was to evaluate the performance of ID RHD XT genotyping assay (Progenika, Grifols) in the detection of high-prevalence D negative and weak D types by challenging it with an extensive number of *RHD* gene variants.

Methods: A cohort of 160 samples harboring 72 different *RHD* genotypes (59 allelic variants) associated with a Weak D, Partial D or D negative phenotype were selected for the evaluation. The samples were obtained from the Service and R&D labs of Progenika Biopharma.The ID RHD XT assay uses Luminex® xMAP technology for the simultaneous interrogation of *RHD*weak D types 1*, *2*, and *3*, *RHD deletion*, *RHD*Pseudogene* and *RHD*DIIIa-CE(3–7)-D* alleles. Genotypes and predicted phenotypes are reported from the combination of these allelic variants. Results obtained with ID RHD XT automated software were compared with results from three test sites ([1]Progenika Biopharma, Derio, Spain. [2]Progenika Inc., Medford, MA, USA. [3]LifeShare Blood Centers, Shreveport, LA, USA) that used molecular reference methods: in-house SSP-PCR, BAGene Weak D-TYPE kit (BAG Healthcare), RHD kit (GTI), RHD BeadChip kit (BioArray Solutions), BLOODchip Reference (Progenika, Grifols), and bi-directional Sanger sequencing (Progenika Service, Grifols).

Results: All samples with Weak D types 1, 2 and 3 and all with D negative variants: (*RHD deletion*, *RHD*Pseudogene* and *RHD*DIIIa-CE(3–7)-D)* were correctly genotyped by ID RHD XT among the 72 different *RHD* variants tested. The rest of variants were detected by ID RHD XT as: "No weak D types 1, 2 or 3"; "No amplification variant", in *RHD-CE* rearrangements; D- in the cases of *RHD(1)-CE(2–10)* and *RHCE*ceHAR*. *RHCE*ceHAR* variant is an *ID RHD* XT limitation for donor typing, as the associated predicted phenotype is *Partial D*. In addition, 3 samples gave "No call"result: two due to RHD*1157A nucleotidic change, due to its proximity to RHD*1154C_(*Weak D type 2)*, and *RHD*807A* due to its location at the same position as RHD*807G_(*RHD*Pseudogene)*. All the *Weak D types 1, 2* and *3* phenotyped samples are correctly detected by ID RHD XT and can be considered as RhD positives for transfusion. The rest of the RHD variants tested encode Weak D, Partial D or D negative phenotypes, for which transfusion of D negative blood is recommended.

Summary/Conclusions: ID RHD XT is an accurate test for the detection of high-prevalence weak D and D negative alleles. That makes it a useful tool for the implementation of the recent recommendations by the Work Group for RHD Genotyping on blood transfusion and anti-D prophylaxis.

P-394

PREVALENCE OF RHD ALLELES IN JAPANESE INDIVIDUALS WITH WEAK D PHENOTYPE

K Ogasawara[1], K Isa[1], K Sasaki[1], M Saito[2], H Tsuneyama[2], R Yabe[2], M Uchikawa[2], T Nagai[1] and M Satake[1]

[1]Japanese Red Cross Central Blood Institute, Koto-ku, Japan [2]Japanese Red Cross Kanto-Koshinetsu Block Blood Center, Tokyo, Japan

Background: The molecular basis of the weak D phenotype has been investigated for many years, and more than 80 different *RHD* alleles producing weak D phenotypes have been identified. Most alleles producing weak D phenotypes have a single missense mutation in exons corresponding to a transmembrane domain of the RhD polypeptide. In addition, some *RHD* variant alleles have a silent mutation that causes an exon skipping of the *RHD* transcript resulting in weak D expression.

Aims: We report here *RHD* alleles in Japanese accounting for weak expression of D antigen.

Methods: D typing was performed using an automated blood grouping system (PK7300) and anti-D reagent (Wako, clones TH28/MS26). We selected the samples for *RHD* gene analysis when hemagglutination strength was 3 + or less using the reagent of monoclonal-polyclonal blend anti-D (Ortho, clone MAD2). Five monoclonal anti-Ds (HIRO-2, -3, -4, -5 and -55) were used to rule out the partial D phenotypes. A total of 226 distinct weak D samples were collected from Japanese blood donors between 2009 and 2014. None of the weak D individuals had alloanti-D. Genomic DNA was extracted from whole blood, and exons 1 to 10 gene and *RHD* genotype were analyzed by PCR and sequencing. Real-time PCR was performed to estimate the relative amounts of the *RHD* transcripts using reticulocyte mRNA.

Results: From our laboratory tests in the year 2010, a weak D phenotype was observed in 75 individuals among 763,408 blood donors and the prevalence of weak D phenotype was calculated to be 0.01%. When the *RHD* gene of the 226 weak D samples collected over several years were analyzed, we identified 46 different *RHD* variant alleles. Among these alleles, the *RHD* alleles with c.960G>A silent mutation in exon 7, c.845G>A (*RHD*15*), or c.1013T>C (*RHD*01W.24*) were major alleles with relative occurrences of 36.7%, 15.9% and 9.7%, respectively. In order to examine the presence or absence of all coding exon of *RHD*, we first analyzed the mRNA transcript of the *RHD* gene from an individual with the c.960G>A silent mutation by cloning. Sequence analysis revealed that only three clones had the complete *RHD* transcript with c.960A, while 43 clones had an incomplete *RHD* lacking exon 7 or exon 7 and 8. Semi-quantitative analysis of the *RHD* transcripts by real-time PCR revealed that the cDNA samples with the c.960G>A mutation showed a significant increment of exon 7 skipping compared with the common *RHD*.

Conclusions: Reduced expression of D antigen is caused not only by missense mutation of the *RHD* gene, but also by silent mutation that may affect splicing. Although the weak D phenotype is rare in Japanese, *RHD* alleles accounting for the phenotype are significantly polymorphic. It remains unclear whether the majority of the weak D individuals in the present study may have a risk of forming alloanti-D after transfusion.

P-395

CASE STUDIES OF DISCREPANT RED CELL GENOTYPING VS SEROLOGICAL PHENOTYPING RESULTS AT THE IMMUNOHAEMATOLOGY REFERENCE LABORATORY, SOUTH AFRICAN NATIONAL BLOOD SERVICES (SANBS)

A Sookraj, L Govender and K Vather

South African National Blood Services, Durban, South Africa

Background: The Immunohaematology Reference Laboratory of the South African National Blood Services (SANBS) has been using manual haemagglutination techniques as well as the enhanced column agglutination method to resolve complex antibody cases. In January 2015 SANBS validated and implemented the ID CORE^{XT} assay for red cell genotyping. Red cell genotyping has now become an additional tool in the battery of specialized tests utilised for the resolution of complex serological cases, genotyping multiply transfused patients and assisting in selecting appropriate blood for patients. The sources of assays for genotyping ranged from mass array assays to bloodchip assays and Luminex bead assays. The Luminex bead assay was selected for validation and implementation due to Luminex instruments already being utilised at SANBS for other applications.

Aims: To describe two cases where the genotyping results obtained were discrepant to the manual serological phenotyping results.

Methods: The IDCORE^{XT} kit and BIDSXT software were validated and implemented in January 2015. The ID CORE^{XT} kit is a qualitative kit that covers the following 10 Blood Group Systems: Rh, Kell, Kidd, Duffy, MNS, Diego, Dombrock, Colton, Cartwright and Lutheran comprising of 37 red cell antigens in one test (Grifols/Progenika IDCORE^{XT}Conventional serology using rare antisera and cells. Phenotype confirmed using the enhanced column agglutination technique and poyclonal and monoclonal reagents.

Results: SANBS Rare Donor 1 has been serologically repeatedly confirmed using polyclonal reagents as hr^s antigen negative and has been crossmatch compatible to various patients with confirmed anti-hr^s. Genotyping results has typed this donor as predicted phenotype hr^s antigen positive. The ID CORE^{XT} kit covers RH:19 but does not cover RH:18. Rare Donor 1 has also been serologically confirmed using monoclonal reagents and the column agglutination technique as Fy^a antigen negative and Fy^b antigen negative (Fy-3) and genotyping results has typed this donor as predicted phenotype Fy^a antigen positive and Fy^b antigen negative. SANBS Rare Donor 2 has been serologically repeatedly confirmed using monoclonal reagents as Rh:34 antigen negative and has been crossmatch compatible to various patients with confirmed anti-Rh34. Genotyping results has typed this donor as predicted phenotype Rh:34 antigen positive. According to the literature Rh:31 negative donors are also RH:34 negative.

Table 1: SOR 2034_15 Genotype Report Results
Table 2: SOR 2034_15 Genotype: Predicted Phenotype Report Results
Table 3: SOR 2034_15 Manual Serological Phenotype Report Results

Antigen	Rh	Duffy	MNS	
Genotype Report	RHCE*ce,RHCE*ce[733G,1006T]	FY*A,FY*B_GATA	GYPA*M	GYPB*s

Antigen	C	E	c	e	hrS	hrB	Fya	Fyb	M	N	S	s	U
	RH:2	RH:3	RH:4	RH:5	RH:19	RH:31	FY:1	FY:2	MNS:1	MNS:2	MNS:3	MNS:4	MNS:5
Phenotype Report	0	0	+	+	+	+	+	0	+	0	0	+	+

Antigen	C	E	c	e	hr*	hrB	Fy^a	Fy^b	M	N	S	s	U
Serologic Report	0	0	4	4	4	4	4	0	4	0	0	4	4

Caption 1: Rare Donor 1: Known Rh:34 Antigen negative

Table 1: SOR 0755_15 Genotype Report Results
Table 2: SOR 0755_15 Genotype: Predicted Phenotype Report Results
Table 3: SOR 0755_15 Manual Serological Phenotype Report Results

Antigen	Rh	Duffy	MNS	
Genotype Report	RHCE*cE,RHCE*ce[712G]	FY*A,FY*B_GATA	GYPA*M	GYPB*S

Antigen	C	E	c	e	hrS	hrB	Fya	Fyb	M	N	S	s	U
	RH:2	RH:3	RH:4	RH:5	RH:19	RH:31	FY:1	FY:2	MNS:1	MNS:2	MNS:3	MNS:4	MNS:5
Phenotype Report	0	+	+	+	+	+	+	0	+	0	+	0	+

Antigen	C	E	c	e	hr*	hrB	Fy^a	Fy^b	M	N	S	s	U
Serologic Report	0	4	4	4	0	4	0	0	4	4	0	4	4

Caption 2: Rare Donor 2: Known hrs Antigen negative

Conclusion: All anomalies have been raised as technical queries with the manufacturer of the red cell genotyping kit for further investigation such as sequencing of the specific allele to determine if there is a difference to the consensus sequence that is causing the false positive genotype. Further data has also been requested from the manufacturer of overlaps with other genotypes that could cause false positive results. Immunohaematology Reference Laboratory is interrogating all its in-house specialized serology reagents and commercial anti-sera to ensure sufficient coverage of the rare antigens. The anomalous results does highlight the need to confirm initial genotype results by serology methods and shows that test kits geared toward Caucasian genotypes/phenotypes may not be 100% suitable for African countries. Although genotyped blood typing does provide a valuable alternative it does not replace traditional methods of pre-transfusion testing.

P-396

GENOTYPING RHD VARIATIONS IN TURKISH POPULATION AND INVESTIGATE THE CORRELATION OF MOLECULAR RESULTS WITH SEROLOGIC IDENTIFICATION

LT Kumas, SH Bal, Y Heper, F Budak, HB Oral and G Göral
Uludag University Medical Faculty, Bursa, Turkey

Background: "D"antigen of the Rh system is one of the most important antigens because of its high immunogenicity. Some mutations of the *RHD* gene cause D variant phenotypes. To date more than 200 *RHD* alleles are defined by mutations that lead to qualitative and/or quantitative changes in serologic expression of the D antigen. And the frequencies of these variant D types vary among populations. D variants are categorised mainly in two groups:
Weak D: A serologic weak D phenotype is defined as reactivity of RBCs with an anti-D reagent giving no or weak reactivity in initial testing, but agglutinating moderately or strongly with antihuman globulin. Weak D phenotypes are associated with decreased antigen expression on the RBC surface. Transfusion recipients with weak D phenotype are not at risk for forming alloanti-D when exposed to RhD-positive RBCs.
Partial D: Partial D phenotypes are associated with amino acid substitutions in the RhD protein on the RBC surface and lack D epitopes. There are many reports of partial D phenotype patients who formed anti-D.
It is important to protect RhD-negative persons from inadvertent alloimmunization to the D antigen by exposure to RhD-positive red blood cells, including RBCs expressing a D variant phenotype. For donor purposes a D variant blood component can be considered as RhD positive. But transfusion recipients / antenatal patients should be treated as RhD negative when they are typed as D variant. Also to make a reliable discrimination between weak / partial D is important to reduce unnecessary administration of RhIG in women with a serologic weak D phenotype, and decrease unnecessary transfusion of RhD-negative RBCs to recipients with a serologic weak D phenotype. Molecular typing is the reference method for specific typing of weak and partial D. There are also commercially marketed panels of monoclonal reagents that are supposed to differentiate a number of D variant types.
Aims: The objective of this study was to estimate the frequency of weak D / partial D types among the Turkish population and to determine the effectiveness of serologic method compared with molecular analysis for D variant typing.
Methods: The study was conducted among blood donors admitted to Uludag University Blood Bank between 2012 and 2014. Microcolumn gel method (Bio-Rad Laboratories 1785Cressier FR Switzerland) was used for blood group testing. Anti-D (DVI-) reagent was used for RhD typing and weak D test was performed for RhD-negative samples. 46 samples are cofirmed as D variant. A panel of 12 different monoclonal antibodies (Extended Partial RhD Typing Set, Bio-Rad Laboratories 1785Cressier FR Switzerland) was used for serologic typing of D variants. PCR-SSP (inno-train Diagnostik GmbH Kronberg/Taunus Germany) was performed for *RHD* genotyping of the samples.
Results: Test results obtained with serologic and molecular methods are shown in Figure 1.
Summary/Conclusions: This is the first study for molecular typing of RHD variants in Turkey. Two of the samples was determined as Rh-positive and one Rh-negative with genotyping. 11 different D variant types were identified among the 43 samples. Weak D type 1 was determined as the most frequent RhD variation; 19 (%44,18), weak D type 15; 10 (%23,26) and weak D type 11; 4 (%9,3). A correlation analysis with Fisher-Freeman-Halton Test showed that when compared with molecular analysis, serologic method was not found to be effective for D variant typing ($P < 0,001$).

	Serology	Molecular
Rh (-)		1
Rh (+)		2
Weak D (Type 1)		19
Weak D (Type 3)		1
Weak D (Type 4.2)		1
Weak D (Type 11)		4
Weak D (Type 15)		10
D III	17	
D cat IIIc		1
D V		1
D VI	1	1
D VII	1	1
DAU (0, 1, 2, 3)		1
DAR-E	2	
DAR		1
DHAR	1	
DFR	14	
Unidentified	10	2

Caption 1 RhD variations determined by serology and molecular methods

P-397

CHANGES IN ERYTHROCYTE ANTIGEN IN REPUBLIC OF KOREA; BECOMING MULTICULTURAL SOCIETY

KH Shin, HH Kim and HJ Lee

Pusan National University Hospital, Busan, South-Korea

Background: It is important to check the blood group antigens of blood donors and recipients for ensure safe blood transfusion. Recently, the number of multicultural family have been increased in republic of korea, multicultural families will be blood donor or recipient. Therefore, the survey for red blood cell antigen, antibody for foreigners and multicultural families in republic of korea is need to establish national blood policy of upcoming multicultural era. In addition, children from multicultural families have the possibility of changes in the erythrocyte antigen of their parents.
Aims: We performed genotyping and phenotyping of the red blood cell antigens in Republic of Korea youth and find of changes in erythrocyte antigen between this study and previous studies in Korean.
Methods: We recruited young people under the age of 25. The participants were divided 2 groups, single-cultural youth group and multicultural youth group. The subjects were performed genotyping and phenotyping of erythrocyte antigens. DNA was extracted from whole blood in EDTA with Qiagen methodologies. ID CORE ^{XT} (Progenika Biopharma, S.A, Spain), which based on Luminex(®) xMAP technology was used for genotyping of 37 red blood cell antigens. Phenotyping including the ABO, Rh, Kell, Kidd, Duffy, MNS, Diego blood type was performed using the test kit for phenotyping (Diagast, France).
Results: Total 214 subjects (84 multicultural youth group, 130 single-cultural youth group) were recruited from September, 2015 to February, 2016. The frequencies of blood group antigens in single-cultural youth group were similar results with the previous studies in Korean. Blood groups with significant difference between multicultural youth group and single-cultural youth group were Kidd (Jka), MNS (Mia) (P value<0.05). Frequency of type B in the ABO blood group is higher in multicultural youth group, type A is higher in single-cultural youth group. The frequency of DCe expression in Rh blood group was higher in multicultural youth group. Mi(a) and Yt (b), which were not expressed in single-cultural youth group, were identified 8.3% and 4.2% in multicultural youth group, respectively. Fy(a-b+) and S+s- were confirmed in multicultural youth group, which were rare blood type in Korean.
Summary/Conclusion: The difference in frequency of blood group antigens between multicultural youth group in this study and previous studies in korean and single-cultural youth group have been identified. These results suggest that national blood policy reflect increasing number of multicultural family and changing in the population and society in Republic of Korea should be prepared. Therefore, further research to predict of problem in blood transfusion safety is necessary. And we need to review of racial presentation of blood donor and blood drive of multicultural family.

P-398

This abstract has been withdrawn.

P-399

INTRODUCTION OF A SOUTH AFRICAN RARE DONOR DATABASE DESCRIBING THE FREQUENCIES OF RARE ALLELES BY RED CELL GENOTYPING AT THE SOUTH AFRICAN NATIONAL BLOOD SERVICES (SANBS)

L Govender, K Vather, U Jentsch and A Sookraj

South African National Blood Services, Durban, South Africa

Background: The South African National Blood Services (SANBS) implemented red cell genotyping to accurately screen and identify rare blood types and determine the frequency of rare red cell antigens in South Africa. At SANBS the serological screening methods for rare donors are restricted due to limited in-house reagents, lack of available rare antisera and expensive commercial antisera. Red cell genotyping based on molecular methods overcomes these limitations by identifying the genes coding for the rare red cell antigens. In January 2015, the Grifols/Progenika IDCORE^{XT} kit covering 37 red cell antigens from 10 blood group systems was implemented for red cell genotyping of donors deemed 'rare' by serology. Genotyping of rare donors is an international requirement as South Africa is part of the international rare donor programme and as such must provide rare blood upon request.

Table 1 – Rare Allele Genotypes and Inferred Phenotypes in South Africa

Rare Allele Genotypes		
Ψ Genotype	*Phenotype	Frequency
RHCE*ceAR, RHCE*ce[712G]	hrS-	0.02% (2/100)
RHCE*ce[733G,1006T], RHD*r's-RHCE*ce[733G,1006T](30)	hrB-	0.03% (3/100)
KEL*K_KPB_JSB	k-	0.02% (2/100)
KEL*k_KPB_JSA	Js(b-)	0.04% (4/100)
GYPB*S_null(IVS5+5t)	Uvariant	0.02% (2/100)
GYPB*deletion	S-s-U-	0.02% (2/100)
YT*B	Yt(a-)	0.02% (2/100)
FY*B_*GATA *GATA mutation in gene	Fy(a-b-)	0.33% (*33/100)

Note:
Ψ Genotyping by the IDCORE^{XT} assay
* Inferred phenotype from the IDCORE^{XT} assay

Accurate screening of these units by genotyping is necessary to find compatible units of blood thus preventing transfusion reactions due to possible alloimmunization. Once blood donors are identified as rare by genotyping, the genotypes and inferred phenotype results are added to the recently established novel South African rare donor database. **Aims:** To introduce a novel South African rare donor red cell genotyping database and determine the frequency of rare alleles amongst the rare donors in South Africa.
Methods: This is a descriptive study based on the results obtained by the IDCORE^{XT} assay genotyping of 100 samples deemed 'rare' by serology. The genotypes and inferred phenotypes were entered into a laboratory information system and used to create the rare donor red cell genotyping database and subsequently determine the frequency of rare alleles. The database is limited to the rare blood types covered by the IDCORE^{XT} kit.
Results: The rare donor database was established and the frequencies of rare alleles as per the genotypes and inferred phenotypes are summarized in Table 1 - Rare Allele Genotypes and Inferred Phenotypes in South Africa. Based on a comparative analysis against global antigen frequencies and within the limitations of the IDCORE^{XT} kit, six genotypes corresponding to high frequency antigens k-, Js(b-), Uvariant, U-, Yt(a-), Fy(a-b-) was found. In addition two other negative for high frequency antigens hrS- and hrB- was identified and is common in South Africa and not globally. The FY*B_GATA genotype was found in 33 of 100 rare donors. This mutation seems to be a common phenomenon amongst the South African rare donor population. It was also noted that several donors had more than one high frequency antigen.
Summary/Conclusions: A novel rare donor red cell genotyping database for South Africa was established. The allele frequencies will be compared to frequency data from around the world as rare blood types are frequently population specific making sourcing of blood units simpler. This database will be expanded as more donors are identified and the database will no longer be restricted to rare donors but may include routine blood donors. This will be in line with the mission of SANBS to provide all patients with sufficient safe, quality blood products and medical services related to blood transfusion.

P-400

SETTING UP BLOOD GROUP GENOTYPING IN A BLOOD CENTRE IN RABAT

A Benahadi, R Alami, S Achargui, A Abouyoub, K Hajjout and M Benajiba

Centre National de Transfusion Sanguine, Rabat, Morocco

Background: Genotyping a patient's blood group is crucial for a safe transfusion. To date, it is mainly performed since the serological methods available are not always reliable.
Aims: The aim of this study was to improve the immunological safety of transfusion. For the first time in a Rabat blood Centre, genotyping was performed by molecular biology methods for *KEL (K1/K2), JK (JKA/JKB) and FY/GATA (FYA, FYB, FY null)* alleles.

Genotype	Phenotype					Number of Successful genotyping	Number of subjects studied
KEL allele	K+k+	K-k+	K+k-	UD		31	36
K1K1	0	0	2	1			
K1K2	7	0	0	0			
K2K2	0	21	0	0			
JK allele	Jk(a+b-)	Jk(a+b+)	Jk(a-b+)	UD		22	30
JKA/JKA	9	0	0	0			
JKA/JKB	0	7	0	1			
JKB/JKB	0	0	4	1			
FY allele	Fy(a+b-)	Fy(a+b+)	Fy(a-b+)	Fy(a-b-)	UD	9	9
FYA/FYA GATA(W/W)	1	0	0	0	0		
FYA/FYB GATA(W/M)	1	0	0	0	0		
FYA/FYB GATA(W/W)	0	2	0	0	1		
FYB/FYB GATA(W/W)	0	0	2	0	0		
FYB/FYB GATA(M/M)	0	0	0	2	0		

UD=undetermined

Table 1. Genotyping results for KEL, JK and FY alleles

Methods: The protocol used was adjusted from Rios, Immunohematology, 1999, Reid, Transfusion, 2000 and Castilho, Hematology, 2000. We used polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) assay to genotype 75 subjects (67 healthy blood donors and 8 patients with β-thalassemia). This technique was selected since it requires simple, inexpensive equipment which is easy to set up in a laboratory with limited resources. The samples were phenotyped by the haemagglutination assay. For 4 patients, however, the phenotype could not be determined and was difficult to interpret.
Results: We observed 100% correlation between phenotype and genotype results for 58 samples. Also 4 patients, without phenotype, were predicted the genotype. However 13 cases didn't succeed. Probably the quality of DNA was involved.
Conclusion: In the Rabat Blood Centre, Blood group genotyping is important for transfusion safety, especially for patients with transfusion-dependent β-thalassemia. The PCR-RFLP is a reliable and easy technique introduced in a blood establishment to improve blood safety.

P-401

METHOD COMPARISON STUDY BETWEEN PHENOTYPING AND GENOTYPING FOR THE DEL DETECTION

MJ KIM

Seoul National University Bundang Hospital, Seongnam, South-Korea

Background: DEL is very difficult to detect because it has extremely low amount of RhD antigens. DEL can be only detected by adsorption elution or RHD genotyping. When RhD negative recipient receives DEL red cell, there is a risk of forming anti-RhD allo-antibodies. Therefore differentiating between DEL and D negative is clinically significant.
Aims: In this study, we want to evaluate result of phenotyping and genotyping of DEL.
Methods: Total of 538 RhD negative recipient samples were tested for RhD phenotype and genotype. Phenotype were determined by an adsorption elution. RHD genotype were determined by three real time PCRs method. The first assay targeted 1227G>A (exon 9), the second assay targeted exon 10 3'(UTR), and the third targeted the β-globin gene.
Results: Among total of 538 RhD negative recipient samples, 117 cases (21.7%) were determined as DEL by phenotyping. RHD genotyping revealed DEL (79 cases, 14.7%), hybrid (36 cases, 6.7%) and deletion (423 cases, 78.6%). Phenotype and genotype result was identical in the 74 cases (63.2%). This study delivers the fact that cases with the phenotype DEL included hybrid (8 cases, 1.5%) or deletion (35 cases, 6.5%) of genotype.
Summary/Conclusions: Our result shows that the number of DEL detected by phenotyping is greater than that by genotyping. Phenotyping takes total 130 min of complex serology procedure including adsorption incubation, multiple cycle of washing step, elution, and column agglutination. However, RHD genotyping takes only 80 min including DNA extraction and assay. Also we learned that phenotyping may produce higher number of false positive result by human manipulation due to complicated serology procedure. RHD genotyping is not only delivers higher accuracy but also reduces turnaround time (TAT) for DEL detection. Accurate identification of DEL donor is crucial to prevent forming anti-D allo-antibodies in true Rh negative recipient population by DEL red cells transfusion. Result of our method comparison study for DEL detection is suggesting that RHD genotyping is right approach for DEL detection for hospital blood bank and blood donor center to ensure the transfusion of safe blood to patients.

P-402

BIOARRAY™ HEA BEADCHIP™ DNA TYPING KIT THE INTRODUCTION OF METHOD AND COMPARATIVE STUDY WITH SEROLOGICAL PHENOTYPING METHODS

M Bohonek[1], L Landova[1], J Kralova[2] and M Pisacka[2]

[1]Central Military Hostpital - Military University Hospital Prague, Prague, Czech Republic [2]Institute of Hematology and Blood Transfusion, Prague, Czech Republic

Background: Creation National Bank of rare RBCs in the Central Military Hospital-University resulted in the necessity to implement the effective testing of a wide range of RBCs antigens. Molecular biological methods, based on rapid PCR testing are an alternative to traditional serological phenotypic methods, are labour intensive and use expensive rare antigens. Although these methods require a certain initial investment, self-examination and evaluation, implementation into routine use is straightforward and less expensive than serological tests.
Aims: Comparative studies to demonstrate the comparability of serological methods with blood group genotyping kit characters, using BioArray[TRADEMARK] HEA BeadChip [TRADEMARK] which aims to establish the routine investigation of a wide range of blood group systems in blood donors based on a parallel examination of genotypes and phenotypes.
Methods: The study included 38 normal blood donors and 20 donors with known allelic combinations of rare blood group characteristics. All donors were serologically typed manually (test tube or column agglutination) to these group features Kell, MNSS, Jkab, Fyab, Luab, P1 or by the automatic analyzer Galileo (ImmucorGamma, Inc. Norcross, GA) on signs Kpab, Cc, ee, Cw, D. PCR methods using a human genomic DNA (BioArray [TRADEMARK] HEA BeadChip [TRADEMARK], ImmucorGamma, Inc.Norcross, GA), were determined by these allelic variants blood goups systems of human erythrocytes: Rh (C, c, e, e, V VS), Kell (K, k, Kpab, Jsab), Duffy (Fyab, GATA, Fyx), Kidd (Jkab), MNS (M, N, s, U, boil), Lutheran (Luab) Dombrock (Doab, Hy, Jo), Landsteiner-Wiener (LWab), Diego (Diab), Colton (Coab) Scianna (Sc1, Sc2). Genomic DNA was isolated from donor whole blood using isolation kit QIAGEN spin column kit QIAcube (Qiagen, Germany).
Results: The results demonstrated a high concordance between the test using HEA BeadChip [TRADEMARK] and serological testing in the analyzer Galileo. Given that manual methods are fraught with subjectivity, and depend on the experience technician - consistency with the other two methods was 100%.
Conclusion: Genotyping allelic variants RBCs system of blood donors is a optimal option for rapid detection of various combinations of alleles. This knowledge is required especially, when administering RBCs transfusions to patients with positive anti-erythrocytes antibody screening. The Bio Array [TRADEMARK] HEA BeadChip [TRADEMARK] tested with parallel serological phenotype was thus introduced as a routine process of blood donors to the search of rare allelic combinations of blood groups systems.

P-403

FREQUENCY OF ABO, RHESUS PHENOTYPES AND MOST PROBABLE GENOTYPES AND THE TYPE OF RHD NEGATIVE VARIANTS AMONG UAE NATIONALS IN AL AIN DISTRICT

M Al Meqbali[1], R Al Hammadi[2] and AMH Ahmed[2]

[1]Al Ain Regional Blood Bank, Al Ain, United Arab Emirates [2]UAE University, Al Ain, United Arab Emirates

Background: Knowledge of the frequencies and the phenotypes of ABO and rhesus system is very important for blood bank and transfusion services policies.
Aim: The objective of this study was to determine the frequency of ABO, Rhesus phenotype and most probable genotypes and the type of RhD negative variants in Al Ain area (UAE).

Table 1 & Figure 1-3: Distribution of different ABO blood groups among male, female and total studied population.

Blood Group	Male		Female		Total	
	Number	%	Number	%	Number	%
A	117	26.5	15	25	132	26.4
B	64	14.5	7	11.6	71	14.2
AB	17	3.8	1	1.6	18	3.6
O	242	55	37	61.6	279	56

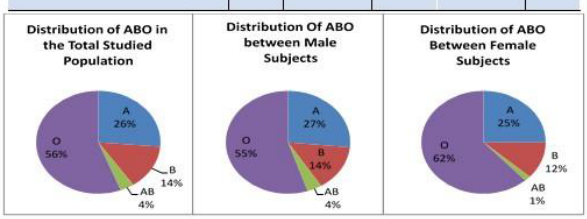


Figure: 1 Figure: 2 Figure: 3

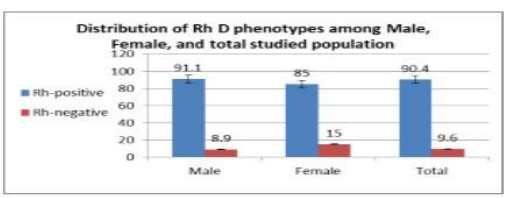


Figure: 4

Table 3 & figure 5: Distribution of Rh phenotypes and most probable genotype

Reaction with Anti-					phenotypic Designation		Most Probable Genotype		%
D	rh′C	rh″E	hr′c	hr″e	Wiener	Fisher-Race	Wiener	Fisher-Race	
Pos					Rh0-pos	D-pos	Rh0-pos	D-pos	90.4
	Pos				rh′– pos	C-pos	rh′– pos	C-pos	76.2
		Pos			rh″–pos	E-pos	rh″–pos	E-pos	27.2
			Pos		hr′-pos	c-pos	hr′-pos	c-pos	71.6
				Pos	hr″-pos	e-pos	hr″-pos	e-pos	97.8
Neg	Neg	Neg	Pos	Pos	rh rh	ccddee	rr	cde/cde	8.4
Neg	Pos	Neg	Pos	Pos	rh′rh	Ccdee	r′r	Cde/cde	1.2
Neg	Neg	Pos	Pos	Pos	rh″rh	ccdEe	r″r	cdE/cde	0
Neg	Pos	Pos	Pos	Pos	rh′rh″	CcdEe	r′r″	Cde/cdE	0
Neg	Pos	Neg	Neg	Pos	rh′rh′	Ccdee	r′r′	Cde/Cde	0
Pos	Neg	Neg	Pos	Pos	Rhorh	ccDee	Ro r	cDe/cde	5
Pos	Pos	Neg	Pos	Pos	Rh_1 rh	CcDee	R_1 r	Cde/cde	29.8
Pos	Neg	Pos	Pos	Pos	Rh_2 rh	ccDEe	R_2 r	cDE/cde	8.2
Pos	Pos	Neg	Neg	Pos	Rh_1 Rh_1	CCDee	R_1 R_1	Cde/Cde	28.4
Pos	Neg	Pos	Pos	Neg	Rh_2 Rh_2	ccDEE	R_2 R_2	cDE/cDE	2.2
Pos	Pos	Pos	Pos	Pos	Rh_1 Rh_2	CcDEe	R_1 R_2	Cde/cDE	16.8
Pos	Pos	Pos	Neg	Pos	Rh_2 Rh_1	CCDEe	Rz R_1	CDE/Cde	0
Pos	Pos	Pos	Pos	Neg	Rh_2 Rh_1	CcDEE	Rz R_1	CDE/cDE	0

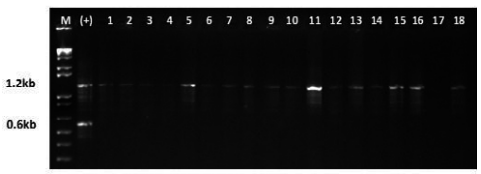
Table 4: Other Red Cell Antigen: Kell Antigens

Anti-K	Frequency	Percent
Neg	448	89.6
Pos	52	10.4
Total	500	100

Methods: The studied group consisted of 500 locals of both gender from different age groups (17–58 years old). ABO and Rh-hr phenotype reactivity were determined using conventional tube method and gel technology, different molecular techniques were also applied to determine the type of RhD negative variants.

Results: Our study showed that the distribution of ABO phenotypes was as follows: O = 56%, A = 26.2%, B = 14.2% and AB= 3.6%. The distribution of Rh antigens

Figure 6: PCR products gel electrophoresis results of the tested Rh negative samples



was as follows: D = 90.4%, C = 76.2%, E = 27.2%, c = 71.6% and e = 97.8%. And the distribution of most probable genotypes was as follows: R1 r = 29.8%, R1 R1 = 28.4%, R1 R2 = 16.8%, rr= 8.4%, R2 r = 8.2%, Ro r = 5%, R2 R2 = 2.2%, and r′r= 1.2%. Our study showed that the most frequent antigen amongst five major antigens of Rh system was RhD while the least common was E antigen.

Conclusion: This study provided serological and molecular frequencies of ABO and Rh phenotypes and most probable genotypes in order to ensure safe blood transfusion in Al Ain area. And it considered as a first study in this area up to our knowledge, which would be of great value for the UAE specially Al Ain area as it provides a reference figure about ABO and Rh-hr antigens which would be applied in blood transfusion practices. Moreover, this study combines molecular analysis and serological testing of Rh genes to be applied in blood bank environment. The challenge lies in integrating such testing into blood bank environment, standardizing methods, obtaining Food and Drug Administration approval for labeling donor units, and enhancing information systems to incorporate and use this new information effectively.

P-404

'RH PROJECT: RHD VARIANTS DEFINITION THROUGH ADVANCED SEROLOGICAL AND MOLECULAR INVESTIGATION' – A RESEARCH PROJECT FUNDED BY THE ITALIAN MINISTRY OF HEALTH

A Matteocci

A.O. S. Camillo - Forlanini, Rome, Italy

Background: With 25 participating Transfusion Services (TS) in 9 Italian Regions, the 'RH Project' (code RF-2010–2321465) is a multicentric research project funded by the Italian Ministry of Health for a period of three years.

Aims: The project aims to conduct a serological and molecular evaluation of *RHD* alleles to agree on some integrated and shared protocols for RhD typing, also taking into account migration flows and the globalization of health care with changing demographics. It considers the general difficulties of RhD testing and takes into account the implementation of new molecular techniques, such as real-time PCR *RHD* genotyping on cell-free fetal DNA from maternal plasma.

Methods: Our Laboratory coordinates this project and carries out the advanced tests for the D-variant definition in collaboration with the other TS. According to the operating protocol, the TS identify samples with weak or doubtful reactions: samples with negative results with anti-D monoclonal IgM and/or IgM+IgG and positive results in the indirect antiglobulin test (IAT), samples typed as RhD positive but presenting antibodies with anti-D specificity and samples with discrepant results and/or weak reactivity with different anti-sera are all considered eligible for entering the project. The following tests are carried out in our lab to analyze the selected samples: liquid phase with different IgM or IgG anti-D sera (Biorad, Astra Formedic, Albaclone, ecc), solid phase with 12 IgG anti-D sera (Albaclone), gel card with 6/12 sera (Biorad) and molecular techniques by SSP-PCR (BAGene, InnoTrain) and by DNA microarray (Immucor/Bioarray Solution).

Results: Up to the end of September 2015 a total number of 559 samples, collected in 25 different TS, were analyzed. Of these, 453 (81.0%) were found to have Weak D variants, 51 (9.1%) had a Partial D, 7 (1.2%) were double variants, 1 (0.2%) was a Del Ex9 and 47 (8.4%) remained unresolved discrepancies that require further investigations (sequencing). Of interest, 31 cases (5.5%) clearly showed D variants of African origins like DAU or D weak type 4. Four cases, 3 with Weak D type 1 variants and 1 with Weak D type 15, presented allo-immunization with anti-D antibodies. A commercial kit for fetal RHD genotyping from maternal plasma (Inst. de Biotechnologies Jacques Boy) was also validated; this test should be used to rationalize the

administration of anti-D immunoglobulin and for an appropriate monitoring of pregnancies with anti-D antibodies.
Conclusion: An incorrect typing of RhD or its variants still represents one of the most frequent causes of immunization, especially in pregnancy. The 'RH Project' has a relevant impact on the NHS because it aims to identify more than 1.000 variants and to define type, frequency and distribution of the D variants with a global map and territorial stratification in Italy. Although we present preliminary data here, our study has already put in evidence some crucial points in routine and secondary level RhD analyses: 1) each lab should evaluate technical features of anti-D sera and technologies used in routine typing; 2) for a proper management and prevention of HDN, fetal *RHD* genotyping in D-negative women is indispensable; 3) it is necessary to catch up on the typing of novel alleles and to have more up-to-date kits and techniques for genomic examination; 4) last but not least, we have to learn which variants produce antibodies most readily.

P-405

A PILOT STUDY TO EVALUATE A GENOTYPING ASSAY, ID RHD XT, FOR DONOR SCREENING APPLICATION

M López[1], I Apraiz[1], A Molano[1], F Puente[2] and D Tejedor[1]
[1]Progenika Biopharma, A Grifols Company, Derio, Spain [2]Servicio Inmunohematologia Banco de Sangre y Tejidos de Aragón, Zaragoza, Spain

Background: Routine serological tests cannot detect some potentially immunogenic D variants, such as DEL, Partial D, and some Weak D types (Daniels G. Human Blood Groups. 3rd ed. Blackwell Science; 2013). D negative patients transfused with D positive blood from mistyped D negative donors may be at risk of anti-D alloimmunization. Molecular screening of blood donors can overcome this serology limitation.
Aims: The aim of this proof of concept study was to demonstrate the feasibility of ID RHD XT, a genotyping assay, to be used as a tool for RHD screening in D negative sample pools.
Methods: Randomly, whole blood and plasma from blood donations and routine RhD serologic typing data were collected. Twenty blood samples were stored at a temperature between 2°C and 8°C while twenty plasma samples were stored at −20°C, until DNA extraction. DNA was extracted from blood and plasma, individually and pre-pooling one D positive in 19 D negative samples, using the QIAamp DNA mini kit (QIAGEN). The DNA of the pools was extracted in triplicates. Extracted DNA samples were processed with ID RHD XT for *RHD* gene detection and the results were automatically analyzed by the proprietary software. The ID RHD XT assay uses Luminex® xMAP technology for the simultaneous amplification of *RHD* exons 1, 6 and 9 and intron 3 for the identification of *RHD*weak D types 1*, *2*, and *3*, *RHD deletion*, RHD*Pseudogene and RHD*DIIIa-CE(3–7)-D alleles. An internal Human Growth Hormone (*HGH*) amplification control is also included in the test.
Results: DNA samples, individually and pooled (three extraction replicates) from blood and plasma (n = 46), gave the correct *RHD* genotyping result (*RHD* gene positive or negative) in comparison to serology typing. The internal amplification control was also detected in all DNA samples. The signal of *RHD* exons 1, 6 and 9 obtained with ID RHD XT using DNA samples extracted from plasma and blood samples, in individual and pool extractions were equivalent. The pool extraction replicates yielded identical results.
Summary/Conclusions: ID RHD XT can detect *RHD* gene in biological samples, plasma and whole blood, in pools of twenty D negative samples. A study with D variant samples is in progress to confirm that ID RHD XT is a sensitive tool and yields reproducible results for cost-effective *RHD* screening in serologically D- typed donors to prevent potential alloimmunization of receptors.

P-406

RELATIONSHIP BETWEEN ABO BLOOD GROUPS AND LEUKEMIA (AML AND ALL)

S Bohisi, D Tamara, S Maher and S Rabie
King Hussein Cancer Center, Amman, Jordan

Background: Associations between ABO blood groups and certain diseases, e.g. gastric carcinoma and duodenal ulcers have previously been studied and were scientifically proven [(1)]. This study investigates the possible relationship between different ABO groups and the occurrence of certain hematological malignancies (Acute lymphoid leukemia (ALL) and Acute myeloid leukemia (AML) in specific). Many recently conducted studies have discussed a possible relationship, most of which concluded that B blood group is associated with a higher risk of leukemia. Yet, other studies still oppose this correlation and fail to approve.
Aim: Proving a relationship between a specific blood group and certain blood malignancies, can help in early detection if the word is spread in awareness campaigns.
Reinforce the universal database that considers this hypothesis by providing more data from King Hussein Cancer Center (KHCC).
Material and Method: This was a retrospective study that was carried out at KHCC in Amman, Jordan. The ABO blood groups data were collected from patient records for patients admitted to King Hussein Cancer Center over a period of three years (January 2013 to December 2015) with ALL or AML.
Results: The study examined the data 515 ALL and AML patients; for those the overall distribution of ABO blood groups did not differ significantly with respect to the normal (source) population (P value= 0.9638) as the distribution was 39.22 (O), 35.73 (A), 19.03 (B), 6.02 (AB). This was also seen, when the distribution of ABO blood groups in ALL patients 37.8 (O), 37.5 (A), 19 (B), 5.6 (AB) (P value= 0.9768) and AML patients 42.4 (O), 31.6 (A), 19 (B), 7 (AB) (P value= 0.7769) was inspected separately. It is worth noting that an association of ABO blood groups distribution with respect to gender (the study examined 204 females and 311 males) was also insignificant (P value= 0.31723).
Conclusion: Up to date studies are failing to establish a clear relationship or association between leukemia patients and their ABO blood groups distribution, this also applies to the association between ABO blood groups distribution and sex of leukemia patients. This result must await further research to confirm an association or oppose it, as the case in our study.

P-407

A SEROLOGICAL AND MOLECULAR ANALYSIS TO DETERMINE RH VARIANTS

C Través[1], M López[2], A Salazar[1], A Molano[2], J Farré[1], D Martorell[1] and D Tejedor[2]
[1]R&D Immuhohematology Area, Diagnostic Grifols, Barcelona, Spain [2]R&D Department, Progenika Biopharma, A Grifols Company, Derio, Spain

Background: Identification of highly immunogenic DCE antigens on the red blood cell plays an important role in transfusion medicine. Serologic testing is routinely used for DCE antigens typing, but some *RH* variants are not correctly detected. Genotyping is a powerful adjunct to serology for detecting the presence of D, C, c, E or e variants, especially in chronic transfusion patients to avoid alloimmunization.
Aims: The aim of this study was to compare the performance of serological direct typing agglutination gel test with molecular tests to detect clinically relevant *RH* variants.
Methods: Twenty eight patient blood samples with rare *RH* variants were tested in Grifols for phenotype and molecular analysis. Serological typing methods were performed using different DG Gel reagents by direct agglutination gel test (Diagnostic Grifols). The samples were tested manually by two reagents for each phenotype specificity: Anti-DVI- (clone P3 × 61 and clone MS-201),), Anti-DVI+ (clones P3 × 290 + P3 × 35 + P3 × 61 + P3 × 21223B10 and clones RUM+ ESD1M), Anti-C (clone MS-24 and clone P3x25513G8), Anti-E (clone MS-260 and clone 906), Anti-c (clone H-48 and clone MS-33) and Anti-e (clones MS-21 + MS-63 + MS-16 and clones MS-63 + MS-16). Molecular tests were performed after DNA extraction using BLOODchip v4.2, ID CORE XT, ID RHD XT and/or Sequencing Service (Progenika, a Grifols company). These products are designed for detecting rare allelic variants in *RHD* and *RHCE* genes.
Results: The phenotypes obtained by serology and the *RH* alleles obtained by molecular tests agreed with those previously reported by the hospital. Nineteen samples with *RHD* variants were identified using molecular tests: eight weak type 4.0, four DAR, two DV, two weak D type 1, one weak D type 2, one weak D type 3 and one DWN. All these samples give positive results by serological methods using four different Anti-D antibodies. In 12 out of 19 samples it was possible to suggest the presence of a variant due to a decrease in the agglutination intensity (lower than 4 +) depending on the Anti-D reagent, this fact is more evident in case of variants that express low levels of D antigen on the red blood cell membrane like weak D type 1, weak D type 2 and DAR. Fourteen samples with *RHCE* variants were identified using molecular tests: four ceAR, three CeRN, three ceMO, two r′s, one Ce602 and one ce48. Three samples gave discordant results in serology using two different Anti-C reagents (clone MS-24 and clone P3x25513G8) suggesting the presence of a partial C. Two samples were identified as *RHD**r's-*RHCE**ce[733G,1006T] allele carriers by genotyping predicting a

partial expression of C antigen, despite these samples gave normal positive results in serologic typing. Five *RHCE* variants that affect to the expression of E and e antigens showed a normal pattern of reactivity with the Anti-E anti Anti-e typing reagents.
Summary/Conclusions: Serological techniques can be complemented by molecular methods to identify variant *RH* alleles that affect antigens expression and to solve serological discrepancies. The use of both techniques, as complementary, can prevent alloimmunization in frequently transfused patients.

P-408

JKA-IMMUNIZATION IN A PATIENT WITH A PARTIAL DELETION OF THE JK LOCUS

A Matteocci, A Moscetti, A Collaretti, K Castagna, L Rogai, F Pirelli and L Pierelli
A.O. S. Camillo - Forlanini, Rome, Italy

Background: The Kidd locus (JK) encodes for a trans-membrane, urea-transporter protein commonly present on red blood cells. JK presents 2 major alleles, Jk^a and Jk^b, that result from a single nucleotide polymorphism in the *SLC14A1* gene (c.838 G>A), causing an amino-acid substitution at position 280 (Asp280Asn). An inhibited expression of these antigens is characteristic of the rare Jknull phenotype, which can be obtained through the inheritance of silent JK alleles or by the presence of a dominant inhibiting gene not linked to the JK locus. Determining the Jknull status in patients is something relevant, since an exposure to Jk^a or Jk^b antigens (due to blood transfusions or pregnancy, for instance) may cause allo-immunization and immediate or delayed hemolytic reactions.
Aims: We report the case of a multiply transfused patient with anti-Jka antibodies and DAT-positivity, that was directed to blood group genotyping for a correct determination of his erythrocyte antigen profile.
Methods: DNA was extracted by using Qiagen columns and analyzed with the HEA BeadChip kit (Immucor-BioArray Solutions) and the KKD Ready Gene SSP kit (Inno-Train). The region close to the $Jk^{a/}Jk^b$ polymorphism (c.838 G>A) in the *SLC14A1* gene was amplified and analyzed through direct bi-directional sequencing. An allele-specific PCR analysis was finally used to determine the presence of a specific deletion that leads to the *JK*01N.01*null phenotype.
Results: Both the HEA BeadChip kit and the KKD Ready Gene SSP kit highlighted the status of heterozygosity at the polymorphic site c.838 G>A in the *SLC14A1* gene. Bi-directional sequencing also confirmed these findings. An allele-specific PCR test, designed to put in evidence the *JK*01N.01*null phenotype, revealed the presence of a deletion involving exons 4 and 5 of one of the alleles in the *SLC14A1* gene. Since the starting codon of *SLC14A1* coding sequence is located in exon 4, no protein can be obtained from the allele with this deletion. Hence the patient was classified as heterozygous *JK*01N.01/02*.
Conclusions: Our study and the current literature show the association between the deletion of exon 4 and 5 in the JK locus and the *JK*A* allele, with subsequent inhibition of Jk^a expression. In the case reported here the presence of anti-Jk^a antibodies was the result of allo-immunization due to recurrent administration of Jk^a-positive blood units in an individual with a partial deletion of the JK locus. This put in evidence that genotyping methods that include commercially available kits, sequencing analyses and specifically designed PCR tests are becoming nowadays an essential support in helping to correctly type patients, solving complex cases and characterize antibody specificities.

P-409

BLOOD GROUP DETERMINATION IN A PATIENT WITH SHWACHMAN-DIAMOND SYNDROME: A CASE REPORT

I Bricl, A Lukan and T Dovc Drnovšek
Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

Background: Blood group of a 2-year-old child diagnosed with Shwachman-Diamond syndrome was determined at birth. Six days after birth the child received his first blood transfusion, followed by additional transfusions in the next two years. No transfusion reaction was reported at any time. When the blood sample was submitted to the Blood Transfusion Centre of Slovenia for another determination of blood group and pretransfusion testing the ambiguity of results was noted.
Aims: We aimed to resolve the cause of the discrepancy in ABO blood group determination in order to provide suitable blood components for the patient.
Methods: For serological determination of blood group, gel cards ID-Card-DiaClone ABO/Rh for Newborns (Bio-Rad) and ID-Card-DiaClone ABO/D+Reverse Grouping with ID-DiaCell ABO test cells A1 and B for reverse grouping (Bio-Rad) were used. ABO genotyping was performed with SSP-PCR method using RBC-Ready Gene ABO and RBC-Ready Gene ABO-Subtype kits (Inno-Train).
Results: Child's blood group, determined at birth with gel card for newborns was 0, RhD-pos. The blood group from fresh blood sample was routinely tested with gel card for patients comprised of forward and reverse part. The results showed the discrepancy between forward and reverse grouping. No agglutination was observed with monoclonal anti-A and anti-B sera, suggesting blood group 0, but in reverse grouping, there was agglutination with A1 erythrocytes and no agglutination with B erythrocytes. Blood was additionally retested using the gel card for new-borns that again showed the same results, no agglutination with human anti-A and anti-B sera. To resolve the matter, ABO genotyping was performed with SSP-PCR method. The patient was determined as homozygous for *O1* allele, with no additional allele for ABO-subtype.
The overview of literature on Shwachman-Diamond syndrome shows that the patients with this diagnosis exhibit exocrine pancreatic dysfunction and skeletal abnormalities as well as bone marrow dysfunction, which is usually reflected in severe neutropenia. The immunodeficiency pattern varies among patients and lymphopoietic functional capacity may also be compromised. It was shown that anti-A and anti-B antibodies can be low or absent in Shwachman-Diamond syndrome patients with the lack of anti-B antibodies being far more common.
Conclusions: The blood group of the patient was determined as O, with the comment that no anti-B antibodies are present, which is in concordance with the patient's diagnosis. In this case, knowing the patient's diagnosis and literature data was crucial for the elimination of blood group determination ambiguity.

P-410

This abstract has been withdrawn.

P-411

RHD*07.02 ALLELE CAUSES DISCREPANT GENOTYPING RESULTS FOR RHCE SMALL C

S Scholz[1] and V van Sandt[2]
[1]Inno-Train Diagnostik GmbH, Kronberg, Germany [2]Rode Kruis Vlaanderen, Mechelen, Belgium

Background: In the human Rh blood group system the c, C, E, e and D antigens are expressed by the two highly homologous genes *RHCE* and *RHD*. After D, c is the most immunogenic Rh antigen. The difference between c (307C) and C (307T) is caused by the SNP on position 307 on the *RHCE* gene. The RHD*07.02 allele (also known as RHD cat VII type 2) carries the SNP 307T>C on the *RHD* gene and additionally the SNP 329T>C. This *RHD*07.02 allele has been described to partially express RHc on the D polypeptide (Faas, Transfusion, 2001).
Aims: Genotyping was performed to clarify the cause of the weak c expression. Serology of a patient sample (Male, °1938) indicated a partial c phenotype with a CDe.
Methods: RhD and RhCE phenotyping was done by accredited routine protocols (monoclonal AB ID card: Diaclon Rh subgroups, seraclone anti-c). Genotyping was performed with a TaqMan Probe assay (RBC-FluoGene vERYfy, inno-train Diagnostik GmbH), SSO (RBC-Lifecodes, Gen-Probe Inc.), in-house SSP-PCR (HILA, Rode Kruis-Vlaanderen) and commercial SSP-PCR (RBC-Ready Gene CDE, inno-train Diagnostik GmbH). Sanger sequencing of the *RHD* gene was performed using an in-house method (inno-train Diagnostik GmbH).
Results: Discrepant genotyping results were generated by different test systems: the TaqMan Probe based assay showed in repetition a CCee genotype, while the SSO system RBC-Lifecodes predicted in repetition a Ccee phenotype. In SSP-PCR the sample showed a weak c band with the in-house method, while there was no band visible with the commercial test kit. The parallel analysis of the *RHD* gene with RBC-Ready Gene CDE test system revealed a variant D cat VII RHD allele. Sequencing of the DNA sample identified two SNPs on one of the RHD alleles (307T>C, 329T>C) confirming a RHD*07.02 and one RHD*01 allele.
Summary/Conclusions: Usually genotyping provides clear answers for conspicuous serology. However, in a few cases PCR does not offer conformable results e.g. where mutations on the primer binding sites prevent the amplification. As described here

there must be differences in the primer and/or probe design (in this case for the *RHCE*c* detection) of different test systems causing either correct or erroneous genotyping results. In this example the high homology between the *RHD* and *RHCE* genes in combination with the presence of the D cat VII SNP 307T>C lead to false positive RHCE*c (SNP 307C) SSO and SSP genotyping result. We therefore suggest to genotype of both RHCE and RHD to resolve the true nature of weak RHCE serology.

References: Faas, Transfusion, 2001

P-412

FUNCTIONAL ANALYSIS OF RHD VARIANTS: A NOVEL PLASMID TOOL TO INVESTIGATE SPLICING ALTERATION

Y Fichou[1], C Bernard[1], G Le Gac[2], C Le Maréchal[1], C Ka[2] and C Férec[1]

[1]Inserm UMR1078 - EFS, Brest, France [2]Laboratoire de Génétique Moléculaire et d'Histocompatibilité, CHRU Morvan, Brest, France

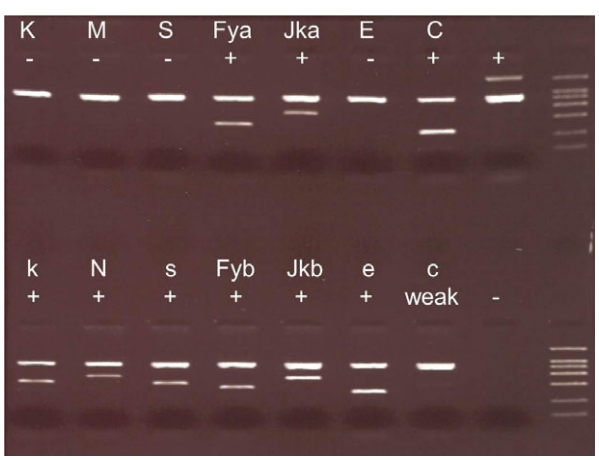
Background: We previously engineered and experienced the plasmid construct pMG1.1, including part of the human *POLR2G* gene, to study the functional impact of genetic variations affecting the cellular splicing by a minigene splicing assay. While this molecular tool proved to be useful for studying the splicing effect of variants in "internal"exons, its structure prevents from the analysis of gene variants affecting the donor site of exon 1.

Aims: We sought 1/ to create a novel plasmid construct to analyze variations within/in the vicinity of intron 1 donor site that potentially affect splicing and 2/ to test our model with reported variations of the *RHD* gene (Rh blood group system).

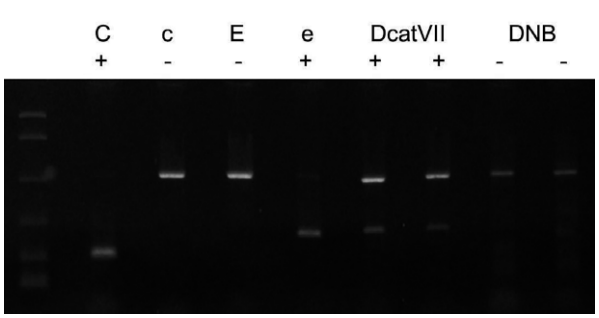
Methods: A novel plasmid construct (pMG2.1) derived from our initial vector was built by a single site-directed mutagenesis experiment in the pMG1.1 vector. The plasmid DNA region including *POLR2G* exon 1 was removed by restriction enzyme digestion and replaced by the corresponding region of the *RHD* gene (i.e. 5'-UTR and exon 1) by a simple homologous recombination-mediated, subcloning step. The novel recombinant plasmid served as a template for subsequent site-directed mutagenesis to generate three constructs carrying the missense c.146A>G, silent c.147A>G, and intronic c.148 + 1G>A variants, respectively. Functional impact of these variations was tested in the HEK 293T eukaryotic cell model. Transcript structure was analyzed by RT-PCR, cloning and sequencing.

Results: The novel alteration created within pMG1.1 to generate pMG2.1 does not alter splicing, suggesting that this latter vector may be used for minigene splicing assay. Splicing is very significantly impaired by c.148 + 1G>A in accordance with bioinformatics predictions. While this *DEL* variant is assumed to generate minute amount of "normal"transcript, this structure could not be detected. The two other substitutions do not appear to alter splicing in our model. These results suggest that 1/ the weakened D expression associated to c.146A>G is likely directly linked to the p.Gln49Arg amino acid substitution; and 2/ additional studies are mandatory to characterize both the molecular effect of and the phenotype resulting from c.147A>G.

Summary/Conclusions: Our novel plasmid construct pMG2.1 is relevant to assess easily the functional effect of variations in the vicinity of intron 1 donor site that potentially disrupt splicing. Future applications of this technology obviously extend to molecular diagnostics of genetic diseases. While the qualitative data are mainly conclusive in our model, a main effort to focus on for a future development is the quantitative aspect of the analysis, as suggested by our examples. Semi quantitative fluorescent PCR may help to assess the quantitative effect of specific variants, which is critical to understand the pathophysiology of numerous disorders.



Caption 1 Faint c band detected with in house SSP test system



Caption 2 No c band visible with RBC-Ready Gene CDE test system

P-413

This abstract has been withdrawn.

P-414

PROVISION OF ANTIGEN-MATCHED BLOOD FOR CHRONIC PATIENTS WITH SICKLE CELL DISEASE (SCD) AND β-THALASSEMIA

L Castilho, EA Sippert, MA Botelho, MD Macedo, AC Gaspardi and J Pellegrino Jr

University of Campinas, Campinas, Brazil

Background: Transfusions are essential for patients with SCD and thalassemia to maintain growth and development during childhood and to sustain good quality of life during adulthood; however, the development of red blood cell (RBC) alloantibodies and autoantibodies complicates transfusion therapy in such patients. Routine phenotyping of blood recipients and the use of phenotype-matched blood units for transfusion has been useful to lower the occurrence of red cell alloantibodies in chronically transfused patients with thalassemia and SCD. Nevertheless, extensive phenotyping is expensive, laborious and cannot be performed in certain situations. The molecular understanding of blood groups has enabled the design of assays that are being used to better guide matched red blood cell transfusions and to maintain an inventory of units DNA typed.

Aims: Based on this, our aim was to perform molecular matching at different levels for patients with SCD and thalassemia in order to identify compatible donors for the transfusion needs of the patients.

Methods: Blood group genotypes covering a set of 15 clinically relevant red cell antigens and RH variants were determined in 67 DNA samples from chronically transfused patients with SCD (38 alloimmunized and 29 non-alloimmunized), in 65 patients with thalassemia (25 alloimmunized and 40 non-alloimmunized) and in 1032 DNA samples from Brazilian donors. Laboratory developed tests (LDTs), HEA BeadChip™, RHD BeadChip™, RHCE BeadChip™, and sequencing were used to determine the genotypes among patients and donors. Molecular matching was performed in 3 levels: (1) RH and K matching; (2) extended matching and (3) extended matching including RH variants. We considered the total of red blood cell units requested for each patient and a number of 2 donations per year for the compatible donors.

Results: We found compatible donors for 100% of our thalassemia and SCD patients in level 1 of matching, 90% for SCD patients and 70% for patients with thalassemia in level 2 and 50% for patients with SCD and 90% for patients with thalassemia in level 3. Although we found compatible donors for most of our patients in level 2, the number of available units was not enough to fulfill the needs of thalassemia patients with multiple negative antigens and alloantibodies. For SCD patients with

RH variants we observed that even having donors with the same *RH* genotypes, we need to screen much more donors to fulfill the needs of patients requiring long-term transfusion support.
Summary/Conclusions: The distribution of blood group genotypes in Brazilian blood donors is more like that found in SCD patients than in thalassemia patients. As the number of *RH* genotyped donors available is limited at present to fulfill the needs of SCD patients with variants and confirmed alloantibodies, it is important to develop an approach to identify donors with RH variants to establish an inventory of RH typed blood to ensure transfusion safety for the patients.

P-415

This abstract has been withdrawn.

Platelet Immunology

P-416

THE IMPACT OF LOW FREQUENCY PLATELET ANTIBODY IN CASES OF NEONATAL ALLO-IMMUNE THROMBOCYTOPENIA IN KUWAIT

SM Al-Shatti, M Tatoyan, H Ashkanani and H Al-Shatti
Kuwait Central Blood Bank, Kuwait city, Kuwait

Background: Allo-immune thrombocytopenia are disorders in which the platelet life span is shortened by allo antibodies elicited during the recipient's immune response against platelet from a genetically different individual .The major clinical conditions are recognized as neonatal allo-immune thrombocytopenia and post transfusion purpura. Neonatal Alloimmune Thrombocytopenia(NAIT) results from the maternal immune response against fetal - specific platelet antigens inherited from the father lacks in the mother. Fetal platelet destruction is mediated following transplacental passage of specific antiplatelet maternal allo- antibodies. (NAIT) is the most common cause of severe fetal thrombocytopenia and the most feared complication of (NAIT) is the occurrence of intra cranial hemorrhage (ICH) leading to death or neurological sequelae. The most common antigen associated with (NAIT) is HPA-1a reported in up to (83%) followed by HPA-5b (20%).
Aim: The aim of this study is to focus on cases of (NAIT) developing low frequency platelet antibodies.
Method: Retrospective study from two mothers samples with affected baby with thrombocytopenia has been tested in platelet testing laboratory in the blood transfusion centre. Investigations were done using life-codes immucore PAK -12 for the presence or absence of human platelet antibodies,Bloodchip genotyping method were also used to confirm the serological results obtained.
Results: A total of 109 mother samples were tested for (NAIT),a low frequency platelet allo antibodies HPA-3a were found in 3 mothers samples (2.75%), two of them having severely affected babies with bleeding and (ICH).
Case (1) having anti-HPA -3a and anti-HLA class I
Case (2) having anti-HPA-3a and HPA-Ib
Case (3) having anti-HPA-3a
Conclusion: In our practice, allo-immunization against rare platelet antigens should not be ignored as a cause of (NAIT) and as important tool for better management of the disorder and to enable appropriate achievement and accurate diagnosis in the index case and future pregnancies.

P-417

A CASE OF STANDARD HEPARIN-INDUCED THROMBOCYTOPAENIA DURING TREATMENT OF GASTRIC STROMAL TUMOR

J Liu, WJ Xia and X Ye
Guangzhou Blood Centre, Guangzhou, China

Background: Heparin-induced thrombocytopenia (HIT) is a prothrombotic side effect of heparin therapy caused by anti-platelet factor 4 (PF4)/heparin complex antibodies and occurs in 1–3% of Heparin treated patients. It is a life-threating complication, and affects the morbidity and mortality of the patient.

treatment			H					PT		H				
date	D 1	D 2	D 3	D 4	D 5	D 6	D 7	D 8	D 9	D 10	D 11	D 12	D 13	D 14
Platelet count(*10^9)	87	-	84	60	48	53	39	68	67	58	52	45	45	20

PT : platelet transfusion
H : Heparin Sodium
D : Day

Table 1. The platelet count after different treatment in the patient.

Aims: To detect the anti-PF4/heparin complex antibodies induced by standard heparin in serum.
Methods: The male patient was 37 years old, with gastric stromal tumor. The platelet count was decreased when using of heparin, and increased after the platelet transfusion (detail see table 1). We used the HiTII-Ab ELISA kit (IBL international GmbH) to detect the anti-PF4/heparin IgG. In patient's serum OD > 1.2 was positive, and for semi-quantitative interpretation concentration, the values >18U/ml was positive.
Results: PF4/heparin IgG were both positive for semi-quantitative and qualitative in this patient. For semi-quantitative interpretation, the concentration values was 19.957 U/ml. For qualitative interpretation, OD values was 1.205. The variation of platelet count was in conformity with the usage of heparin.
Conclusions: In this case, the positive heparin induced platelet antibodies test and dramatic fall in platelet count confirmed the diagnosis of heparin induced thrombocytopaenia type II.

P-418

This abstract has been withdrawn.

P-419

A CASE REPORT: ANTI-HPA-1B AND ANTI-HPA-5B MEDIATED POST TRANSFUSION PURPURA

M Hamed, S Al-Shatti, H Abdullah, H Al-Ansari, H Al-Shatti and R Al-Radwan
Kuwait Central Blood Bank, Kuwait, Kuwait

Background: Post-transfusion purpura (PTP) is a rare but potentially fatal immunohematological disorder characterized by severe thrombocytopenia following transfusion of blood components and induced by an alloantibody against a donor platelet antigen. It is most commonly caused by anti-human platelet antigen-1a alloantibodies. The incidence of PTP caused by anti-HPA-1a antibody has been reported as one case per 50,000 to 100,000 units of transfused blood components, but this is probably an underestimate and the majority of cases occur in multiparous women. Other antibodies, like HPA-5b and HPA-1b, appears even rarer, however can cause potentially life-threatening bleeding and should be tested for if PTP is suspected.
Aim: To illustrate a case of post-transfusion purpura secondary to HPA-1b and HPA-5b alloantibodies after receiving multiple blood products.
Case Report: Female Kuwaiti patient 78-years-old, diabetic, hypertensive, with chronic kidney disease, initially admitted with chest infection and melena. Her transfusion history is not clear, however she had history of pregnancies. During her current admission, red blood cells transfusion was requested, and she received 4 units red blood cells over 3 days. They started IV antibiotics and the chest infection resolved. However her platelet count began to drop rapidly on day 6 till reaching 50×10^9/L on day 7. She was afebrile with no more signs of chest infection. The patient deteriorated with development of purpuric skin lesions while the progressive drop in the platelet count continued,till reaching 7×10^9/L on day 11. Thus one random apheresis platelet dose was requested and transfused with poor improvement. PTP was suspected and patient started 0.5 mg/kg intravenous immunoglobulin (IVIG) for 5 days with clinical improvement. At the time of discharge, her platelets had increased to 165×10^9/L, with no further evidence of active bleeding or purpura and her hematologic profile gradually returned to normal values.
Methods: Patient's platelet rich plasma was tested by enzyme-linked immunoassay commercial kit (Immucor / Lifecodes/ PAK12). The platelet genotyping was

performed by polymerase chain reaction (BloodChip- Reference/ by Progenika Biopharma/ Grifols).
Results: Alloantibodies against HPA-1b and HPA-5b were detected. Antigen typing performed by molecular testing demonstrated that the patient is: HPA 1a/1a and HPA 5a/5a.
Conclusions: PTP is considered a self limiting condition, where in untreated patients, thrombocytopenia usually resolves in approximately 20 days. Random platelet transfusion is generally unhelpful in the management of this condition as the platelet count drops further even with platelet transfusions. In our patient, the response to platelet transfusion was poor, as expected. Thus IVIG is considered the first line of treatment and now replacing therapeutic plasma exchange.
This case highlights the importance of maintaining a high level of suspicion for PTP in the differential while evaluating life-threatening thrombocytopenia in patients with a recent history of blood transfusion. Thus prompt diagnosis and initiation of appropriate therapy can shorten the duration of thrombocytopenia and reduce both morbidity and mortality.

P-420

A NEW WAY TO STUDY HPA GENOTYPING FOR NEONATAL ALLOIMMUNE THROMBOCYTOPENIA (NAIT) MANAGEMENT USING DNA EXTRACTED FROM BUCCAL SWABS WHEN BLOOD SAMPLE COLLECTION IS IMPOSSIBLE

R Petermann, C Chenet, J Quesne, Y Mammasse and C Martageix
Institut National de la Transfusion Sanguine, Paris, France

Background: The HPA Beadchip genotyping kit (CE-IVD BioArray Solutions, Immucor, Warren, NJ) allows to test for 11 human platelet antigen (HPA) groups simultaneously in a single platform.
Aims: Although the current protocol for genotyping required DNA at a concentration ranging from 10 to 80 ng/μL, our goal is to validate this method for low DNA concentration.The 1st part of the study (2013 and 2014) concerned 112 samples comparing HPA genotyping on DNA extracted from blood and buccal swabs in patients suffering from severe thrombocytopenia such as NAIT and particularly pre-term neonates.
Methods: In the second part of the study, 94 patients have been analysed. 94 DNA samples from blood and 100 buccal swabs have been genotyped and compared. Two CE marked *in vitro* diagnostics techniques were used for DNA extraction: i) the automated extraction on MagNA PURE Compact with the MagNA PURE Compact Nucleic Acid Isolation Kit I (Roche Diagnostics,Gmbh); ii) and the manual QIAamp DSP DNA Blood Mini kit (Qiagen,Gmbh). All swabs samples plus 21 of the 1st study were tested in q-PCR (Quantifiler trio DNA Quantification Kit, Life Technologies).
Results: On the 100 swabs' samples, the range of DNA concentration in q-PCR is from 0,03 to 39,22 ng/μL. 88 samples (88%) are lower than 10 ng/μL required for genotyping protocol: 63 from 0 to 5 ng/μL and 25 from 5 to 10 ng/μL. It may be noted that 20 samples had a DNA concentration under 1 ng/μL (10 times lower than required) and HPA genotyping results were interpreted in 90%. On the 100 samples tested, one sample has been excluded related to a mutation detection. So HPA genotyping results were 98,9% concordant. Three HPA genotyping buccal swabs results were non-interpretable.
Conclusions: 189 patients were studied in this global study whether a total of 384 DNA samples from blood and buccal swabs. The HPA Beadchip genotyping kit allows a suitable genotyping of platelets antigens even at low DNA concentration. On 189 patients tested, only 4 samples gave IC or LS results. So HPA genotyping results were 97,6% concordant between blood and swabs (excluding 1 discordance and 4 indeterminated samples). Then, these results suggest that this easy and non-invasive collecting method could be introduced in routine screening but on account of low DNA concentration, HPA genotyping on swabs might be indicated in first intention for NAIT and pre-term neonates. In a second intention, when it is possible, HPA genotyping on blood must be performed to confirm swabs results.

P-421

INTEGRATED TRANSCRIPTOME AND PROTEOME ANALYSIS REVEAL CELL DEATH AND INFLAMMATORY RESPONSE AS THE MOST SIGNIFICANT BIOLOGICAL DISORDERS IN PLATELET COMPONENTS INVOLVED IN TRANSFUSION REACTIONS

O Garraud[1], C Aloui[2], S Claverol[3], F Salin[4], I Lesur[5], J Fagan[6], T Chakroun[2], A Benamara[6], C Mounier[7], F Cognasse[6] and S Laradi[6]
[1]INTS, Paris, France [2]University of Lyon-Saint-Etienne, Saint-Etienne, France [3]Proteome Platform, CGFB, University of Bordeaux Segalen, Bordeaux, France [4]INRA, UMR 1202 Biodiversité Gènes et Ecosystèmes, Genome Transcriptome Facility, Cestas, France [5]HelixVentur, Merignac, France [6]EFS Rhone-Alpes-Auvergne, Saint-Etienne, France [7]ICLN, Saint-Etienne, France

Background: Blood platelets destined for transfusion release inflammatory molecules during preparation and storage that are occasionally associated with transfusion adverse events (AEs).
Aims: The rationale of this study is to decipher the transcriptome and the proteome of Platelet Components (PCs) involved in severe and immediate AEs (SAEs).
Methods: We analyzed 5 leukodepleted PCs implicated in SAEs vs 5 matched PC controls.
The platelet RNA transcriptomes were acquired by RNA-Seq using the IonProton platform and data were mapped using CLC Bio software. Transcripts were counted using HTSeq-count software and differentially expressed genes were identified using DESeq2 package (Bioconductor).
For quantitative proteomic analysis of the platelet pellets and the supernatants respectively, we performed a Label-Free LC-MS/MS method: LC system (Dionex, Amsterdam, The Netherlands) coupled to an Electrospray Q-Exactive quadrupole Orbitrap benchtop mass spectrometer. Subsequently, data were searched by SEQUEST through Proteome Discoverer 1.4 against the Homo sapiens Reference Proteome Set (Uniprot version 2015–07; 68482 entries). Raw LC-MS/MS data were imported in Progenesis QI 2.0 for peptide quantification and statistical comparison (ANOVA test).
Differentially expressed genes /proteins ($P < 0.05$; absolute Fold Change >2) were analyzed by the Ingenuity Pathway Analysis (IPA) bioinformatics software.
Results: For the transcriptome study, out of 19.143 genes, 39 genes were differentially expressed. From the platelet pellet and the supernatant proteome study, 1000 and 187 proteins, respectively, were identified from which 430 and 83, respectively, were differentially expressed within the two studied groups.
Cell death and inflammatory response were globally the most significant biological functions associated with ATR after platelet transfusion. Moreover, inflammatory disorders were among the most relevant disease mechanism associated with ATR after platelet transfusion.
Conclusion: The integrating transcriptome and proteome data of PC supernatants reveal a significant association with apoptosis and inflammatory mechanisms which may be involved in platelet transfusion reactions. This better understanding of SAEs process may help prevention means.

P-422

HUMAN PLATELET-SPECIFIC ANTIGENS FREQUENCIES AMONG BLOOD DONORS IN BIRJAND-IRAN

G Anani Sarab[1], M Moallemian[1] and AM Varzi[2]
[1]Birjand University of Medical Sciences, Birjand, Iran [2]Lorestan University of Medical Sciences, Khorramabad, Iran

Introduction: Human platelet antigens (HPAs) are antigenic determinants on platelet membrane glycoproteins. The frequencies of human platelet antigens (HPAs) vary between different populations. and are major determinant for the prevalence of HPA alloimmunization and its clinical associated entities following pregnancy or upon a transfusion. A total of 104 blood donor individuals from Birjand in East of Iran were studied for the frequency distribution of HPA-1,-2,-3,-4 and -5 systems.
Methods: DNA extraction was performed from peripheral venous blood samples. DNA-based polymerase chain reaction with sequence-specific primers genotyping method was used for HPA genotyping. The HPA bands were visualized by using GelRed-stained agarose gel, after electrophoresis. Genotypes 1a/1a, 1a/1b, and 1b/1b were assigned accordingly.
Results: The frequencies obtained from blood donors were 0.95 and 0.05 for HPA-la and -Ib, 0.81 and 0.19 for HPA-2a and -2b, 0.605 and 0.395 for HPA-3a and -3b and 1.00 and 0.00 for HPA-4a and -4b and 0.933 and 0.067 for HPA-5a and -5b.

Conclusions: The HPA-1b, -4b and -5b homozygous donors were detected at low frequencies but HPA-2b frequency was higher than expected.

P-423

INDIVIDUAL SELECTION OF A DONOR/RECIPIENT PAIR BY CROSS-MATCHING IN HEMATOLOGICAL PATIENTS WITH REFRACTORINESS TO PLATELET TRANSFUSIONS CONCENTRATES

AF Rakhmani, IV Dubinkin, EA Mikhailova, OS Kalmikova, VV Zhuravlev, TV Gaponov, VV Troitskaya and EN Parovichnikova

National Research Center for Hematology, Moscow, Russian Federation

Background: Transfusion of platelet concentrates (PC) is an efficient treatment and prevention of hemorrhagic complications in hematology patients. However, multiple blood transfusions, pregnancy history lead to allosensibilization which is the main reason for the development of immunologic refractoriness and treatment failure. The development of refractoriness might be due to non-immune (fever, sepsis, splenomegaly, hemodilution, disseminated intravascular coagulation syndrome), and immune factors. Immune factors are associated with the development of antiplatelet allo, auto and hapten antibodies. The appearance of the patient's anti-HLA and anti-HPA antibodies may be the cause of non-hemolytic type immune responses and lead to a complete lack of clinical effect of platelet transfusion. Due to the high polymorphism of HLA and HPA alloantigens of platelet genotyping of donors and recipients for the purpose of selection of incompatible pairs is inappropriate. Selection of compatible donor/recipient pairs is recommended to be performed by cross-matching tests.
Aims: To evaluate the PC transfusions efficacy using individual selection by cross-matching in hematological patients with refractoriness to platelet transfusions concentrates.
Methods: Eighteen patients with refractoriness to PC transfusions (2 inefficient transfusions) were observed in the clinics of Hematological Scientific Center between July 2015 and March 2016. The average age of the patients was 35 years (22–58; M/F-6/12). Among them 5 patients had aplastic anemia (AA), 8 - acute myeloid leukemia (AML), 3 - myelodysplastic syndrome (MDS), 2 - acute lymphoblastic leukemia (ALL). 10 patients underwent chemotherapy, 6 - allogeneic bone marrow transplantation, 3 - splenectomy and 5 - anti-thymocyte globulin therapy; 4 patients underwent plasmapheresis procedures. The remission of the underlying disease was achieved in 14 patients, resistance to therapy was observed in 4. All patients received hemocomponent therapy with individual selection using analyzer Galileo-Neo (Imucor Gamma).
The efficacy of PC transfusion was evaluated by the absolute platelet increment (API) and the corrected count increment (CCI) in the groups with refractoriness (with matching) compared to the controls without refractoriness (without matching). Also, we evaluated the interval between transfusions, which allowed for adequate hemostasis.
Result: Ninety-six PC transfusions were performed, including 72 with matching and 24 without matching (Figure 1.)
Intervals in days between the transfusions among the diseases: AML M = 3.5 (9–3), MDS M = 10 (10-7), AA M = 6 (10-2), ALL M = 4 (7-3). 14 out of the 18 patients had efficient PC transfusion followed by hemostasis correction and decontinuation of bleeding, 4 out of 18 had inefficient outcome, probably due to the increased consumption *of* blood-clotting factors.
Conclusions: Implementation of individualized selection of a donor/recipient pair, taking into account anti-HLA/HPA by cross-matching method with application of an automated immune analyzer Galileo-Neo (Imucor Gamma), enhances clinical efficacy and immunological safety, increases the absolute and corrected post-transfusion platelet increment, and reduces PC transfusion frequency. In case of non-efficient matching we should take into account non-immunological nature of refractoriness, in particular, increased consumption of blood-clotting factors syndrome.

Parameter	With matching n=72			Without matching n=24		
	M±m	Max	Min	M±m	Max	Min
API x10^9/l	23±2.9	107	-13	35±2.9	79	2
CCI x10^9/l	6,6±2.4	31	-8	14,7±0.8	34	1

Caption 1 Findings

P-424

IMMATURE PLATELET FRACTION TEST IN PATIENTS WITH LIVER DISEASE

HH Hsieh[1], KM Lu[2], JH Lin[2] and TH Lin[2]

[1]Taichung Veterans General Hospital, Taichung, Taiwan [2]Section of Transfusion Medicine, Taichung Veterans General Hospital, Taichung, Taiwan

Background: Thrombocytopenia (TP) is a common complication in patients with liver disease. The causes of TP can be increased destruction or decreased production. Human leukocyte antigen (HLA) and human platelet antigen (HPA) antibodies could increase platelet destruction when platelet transfusion refractory occurs in patients with TP. Reticulated platelets (RP) are immature platelets which reflect decreased platelet production in the bone marrow. The measurement of immature platelet fraction (IPF) is recognized as a useful marker of flow cytometric analysis of RP. Low IPF value may increase the risk of treatment for TP patients with liver disease.
Aims: To help clinicians evaluate and treat TP patients with liver disease, this study aimed to investigate the difference of IPF between patients given transfusion and patients not given transfusion.
Methods: From April to December 2015, 60 inpatients with liver disease at one medical center in Taichung were included. All inpatients were divided into two groups: non-transfused and transfused (patients receiving 1 unit of apheresis platelets within 24 h). The study also included 40 healthy adults as a control group. IPF and complete blood cell count (CBC) within 24 h prior to transfusion was performed with Sysmex XE5000 analyzer. ANOVA analysis was utilized to examine the association between platelet transfusions, liver diseases, platelet production and IPF values. This study was approved by the hospital institutional review board.
Results: Sixty inpatients included 35 liver cancers, 17 cirrhosis, and 8 other diseases. 24 hepatitis C and 21hepatitis B were positive in those patients. Platelet transfusions doesn't affect the IPF values ($P = 0.296$). However, platelet count in patients with hepatitis B, hepatitis C, liver cancer were significantly lower than control group ($P < 0.001$). The median absolute IPF significantly decreased in patients with liver cancer ($P < 0.001$).
Summary/Conclusions: Our preliminary results suggest that platelet transfusion does not alter the IPF in patients with liver disease. The platelet counts were significantly decreased in patients with viral hepatitis and liver cancer. Virus infection and tumor progression causes hepatocyte damage, which may result in decreased serum levels of thrombopoietin. The department of transfusion medicine should have appropriate medical intervention when IPF was within normal range in the patients with TP. To reduce the risks of medical intervention, a sufficient supply of HLA/HPA-matched platelet units should be prepared to meet clinical needs.

P-425

DENATURING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY/SEQUENCING METHOD FOR MUTATIONAL ANALYSIS OF THE ITGA2B AND ITGB3 GENES IN TUNISIAN PATIENTS WITH GLANZMANN THROMBASTHENIA

O Garraud[1], T Chakroun[2], C Aloui[2], J Fagan[3], S Jemni Yacoub[4], V Granados[5], A Khelif[6], N Kalhoul[6], S Hammami[7], F Cognasse[3] and S Laradi[3]

[1]INTS, Paris, France [2]University of Lyon-Saint-Etienne, Saint-Etienne, France [3]EFS Rhone-Alpes-Auvergne, Saint-Etienne, France [4]Centre régional de transfusion sanguine, CHU Farhat Hached, Sousse, Tunisia [5]Department of Genetics, University Hospital, Saint-Etienne, France [6]Clinical Hematology, Sousse, Tunisia [7]Department of Pediatrics, University hospital of Monastir, Tunisia

Background: Glanzmann Thrombasthenia (GT) is a rare inherited severe bleeding disease caused by a quantitative or qualitative defect of the αIIbβ3 integrin platelet membrane receptor. Mutations associated with GT are highly heterogeneous, mostly sporadic and occur across the *ITGA2B* or *ITGB3* genes, coding for the αIIb and β3 subunits, respectively.
Aim: This study aimed at identifying GT associated mutations in Tunisian patients using an easily accessible method such as Denaturing High-Performance Liquid Chromatography (DHPLC) associated with direct sequencing.
Methods: Three patients with GT issued from consanguineous marriage. The 3 patients are unrelated and originate from different Tunisian regions. GT diagnosis was based on patients and family bleeding histories, lack of platelet aggregation induced by ADP, collagen and TRAP and presence of normal or cyclical ristocetin-induced agglutination. 10 ml of EDTA-anticoagulated whole blood was used for DNA extraction. A hot-start/touch-down PCR program was performed followed by a heteroduplexing reaction with a known sequenced control DNA. DHPLC analysis was performed on a wave DNA fragment analysis system. To identify the type and

position of the genetic variants, amplicons with abnormal elution profiles were re-amplified, and then subjected to both forward and reverse direct sequencing.
Results: Using the same PCR program, we successfully amplified the 21 amplicons of *ITGA2B* gene and the 14 amplicons of *ITGB3* gene. Interestingly and for the three patients, DHPLC analysis of the 21 obtained amplicons of *ITGA2B* gene showed an abnormal elution profiles for two amplicons named A1 (covering exon 25-intron 25- exon 26) and A2 (covering: Ex14- In14-Ex15-In15-Ex16). Nucleotides sequence analysis of A1 identified the same homozygote point mutation in exon 26 at position **c.2702C>A**, inducing a nonsense mutation **S901X**. Sequencing of A2 revealed a new intronic homozygote SNP at position **g.8945G>A**. DHPLC analysis of the 14 amplicons of *ITGB3* gene revealed abnormal elution profiles for only one amplicon that includes exon 3. This anomaly was observed in only one patient. Sequence analyses identified a heterozygote mutation at position **c.176T>C**, resulting in a **Leu33Pro** substitution. This mutation corresponds to the HPA1a/HPA1b polymorphism of the Human Platelet Antigen 1.
Conclusion: Since the S910X mutation was previously reported in a Tunisian GT patient and not in other populations, this mutation can be frequent and characteristic of Tunisian GT patients. Further validation studies including GT patients are needed to confirm these results.

P-426

This abstract has been withdrawn.

P-427

THE ROLE OF PLATELET MICROPARTICLES IN THE TRANSMISSION OF CXCR4 CO-RECEPTOR TO DIFFERENT CELL LINES

Z Sharifi, F Yari, T Manoochehrabadi and S Soleimani

Blood Transfusion Research Center, High Institute for Research and Education, Tehran, Iran

Background: Platelet-derived microparticles (PMPs) are the most abundant microparticles in the blood and their ability to transfer materials such as arachidonic acid has been shown.
Aims: This study attempted to examine the role of PMPs as a transporter of CXCR4 co-receptor to the cell lines; U937, K562 and Nalm-6.
Methods: PMPs were isolated from platelet concentrate bags at the third day of storage by centrifugation and their concentrations were determined by Bradford method. 1 × 105 numbers of cells were incubated with different concentrations of 125,250,500 μg / ml of PMP for an hour at 37° C with 5% CO2. Nalm-6 (human B cell precursor leukemia), U937 (human macrophage, Mφ, cell line) and k562 (human erythromyeloblastoid leukemia) cell lines were cultured in RPMI1640 medium supplemented with, 10% FBS. Then, cell lines were separately incubated for an hour at 37° C with 5% CO2 with different concentrations of PMP(10–250 μg / ml). Both cells and PMP were evaluated for the presence of CXCR4 and analyzed by Partec flow cytometer and flow max software. Statistical analysis was done by with SPSS 22 software and paired-T test.
Results: Platelet surface receptor CXCR4 was transferred in co-culture of Nalm-6, K562 and U 937 with PMP. This transfer was increased with increasing the concentration of PMP, but it was not statistically significant (P-value >0.05).
Conclusion: This study demonstrated the transfer of CXCR4 into different cell Lines by various concentrations of PMPs.

P-428

INCLUSION OF MAST CELL TRYPTASE ESTIMATION DOES NOT IMPROVE QUALITY OF STORED PLATELET CONCENTRATES IN PREVENTION OF ALLERGIC TRANSFUSION REACTIONS

S Khan and SS Das

Apollo Gleneagles Hospital, Kolkata, India

Background: Allergic transfusion reactions are common in blood transfusions and occur in 1 of 50 platelet concentrate products, despite optimization of platelet quality. The causes for most of these reactions remain ill understood, including possible immunological factors such as *in-vitro* mast cell activation during storage.
Aims: To understand if mast cell activation occurs during standard storage conditions of single donor platelets over 5 days by serial measurement of tryptase levels.
Methods: Whole blood (450 ml) was collected from voluntary healthy donors in the 'Top & Bottom' quadruple bag system (Terumo Penpol, India) and separated within 6 h using the T-ACE II + automated component extractor (Terumo Penpol, India). Platelet concentrates were prepared using "buffy-coat"method and stored in platelet agitator-incubator at 20–24°C for a maximum of 5 days. Samples were collected after thorough stripping of bag tubing so that each sample optimally represented the bag sample. Samples from 24 bags of platelet concentrates for routine platelet quality control were sent to Haematology and mast cell tryptase estimation to Immunology on days 1, 3 and 5 of storage. All platelet concentrates subjected to study were released on day 5 after the sampling. Mast cell tryptase level was measured using ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden) fluoroenzyme immunoassay after a 6-point calibration with detection range 1–200 ug/l (normal plasma level non-activated <11.5 ug/l).
Results: Platelet quality control checks showed that all units were within normal pH range, retained optimal morphology (median swirling score 3), with mean volume and platelet yield of 61 ml and 5.67×10^{10} respectively per bag on day 5 of storage. Mean hematocrit on days 1, 3 and 5 were 0.8, 0.9 and 0.85 suggesting no red cell contamination in any of the platelet bags (Hct <1%). Mean (±SD) mast cell tryptase concentration in platelet concentrates on D1, D3 and D5 were 4.23(±0.74), 4.25 (±0.76) and 4.07(±0.76) ng/ml respectively with no storage associated increment (P-value 0.97 D1 vs D3; 0.76 D1 vs D5). No significant inter-bag variation was noted with respect to routine QC parameters and tryptase levels over the storage period. There were no reported adverse events on transfusion of any of the platelet concentrates. One-hour post-transfusion mean corrective count increment (CCI) in 3 patients who received the platelets under study was 14820 platelet x $m^2/\mu L$ which could be well appreciated in terms of clinical benefit.
Conclusions: Stored platelet concentrates destined for transfusions retain optimum quality until the last day of storage using currently accepted guidelines. Mast cell tryptase even on day 5 of platelet storage remained within normal range (i.e., non-activated state) and would not be considered as a transfusion reaction marker in platelet therapy. However, donor/recipient-specific mechanisms that cause allergic transfusion reactions can only be addressed via well-designed, long-term prospective studies.

P-429

This abstract has been withdrawn.

P-430

IDENTIFICATION OF ANTIPLATELET AUTOANTIBODIES BY FLOW CYTOMETRY IN PATIENTS WITH HEMATOLOGICAL DISEASES

AF Rakhmani, IV Dubinkin, EA Mikhailova, IV Galtseva, TV Gaponov, JO Davidova, NV Tsvetaeva, VV Troitskaya, EI Pustovaya and EN Parovichnikova

National Research Center for Hematology, Moscow, Russian Federation

Background: Hemocomponent therapy in patients with hematological diseases is a necessary and highly efficient method of treatment, but there are a number of complications associated with sensitization and development of refractory to platelet concentrate transfusion, which entails a chain of immunologically-mediated effects. This leads to undesirable immune processes associated with the production of antiplatelet alloantibodies (anti-HLA/HPA). Multiple transfusions can lead to a disruption of immunological tolerance which is accompanied by the production of autoantibodies (IgG, IgM) to platelets. Also, a complement system is involved in the cytolysis, which proves the importance of the identification of C3 complement component on platelets.
Aims: To evaluate the presence of the autoimmune process against the platelets by flow cytometry in patients with hematological diseases.
Methods: Twenty-six patients in the clinics of Hematological Scientific Center were followed up between September 2015 and March 2016. The average age was 39 years (19–75; M/F-8/18). Among them there were 8 patients diagnosed with aplastic anemia (AA), 7-acute myeloid leukemia (AML), 3-myelodysplastic syndrome (MDS), 6-idiopathic thrombocytopenic purpura (ITP), 2 -lymphoproliferative disease (LPD). 6 patients had no blood transfusions, 11 patients had a history of multiple transfusions of donor blood components. 3 patients with AA, 2 - with MDS, 2 - with

ITP, 2 - with LPD had splenectomy (SE), 5 patients with AA and 1 with MDS received anti-thymocyte globulin therapy, 7 patients with AML received chemotherapy, 6 patients with ITP and 2 with MDS received glucocorticoid therapy, one patient with AA underwent the plasmapheresis procedure.
C3 complement component on platelets and antiplatelet antibodies were identified by immunofluorescence, using as conjugates Goat anti-Human IgG, IgM and anti-C3, labeled with FITC. Healthy donors (n = 45) were included into the control group. The study was performed on a flow cytometer BD FACSCanto II.
Results: In patients with AML, IgM on the surface of platelets were detected in 3 out of 11 cases (27%), IgG -2/11 (18%), C3- 11/11 (100%). In patients with AA: IgM 7/13 (50%), IgG 2/13 (21%), C3 13/13 (100%). In the group of patients with MDS: IgM 4/9 (44%), IgG 4/9 (44%), C3 9/9 (100%). In patients with LPD: IgM 2/5 (40%), IgG 1/5 (20%), C3 5/5 (100%). In the group of patients with ITP: IgM 3/8 (38%), IgG 2/8 (25%), C3 7/8 (88%). Simultaneously we identified IgG, IgM and C3 in AML patients: 3 of 7, AA: 4 of 8, MDS 2 of 3, LPD 1 of 2, ITP 1 of 6. In healthy donors only in one case was registered with the presence of component C3 on the platelet surface (22%). Anti-HLA/HPA alloantibodies were also identified in patients with a history of multiple transfusions: 5 with AA: 84%, 1 with MDS: 79%, 1 with AML: 44%.
Conclusions: In patients with hematological diseases C3 complement component on the platelet surface can be used as a cytolysis marker with greater sensitivity than the IgG and IgM.

P-431

This abstract has been withdrawn.

P-432

STEM CELL TRANSPLANTS RESULT IN ANTI-CD36 THAT CAUSED PLATELET TRANSFUSION REFRACTORINESS

Y Zhou, L Li, ZL Zhong, JL Liu, M Yu, NY He, WD Shen and GG Wu
Nanning Institute of Transfusion Medicine, Nanning, China

Background: CD36 is broadly expressed on platelets, monocytes, endothelial cells etc. The incidence of CD36 deficiency was 4.13% in guangxi, China. CD36 deficiency people can generate anti-CD36 antibodies through blood transfusion, gestation and transplantation. It is rare case that stem cell transplants result in anti-CD36. A case of acute leukemia female patient who was CD36 deficiency showed platelet transfusion refractoriness after received hla-matched unrelated donor hematopoietic stem cell transplantation.
Aims to Study: The expression of CD36 on both of patient and donor. To analyze and identify anti-CD36 antibody from the patient. To identify platelet transfusion refractoriness caused by anti-CD36 antibody. And to monitor the efficacy of platelet transfusion by giving matched CD36 deficiency platelets transfusions.
Methods: The CD36 expression on platelet and monocyte was analyzed by flow cytometry (FCM) in both patient and donor. SBT-PCR was used to analyze CD36 exons sequence and HPA sequence. Fast monoclonal antibody-specific immobilization of platelet antigen (F-MAIPA) and fcm were used to identify platelet antibodies in the patient. STR-PCR was applied to monitor engraftment evidence. The platelets level was monitored by laboratory. CD36 deficiency donor platelets were chosen from CD36 deficiency donor blood bank.
Results: The donor was CD36 positive and the patient was typed I CD36 deficiency. The anti-CD36 antibody was identified in patient's serum. CD36 exons sequence analysis in the patient was shown exon 6 -1 g>c (change in splicing site) homozygote, which was a novel CD36 mutation. The patient platelet was first observed the gene types of donor STR, HPA and CD36 (complete chimerism) on day 18 after allo-HSCT. The CD36 expression on platelet and monocyte in patient was showed the positive on day 96 after allo-HSCT. The patient was showed the platelet transfusion refractoriness and significantly improved after received platelets transfusions from CD36 deficiency donors. Summary stem cell transplants result in anti-CD36 that caused platelet transfusion refractoriness was first reported in china. To ensure the efficacy of platelet transfusion, the patient should be given cd36 deficiency platelets for transfusion.

Fetal-Maternal Immunology

P-433

NEONATAL ALLOIMMUNE THROMBOCYTOPENIA CAUSED BY HLA -I ALLOANTIBODIES: CASE REPORT

X Ye, J Deng, X Xu, JL Wang, JL Liu, Y Sao, WJ Xia, YK Chen, HG Ding and DW Chen
Guangzhou Blood Center, Guangzhou, China

Background and Aims: Analyze and report the case of neonatal alloimmune thrombocytopenia caused by anti-HLA antibodies.
Methods: Four children clinically diagnosed as neonatal alloimmune thrombocytopenia were included in this study. Platelet alloantibodies (including HPA and HLA) and autoantibodies in sera of patients and their maternal were measured by ELISA method. Using PCR-SSP, we got the HPA/HLA genotype of patients and their parents. HLA-class I antibodies specificity was also tested with the luminex detection platform.
Results: We did not find platelet-specific antibodies and autoantibodies from sera of four children and their maternal. Only HLA-class I antibodies, corresponding to paternal hla antigen, were found from their sera.
Conclusion: There are many times of pregnancy, a history of miscarriage in pregnant women or foetuses suspected nait. For them, platelet antibody testing in lab should consider HLA-class I antibodies factors except HPA antibodies.

Figure 1. Results of HPA genotype in 4 children and their mothers

No.		HPA1～15 (No listed were homozygous for AA)
1	Children 1	3ab, 15ab
	Mother 1	3ab, 15ab
2	Children 2	15ab
	Mother 2	3ab, 15bb
3	children 3	All aa Homozygous
	Mother 3	3ab
4	Children 4	3bb, 15bb
	Mother 4	3ab, 15bb

Figure 2. Results of HLA antigen and HLA antibody in 4 patients with NAIT

Case No.		HLA-A	HLA-B	HLA-DR	- HLA class I ab
1	Children 1	02.11	40(60),46	07.09	-A11,-B40
	Mother 1	02.33	13.46	09.15	
2	Children 2	02.24	13.15(75)	04.11	-A02
	Mother 2	11.24	15(75),46	04.08	
3	children 3	11.33	15.27	09.12	-A11,-B15
	Mother 3	24.33	27.55	11.12	
4	Children 4	11.24	15(75),40(60)	12.12	-B07,-B27
	Mother 4	02.11	15(75),51	04.12	

P-434

RHESUS ALLOIMMUNIZATION IN PREGNANCY: ABOUT A CASE

F Adda, K Taouli and N Kadri
Faculty of Medicine, Tlemcen, Algeria

Rhesus alloimmunization in pregnancy still a major cause of fetal anemia. The most classic situation of feto-maternal alloimmunization concerns the D antigen. We bring a case of fetal maternal allo immunization in RH system due to inadequate prevention (failure to administer Rh IG) is responsible for hemolytic disease of the newborn. Aims:The goal is to optimize RH alloimmunization prevention in revealing the importance and obligation of immuno hematological surveillance pregnant woman; respecting RAI calendar and injecting adequate dose.
Patient and Observation: Mrs S., 33 years, is third gesture, third pare. She is B RH -1 group has never been transfused. Two living children were all born via a vaginal approach, Group O RH 1, but the anti-D was not received after first delivery following an blood grouping error of mother which has been corrected one week after by

a double determination of blood group ABO-RH1 (from O RH 1 to B RH -1). During her second pregnancy, she was followed regularly and any complications were reported, RAI negative and anti-D injection was administered after the birth of her second daughter.

Mrs S. gives birth to a boy, 4000 g, asymptomatic. The newborn present in D2, paleness, a significant icterus with a total serum bilirubin at 247 μmol/L, without hepatosplenomegaly, neither alteration of the general state. NFS at D1 showed no anemia; there are macrocytoses, high reticulocytosis, accompanied by a very important erythroblastosis. The leukocyte count is normal, a normal platelet count. The newborn was determined group B RH 1. The direct antiglobulin test using a polyvalent antiglobulin is negative. RAI among mother positive identify tow antibodies anti D and anti C. The diagnosis of hemolytic anemia by maternal-fetal incompatibility RH D and RH C is retained. The child is treated with intensive phototherapy.

In conclusion, red cell feto-maternal alloimmunization remain actuality because of their persistence despite prophylactic measures. Immuno hematological surveillance of pregnant woman should be obligatory in Algeria, it can detect and identify feto-maternal alloimmunization and identify the most affected children for a better therapeutic care.

P-435

ANTI-D ANTIBODIES IN PREGNANT D VARIANT ANTIGEN CARRIERS INITIALLY TYPED AS RHD POSITIVE

S Dajak[1], J Lukacevic Krstic[1], P Erceg Maglic[1], J Bingulac Popovic[2], V Dogic[2] and J Mratinovic Mikulandra[1]

[1]University Hospital Split, Spilt, Croatia [2]Croatian Institute of Transfusion Medicine, Zagreb, Croatia

Background: RhD antigen could be presented in more than 200 variants, D variants with reduced expression of the D antigen is traditionally referred to as "weak D", while "partial D"represents variants in which the mutations and gene rearrangements result in the altered protein sequence lacking certain polypeptides on the outer RBC surface. Anti-D alloimmunization are prone in some carriers of D variants, mostly partial D. The distribution of D variants varies among different populations, and their recognition depends on the choice of D typing reagents used.

Aims: The aim of the study was to evaluate the incidence and the consequences of anti-D alloimmunizations of D variant carriers in Split-Dalmatia County obstetric population.

Methods: RhD immunization events were evaluated retrospectively for a period of 20 years (between 1993 and 2012). For women who were not serologically D negative or D variant women we analyzed RHD genotype and serologic reactivity of RhD antigen. RhD alloimmunized D variant mothers were evaluated for immunization events which could cause RhD immunization, pregnancies, transfusions and RhD immunoprophylaxis. Also, we analysed the titer of anti-D antibody during the pregnancy. The neonates of D varians mothers were evaluated for RhD status, DAT, hemoglobin and bilirubin levels, transfusion therapy, phototherapy and outcome.

Results: In the observed period, out of 102,982 pregnancies tested for ABO and RhD antigens and irregular antibodies there had been 184 women affected by RhD alloimmunization, which accounts for ~1% of all pregnant women at risk for RhD alloimmunization. Of those, 181 women were serologically typed RhD negative, and 3 immunizations occurred in women who were carriers of RhD variants. RHD genotyping defined that two of three women were partial D type Va, while one woman was carrier of partial D type DNB. All three women were labeled as RhD positive in their initial serology typing of RhD antigen, due to the choice of reagents which were used at the time. Therefore, two out of three received RhD positive RBCs, and neither of them received antenatal or postnatal anti-D immunoprophylaxis. There had not been RhD immunization cases in pregnant women initially typed as D variant.

Pregnant women with partial D variants and anti-D antibody had nine pregnancies and six RhD positive children. Out of six RhD positive infants four infants had positive DAT, two needed phototherapy, but there had been no cases of severe HDFN. Titer of anti-D antibody during pregnancies were from 1:1 to 1:16 in D variants women.

Conclusion: Anti-D alloimmunization occurred in pregnant partial D carriers (DVa, DNB). RhD positive children had serologic markers of HDFN, with no cases of severe HDFN. Anti-D alloimmunized D variant mothers were labeled as RhD positive in their initial serology typing of RhD antigen, due to the choice of reagents. That implies the need for further investigation in order to make appropriate choice of reagents for serologic RhD typing which will detect D varants at risk of anti-D alloimmunization in our population.

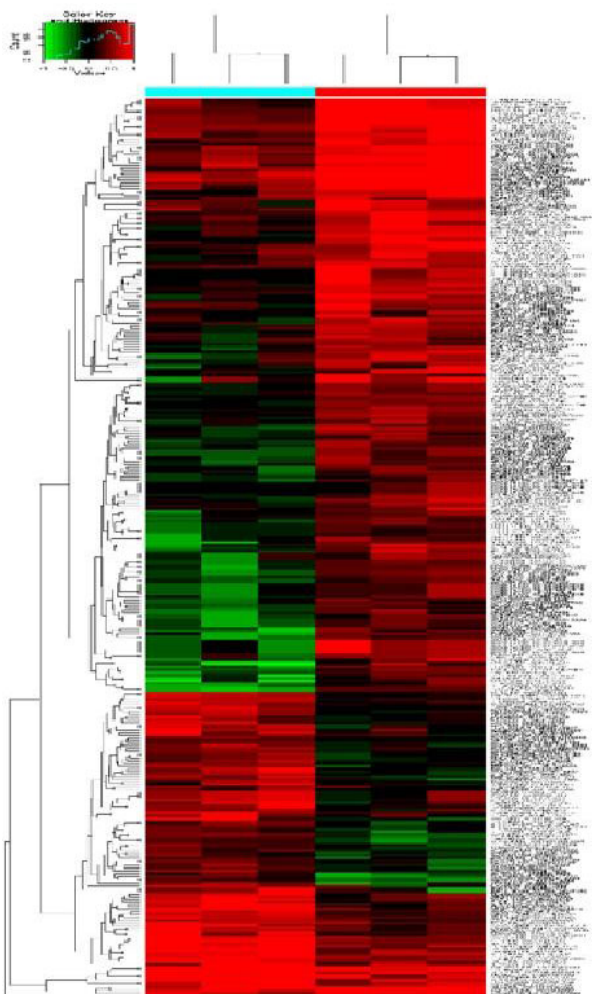
P-436

SCREENING AND IDENTIFICATION OF DIFFERENTIALLY METHYLATED MARKERS BETWEEN MATERNAL AND FETAL DNA

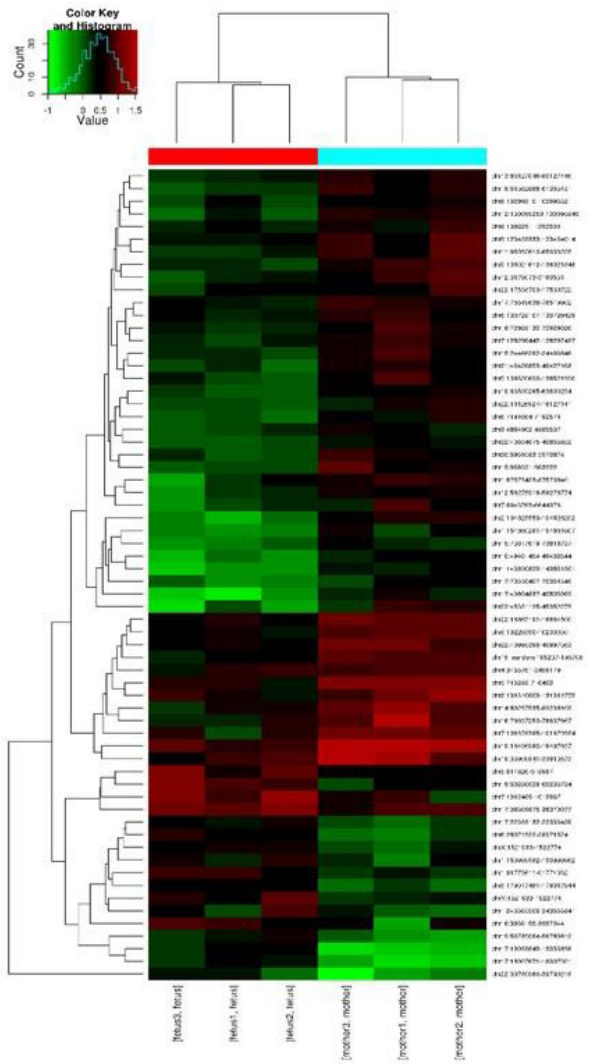
J Yu[1], C Xiao[2] and DX Huang[2]

[1]Wuhan Blood Center, Wuhan, China [2]Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China

Background: In recent years, the cases of prenatal paternity testing gradually increased in forensic practice. The traditional prenatal paternity analysiscan be performed only after invasive sampling of chorionic villi or amniotic fluid, which can result in a risk of miscarriage.The existence of circulating cell-free fetal nucleic acid in maternal plasma has brought new opportunities for the noninvasive prenatal paternity testing.



Caption 1 413 DMRs located in the promoter region heat map clustering analysis



Caption 2. 75 DMRs located in the CpG island heat map clustering analysis

Aims: To screen the DMR with significant differences between maternal fetal DNA methylation and with better frequency of SNP sites in genome-wide to provide candidate marker for noninvasive fetal prenatal paternity testing by using epigenetics.
Methods: Maternal blood cells (MBC) and fetal placental chorionic (CVS) were as the research object to systematically detect the difference of methylation level of maternal DNA by high throughput NimbleGen HG18 CpG Promoter methylation chip. The results of the chip were verified by the method of the pyrosequencing. A number of SNP markers and methylation sensitive restriction sites of the enzyme in accordance with the amplification system were selected. Finally, further validation was made in maternal plasma to evaluate the specificity, stability and sensitivity of the fetal epigenetic markers in maternal circulation.
Results: (1) A total of 185 highly methylated regions and 303 methylated regions were found in PeakDMvalue >0.5. In these 488 differentially methylated fragments, 75 (15.37%) were located in the CpG island region and 413 (84.63%) were located in the promoter region. The promoter region occupies most of the differentially methylated fragments.Differentially methylated fragments were randomly distributed on all chromosomes. Database was used to search for SNPs in the screening of 488 maternal DMR, and the popuUlation's gene frequency distribution was investigated to search for SNP sites with high polymorphism and non linkage in maternal DMR. 488 maternal DMR were screened one by one in NCBI and SPS database by us. The number of SNP sites that DM value >0.5 and SNP frequency >0.2 was 91. (2) We considered the DM values, differences in maternal and fetal fragments, SNP frequencies and enzyme cutting site positions, and eventually 15 fragments were selected to validation analysis of the subsequent pyrosequencing. In these 15 fragments, there were 3 fragments that consistent with the microarray results andcontaining the enzyme sites close to the SNP site. (3) For the chr10:101281722-101282479 (NCBI36/hg18) piece of six samples, the maternal fragments were completely cut by *Bst*UI and *Hin*P1I while the fragments of fetal villi are not cut, clear difference between them can make effective distinction. The other two pieces chr9:138021600-138022048 (NCBI36/hg18) and chr17:34251133-34251505 (NCBI36/hg18) of enzyme situation reflects a certain number of individual differences.
Conclusions: This subject has found and validated 1 methylation fragments with maternal differences in the whole genome wide range by methylation microarray screening. This fragment can be used in prenatal paternity testing as a specific marker for distinguishing between the fetus and the mother. Other two DMR have a certain number of individual differences only meet part of prenatal paternity testing requirements.

P-437

SNP-STR: A NOVEL MARKER FOR IMPROVED ANALYSIS OF UNBALANCED DNA MIXTURES

C Pan
Forensic Medicine, WuHan, China

Background: Autosomal short tandem repeats (STR) markers and single nucleotide polymorphism (SNP) are widely applied to forensic individual identification and paternity testing. However, in the samples that containing unbalanced DNA mixtures from two individuals commonly happen both in forensic mixed stains and in peripheral blood DNA microchimerism caused by pregnancy or following organ transplant. Traditional forensic DNA analysis has limited power in addressing this problem. Hall et al. had established a new compound marker, Deletion/Insertion-STR (DIP-STR), to analysis unbalanced DNA mixtures. Unfortunately, DIP are fewer than SNP in human genomes, therefore, it is necessary to find a new genetic marker including a large number of loci with high polymorphism that allows detection of the minor DNA contributor in the DNA mixtures. To this end, we propose a new type of genetic marker named SNP-STR, which is a compound genetic marker combining a STR locus with tightly linked SNP.
Aims: To build a 6-loci SNP-STR fluorescent-multiplex PCR for forensic purpose, investigate the genetic polymorphism of these loci in Wuhan Han population, explore its sensitivity and assess its capacity of resolving unbalanced DNA mixtures.
Methods: Six SNP-STR markers were selected from the UCSC genome browser. To increase the specificity of primers, a deliberate mismatch was introduced to the allele-specific primers at 1–2 bp from the 3'-terminus respectively. Pairs of SNPs and STRs linked to each other and at a distance of less than 150 bp. The method amplifies the target SNP using the amplification refractory mutation system. The two allele-specific primers are labeled by different fluorescent and the reverse primer is located at the other side of the corresponding STR sequence which is linked to the SNP. Through this method, alleles of the STR can be genotyped by the size of the amplicons and the SNP can be genotyped by the different colors of the amplicons in one reaction. Then we genotyped 300 unrelated individuals from Wuhan Han population with the established multiplex. Finally, we calculated its polymorphic parameters and explored the capacity of identifying unbalanced DNA mixtures.

Caption 1 Sample electropherograms of the SNP-STRs multiplex run with 1uL PCR product of one sample

Results: A 6-loci fluorescence-multiplex PCR was established successfully. Compared with the single-loci genotyping results, it was consistent with the corresponding results from the multiplex. The minimal amount of DNA template required for successful amplification using the multiplex is 0.1 ng. Six SNP-STR loci were highly polymorphic in Wuhan population. In mixture study, this novel analytical approach allows for the unambiguous genotyping of a minor component in the presence of a major component, where SNP-STR genotypes of the minor were successfully acquired at the ratios up to 1:130.
Conclusions: We set up an effective methods to genotype for DNA mixture. A new 6-loci SNP-STR multiplex PCR was successfully developed in this study. The results of population genetics investigation on these loci in Wuhan population provide the basic data for the forensic applications. The multiplex has such advantages as high polymorphism, high stability, repeatability and satisfactory sensitivity. We expect that the multiplex will detect the DNA of the baby even in early pregnancy despite the slight reduction of the expected DNA quantity. This new genetic maker will provide more significant clues for forensic individual identification.

P-438

ESTIMATING FETO-MATERNAL HAEMORRHAGE ON MATERNAL SAMPLES USING HBF% BY CAPILLARY ELECTROPHORESIS

S Khan and SS Das
Apollo Gleneagles Hospital, Kolkata, India

Background: Feto-maternal haemorrhage (FMH) mainly occurs due to Rh/ABO incompatibility and responsible for 14% of all unexplained fetal deaths. Accurate determination of numbers of Rh-D positive cells in circulation of Rh-D negative women allows administration of the correct amount of Rh immune globulin.
Aims: We determined the feasibility of measuring HbF% on fetal erythrocytes as an estimate of FMH using capillary electrophoresis (CE) as it is a rapid, analytically simple and completely automated method.
Methods: Serial dilutions of adult D-negative red cells were incubated in the presence of various amounts of fetal D-positive cells (0.06, 0.12, 0.25, 0.50, 0.75, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0 and 7.0%). After incubation, the samples were tested for HbF% using CE (Capillarys2 Flex Piercing, SEBIA). The instrument uses capillary separation technology and has cap piercing capability to provide whole blood analyses for hemoglobinopathies. This method was compared against the previously published gel agglutination technique (GAT). Mothers with hemoglobinopathies were excluded in this proof-of-concept study. Statistical analysis was performed using Microsoft Excel 2013.
Results: HbF peak was not detected at first 3 concentrations (0.06, 0.12, 0.25%) and identified at 0.50% fetal RBC (HbF peak at 0.2%), 0.4 HbF% at 0.75% RBC. The best-fit curve regression analysis showed increase in HbF% at subsequent concentrations ($y = 0.5251x-0.0904$) with R^2=0.9394, suggesting excellent linear reliability. There was good agreement with GAT.
To establish the HbF% that could determine significant FMH (ml), we devised the formula, FMH (ml) = [HbF% x 1.22 x maternal Hct x 100 ml/kg bw)]/1000. Using above formula (considering Hct 0.28 and maternal blood volume at term to be 6L), a HbF% at 0.2% calculates to FMH 0.41 ml and 0.7 HbF% calculates to a significant volume of 1.43 ml FMH.
Conclusions: FMH estimation using HbF% by CE is rapid and simple but prior knowledge of HbF% is required for mothers with hemoglobinopathies. Studies comparing CE with the established flow cytometry technique are required to further explore its utility as an effective screening tool.

P-439

EDTA-DEPENDENT PSEUDOTHROMBOCYTOPENIA (PTCP) IN THE NEONATE DUE TO THE MOTHER: CASE REPORT

M Tomicic[1], T Sotonica Piria[2], V Dogic[1], S Jagnjic[1], N Jurakovic-Loncar[1] and I Jukic[1]
[1]Croatian Institute of Transfusion Medicine, Zagreb, Croatia [2]Virovitica General Hospital, Virovitica, Croatia

Background: Pseudothrombocytopenia (PTCP) is a phenomenon of falsely low platelet count determined on hematology analyzers due to platelet clumping in ethylenediaminetetraacetic acid (EDTA) anticoagulated blood. In EDTA-PCTP platelet autoantibodies directed to the platelet membrane GP IIb-IIIa complex modified by EDTA induce *in vitro* platelet aggregate or agglutinate formation, which finally leads to a falsely decreased platelet count. Blood smear done from EDTA anticoagulated blood searching for platelet aggregates, along with determination of complete blood count (CBC) in EDTA and sodium citrate anticoagulated blood, can help in the diagnosis of EDTA-PTCP.EDTA-PTCP occurs at a frequency of approximately 0.1% in the general population and 0.15% in hospitalized patients. According to literature data, EDTA-PTCP in the neonate due to mother has been reported only in few cases.Aim: We report on a case of EDTA-dependent PTCP in a neonate due to transplacental transfer of maternal EDTA-autoantibodies of IgG class.
Case Report: A female newborn, birth weight 3250 g, born from first uncomplicated pregnancy to a healthy 23-year-old mother in 39th week of gestation. Mild thrombocytopenia (82 to 100 × 109/L) was detected on the first day of the newborn's life on routine laboratory checkup. Other laboratory findings were normal. No signs of hemorrhage were present. On day 3, platelet count dropped to 64 × 109/L and the newborn was transferred to the Neonatology Ward to investigate the etiology of thrombocytopenia. Upon excluding perinatal infection and congenital malformations, serology of alloimmune neonatal thrombocytopenia was indicated. Serologic screening of maternal and neonatal EDTA anticoagulated blood samples using direct and indirect immunofluorescence method for antiplatelet antibodies (manual PSIFT) was positive. Serology of the maternal and neonatal sera for specific antiplatelet alloantibodies (anti-HPA) by using the monoclonal antibody immobilization of platelet antigens (MAIPA) assay was negative. HPA (1–15) genotyping of the mother and the neonate showed no incompatibility. As direct manual IF test of maternal and neonatal platelets showed platelet aggregates, blood smear from EDTA anticoagulated blood sample was done, along with CBC on hematology analyzer. Laboratory screening for EDTA-PTCP was positive. Recollection of the maternal and neonatal blood samples using sodium citrate anticoagulant confirmed the diagnosis. Platelet count in newborn's EDTA blood was 101 × 109/L and in citrate blood 695 × 109/L. Platelet count in mother's EDTA blood was 133 × 109/L and in citrate blood 369 × 109/L. In leukocyte histogram provided by automated cell counter, platelet clumps of less than 35 fl appeared in EDTA blood of the mother and the neonate, but not in the citrate blood. On 3-week follow up, platelet count in newborn's EDTA blood was 46 × 109/L and in citrate blood 382 × 109/L. On 6-week follow up, platelet count in newborn's EDTA blood was normal (288 × 109/L).
Conclusion: Although PTCP is a rare phenomenon, it should always be excluded in patients with newly detected thrombocytopenia to avoid erroneous interpretation of platelet and leukocyte count, unnecessary and false positive serology workup for antiplatelet antibodies and needless platelet transfusions.

P-440

NEONATAL AUTOIMMUNE HEMOLYTIC ANEMIA IN PATIENT WITH DIGEORGE SYNDROME

KK Hellstrom, HS Alhumaidan and E AlZaabi
Sheikh Khalifa medical city / SEHA, Abu Dhabi, United Arab Emirates

Background: The development of warm autoimmune antibody to red blood cell antigens is rarely observed during the neonatal period. However, patients with DiGeorge Syndrome are particularly vulnerable and can develop autoimmune disease at a higher prevalence during childhood and adulthood including autoimmune idiopathic thrombocytopenic purpura (ITP) and autoimmune hemolytic anemia (AIHA).
Aim: Report a case of a neonate diagnosed with DiGeorge Syndrome who presented at birth with warm autoantibody and no evidence of maternal antibodies.
Case Report: This report describes a case of a full term male infant who was born to a 22 year old female after an uneventful pregnancy. Upon birth, his direct antiglobulin test (DAT) was positive and at five days of life, he was transferred to our institution for an interrupted aortic arc repair. Laboratory investigations upon admission revealed a pan-reactive indirect antiglobulin test (IAT) in plasma, in addition to IgG and C3d positive DAT. Immunoglobulin subclass IgG1 was detected in low concentration. Eluate showed strong pan-reactivity with panel cells both by column agglutination and tube techniques. The mother's antibody screening test and DAT were both negative and despite extensive testing with different techniques and enhancement procedures, no antibodies were detected in the mother's plasma. Patient Hemoglobin (Hb) was initially122 g/L which gradually dropped to 70 g/L accompanied by high total and indirect Bilirubin. AIHA was suspected and the patient received steroids and IVIG to control his hemolytic process prior to surgery. Post-operative diagnosis was consistent with Digeorge syndrome characterized by absence of thymus. During the post-operative course, patient received multiple blood transfusions to maintain his hemoglobin, his bilirubin level gradually decreased to normal level (13.7micromol/L) on 22 days of life and his Hb stabilized. Tests for other immunological disorders including ENA, ANA and ANCA tests were all negative. Multiple infectious serology workups were unremarkable on initial admission.

Table 1:
Immunohematology work-up at first week of life

Test	Neonate	Mother
Blood Group	O Rh positive	B positive
Phenotype (Rh and kell)	C+,c+,E-,e+,K-	C+,c+,E-,e+,K-
DAT	IgG 3+, C3d: 3+	Negative
Elution	pan-reactive	Negative
Antibody screening	2+ pan-reactive	Negative with enhancement
Antibody panel	2+ pan-reactive	Negative
Cross-matched	**With group O units:** 2+ incompatible **With mother's red cells:** 2+ incompatible	**With group O units:** compatible

Caption 1 Neonate and maternal blood bank evaluation

Immuno-phenotypic study showed a significantly reduced level of CD4 T-cells and normal CD20 B-cells. Subsequently, he was admitted on multiple occasions for recurrent episodes of infections precipitating coagulopathy, airway problems, and episodes of hypocalcemia. His DAT remained strongly positive with both IgG and C3d during the course of his life. The patient was deceased at the age of eight months due to sepsis and respiratory arrest.

For blood bank evaluation, refer to **Table 1.**

Discussion: AIHA has been described in patients with DiGeorge Syndrome in childhood but rarely during the neonatal period; however, we report an early detection of an autoantibody, which is not maternal in origin, in a neonate who was subsequently diagnosed with DiGeorge syndrome. Not surprisingly, the absence of thymus in this patient would account for lower CD4 + regulatory T-cells, a thymic derived population that is essential for immune self-tolerance and defense against infections. Notably, the hemolytic process which stabilized by IVIG and steroid administration was consistent with a first line therapeutic option been described in similar cases. Our patient had an unfavorable outcome and died at the age of 8 months secondary to severe pneumonia. To our knowledge, two reported cases with neonatal AIHA had a fatal outcome, neither was reported to have DiGeorge syndrome.

Conclusion: The current case depicts the development of possible in-utero IgG-warm autoantibody complicated by a hemolytic episode early in life likely due to DiGeorge syndrome.

P-441

A CASE OF SUSPECTED CONFINED PLACENTAL CHIMERISM FOLLOWING DISCORDANT FETAL RHD SIGNALS IN MATERNAL PLASMA AND THE NEWBORN SEROLOGY

CA Hyland[1], GJ Gardener[2], S Baidya[1], H O'Brien[1], G Millard[1], EC McGowan[1], GH Lopez[1], EM Schoeman[1], YW Liew[1] and RL Flower[1]

[1]Australian Red Cross Blood Service, Brisbane, Australia [2]Mater Centre for Maternal Fetal Medicine, South Brisbane, Australia

Background: Non-invasive prenatal testing (NIPT) for fetal *RHD* genotype assessment has been performed for pregnancies at risk of Haemolytic Disease of the Fetus and Newborn (HDFN) by the Australian Red Cross Blood Service since 2009. A small proportion of these requests arise from cases of fetal maternal haemorrhage (FMH) where results may influence anti -D usage.

In late 2015, an RhD negative woman at 33 weeks gestation was administered a total of 1 × 625 IU and 4 × 1500 IU of anti-D for an estimated FMH of 68 ml by the Kleihauer method (46.6 ml by flow cytometry) following a motor vehicle accident. A maternal blood sample was referred for fetal *RHD* genotyping and testing predicted an RhD positive fetus with 10 of 12 positive *RHD* replicates. A further 4 × 1500 IU of anti-D was given. The following day, the baby was delivered and cord blood serology was RhD negative. The hospital reported the discrepancy to the Blood Service, triggering an investigation.

Aims: To identify the cause of the *RHD* genotype and RhD phenotype discrepancy.

Methods: A second pre-delivery maternal blood sample was forwarded to the laboratory with cord blood and a formalin-fixed section of placenta. Post-delivery maternal and paternal whole blood was collected for DNA blood group evaluation. Cell-free DNA was isolated from maternal plasma for fetal *RHD* genotyping. Genomic DNA was isolated and genotyped using Immucor GAMMA BioArray[TRADEMARK] RHCE and RHD BeadChip [TRADEMARK]. An in-house test to evaluate paternal *RHD* gene zygosity was performed. Placental gDNA was sequenced using Illumina® TruSight One Sequencing Panel with data for non-blood group related genes masked during analysis. The batch of anti-D administered was identified and tested for *RHD* sequences.

Results: The second pre-delivery maternal sample produced inconclusive results for fetal *RHD* (6 of 12 replicates amplified). Post-delivery maternal samples showed complete clearance of plasma *RHD* sequences. SNP genotyping results for maternal gDNA and cord gDNA were *RHD* negative. Paternal gDNA was hemizygous for *RHD*. DNA derived from four sections of placental tissue failed to produce *RHD* signals on qPCR. This was corroborated by massively parallel sequencing which showed a complete lack of *RHD* sequence reads. *RHD* was not detected in the anti-D preparation.

Summary/Conclusions: Discordant blood group genotyping results between fetal DNA and newborn serology can occur due to assay specificity limitations, pre-analytical errors, or presence of *RHD* variants. Another potential cause is confined placental chimerism (Thurik, Prenatal Diagnosis, 2015), the presence of a second cell line in the placenta due to the demise of a co-twin. Detection of 'fetal' *RHD* sequences in independent maternal samples eliminated sample mix-up as a cause for false positive fetal *RHD* results. Absence of maternal and infant *RHD* sequences, and presence of a single paternal *RHD* gene all exclude the presence of an *RHD* variant, and permit a scenario of *RHD* inheritance by a co-twin. While analysis of the placenta did not demonstrate a second cell line, this might have been accomplished via more extensive tissue sampling from the entire placenta. Beyond unnecessary usage of anti-D immunoglobulin, the clinical impact of false positive fetal *RHD* assignment is low, but this case highlights the potential contribution of underlying biology to assay performance.

P-442

FETAL RHD GENOTYPING IN WOMEN WHO RECEIVED A BONE MARROW TRANSPLANTATION

B Veldhuisen[1], FF Thurik[1], GCML Page-Christiaens[2], A Ait Soussan[1], P Ligthart[1], G Cheroutre[1], B Bossers[1], CE van der Schoot[1] and M de Haas[1]

[1]Sanquin, Amsterdam, The Netherlands [2]University Medical Center, Utrecht, The Netherlands

Background: Hemolytic disease of the fetus and newborn (HDFN) caused by maternal alloantibodies against RhD is a potentially life-threatening disease for the fetus. To determine if a fetus is at risk, non-invasive fetal *RHD* genotyping on cell-free DNA isolated from maternal plasma is performed. To prevent allo-immunization and guide targeted anti-D immunoglobulin prophylaxis, non-invasive fetal *RHD* genotyping is performed routinely in the Netherlands for all RhD-negative women in the 27th week of pregnancy. In the majority of the pregnant women, a true-positive or true-negative result is obtained but false-positive and false-negative genotyping results have been described due to a variety of causes. False-negative results are often due to low concentrations of fetal DNA. False positive results occur in case of *RHD* variant genes with extremely low or no RhD expression. We present two cases where non-invasive fetal *RHD* typing was complicated due to previous bone marrow transplantation.

Aims: To elucidate the cause of discrepant results observed during non-invasive fetal genotyping on cell-free DNA isolated from maternal plasma.

Methods: A pregnant RhD-negative woman presenting with anti-D alloantibodies in the first trimester, was genotyped using a duplex real-time polymerase chain reaction (PCR) targeting *RHD* exon 5 and 7 (in triplicate) on two separate maternal plasma DNA isolations to determine if her fetus is at risk to develop HDFN. Multiplex ligation-dependent probe amplification (MLPA) was used to determine the maternal and paternal genotypes and the genotype of the child after delivery. For the second pregnant RhD-negative women, the fetal *RHD* genotype was defined by screening *RHD* exon 5 and 7 in triplo in the 27th week of pregnancy using an automated system of DNA isolation, experimental setup and testing. Both ABO and RhD antigens were determined serologically. Anti-D immunoglobulin prophylaxis is advised in 30th week of pregnancy and post-delivery in case of an *RHD*-positive fetus.

Results: In two women with a history of allogeneic bone marrow transplantation in early childhood, a positive fetal *RHD* genotype was observed. Both were born RhD-positive and received a transplant of their RhD-negative male sibling. In the first case, the woman had developed anti-D shortly after transplantation or after elective abortion during the first pregnancy. The biological father was RhD-positive and genotyped as *RHD* variant (DUC2/d). An RhD-negative male was born (dd genotype).

The false-positive fetal genotyping was caused by the presence of maternal *RHD*-positive cell-free DNA in her plasma. In the second case, we observed a discrepancy upon ABO typing, with a very small population of A, RhD positive red cells amongst B, RhD negative red cells. In this woman, the antibody screen was negative and upon fetal *RHD* typing anti-D immunoglobulin prophylaxis was administered. An RhD-positive female was born (true-positive fetal genotyping).
Summary/Conclusion: False-positive results of non-invasive fetal *RHD* genotyping may occur in RhD-positive women transplanted with bone marrow of an RhD-negative donor, due to circulating cell-free DNA originating from non-hematopoietic tissue. The cases highlight that allogeneic bone marrow transplantation can cause false-positive results in fetal *RHD* genotyping using cell-free DNA from maternal plasma.

P-443
EVALUATION OF PHOCINE HERPESVIRUS (PHHV) AS AN INTERNAL CONTROL FOR PRENATAL RHD GENOTYPING IN MATERNAL PLASMA

A Javadi[1], B Veldhuisen[1], M de Haas[2] and E van der Schoot[1]
[1]*Sanquin Research, Amsterdam, The Netherlands* [2]*Sanquin Diagnostic, Amsterdam, The Netherlands*

Background: RhD-negative pregnant women in the Netherlands are routinely screened by duplex *RHD* real-time PCR (exon 5 and 7) to detect *RHD* in cell-free DNA isolated from maternal plasma in the 27th week of pregnancy to predict the D-blood group status of the fetus and guide anti-D immunoglobulin prophylaxis administration. Maternal plasma contains 3–10% cell-free fetal DNA of extremely low concentration. In the absence of a universal fetal marker, the risk of non-invasive fetal genotyping is the occurrence of false-negative results due to low fetal DNA concentration, the presence of inhibitors during PCR or errors in the test procedure. The inclusion of an internal positive control in the plasma sample prior to automated DNA extraction can prevent some of these false-negative test results.
Aims: Evaluate the clinical performance of phocine herpes virus (PhHV) as an internal positive control to monitor DNA extraction procedure and PCR amplification.
Methods: Cell-free DNA was isolated from 1 ml plasma using the a MagNAPure96 (Roche Diagnostics). The elution volume was 50 µl. A plasmid with a 89 bp PhHV insert was used as internal control. A triplex PCR amplifying PhHV, *RHD* exon 5 and 7 was developed using MGB-probes labeled with NED, FAM and VIC, respectively. A PCR targeting *ALB* was used as a control for DNA input. To determine the effect of the PhHV-PCR on amplification of *RHD* exons, and vice versa, dilutions of the virus (0 to 100,000 copies/PCR) were added to 100 ng genomic DNA of D-pos and D-neg individuals. In addition, a fixed amount of PhHV (100,000 copies/PCR) was added to variable DNA concentrations (5 to 50,000 pg). The Limit of Detection of the triplex genotyping assay was determined in the presence and absence of PhHV. The recovery and reproducibility of the assay was tested using PhHV-spiked (3500copies/ml plasma) plasma pools of D-negative pregnant women.
Results: We showed that simultaneous amplification of PhHV does not influence PCR efficiency of the *RHD* assays. Spiking 100,000 copies PhHV to variable genomic DNA input showed similar amplification of exon 5 and 7 with and without virus. Adding variable amounts of virus to 100 ng of genomic DNA, PhHV could be detected (at least10 copies) and *RHD* exon 5 and 7 amplification was not affected by high PhHV concentrations. The limit of detection was similar with and without PhHV.
We demonstrated that adding PhHV to plasma does not influence the isolation of fetal and total cell-free DNA from maternal plasma. The total DNA, measured by albumin PCR, as well as fetal DNA as measured by *RHD* PCR, was the same in 30 different plasma pools from D-negative women pregnant from a D-positive fetus.
Summary/Conclusions: In conclusion, the triplex real-time PCR assay, in which the internal positive control PhHV is included, has exactly the same sensitivity and specificity as the previously validated *RHD* exon 5/7 duplex PCR. Inclusion of PhHV will prevent false-negative fetal genotyping results due to failures in DNA isolation or to the presence of PCR inhibitors, thereby further increasing the accuracy of non-invasive fetal *RHD* genotyping.

Granulocyte Immunology

P-444

This abstract has been withdrawn.

P-445
DEVELOPMENT OF A NOVEL HNA GENOTYPING USING LUMINEX SYSTEM

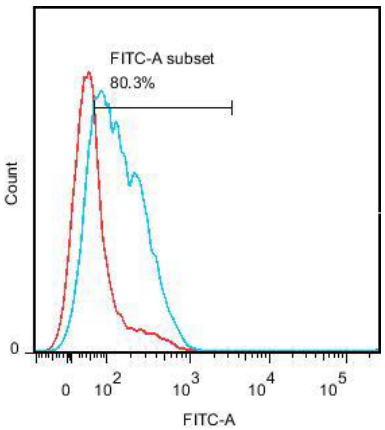
D Takahashi, S Onogaki, T Miyazaki, W Ohashi, S Sato, T Kato, H Ikeda, T Yamamoto, S Kino and S Takamoto
Japanese Red Cross Hokkaido Block Blood Center, Sapporo, Japan

Background: Antibodies against human neutrophil antigens (HNA) were involved in the clinical conditions such as transfusion-related acute lung injury (TRALI), febrile transfusion reaction (FTR) and neonatal alloimmune neutropenia (NAIN). In these conditions, identification of patient HNA type is very important for a determination of the antibody specificity involved in TRALI or NAIN.Instead of phenotyping which sometimes causes nonspecific reactions, the HNA genotyping has become popular. Although the genotyping of HNA-1, -3, -4 and -5 have been reported by using various methods such as PCR-SSP, PCR-RFLP and PCR-SBT, they are not suitable to test a large number of samples because they require the separate amplification and analysis of each allele.
Aims: In this study, we tried to develop multiple genotyping system using a specific array of color -coded fluorescent microspheres from Luminex Technology (Luminex Corporation) to identify the alleles coding for HNA-1a, -1b, -1c, -3a, -3b, -4a, -4b, -5a and -5b.
Methods: Peripheral blood was obtained from healthy blood donors in EDTA tubes, and genomic DNA was extracted using commercial kit. A total of 450 random healthy donors were determined for their alleles of HNA-1, -3, -4, and -5. Forward or reverse primers to amplify the polymorphic regions of HNA-1, -3, -4 and -5 were biotinylated at 5-prime end. Sequence specific probes for HNA-1 were designed using a polymorphism at position 222A, 277A and 266A and HNA-3, -4 and -5 at position 461(G>A), 302(G>A) and 2372(G>C), respectively. A coupling of specific probe to microsphere was performed by recommendation by Luminex Corporation. An identification of HNA alleles was conducted by Luminex rSSO assay with minor modification and the reactivity of each probe was evaluated by fluorescence intensity. Furthermore, HNA-1 and -3 phenotypes were confirmed by GIFT-FCM test using monoclonal antibodies or alloantisera.
Results: The multiplex bead assay was validated using 450 samples and each reactivity of sequence specific probe was very clear. We determined allele frequencies in the Japanese blood donors as follows; 0.603 for HNA-1a, 0.397 for HNA-1b, 0.000 for HNA-1c, 0.582 for HNA-3a, 0.418 for HNA-3b, 1.000 for HNA4a, 0.000 for HNA-4b, 0.892 for HNA-5a and 0.158 for HNA-5b. A HNA null was found in two cases. The gene frequencies of HNA-1, -4 and -5 in Japanese are found to be strikingly different from those of Caucasians which have been previously reported. Difference of frequencies of HNA-1 and - 3 genotypes are rather slight among Asian population. Therefore, risk of HNA alloimmunization would be expected to be different in Asians and Caucasians. A genotype of HNA-1 showed 100% concordance with the phenotype in all samples. However, discordance was observed in 28 cases (6.22%) in HNA-3. These mismatches were probably caused by non-specific reactions in serological tests using aloantisera. Accordingly, we consider that genotyping of HNA-3 is more exact than serological phenotyping.
Summary: A novel method of HNA genotyping using Luminex system was simple, exact, and high throughput and will be useful for TRALI or NAIN study in clinical labotatory settng.

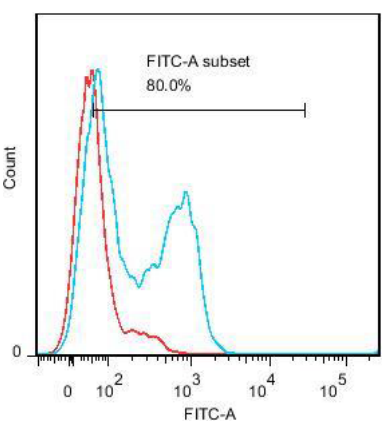
P-446
SCREENING FOR LEUCOCYTE ALLOANTIBODIES IN PATIENTS WITH MULTIPLE HISTORY OF PLATELET TRANSFUSION

W Xia, J Liu and X Ye
Guangzhou Blood Center, Guangzhou, China

Background: Transfusion-related acute lung injury (TRALI) is known as a life-threatening complication of transfusion. HLA and HNA antibodies have been associated with the immune pathway of TRALI.



Caption 1. Anti-HNA-3b antibody express with GIFT



Caption 2 Anti-HNA-1b antibody express with GIFT

Aim: To investigate the association of a history of platelet transfusions with the occurrence of HLA and HNA alloimmunization.
Study Design and Methods: Blood samples were collected from 89 patients with multiple platelet transfusions (n > 5). HLA class I, II antibodies and Anti-HNA-1,-2,-3,-4,-5 antibodies were screened by the LABscreen Assay (One Lambda). Anti-HNA antibodies were confirmed by the granulocyte immunofluorescence test (GIFT).
Results: Screening of the total 89 patients (42 females and 47 males) showed higher prevalence of HLA antibodies. We found antibodies against HLA class I (89.9%), HLA class II (68.6%) and both (66.3%) in these patients.
When sera from 89 patients were screened for neutrophil specific antibodies, two samples were demonstrated HNA antibodies (2.2%). One of the samples was HNA-3b specific (Figure 1) and the other one was anti-HNA-1b antibody (Figure 2).
The two patients HNA antigens were HNA-1aa,3aa,4aa,5aa and HNA- 1aa, 3ab,4aa, 5aa.
Conclusion: In this study, we found high alloimmunization against HLA class I and II antigens in multiple platelet transfusion patients. Alloimmunization against HNA was espressed in these patients, too. These alloantibodies formed when the immune system of individuals with blood cells negative for an antigen was exposed to blood cells carrying the antigen.

Clinical Transfusion: Neonatal and Pediatric Transfusion

P-447

TWO CASE REPORTS OF HAEMOLYTIC DISEASE OF THE NEW BORN DUE TO RHESUS D ANTIBODY

WMI de Alwis, TMGT Priyamali, SIN Wickramasinghe and HDSM Bandara
National Blood Transfusion Service, Ratnapura, Sri Lanka

Introduction: Haemolytic disease of the New born occurs, because of haemolysis of neonatal Red Blood Cells(RBCs) due to RBC defects itself and external causes like placental transfer of maternal RBC antibodies. ABO antibodies accounts for majority of less severe HDN cases, while clinically significant titres of Rhesus D antibody result in moderate to severe disease. In 2009, a study was done at National Reference laboratory, Sri Lanka, detecting 28 antenatal women with clinically significant antibodies. 24 had anti D, 3 had anti D and C, and 1 had anti c.
Aim: To compare the clinical picture and investigation findings of two HDN cases.MethodsCase histories were extracted from records of antenatal clinics (ANC), bed head tickets and Transfusion reports.
Case Reports: Mrs C was an ANC woman of 40 years. She had Anti D and Anti C titres of 128 and 8 respectively, at 26 weeks of the third pregnancy with past 2 uncomplicated deliveries, covered by postpartum anti D immunoglobulin. She was group O rr. Partner was group O R1R1. She had no history of blood transfusions. She was followed by the Obstetrician and Transfusion Physician at 2 weekly intervals with no change in antibody levels till 37 weeks, where she had Anti D and Anti C titres of 512 and16. Therefore she was admitted and labour was induced. Active but pale Baby girl was delivered vaginally. Baby was straightaway taken to Special Care Baby Unit (SCBU). The Baby was given double phototherapy, intra venous immunoglobulin (IVIG) and (as her Haemoglobin (Hb) dropped to 8.1 g/dl with bilirubin; direct 19.4umol/L & total 92.6 umol/L at 20 h) two top up transfusions on Day 1 and 2 (Hb rose to 14.6 g/dl). In the follow up clinic, the baby had Hb of 6.7 g/dl at 7 weeks and corrected to 9.2 g/dl with top up transfusions.Group O rr, Mrs P was 28 years, admitted to Obstetrics ward with labour pain at term in the fourth pregnancy. She had a live child, two abortions (no records of anti D immunoglobulin cover) and no history blood transfusions. She delivered lethargic baby girl with jaundice. She was found to have Anti D titre of 64. The baby was given double phototherapy, IVIG and an exchange transfusion, as the bilirubin; direct 11.5umol/L and total 265 umol/L at 6 h with Hb of 11.8 g/dl. In the follow clinic, the baby remained well.
Results: Baby of Mrs C: grouped as O Rh D negative, direct antiglobulin test (DAT) 4 + with IgG specificity. Elution studies revealed anti D, which has blocked D antigen sites on RBCs, leading to wrong Rh D grouping as D negative. Baby of Mrs P was O Rh D positive, direct antiglobulin test (DAT) 4+ with IgG specificity. Elution studies revealed anti D.DiscussionEven though anti D titres do not directly correlate with the clinical pictures of these two cases, they were higher than 32, which is usually considered as the clinically significant. Higher level of anti D titre, causing more severe anaemia (i.e. less amount of RBCs) in Baby of Mrs C, have led to lesser concentration of bilirubin, needing less invasive top up transfusions.

P-448

GRANULOCYTE TRANSFUSION THERAPY EXPERIENCE IN SAMIC GARRAHAN PAEDIATRIC HOSPITAL

R Alba, G Marcarian, L Trillini, J Boo and AM Pugliese
Juan P Garrahan Paediatric Hospital, Buenos Aires, Argentina

Background: The development of new chemotherapy treatments, immunosuppressant drugs, as well as the advances on stem cell transplantation led to an increase in patients presenting severe neutropenia and life threatening infectious diseases. Granulocyte transfusion was developed as an adequate support therapy for patients suffering from severe neutropenia and broad spectrum of antibiotic resistant infections until the resolution of the infection or neutrophil recovery.
Aim: Describe the experience of granulocyte transfusion therapy in paediatric patients who were admitted in our hospital from September 2012 to May 2015.
Methods: A retrospective study was conducted on 16 patients with severe neutropenia, bacterial or fungal infections resistant to antimicrobial treatment and reasonable hope for bone marrow recovery. Two patients required granulocyte transfusion

therapy in two separate occasions. Severe neutropenia was defined as absolute neutrophil count under 500 cells/mm3.
Results: The women/men ratio was 3 to 1. The median age was 3 years old (range 0.5–15.6). The most frequent underlying disease was aplastic anemia and the reported infections were 27.7%bacterial, 33.3% fungal and 33.3% both; and 37.5% of the patients presented parasitic or viral co-infection (12.5% and 25% respectively). The infections were diagnosed by germ cultivation (94.4%), local biopsy (66.6%) and diagnostic imaging (72.2%). In 66.6% of the cases, the infection was diagnosed by more than one method. 72.2% of the patients were admitted at the intensive care unit (ICU) from which 92.3% was on mechanical ventilation. A total of 289 granulocyte transfusions were performed with a median of 13.5 transfusions per patient (range 4–40). The median dose was 2.63xE10 (range 0.3–8.48xE10). The reasons for interrupting the treatment were 16.6% recovery of neutrophil count, 44.4% death of the patient, 16.6% insufficient volunteer donations and 22.2% due to infection resolution.
Conclusions: There has been a great improvement in the granulocyte transfusion therapy. To date, we have had positive results with only 2.1% of adverse effects to the granulocyte transfusions and 65% of survival.

P-449

TRANSFUSION THRESHOLDS FOR BLOOD COMPONENTS IN A PEDIATRIC INTENSIVE CARE UNIT

FY Ayhan, O Sarac Sandal, F Sari, G Ceylan, H Sarihan, R Isguder and H Agin
Dr Behcet uz Children's Hospital, Izmir, Turkey

Background: Critically ill children hospitalized in the pediatric intensive care unit (PICU) are candidates for transfusion of blood components because of severe complications of underlying diseases and sepsis. Transfusion thresholds are important markers for development of the transfusion strategies and implemention of the transfusion policies within the establishment.
Aims: In this study, transfusion thresholds for blood components in patients followed and treated in a PICU are investigated, retrospectively.
Methods: *Patients*. Thirty six patients with at least one transfusion of any blood components in the first 3 months of the 2016 were evaluated. The age distribution and gender of the patients were shown in Figure 1.
Blood components. Red blood cell (RBC) concentrates, fresh frozen plasma (FFP), random platelet (RP) and apheresis platelet (AP) concentrates supplied by regional blood center. Prestorage leucoreduction of RBC concentrates was performed by the affiliated regional blood center while the irradiation of RBC and platelet concentrates was applied in the transfusion center of the hospital. Totally, 269 units of FFP, 103 units of RBC, 51 units of RP and 20 units of AP concentrates were transfused.
Transfusion thresholds. Pretransfusion hemoglobin levels, pretransfusion platelet counts and dissemine intravascular coagulation (DIC) scores were assessed for transfusion thresholds of RBC, RP/AP concentrates and FFP, respectively.
Statistical analysis. It was performed using the SPSS/*PASW version 18* to present descriptive statistics.
Results: Sixteen (16.2%) RBC concentrates were transfused to patients with hemoglobin threshold of less than 7.0 grams per deciliter. The mean of hemoglobin level for this group was 6,2 g/dl (min.3,1 g/dl, max. 7.0 g/dl) Patients having a DIC score higher than 5 were transfused with 114 units of FFP (54.5%). Eleven (25.0%) RP/AP transfusions were given to patients with platelet counts below 10.000/mm^3, 17 units (38.6%) transfused to patients with platelet counts between 10 and 20.000/mm^3 and 16 units (36.4%) transfused to patients with platelet counts higher than 20.000/mm^3 (Figure 2). No significant difference was determined for the transfusion thresholds of blood components with the presence of bleeding, mechanical ventilation, cardiac failure and bacteremia or fungemia ($P > 0.5$).
Conclusions: Usually, an agreement is reached on transfusion thresholds for adult patients but still there are divergencies for children. Decision of transfusion is affected by multipl factors in critically ill children such as bleeding, mechanical ventilation, sepsis, etc. It is necessary to reckon with these factors for development of reasonable transfusion strategies in PICUs.

AGE	GENDER	
	Male	Female
<1	11	9
1-4	5	5
>5	3	3

Caption 1. Age distribution and gender of the patients

Platelet Thresholds (x10^3)	Platelet transfusions n (%)	Pretransfusion Platelet Counts (95% Confidence Interval)			
		Mean (x10^3)	Standart Deviation	Minimum (x10^3)	Maximum (x10^3)
<10	11 (25.0)	5,91	2,38	1	10
10-20	17 (38.6)	16,18	2,67	11	20
>20	16 (36.4)	34,63	11,5	21	55

Caption 2 Platelet transfusion thresholds

P-450

ASSESSMENT OF PATTERN AND EFFECTIVENESS OF NEONATAL TRANSFUSION PRACTICES

K Dogra[1], S Basu[2], G Kaur[3] and D Chawla[3]
[1]*All India Institute of Medical Sciences, New Delhi, India* [2]*TMC, Kolkata, India*
[3]*GMCH, Chandigarh, India*

Background: Transfusion practices demand special precautions due to the small blood volume and immature organ systems in neonates. Although physiologic anaemia of infancy is self-limited, anaemia due to repeated phlebotomy, sepsis, on-going bleeding, etc., requires transfusion of blood/blood components. Although neonatal transfusion guidelines exist, many issues pertaining to transfusion triggers, dosage and clinical outcomes still remain debatable. Hence, we conducted a study to get an insight into the neonatal transfusion practice, prevailing at our centre.
Aim: The study aims to evaluate the usage of blood products, donor exposure among neonates and efficacy of transfusion to neonates admitted in the neonatal intensive care unit (NICU).
Materials and Methods: We conducted a prospective cohort study in the NICU, Government Medical College and Hospital, Chandigarh, India by recruiting neonates of gestational age ≥ 26 weeks, birth weight >700 g and duration of stay in NICU >6 h and excluding neonates with major congenital malformations. Each of the transfusion events was assessed by product details, indication of transfusion, haematocrit, donor exposure, platelet count and PT/INR. Packed red blood cell (PRBC) transfusion affecting clinical parameters was assessed by pre- and post-transfusion parameters viz. respiratory support, lactate levels, weight gain, inotropic support, respiratory distress and haematocrit. Platelet and FFP transfusions were assessed by presence or absence of bleeding.
Result: Of total 291 neonates admitted in NICU, 2 neonates had congenital malformations and were excluded. The mean gestational age and mean birth weight of neonates were 33.2 ± 3.2 weeks and 1776 ± 483.4 gms, respectively. Of 289 neonates, 61 newborns received blood product transfusion. The average number of donor exposures per patient was 1.6.
The pre-transfusion and post transfusion hematocrits were 18–36% and 21–39%, respectively. The pre-transfusion platelet count ranged from 10,000 to 86,000/μl (Mean: 36,881 ± 17,579/μl). The post transfusion platelet count of these neonates ranged from 12,000 to 2,00,000/μl (Mean: 55,775 ± 37,407/μl). The pre-transfusion and post transfusion INR values were 1.19–2.96 and 1.00–2.43, respectively. Increment in lab parameters was seen in all newborns ($P < 0.05$) except for 3 neonates. These results may be attributed to the presence of sepsis, ongoing thrombocytopenia and bleed.
It was also found that the effects on clinical parameters like weight gain, respiratory support, apnoeic episodes were not significant ($P > 0.05$). The mean difference between post-transfusion and pre-transfusion lactate levels was 0.6 mmol/L ($P < 0.05$).
Conclusion: Based on observations, we concluded that no clinical benefits of PRBC transfusions were seen on parameters like weight gain, episodes of apnea and respiratory support. Also no benefits of platelet and FFP transfusions were seen on bleeding. Hence, severity of complications and ongoing bleeding determine the clinical outcome. As the neonates were not followed up for long term, complications of transfusion might have been missed.

P-451

INVESTIGATION AND ANALYSIS OF THE EFFICACY OF APHERESIS PLATELETS TRANSFUSION IN 21545 UNITS

J Wang and Q Fu

Shanghai Children's Medical Center,Shanghai Jiaotong University Medical College, Shanghai, China

Background: The history of platelet transfusion has lasted for over 60 years. As one of the major means to prevent and treat hemorrhagic symptoms caused by thrombocytopenia or platelet function defects, platelets transfusion has become an important supportive therapeutics in hemopathy, oncology and surgery.

Objective: 21545 units of apheresis platelets transfusions in Shanghai Children's Medical Center from 2010 to 2015 were enrolled for this study and efficacy of platelet transfusion was retrospected.

Methods: 21545 units of apheresis platelet transfusions were investigated. The efficacy of platelet transfusion was judged by Corrected Count Increment (CCI). CCI $\geq$ $4.5 \times 10^{\wedge}9$/L is considered to be effectively responses to platelet transfusion after 24 h. In contrast, CCI < $4.5 \times 10^{\wedge}9$/L is considered to be ineffectively. A single apheresis of transfused platelet generally contains approximately $2.5 \times 10^{\wedge}11$ platelets. CCI= corrected count increment×body surface area of the patient(m^2) /dose of platelets transfused.

Result: A total of 21545 units of apheresis platelets were transfused for 22602 patients in Shanghai Children's Medical Center within the 6 years, the children with a small age and light weight could be transfused 0.5 units selectively. 20239 units (93.94% of total issues) were given for patients in the department of hematology-oncology. The following was department of cardiothoracic surgery, 1061 units (4.92%) were transfused. 152 units (0.71%) for general surgery, 54 units (0.25%) for neonatology, 17 units (0.08%) for nephrology, 5.5 units (0.03%) for gastroenterology, 16.5 units (0.08%) for the other department of medicine (table 1).

Among the 21545 units, 6981 units were γ-irradiated apheresis platelets, all of which were transfused to 7450 patients (938 patients accepted 0.5 units) for stem cell transplantation in the department of hematology-oncology to prevent TA-GVHD.

There were 17629 cases of prophylactic platelet transfusions (platelet count was $10\sim50 \times 10^9$/L for medical indications and $50\sim100 \times 10^9$/L for surgical indications) with the usage of 16805 units (78.00%). 4973 cases of therapeutic transfusions with the usage of 4740 units (22.00%).

Conclusion: The efficacy of single transfusion is better than multiple transfusion. The efficiency of transfusion in hematology-oncology patients was 72.8% with multiple transfusion, while the others was more than 88% with single transfusion. The platelet antibody was induced by randomly apheresis platelets transfusion for many times, causing the immunological tolerance and influencing the efficiency of platelet transfusion.

There is no difference in increasing platelet count for a short time transfusion between γ-irradiated apheresis platelet and ordinary apheresis platelet. Whereas, the efficiency of γ-irradiated apheresis platelet transfusion is better than the ordinary transfusion for a long term. Maybe γ-ray could kill the lymphocyte and karyocyte, that make patients contact with HLA antigen of donors as little as possible and keep the validity of CCI for a long time. While the CCI of ordinary apheresis platelets transfusion declined earlier and the long-term outcome was poor.

Table 1. The efficacy of apheresis platelets transfusion of 21545 units in 2010~2015

Department	Amount (U)	Percentage (%)	Cases	Efficiency cases	Efficiency (%)
Hematology–Oncology	20,239	93.94	21,164	15,407	72.80
Cardiothoracic surgery	1061	4.92	1177	1177	100
General surgery	152	0.71	154	154	100
Neonatology	54	0.25	65	62	95.38
Nephrology	17	0.08	17	15	88.23
Gastroenterology	5.5	0.03	6	6	100
Other Department of Medicine	16.5	0.08	19	17	89.47
Total	21,545	100	22,602	16,838	74.50

P-452

SAFETY AND EFFICACY OF REPLACED PLATELET CONCENTRATES CONTAINING BICARBONATED RINGER'S SOLUTION, A NEW PLATELET ADDITIVE SOLUTION, IN PEDIATRIC PATIENTS

R Yanagisawa[1], J Kobayashi[1], T Ono[1], Y Tatsuzawa[1], N Kubota[1], E Hidaka[1], S Kojima[2], K Sakashita[1] and S Shimodaira[2]

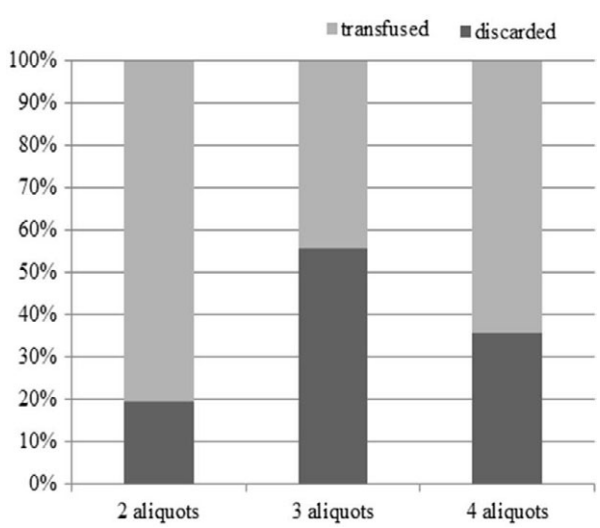
[1]Nagano Children's Hospital, Azumino, Japan [2]Shinshu University Hospital, Matsumoto, Japan

Background: Prestorage leukoreduction and diversion of the first aliquot of blood may decrease several transfusion-related adverse reactions (TRARs) even in pediatric patients. However, allergic transfusion reactions (ATRs) caused by plasma-rich platelet concentrates (P-PCs) remain problematic. Washed PCs (W-PCs) have been recommended in cases with recurrent TRARs. However, concerns such as complicated manipulation during the washing process and decreased efficiency of transfusion of washed products remain. Recently, replaced PCs (R-PCs) were introduced as an adequate alternative preparation of W-PCs to conventional washed and replaced PCs (W/R-PCs). Furthermore, bicarbonated Ringer's solution (BRS) was reported as an excellent platelet additive solution (PAS) that retains platelet quality for longer durations.

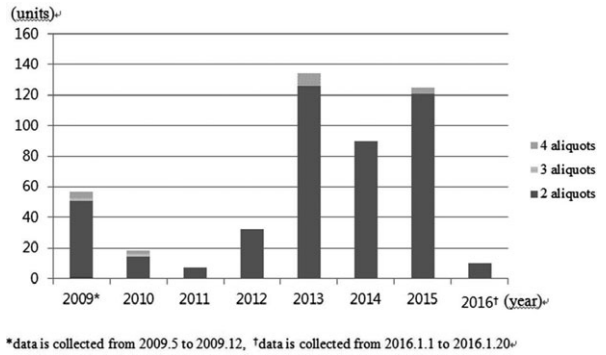
Aim: To determine whether R-PCs with BRS (BRS-R-PCs) transfusion decreases TRARs frequency in pediatric patients, to confirm its transfusion efficacy, and to ascertain consistent quality even when preparation involves different technicians.

Methods: Between June 2015 and March 2016, four technicians prepared BRS-R-PCs for pediatric patients experiencing repeated TRARs. PAS was prepared by mixing BRS and ACD-A solution just before R-PC preparation (ratio, 20:1). We analyzed platelet count (PLT) recovery, fall in plasma protein levels, pH, electrolytes, glucose, and P-selectin while using BRS-R-PCs. Next, we determined hemorrhagic symptom incidence and post-transfusion corrected count increment at 24 h after transfusion ($CCI_{24\ h}$) to evaluate the efficacy of BRS-R-PCs. Patients transfused with P-PCs during the same period were used as controls. Furthermore, we compared $CCI_{24\ h}$ of W/R-PC with G-sol (which had been used as PAS before introduction of BRS during December 2010-May 2015). All adverse events and TRARs were monitored during and after transfusions.

Results: During the study period, 36 BRS-R-PCs were prepared and transfused in five pediatric patients with hematological or oncological diseases; no adverse events were observed. Although these patients had several TRARs during transfusions with P-PCs (nine ATRs; one febrile nonhemolytic transfusion reaction), no TRARs occurred with BRS-R-PCs (P = 0.024). $CCI_{24\ h}$ with BRS-R-PCs was similar to that with P-PCs (2.53 ± 0.96 and 2.17 ± 0.58, respectively; P = 0.288), whereas that with G-sol-W/R-PC was significantly lower than that with both BRS-R-PCs and P-PCs (1.30 ± 0.73, P < 0.001). However, no patients developed hemorrhagic symptoms with any product. The quality analysis results for BRS-R-PCs (shown together



Caption 1 The percentage of transfused post-aliquots and discarded post-aliquots among requested aliquots



Caption 2 The number of requested neonatal aliquots in each day

with reference data for BRS made as PAS) were as follows: PLT recovery, 83.7 ± 5.8%; fall in plasma protein levels, 94.4 ± 1.8%; pH, 7.00 ± 0.06 (6.82 ± 0.06); Na^+, 133.5 ± 1.6 mmol/L (134.0 ± 0.00 mmol/L); K^+, 3.72 ± 0.08 mmol/L (3.68 ± 0.01 mmol/L); $Cl^{+'}$, 89.8 ± 1.7 mmol/L (87.3 ± 0.6 mmol/L); Mg^{2+}, 0.88 ± 0.02 mmol/L (0.91 ± 0.00 mmol/L); glucose, 6.43 ± 0.33 mmol/L (5.80 ± 0.00 mmol/L); and P-selectin, 5.11 ± 5.98%. All products showed good swirling.
Summary/Conclusions: Transfusion using BRS-R-PCs is safe in pediatric patients with repeated TRARs without loss of transfusion efficacy. Furthermore, the quality was maintained even when different technicians prepared PAS and R-PCs. However, further studies are required for validation of these findings.

P-453

ANALYSIS OF UTILIZATION OF NEONATAL ALIQUOTED RBC AND DEVELOPMENT OF NEW COMPUTERIZED PROGRAMS

MJ Nam[1], MJ Kim[2], SH Choi[2], YJ Hong[2], HS Kim[1], SM Hwang[2], JS Park[2], CW Choi[2], KU Park[2], JH Song[2] and KS Han[1]

[1]Seoul National University College of Medicine, Seoul, South-Korea [2]Seoul National University Bundang Hospital, Seongnam, South-Korea

Background: Although neonates are vulnerable and sensitive for transfusion side effect, no studies have been conducted in aliquoted transfusion for neonates. Even worse, in blood bank, there was no independent system to manage neonatal aliquoted red blood cell.
Aim: To use the neonatal aliquoted red blood cell properly, this study point out current problems and suggest new systems in neonatal transfusion.
Methods: A total red blood cell (RBC) aliquots was analyzed from May of 2009 to January of 2016 in neonate intensive care unit. We investigated the aliquot number, issued day interval among post-aliquots, patients' blood type, and discarded RBC units among requested RBC units. To reset the shelf-life of irradiated post-aliquots, we applied new label on the bag and developed computerized programs.
Results: Of 472 RBC aliquots, 95.4%(450/472) were divided into two units. The distribution of patients' blood type was similar with that of Korean population: A blood group is highest (34.3%), followed by B group having 28.2%, and O group at 27.5%. The second, third, and forth unit of post-aliquots were taken after average 4.3 days. Among post-aliquots, the number of units discarded without use was 22.5%.
Conclusions: Patients' on the neonatal intensive care unit (NICU) received a greater number of RBC aliquots. 95.4% (450/472) of the pre-aliquots divided into 2 units and 22.5% (208/925) of the post-aliquots were discarded. In order to reduce the wastage of residue from aliquoted red blood cells and decrease the number of near miss that it is possibly happen, we propose new label attached on the blood bags and new blood management system.

P-454

RED BLOOD CELL ALIQUOTS TRANSFUSION IN A NEONATAL INTENSIVE CARE UNIT IN A TERTIARY UNIVERSITY HOSPITAL

HS Chung[1], SY Shim[2], SJ Cho[2], EA Park[2] and M Lee[1]

[1]Department of Laboratory Medicine, Ewha Womans University School of Medicine, Seoul, South-Korea [2]Department of Pediatrics, Ewha Womans University School of Medicine, Seoul, South-Korea

Background: Transfusion practice in neonatal and pediatric patients quite differ from that in adults. Transfusion of small-volume red blood cell (RBC) aliquots in neonatal and pediatric patients can limit donor exposures, prevent circulatory overload, and potentially decrease donor-related risks. Because of small discarded amount of aliquoted RBC (only one-half or one-third of non-aliquoted RBC), total discarded amount of RBC components can be reduced. A hospital blood center should understand special characteristics of transfusion practices in neonatal and pediatric patients and provide optimal services. However, not much is known on the current state of transfusion practice of small-volume RBC aliquots for neonatal and pediatric patients, especially in our region, and the number of studies on this important issue is limited.
Aims: Our study designed to investigate the current state of transfusion practice of small-volume RBC aliquots for neonatal and pediatric patients in a neonatal intensive care unit (NICU) in a tertiary university hospital, which is important not only for safe and appropriate transfusion in the patients but also for rational management and proper service of hospital blood center.
Methods: We analyzed RBC component units requested for transfusion to patients who admitted to the NICU in a tertiary university hospital between January 2006 and December 2014. The original RBC component units were equally divided into two or three bags using a sterile connection device (TCD B40; DENCO, ICN., UK) at the request of the pediatricians. The aliquoted RBC units from one single donor RBC unit were dedicated to one patient.
Results: The number of RBC components issued during the study period was 4414 units. Among them, aliquoted RBCs were 3303 units (68.6%); 1041 units and 316 units were divided into two and three units, respectively. A total of 4252 units (2872 aliquoted RBC and 1380 non-aliquoted RBC) from 2663 donors were transfused to 560 patients. Overall, transfusion of aliquoted RBC units decreased 55.3% of donor exposure (2,872 aliquoted RBC from 1283 donors), and donor exposure of each patient was significantly decreased. For the patients who transfused more than two RBC units, median decrease of donor exposure was three. At the same time, total utilization of RBC components reduced. The number of discarded RBC components was 162 units and the main component was aliquoted RBC (158 units). The wastage rate of aliquoted RBC (5.2%) was significantly higher than non-aliquoted RBC (0.3%). Among 2082 2-unit-aliquots, 129 units were discarded (the wastage rate of 4.3%), whereas among 948 3-unit-aliquots, 29 units were discarded (the wastage rate of 3.1%).
Summary/Conclusions: Transfusion of small-volume RBC aliquots in the patients in NICU is effective strategy to reduce donor exposure and blood utilization. Even though total amount of discarded RBC aliquots is small, further efforts are needed for minimizing the number of discarded units.

Therapeutic Apheresis

P-455

THERAPEUTIC APHERESIS AT THE INSTITUTE OF HEMATOLOGY AND TRANSFUSION MEDICINE IN WARSAW – A GLIMPSE OF RECENT TENDENCIES

A Rosiek, R Poglod, E Lachert, J Antoniewicz-Papis, L Rzymkiewicz and M Letowska

Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Background: For several decades now therapeutic apheresis has been used for numerous clinical conditions although indications to perform the procedure are often controversial and frequently modified by alternative forms of therapy.
Aims: The aim of the study was analysis and evaluation of the therapeutic apheresis procedures performed at the Institute of Hematology and Transfusion Medicine in Warsaw in the period 2007–2014.
Methods: Retrospective analysis of the documentation on therapeutic apheresis procedures with special emphasis on the number and types performed as well as the clinical indications for which they were recommended.

Results: In the study period, a total of 1625 therapeutic apheresis procedures were performed (plasmapheresis - 1241, leukapheresis - 362, plateletpheresis - 22), using Cobe Spectra and Cobe Trima cell separators. The number of procedures was observed to gradually decrease from 347 per 90 patients in 2007 to 61 per 25 patients in 2014. In the same period, most patients (165) underwent leukapheresis for elimination of the excess of leukocytes in the course of leukemia, both in leukostasis and for prophylaxis. Almost the same number of patients (160) underwent plasmapheresis for elimination of the excess of pathogenic proteins for such conditions as myeloma but for this indication a decreasing tendency was observed over the study period. Plasmapheresis procedures in TTP were performed in 24 patients. Plateletpheresis for elimination of the excess of platelets was performed in 22 patients. In 49 patients plasmapheresis procedures were performed for neurologic disorders, mostly Guillain-Barré syndrome (33 patients) polyneuropathies, myasthenia gravis etc. Other indications included single cases of Wegener's Granulomatosis, Goodpasture's syndrome and pemphigus vulgaris.Most of the above conditions are accepted as criteria for apheresis by the American Society for Apheresis, though not always I category.The number of procedures per patient ranged from 1 to 161 as in the case of one TTP patient, where procedures ran in cycles over a period of several years. In most cases, therapeutic apheresis contributed to alleviation of symptoms related to pathogenic blood components. The effects were usually short-term with no significant impact on prognosis. However, in cases of apheresis combined with chemotherapy some long-term remissions were observed. Therapeutic procedures were generally well tolerated and complications were usually vasovagal events, mild symptoms of hypocalcemia and vascular access problems.
Conclusions: Therapeutic apheresis remains an adjunctive and palliative procedure helpful for rapid elimination of pathogenic agents from the patient's circulation. In some clinical conditions such as TTP it is an important therapeutic modality in others however it was observed to become less useful over the study period (eg. in multiple myeloma - probably due to bortezomib-based therapy). In any case it is worth noting that therapeutic apheresis is a costly procedure not without risk to the patient and should therefore be applied with sound clinical judgement and careful consideration.

P-456

ROLE OF LOW PLASMA VOLUME TREATMENT ON CLINICAL EFFICACY OF PLASMAPHERESIS IN NEUROMYELITIS OPTICA

A Batra, S Periyavan and P Satishchandra

National Institute of Mental Health and Neurosciences, Bengaluru, India

Background: Neuromyelitis optica (NMO) is an autoimmune inflammatory demyelinating disease preferentially targeting the optic nerves and spinal cord. Plasmapheresis is an effective adjunct therapy in severe NMO attacks (American Society for Apheresis (ASFA) Category 2). The recommended minimum plasma volume to be treated per session of plasmapheresis is equivalent to total plasma volume (TPV) of the patient (Schwartz, Journal of Clinical Apheresis, 2013).
Aim: To study the effect of lower plasma volume treated in patients with NMO on clinical efficacy of plasmapheresis in comparison to ASFA guideline at a Tertiary Neurosciences Center in Southern India.
Methods: Retrospective data of patients who were managed acutely for NMO with plasmapheresis in the last 1 year at our center was collected. Patients who had 5 sessions of plasmapheresis spread over 10 days were included. Clinical outcome was defined as per criteria of Keegan *et al* (Neurology, 2002). Patients who were on maintenance therapy for NMO, were excluded. Data analysis was done using Microsoft® Office 2007. Statistical significance was accepted when $P < 0.05$ (95% CI).
Results: We retrospectively collected the data of 24 patients who underwent plasmapheresis for acute attack of NMO, and met our exclusion and inclusion criteria. Females (age; mean (SD) 33.7 (11.2) years) were more common (n = 18). Against the minimum recommended plasma volume that was supposed to be treated during entire acute therapeutic period (mean (SD) 195.5 (14.6) ml per kg-bw), lower plasma volume (112.7 (17.0) ml per kg-bw) was treated per patient (n = 24); the difference was significant ($P < 0.05$). The volume treated was same across the gender ($P > 0.05$). Significant clinical improvement (in neurological status and function) was observed in 79% of patients (n = 19) (13 with moderate improvement, and 6 with marked improvement); 6 months after plasmapheresis. There were no significant differences in volume of plasma treated between patients who had moderate improvement and patients who had marked improvement; and between patients who did, and did not have significant clinical improvement ($P > 0.05$; for both).
Conclusions: Plasmapheresis is a safe and efficient add-on therapy in NMO, especially in steroid-resistant cases. Although the volumes of plasma treated during acute plasmapheresis were less than minimum recommended volumes (Table 1), majority of patients had significant clinical improvement (Table 2). Further randomized therapeutic trial that compares clinical improvement in patients with NMO who would, and would not have their plasma volume treated as per ASFA recommendation; is required.

Plasmapheresis	Plasma Volume Treated (Mean (SD), mL/kg-bw)		t test	
	Recommended (Minimum) as per ASFA Guidelines	Actual	t	p value
Session 1	39.1 (2.9)	21.4 (5.1)	13.7	0.00
Session 2		22.6 (3.9)	18.6	
Session 3		22.2 (4.6)	14.7	
Session 4		23.4 (4.1)	13.8	
Session 5		23.1 (4.4)	14.7	

Table 1. Comparison of recommended and actual plasma volume treated per session in patients with NMO at our center (n = 24)

Plasmapheresis	Plasma Volume Treated (Mean (SD), mL/kg-bw)		t test	
	Recommended (Minimum) as per ASFA Guidelines	Actual	t	p value
Session 1	39.3 (3.1)	21.0 (4.3)	14.0	0.00
Session 2		22.3 (3.6)	16.8	
Session 3		22.2 (4.6)	12.2	
Session 4		23.2 (4.4)	11.5	
Session 5		23.1 (4.9)	11.9	

Table 2. Comparison of recommended and actual plasma volume treated per session in patients with NMO that had significant clinical improvement (n = 19)

P-457

THERAPEUTIC PLASMA EXCHANGE IN NEUROLOGICAL PATIENTS TREATED FROM JANUARY TO DECEMBER 2015

V Srejic[1], G Bogdanovic[1], B Balint[2], D Lavrnic[3], V Rakocevic Stojanovic[3], I Dujmovic Bašuroski[3], S Milutinovic[1] and D Miljuš[1]

[1]Blood Transfusion Institute of Serbia, Belgrade, Serbia [2]Institute of Transfusiology and Hemobiology, Belgrade, Serbia [3]Neurology Clinic, Belgrade, Serbia

Background: Therapeutic plasma exchange (TPE) is a nonselective, nospecific method used for the separation of circulating agents. Recommendations of the Amercan Neurology Association and Recommendations of the Council of Europe are applied in the TPE procedures.
Objective: Objectiv of this study was to demonstrate justified use of thei therapeutic procedure in the treatment of neurological patients, in respect with the corresponding classification of neurological diseases.
Material and Methods: From January 2015 till December 2015, total of 492 TPEs were performed at the Neurology Clinic of the Clinical Center of Serbia. Device based on filtration technique for plasma exchange using disposable plasma exchange sets was applied. Plasma supstitute used in these cases was 20% combination of albumin and Ringer solution. Protocol using 3 to 5 (or more) TPEs in two day intervals was applied, and later on one TPE procedure in 28 day intervals in order to mainytain the effects of TPE. At each TPE procedure, one estimated plasma volume was removed. Patients were controlled right before and after TPE (neurological check up and laboratory testing - blood count, total protein content, albumins, electrolites and INR).

Patients were classified according to categories into class I with Guillan - Barre syndrome (11 patients), CIDP (6), polyneuropathy type IgG, IgA, IgM, class II with Lambert-Eaton syndrome (4), multiple sclerosis (35), Mb Devic (12), class Ic with myasthenia gravis (27), cryoglobulinemic polyneuropathy (2), neurolupus and dermatomiositis (2), acquired neuromyotomy (1). Classification of indications of neurological diseases according to 2014 AABB recommendations was used. The effect of TPEs was estimated In the above stated patients' categories. Average age of patients was 31,4 years.

Results: In the Guillan - Barre syndrome group, class Ia, 49 TPEs were performed resulting in cure, in patients with CIDP class Ia, 20 TPEs were performed (in 83% with improvement), in polyneuropathy, class II with Lambert-Eaton syndrome, 11 TPEs were performed (in 50% with improvement), in multiple sclerosis class I/II 192 TPEs were performed (with slow development of symprtomes in 89%), in Mb Devic class 60 TPEs were performed (condition was stabilized), in class Ic with myasthenia gravis 147 TPEs were performed (with improvement in 94%), in cryoglobulinemic polyneuropathy 3 TPEs were performed, in cases of neurolupus and dermatomiositis 7 TPEs were done, and in acquired neruomyotomy 5 TPEs were performed.

Conclusion: In most patients in which TPE protocol was fully (3–5 procedures every second day) clinical condition improved, and in patients with Guillain-Barre syndrome complete cure was achieved. In patients with Mb Devic condition was stabilized, and in those with multiple sclerosis the development of symptomes was slowed down. TPE is an inseparable part of the overall therapy in a significant number of patients with autoimmune neurological diseases.

P-458

THERAPEUTIC PLASMA EXCHANGE: A TERTIARY CARE CENTER EXPERIENCE

MA Badawi[1], F Almansouri[2], AH Alzahrani[1], H Bukhari[1], I Khalil[1], K Gholam[1], GF Zaher[1] and SI Hindawi[1]

[1]King Abdulaziz University, Jeddah, Saudi Arabia [2]King Fahad General Hospital, Jeddah, Saudi Arabia

Background: Therapeutic plasma exchange (TPE) is a treatment option used in many medical conditions. Response rates are variable as some disorders clearly benefit from TPE as first or second line therapy, while the optimum role of TPE in many other conditions is not established because of inadequate or contradicting evidence.

Aims: To summarize the experience of our center over 11 years and to describe response rates of this treatment modality in diseases in various categories according the American Society for Apheresis (ASFA) guidelines (2013).

Methods: King Abdulaziz University Hospital (KAUH) is 600-bed academic center that provides tertiary care to adults and pediatric patients. TPE is performed after discussion between the primary physician and apheresis physician. TPE in KAUH is performed using Spectra Optia® and Haemonetics® systems. Plasma is used as replacement fluid in patients with thrombotic thrombocytopenic purpura (TTP)/hemolytic uremic syndrome (HUS), while albumin and normal saline are used as replacement for other disease entities.

This retrospective review was performed through reviewing patients' and apheresis service's records in both electronic and paper formats.

ASFA Category	Disease	Number of patients	Percentage of patients with partial or complete response
I (n= 66)	Myasthenia gravis	22	100
	Guillain-Barre Syndrome	20	75
	Thrombotic thrombocytopenic purpura	18	78
	Hyperviscosity in monoclonal gammopathies	3	100
	Wegner's granulomatosis	2	50
	Anti-glomerular basement membrane disease, with diffuse alveolar hemorrhage	1	100
II (n=11)	Familial hypercholesterolemia	1	100
	Neuromyelitis optica, acute	3	33
	Multiple sclerosis	1	0
	Systemic lupus erythematosus, severe	3	0
	Catastrophic antiphospholipid syndrome	3	33
III (n= 4)	Hypertriglyceridemic pancreatitis	4	100
IV (n= 12)	Systemic lupus erythematosus, nephritis	12	50
Undetermined (as mutation analysis is not available)	Hemolytic uremic syndrome, atypical	1	0
Uncategorized	Membranoproliferative glomerulonephritis type 1 with advanced glomerulosclerosis	1	0
	Methemoglobinemia, acquired	1	100
	Sideroblastic anemia (later diagnosed as Pearson syndrome)	1	0
Total		97	

Table 1: Patients distribution according to ASFA categories and response rates to TPE in KAUH.

Results: Between January 2005 and the December 2015, 97 patients underwent a total of 539 sessions of TPE. The majority of patients had neurologic disorders. Most disease entities belonged to ASFA categories I and II, while 3 patients with uncategorized disease entities in ASFA guidelines underwent TPE in our center. We present a patient who underwent TPE for acquired methemoglobinemia secondary to ingestion of a toxic substance and had a partial response to TPE used with other treatment measures. Summary of disease entities and response rates to TPE are presented in Table 1.

Summary: The majority of patients undergoing TPE in our center are of categories I and II according to ASFA guidelines (2013). Overall response rates in these disease categories were good. One patient with acquired methemoglobinemia had partial response to TPE in our center.

P-459

THERAPEUTIC PLASMA EXCHANGE WITH 5% ALBUMIN REPLACEMENT IN A PATIENT WITH CATASTROPHIC ANTIPHOSPHOLIPID SYNDROME ON FONDAPARINUX

Y Li[1], S Liu[1], X Luo[1], K Simmons-Massey[2], JH Lin[3], N Songdej[1] and DN Moolten[4]

[1]Lewis Katz School of Medicine at Temple University, Philadelphia, United States of America [2]Temple University Hospital, Philadelphia, United States of America [3]Naussa University Hospital, East Meadow, United States of America [4]American Red Cross Penn-Jersey Region, Philadelphia, United States of America

Background: Catastrophic Antiphospholipid Syndrome (CAPS) is a rare life-threatening condition. Interventions for CAPS include anticoagulants, steroids and therapeutic plasma exchange (TPE). However, the optimal protocol for treating CAPS with TPE, has not yet been established. The commonly used replacement fluid fresh frozen plasma (FFP), contains natural anticoagulants such as anti-thrombin III (AT-III), which may have a salubrious effect since it is through AT-III that heparin-based anticoagulants inactivate proteases involved in blood clotting, most notably Factor Xa. However, FFP is also made up of clotting factors, complement activation products and cytokines which could variously aggravate complications of CAPS including "thrombotic storm". Current American Society for Apheresis guidelines suggest FFP replacement in TPE for CAPS. In contrast to FFP, human albumin, while it lacks AT-III, c ontains no provocative substances, and therefore may be a superior alternative replacement fluid in some patients with CAPS. There are reports of CAPS managed successfully by TPE using albumin replacement, but the approach has not been established.

Aims: We report a case of a 36-year-old female diagnosed with APS [lupus anticoagulant and anticardiolipin (aCL) IgA positive] on fondaparinux who improved after TPE with albumin replacement.

Methods: The patient developed left posterior tibial artery thrombosis with gangrene of the left toes and abdominal pain with possible liver ischemia. On admission, her laboratory tests (not on heparin) showed PT 27.4 (10.3–13.4)s, INR 2.3, aPTT 114.5 (26.7–36.2s)s, and fibrinogen 441 (200–385)mg/dl, aCL IgA 46.4 (0–12.0)APL (Table). She was initially anticoagulated with heparin with monitoring of anti-Xa levels, but was switched to subcutaneous fondaparinux 7.5 mg daily due to concern for heparin-induced thrombocytopenia. After approximately 2 weeks of anticoagulation, there was no clear improvement clinically. A course of TPE was initiated. A total of four 1.0 plasma volume TPEs were performed every other day with 5% albumin replacement in TPEs #1-#3 and FFP in TPE #4. At same time, she was started on steroids and received subcutaneous fondaparinux 7.5 mg every other day (omitting on TPE day). Her coagulation profile was monitored daily. After the completion of procedure #4, daily fondaparinux was reinstituted.

Day from admission	PT 10.3-13.4s	INR NA	aPTT 26.7-36.2s	Fibrinogen 200-385 mg/dl	aCL IgA 0-12.0 APL
0 (admission)	27.4	2.3	114.5	441	46.4
7 (Pre-TPEs)	15.0	1.3	>240	545	88.5
21 (Post-TPEs)	11.6	0.9	37.1	209	11.4

Note: PT, Prothrombin time; INR, International normalized ratio; aPTT, Activated partial thromboplastin time; aCL IgA, anticardiolipin IgA; NA, Not applicable.

Results: The patient reported marked alleviation of left foot pain the day following the first TPE. The area of dark red discoloration on her left foot had notably diminished after the 4th TPE. Her coagulation profile showed PT 11.6s, INR 0.9 and aPTT 37.1s (Table) post TPE treatment. A repeat anti-cardiolipin antibody test showed a decrease of aCL IgA from 88.5APL before TPE to 11.4APL after TPE (Table). However, the repeat angiogram showed severe left sided distal peripheral vascular disease with no significant improvement. The patient underwent a below knee amputation 7 days after TPE.

Summary/Conclusions: This patient improved after TPE with albumin replacement alternating with fondaparinux every other day. Our experience suggests that the use of 5% albumin replacement is safe and effective for CAPS patients. On the TPE day, anticoagulated status was maintained without fondaparinux due to anticoagulating effects of TPE with albumin. Since this is a single case experience, further study is needed.

P-460

ANALYSIS OF 1-YEAR EXPERIENCE WITH PLASMAPHERESIS IN PATIENTS WITH NEUROLOGICAL DISEASES AT A TERTIARY NEUROSCIENCES CENTER IN SOUTHERN INDIA

A Batra and S Periyavan

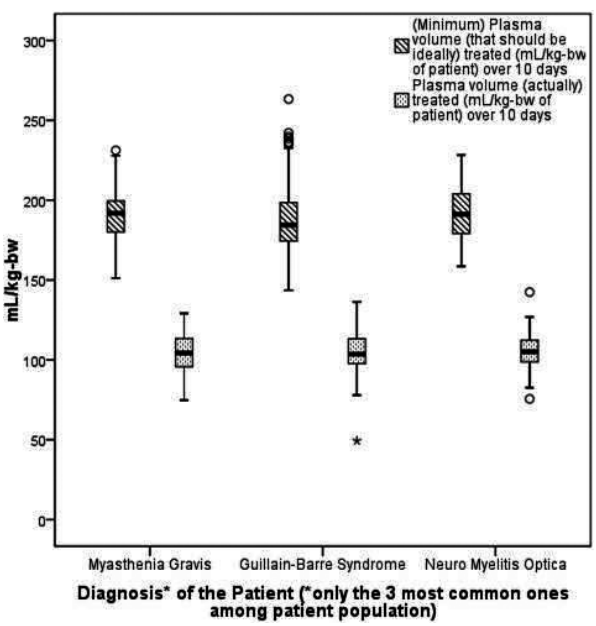
National Institute of Mental Health and Neurosciences, Bengaluru, India

Background: Plasmapheresis is an established therapeutic procedure used in neurological disorders of autoimmune etiology. Randomized controlled studies have demonstrated its clinical efficacy.

Aim: To analyse our experience with plasmapheresis in patients with neurological disorders at a tertiary neurosciences center in southern India during 1-year period.

Methods: We retrospectively collected demographic data, baseline investigations (before the start of the first plasmapheresis session) and volume of plasma treated; of patients with various neurological illnesses, for whom plasmapheresis was done either as a primary or an adjunctive therapy; either at specially designated Plasmapheresis Suite or at Intensive Care Unit (ICU) during 1-year period. Patients who had 5 sessions of plasmapheresis over 10 day period were included. Outpatients were excluded.

Results: Two hundred and eighty-eight patients met our exclusion and inclusion criteria. Between them, they had 300 sittings of therapy; each sitting included 5 sessions of plasmapheresis. The 3 most common diagnoses were Guillain Barré syndrome, myasthenia gravis and neuromyelitis optica (Table 1). The remaining included autoimmune encephalitis (n = 12, 4.2%); chronic inflammatory demyelinating polyradiculoneuropathy (n = 8, 2.8%); vasculitic neuropathy and Miller Fisher syndrome (n = 2, 0.7%; respectively); and hypokalemic periodic paralysis, Morvan syndrome, Sjögren syndrome with neuropathy, transverse myelitis, and mitochondrial cytopathy (n = 1, 0.3%; respectively). 42 (14.6%) patients (males 30 (71.4%)); and 55 (19.1%) patients (females 30 (54.5%)) had high and low pre-procedure hematocrit (mean (SD) %; 50.7 (3.5), and 32.8 (3.5)), respectively. 21 (7.3%) had low platelet count (mean (SD), 115 (30) $*10^3/\mu L$). 100 (34.7%) patients had high leukocyte count (mean (SD), 14.8 (3.6) $*10^3/\mu L$). Among these 100 patients, there were no significant differences in leukocyte counts who had plasmapheresis in ICU (n = 12; 17.3 (5.1)) and Suite (n = 88; 14.5 (3.3)); and who had central (n = 5; 17.5 (6.7)) and peripheral (n = 95; 14.7 (3.4)) access for the procedure (P > 0.05; respectively). 4 (1.4%) patients had mildly deranged liver function tests (mean (SD); serum total bilirubin 2.4 (1.0) mg/dl and serum total protein 6.1 (0.3) g/dl) before the start of plasmapheresis; but did not have increased bleeding tendency (INR, mean (SD), 1.09 (0.08)). 6 (2.1%) patients had deranged renal function tests (mean (SD); serum creatinine 1.4 (0.2) mg/dl; serum urea 79 (47) mg/dl); though these patients had less fluid infusion (21.0 (2.3) ml/kg-bw) compared to other patients with normal renal function tests (21.8 (2.8) ml/kg-bw) per session of plasmapheresis, the difference was not significant (P > 0.05). 24 (8.3%) patients had pre-procedure low calcium levels (mean (SD), 8.2 (.9) mg/dl); only 2 (8.3%) developed clinical features of



Graph 1. Box plot showing total volume of plasma treated per patient during 10 days of alternate-day plasmapheresis therapy

Diagnosis*	Patients (%)	Age (Mean (SD); years)	Females (%)	Sessions (%)	Site (per session)		Venous Access (per session)		Plasma volume treated (Mean (SD); mL/kg-bw) per session		
					ICU (%)	Suite (%)	Central (%)	Peripheral (%)	Actual	"Ideal" (Minimum#)	p value$
Total	288	36.4 (15.0)	112 (38.9)	1500	145 (9.7)	1355 (90.3)	75 (5.0)	1425 (95.0)	21.0 (2.5)	37.8 (3.9)	0.000
Guillain Barré Syndrome	191 (66.3)	36.4 (14.9)	61 (31.9)	965 (64.3)	100 (10.4)	865 (89.6)	40 (4.1)	925 (95.9)	21.0 (2.3)	37.4 (3.9)	0.000
Myasthenia Gravis	34 (11.8)	42.7 (15.6)	16 (47.1)	190 (12.7)	30 (15.8)	160 (84.2)	30 (15.8)	160 (84.2)	20.9 (2.6)	38.5 (3.8)	0.000
Anti-AChR Antibody											
Positive	29 (85.3)	42.8 (15.1)	13 (81.3)	160 (84.2)	25 (83.3)	135 (84.4)	25 (83.3)	135 (84.4)	20.9 (2.5)	38.9 (3.9)	0.000
Negative	5 (14.7)	42.4 (20.1)	3 (18.7)	30 (15.8)	5 (16.7)	25 (15.6)	5 (16.7)	25 (15.6)	21.1 (3.5)	36.6 (3.2)	0.000
Neuromyelitis Optica	34 (11.8)	33.3 (12.1)	25 (73.5)	170 (11.3)	10 (5.9)	160 (94.1)	5 (2.9)	165 (97.1)	21.0 (2.7)	38.2 (3.3)	0.000
Anti-AQP4 Antibody											
Positive	11 (32.3)	38.4 (12.4)	9 (36.0)	55 (32.3)	5 (50.0)	50 (31.3)	0	55 (33.3)	21.1 (2.0)	38.3 (4.4)	0.000
Negative	23 (67.6)	30.8 (11.4)	16 (64.0)	115 (67.7)	5 (50.0)	110 (68.7)	5 (100)	110 (66.7)	20.9 (3.0)	38.1 (2.8)	0.000

*The 3 most common ones are shown
#As per ASFA Guidelines
$The difference is calculated using Student's t-test (significant when p<0.050)

Table 1. Shows cumulative data of patients who had plasmapheresis during 1-year period

hypocalcemia during the procedure. The plasma volume treated per session and over 10 day period were less (in total; and across all diagnoses) compared to minimum plasma volume that should be treated as per American Society for Apheresis (ASFA) guidelines (Graph 1). 12 (4.2%) patients had more than 1 sitting (each with 5 sessions) of plasmapheresis.
Conclusion: Being a tertiary care neurosciences center, patients with myriad of neurological diagnosis were treated with plasmapheresis. The clinical indications for plasmapheresis were either from ASFA category 1 or category 2. We observed that guidelines regarding volume of plasma treated per patient were not strictly adhered to; though definite clinical improvement was seen in majority of them.

P-461

CHALLENGES IN THERAPUETIC PLASMA EXCHANGE IN PEDIATRIC PATIENTS- EXPERIENCE FROM A TERTIARY CARE CENTRE FROM NORTH INDIA

RR Sharma, R Hans, S Prakash, S Singh and N Marwaha
Post Graduate Institute of Medical Education and Research, Chandigarh, India

Background: Therapeutic plasma exchange (TPE) in pediatric age group is technically demanding because of low blood volume, difficult venous access and poor cooperation of the patient during the procedure. We here present our experience of TPE in pediatric patients from our centre.
Aims: To assess the challenges during TPE in pediatric patients and formulate appropriate strategies.
Materials and Methods: We did retrospective analysis of all TPE procedures performed in pediatric patients over a period of 15 years (2001–2015). TPE procedures were done on two different apheretic devices (CS 3000 plus, Fenwal USA and Cobe spectra, Terumo BCT Lakewood, Colorado) daily or on alternate days depending on clinicial condition of the patient. For all procedures, kit was primed with compatible packed red cells. Adverse events during the procedure were noted and analyzed.
Results: A total of 356 TPE (range 1–22/patient with mean of 6.2 procedures) were performed for 55 pediatric patients with different indications like atypical HUS (category I as per American Society for Apheresis (ASFA) in total 44 patients, Neuromyelitis optica (category II) in 4 patients, Rapid proliferative glomeronephritis (category I), C3 glomerulopathy in 3 patients each and one patient of infective hemophagocytosis. The average age of patient population was 7.8 yrs (1.2–13 years). The Male:Female ratio was 3:1 with an average weight of 25.5 Kgs. Adverse events were observed during 20 (5.61%) procedures. Most commonly observed adverse events were allergic reaction to replacement fluid (1.4%) followed by hypotension (1.1%), line occlusion (0.8%), vasovagal, endotracheal tube blockage and symptomatic hypocalcemia was observed in one procedure each (0.28%).There was no corelation observed between physical parameters of patient with adverse events. All adverse events were managed as per departmental standard operating procedures (SOPs) and procedures were completed successfully except in one where the procedure was abandoned. No mortality was observed during the procedures.
Conclusion: TPE is safe therapeutic modality in pediatric patients when performed under expert technical supervision with proper SOPs in place.

P-462

OPTIMAL MOBILIZATION PREDICTING FACTORS FOR THE YIELD OF ALLOGENEIC PERIPHERAL BLOOD STEM CELL COLLECTION IN HEALTHY DONORS

SN Lee, E Hyeon Seok, L Hyewon, P Hyeon Jin and K Sun-Young
National Cancer Center, Goyang-si Gyeonggi-do, South-Korea

Background: Allogeneic peripheral blood stem cell (PBSC) transplantation is a key therapeutic procedure in hematologic malignancies. The success of allogeneic PBSC transplantation is determined by collection of sufficient amount of PBSCs.
Aims: This study aimed to identify the optimal mobilization prediction factors associated with the amount of collected PBSC in healthy donors.
Methods: The clinical data for 164 procedures of allogeneic PBSC performed on 121 donors from February 2007 to December 2015 in National Cancer Center were reviewed retrospectively. The definition of optimal mobilization was acquiring of total CD34 + cells over 5.0 × 106/kg at first day of apheresis. PBSCs in donors were mobilized with granulocyte-colony stimulating factors(G-CSF) for 4 days before collection. Blood parameters such as total nucleated cells (TNCS), white blood cells (WBCS), hematopoietic cells (HPCS), mononuclear cells (MNCS), hematocrit (HCT), and platelet (PLT) count of donors at the time of baseline of pre-mobilization, pre- and post-PBSC collection assessed by XE-2100 (Sysmex, Kobe, Japan), we also studied the optimal mobilization factors associated with the apheresis procedures and clinical characteristics of the donors.
Results: The donors consisted of 68 (56%) related donors and 53 (44%) unrelated donors. The median age was 37 years (range, 15–62). Medians of cell counts in collected product per procedure at first day were as following: total CD34 + cell count 4.43 × 106/kg, TNCS 10.36 × 108/kg, and MNCS 8.40 × 108/kg. Optimal mobilization yield was obtained from 51.3% (62/121) of donors. In a univariate analysis, younger age (≤30 years) ($P = 0.011$), central venous catheter (CVC) (vs Peripheral vein) ($P = 0.032$), high WBC ($P = 0.004$) and PLT ($P < 0.001$) at baseline, high number of WBC ($P < 0.001$), HPC ($P < 0.001$), PLT ($P = 0.001$) at pre-PBSC collection were significantly correlated with optimal CD34 + cell yield. A multivariate forward and backward stepwise selection regression analysis showed that the factors associated with the CD34 + cell yield were CVC, PLT at baseline and HPC of pre- PBSC collection in all donors and especially PLT of pre-mobilization were significant in optimal mobilization donors.
Conclusions: This study represent CVC, PLT of pre-mobilization and HPC of pre-PBSC collection are important prediction factors for optimal mobilization in allogeneic PBSC collection in healthy donors.

P-463

LDL-APHERESIS IN A PAEDIATRIC PATIENT OF FAMILIAL HYPERCHOLESTEROLEMIA: PRIMI EXPERIENTIA FROM A TERTIARY CARE CENTRE IN NORTH INDIA

K Dogra, D Rout, P Coshic and K Chatterjee
All India Institute of Medical Sciences, New Delhi, India

Introduction: Familial hypercholesterolemia (FH) is caused by mutation of apolipoprotein-B (Apo-B) receptors which leads to decreased hepatic low density lipoprotein (LDL) uptake. This may present either as homozygous (1 in million) or heterozygous (1 in 500) condition. Xanthoma usually occurs by 4 years of age and pre-mature death from congenital heart disease by 20–30 years of age. Management plan dictates lifestyle modification and medical treatment. LDL-apheresis (LAph) comes forth when the aforementioned treatment plan fails to control the blood cholesterol levels or is not tolerated. A severely malnourished female child presented with yellowish skin lesions over different parts of the body viz. bilateral Achilles tendon, both knees, elbows, both pinnae and outer canthus of both eyes. She had a strong family history of borderline hypercholesterolemia and was diagnosed as a case of FH. LA was planned as the cholesterol levels were not controlled with the diet modification.
Aim: The study reports the first LAph in a paediatric case of FH at a tertiary care centre in North India.
Materials and Methods: After being diagnosed with FH at the age of 3 years, the child was maintained on diet modification. LAph was planned as the cholesterol levels could no further be kept under control with the diet modification and/or drug therapy. However, the unavailability of an appropriate kit in Indian market for LAph (P1R, Fresenius Kabi, Hong Kong) as prescribed by the manufacturer; led us to use the PL1 disposable kit (Fresenius Kabi, Hong Kong) with minor modifications in the circuit, available in the Indian market for routine TPE procedures. The LAph was conducted with the apheresis equipment viz. COM.TEC (Fresenius Kabi, Hong Kong) using a fibre cascade filter (Evaflux, Model 5A20, Kawasumi Lab Inc., Japan).
Results: We conducted 2 sessions of LAph. The total blood volume of the child was calculated to be 1120 ml. The small blood volume of the patient was compensated by priming the circuit with 1 unit of group matched and cross-match compatible (B^+) PRBC to reduce the total extracorporeal blood volume at any time. Total 2.7 fold of plasma volume (~3 L) was processed. During these processes the vital functions of the patient were closely monitored. The entire procedure was completed without any technical difficulties nor any discomfort to patient. After the first session, the LDL-C level fell by 75.9% and the total cholesterol fell by 73.5%. Subsequently, a second procedure led to a decline in total cholesterol level by 18.6% and LDL-cholesterol (LDL-C) by 19.46% (Table 1). Subsequently, she was advised diet modification and started on Atorvastatin 20 mg once daily with regular follow-up after every 6 months to evaluate the disease progression.
Conclusion: LAph is a very safe and effective treatment for removing undesirable lipoproteins, such as LDL-C, total cholesterol, and triglycerides, from plasma. Though it removes the good lipoproteins but the benefits most certainly outweigh this side-effect.

P-464

IMMUNOADSORPTION AND IT'S ROLE IN ABO INCOMPATIBLE RENAL TRANSPLANT: A PRELIMINARY EXPERIENCE FROM A TERTIARY CARE CENTER IN INDIA

S Agrawal, RN Makroo and M Chowdhry

Indraprastha Apollo Hospital, New Delhi, India

Aim and Background: ABO incompatible (ABOi) renal transplant has now become a routine procedure for patients with end stage renal diseases, especially in countries like India, where the compatible donor pool is limited. Although conventional therapeutic plasma exchanges (cTPE) is efficacious and widely used, the time taken for attaining target antibody titers (AT) is often long. A recent meta-analysis has shown that, antigen specific immunoadsorption (IA) is efficacious and less time consuming as compared to cTPE. Here, we describe our preliminary experience in 3 consecutive patients undergoing ABOi renal transplantation after IA.

Methods: All three patients were conditioned with rituximab followed by plasmapheresis. cTPE was performed on the Hemonetics MCS+ cell separator (Braintree, MA, USA). IA was done using antigen specific immunoadsorbent column (Glycosorb ABO, Glycorex Transplantation, Lund, Sweden) on Terumo BCT's Spectra Optia®. Combined IgM and IgG isoagglutinin titers were determined by tube technique at baseline, after each cTPE session and after processing 4 and 8 plasma volumes by IA. Procedure was stopped once the target titer of <1:8 was achieved. AT was further monitored daily, till the 14th post-operative day. Post-transplant cTPE was done if, titers rebounded to ≥1: 8. Intravenous immunoglobulin (IVIG) was given after every cTPE session in Patients 1 and 3. Post-transplant immunosuppression consisted of tacrolimus, mycophenolate mofetil, and prednisone.

Results: In Patient 1, cTPE was ineffective in achieving the target titers even after 8 consecutive sessions of one plasma volume each. Although, there was an overall decline in the AT by 2 serial dilutions (SD) (1:32 to 1:8), rebounds in AT were noted after every session. Subsequently, a single session of IA, reduced the antibody titers from 1:8 to 1:1. On the other hand, in Patient 2, IA led to a rapid decline in AT from 1: 64 to 1: 1 (6 serial dilutions) with only two consecutive sessions. In the post-operative period, both patients maintained the AT within the limits and required no additional procedures.

Interestingly, IA in Patient 3 led to a minimal reduction in AT by only one SD (1:32 to 1:16) even after processing 16 plasma volumes in two consecutive sessions. Hence, two cTPE procedures of one volume each, were undertaken leading to a reduction in AT by 3 SD (1:16 to 1:2). However, post-operatively, the patient had a rebound in AT to 1:8, requiring an additional cTPE session with no adverse effect on graft function. Blood group and procedural details are shown in Table 1.

All three patients are under regular follow-up with graft function and patient survival at 5 months being 100%. No hyper-acute rejection or late rebound of AT was noted.

Conclusion: Our study highlights the merits & demerits of IA apheresis in different scenarios. As seen in Patient 1, cTPE was ineffective and was followed by IA which proved to be a success. IA also proved to be advantageous in Patient 2. Whereas, in Patient 3, IA was not effective, probably due to presence of core chain antibodies wherein, cTPE was beneficial.

P-465

PREDICTING THE NUMBER OF TREATMENT PROCEDURES NEEDED TO NORMALIZE SERUM FERRITIN LEVELS IN NEWLY DIAGNOSED HEREDITARY HEMOCHROMATOSIS PATIENTS TREATED BY PHLEBOTOMY OR ERYTHROCYTAPHERESIS

E Rombout-Sestrienkova[1], B Winkens[2], M Van Kraaij[1], CThBM Van Deursen[3], MCH Janssen[4], AJM Rennings[4], D Evers[5], JL Kerkhoffs[6], A Masclee[7] and GH Koek[7]

[1]Sanquin Blood Supply, Amsterdam, The Netherlands [2]Maastricht University, CAPHRI, Maastricht, The Netherlands [3]Zyuderland Medical Center, Heerlen, The Netherlands [4]Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands [5]Leiden University Medical Center, Leiden, The Netherlands [6]HAGA Teaching Hospital, The Hague, The Netherlands [7]Maastricht University Medical Center, Maastricht, The Netherlands

Background: Hereditary hemochromatosis (HH) is the most prevalent inherited metabolic disorder. It is characterized by an increased iron absorption that is inappropriate to body iron stores resulting in progressive accumulation of tissue iron, mainly in the liver, joints, heart, pancreas, skin and gonads. Standard treatment for newly diagnosed patients with HH and iron overload consists of phlebotomy or more personalized erythrocytapheresis. Erythrocytapheresis is more efficient, but infrequently used because of costs and specialized collection equipment.

Aim: The aim of our study was to develop a mathematical model that accurately predicts the number of treatment procedures needed for the initial treatment of HH, by either using phlebotomy or erythrocytapheresis. This model could help physicians to select the most effective treatment modality for the individual patient prior to start of treatment. Moreover, this will on forehand provide insights in expected treatment-related costs, which will be of great value for insurance companies.

Methods: A retrospective data analysis was carried out among 97 newly diagnosed HH patients (C282Y homozygous), who were treated with either phlebotomy (n = 54) or erythrocytapheresis (n = 43) until serum ferritin (SF) reached levels ≤ 100 μg/L. Regarding phlebotomy, 500 ml whole blood was removed once weekly. Regarding erythrocytapheresis, once every two to four weeks, 350–800 ml of erythrocytes was withdrawn, depending on the estimated total blood volume (based on sex, body weight, and height), and hematocrit (Hct) of the patient. Minimal targeted post-procedural Hct values were set at 30%.

To identify predictors significantly associated with the number of treatment procedures, we used multiple linear regression analysis for each treatment method separately. The overall prediction quality of the model was expressed in R-square (closer to 1 represents higher quality) as well as in square root of the mean squared prediction error (√MSPE; closer to 0 represents higher quality).

Results: The mean number of treatment procedures needed to reach a targeted SF value ≤ 100 μg/L was significantly lower among patients treated with erythrocytapheresis (12 ± 6) than among those treated with phlebotomy (23 ± 14; $P < 0.001$). Regarding erythrocytapheresis, initial SF, initial hemoglobin (Hb), and body weight were the best practical predictors, while for phlebotomy these were initial SF and body weight. The established formulas for the number of treatment procedures were: $X_e = 28.6 + (0.004 \times \text{initial SF}) - (1.82 \times \text{initial Hb}) - (0.08 \times \text{body weight})$ for erythrocytapheresis, and $X_p = 21.4 + (0.011 \times \text{initial SF}) - (0.17 \times \text{body weight})$ for phlebotomy. Presented formulas showed better accuracy for the erythrocytapheresis prediction model as compared to phlebotomy ($R^2 = 0.70$, √MSPE = 3.7 for erythrocytapheresis vs $R^2 = 0.69$, √MSPE = 7.9 for phlebotomy).

Conclusions: We here present a practical and feasible clinical prediction rule that might help clinicians in selecting the proper treatment modality for newly diagnosed HH patients. It should be kept in mind that this analysis was based on retrospective data and that further prospective studies validating and optimizing our predicton model may lead to a calculation tool with a higher predictive quality.

Evidence Based Transfusion Medicine Practice

P-466

This abstract has been withdrawn.

P-467

This abstract has been withdrawn.

P-468

OPTIMIZATION OF BLOOD TRANSFUSION SERVICES: ANALYSIS OF BLOOD REQUISITION AND UTILIZATION PRACTICES IN CARDIAC SURGICAL PATIENTS IN A TERTIARY CARE HOSPITAL, INDIA

S Mangwana, N Bedi, P Yadav, R Chugh and D Arya

Sri Balaji Action Medical Institute, New Delhi, India

Background: Blood products are resources vital to healthcare institutions with constant and growing demands. Ability to maintain adequate stocks of blood products to meet its needs can be a challenge to any hospital. Data from many developing countries have shown gross over-ordering of blood in 40% to 70% of patients transfused. This may cause exhaustion of valuable supplies and resources both in

technician time, effort, and biochemical reagents. It also adds to financial burden for each patient undergoing a surgical procedure.
Aims: Objective of this study was to evaluate the blood requisition, utilization and utilization management in cardiac surgical patients in a tertiary care hospital in the National Capital of India.
Methods: A hospital based prospective study was conducted in elective and emergency cases of cardiac surgical patients in the tertiary care hospital over a period of three years from January 2013 to December 2015. Details of blood requisition and transfusion of cases were collected and reviewed. Patient's age, sex, number of units cross-matched and transfused, number of patients cross-matched and transfused, and type of surgery were noted. Blood utilization indices were computed with the following equation.
(i) Cross-match to Transfusion ratio (C: T ratio) = Number of units cross-matched/ Number of units transfused
(ii) Transfusion index (TI) = Number of units transfused/ Number of patients cross-matched.
(iii) Transfusion probability (%T) = Number of patients transfused/Number of patients cross-matched × 100.
C: T ratio of 2.0 and below, TI value of 0.5 or more and %T value of 30% and above were considered indicative of significant blood usage. Excessive cross matching was considered when these indices were above thresholds for appropriate blood usage i.e. C: T ratio >2.0 and TI < 0.5.
Results: During the study period of 3 years, a total of 15,392 patients' requisitions were received in Department of Blood Transfusion Services. 25,190 units of packed red blood cells (PRBC) were cross matched for these patients of which 18741 units were issued (C:T. Ratio 1.34) implying that overall 25.60% of the blood cross-matched was not transfused. Overall TI was 1.22 and % T as 83.07%.
Out of total blood requisition received, requision of cross-matching was received for 558 cardiac surgical patients of which 479 patients were transfused. From a total of 2752 units cross matched, only 1296 units were transfused with C:T Ratio 2.12. This means that 52.91% of the blood cross matched for cardiac cases was not utilized which was much higher than in total patients (C:T ratio-1.34) which is statistically highly significant ($P < 0.001$). TI for cardiac patients was 2.32 with %T as 85.84%.
Conclusions: Blood Transfusion undoubtedly plays a major role in resuscitation and management of cardiac surgical patients but overestimation of anticipated blood loss results in over-ordering of blood leading to wastage of supplies and resources in terms of time and reagents. This study revealed that overall 25.60% of cross matched blood was unutilized but in cardiac surgery cases, it rose to nearly 53%.
Blood Transfusion Services need to adopt blood conserving policies. Efforts should be made to adopt more conservative transfusion thresholds, conduct regular auditing about effectiveness of blood requesting policy using C: T ratio and periodic feedback to improve blood ordering, handling, distribution & utilization practices of this scarce resource. For continued improvement of transfusion practice, continuous surveillance of utilization pattern is needed.

P-469

PREDICTORS OF BLOOD TRANSFUSION REQUIREMENT IN HIP ARTHROPLASTY A SINGLE-CENTRE EVALUATION

H Cruz Gomes, C Monteiro, BD Oliveira Delgado, LG Ricca Gonçalves, A Leite, M Carvalho and C Koch

Centro Hospitalar São João, EPE, Porto, Portugal

Background: Orthopedic surgery is associated with excessive bleeding and high transfusion rate. The prevalence of allogeneic red cell transfusion associated with hip arthroplasty has been reported between 21% and 70%. Patient related and surgery associated factors, such as gender, preoperative hemoglobin level and comorbidities have been associated with the transfusion rate in different surgical settings.
Aims: In the present study, we assessed transfusion requirements after elective or urgent partial and total hip arthroplasty and evaluated potential predictors for excessive blood loss and transfusion in our University Hospital during a three-years period.
Methods: Seven hundred and seventy-six patients who underwent hip arthroplasty were identified from our database through 2012–2014, (40.0% male; median age, 73.0 [range, 16–100] y). To ascertain the most powerful independent factors associated with need for transfusion support, we performed a logistic regression analysis using the 0/1 variable transfusion/no transfusion as outcome and using patient demographics, ASA Score (American Society of Anesthesiologists Score), preoperative and postoperative hemoglobin values, elective vs urgent surgery, partial vs total hip arthroplasty, regional vs general anesthesia and lenght of hospital stay as potential predictors. Statistical analysis was performed using IBM® SPSS® Statistics, version 23. Significance was assumed if p was <0.05.
Results: The rate of transfusion in the present study (27%) is similar to the lower range described in literature.
Hosmer & Lemeshow test of the goodness of fit suggests the model is a good fit to the data as $P = 0.553$ (>0.05).
No statistically significant association was found between age, postoperative hemoglobin values, length of hospital stay or elective vs urgent surgery and transfusion requirements. Female (OR=1.60), lower preoperative hemoglobin (OR=1.54), higher ASA Score (ASA IV vs ASA I, OR=4,85), total hip arthroplasty (OR=2.02) and general anesthesia (OR=1.80) were factors significantly more associated with need for transfusion support.
Conclusion: Identification of patients at higher risk of transfusion is desirable in order to maintain hemoglobin concentration, optimize hemostasis and minimize blood loss, in an effort to reduce transfusion requirements and improve patient outcome.
This study identified some clinical predictors in patients undergoing joint arthroplasty that we believe can be used for implementing more effective blood conservation stategies.

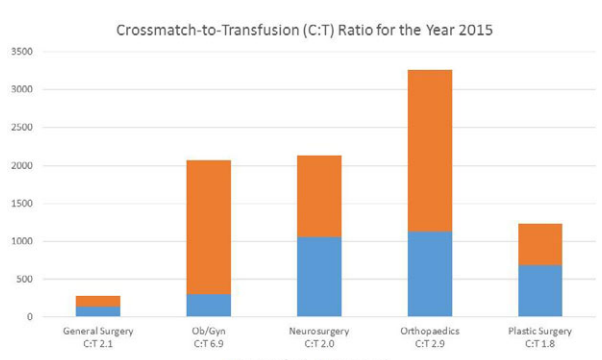
P-470

AUDIT OF CROSSMATCH-TO-TRANSFUSION RATIO OF ELECTIVE PROCEDURES PERFORMED IN 2015 AT TERTIARY CARE HOSPITAL AND TRAUMA CENTER

M Al Zaabi, S Johnson, M Theresa and A Al Harthi

Khoula Hospital Directorate of Laboratories, Muscat, Oman

Background: Patient blood management around time of surgical procedures continues to be one of the main challenges in transfusion practice. Preoperative over-requesting of red cell units is still common and can result in wastage of blood bank resources.
Aim: To evaluate practice of blood ordering for elective surgeries by calculating crossmatch-to-transfusion (C:T) ratio and compare it to existing guidelines and hospital transfusion policies.
Methods: We performed a retrospective collection of red cell units requested for transfusion prior to elective procedures over a 12-month period (from 1st January 2015 – 31st December 2015). Patients were admitted at general surgery, obstetrics/gynaecology (Ob/Gyn), neurosurgery, orthopaedics and plastic surgery wards. C:T ratios were obtained for each ward. Units transfused during preoperative and intraoperative periods were included.
Results: A total of 8986 units were cross-matched over 12-month period. 3299 units were transfused (37%) either preoperatively or intraoperatively. Overall C:T ratio was 2.7:1. There was a significant increase in C:T ratio of Ob/Gyn and orthopaedic procedures (6.9:1 and 2.9:1 respectively). Ratios of remaining 3 department were found to be nearly closed to 2:1 [general surgery (C:T 2.1:1), neurosurgery (C:T 2:1), plastic surgery (C:T 1.8:1)] (figure.1).
Conclusions: Adherence to maximum surgical blood ordering schedule and hospital transfusion policies is essential part in managing blood products. Re-allocation of procedures associated with less than 30% usage into group and save (G&S) is considered cost-effective and safe measure if implemented appropriately.



P-471

NEEDS FOR BLOOD TRANSFUSION IN PATIENTS WITH AUTOLOGOUS STEM CELL TRANSPLANTATION

EB Protopopova, NE Mochkin, VY Melnichenko, EA Shestakov and EB Zhiburt

Pirogov National Medical Surgical Center, Moscow, Russian Federation

Background: More than 50,000 stem cell transplantations are carried out annually. Most of them (57–59%) are autologous stem cell transplantations (ASCT).
Hemopoiesis deficiency after chemotherapy and ASCT often needs correction with blood components.
Aim: To define needs for blood transfusion in various patients after ASCT.
Material and methods: Platelet and red blood cell transfusion have been retrospectively studied in 169 patients with autologous stem cell transplantation. Patients were divided into 2 groups: with autoimmune (n = 87) and hematological (n = 82) diseases. Bleedings were not registered, lethal outcomes - 2.
We performed prophylactic platelet transfusions in patients with platelet count at or below 10,000/μL. 158 units were transfused to 104 recipients. An apheresis platelet concentrate contained not less 2×10^{11} cells.
Red cells were transfused on the base of clinical and laboratory complex of anemia signs. Usually target hemoglobin concentration in patients with ASCT is 8 g/dl and higher. 55 units of red cells were transfused to 37 recipients.
Frequency and needs for transfusion of platelets and red blood cells in various patients after ASCT were evaluated.
Results: It is established that platelets were transfused to 99% of hematological patients, 47% of them received 2 and more units. 26% of patients with autoimmune disease needed platelet transfusion; majority of them received a single unit.
Frequency of platelet transfusions in hematological patients is 3,7 times higher in compare with autoimmune group.
The mean need for platelets is 0,3 unit per patient with autoimmune and 1,6 unit per patient with hematological desease.
It is established that red cells were transfused to 92% of patients with ASCT; majority (70%) of them got a single transfusion, which was enough for hemoglobin deficiency correction. Multiple transfusions required in only 7% of patients.
Negative correlations between concentrations of hemoglobin level at the beginning of ASCT and needs for red cell transfusions were revealed.
6% of patients with hematological and 39% with autoimmune desease need red cell transfusion after ASCT.
The mean need for red cells in patients with ASCT is 0,1 unit per patient with autoimmune and 0,6 unit per patient with hematological disease.
Conclusion: 99% of patients with hematological and 26% of patients with autoimmune disease need platelet transfusion after ASCT.
Frequency of platelet transfusions is 3,7 times higher in hematological patients in compare with autoimmune diseases group.
The mean need for platelets in patients with ASCT is 0,3 unit per patient with autoimmune and 1,6 unit per patient with hematological disease.
39% of patients with hematological and 6% of patients with autoimmune disease need red blood cell transfusion after ASCT.
The mean need for red cells in patients with ASCT is 0,1 unit per patient with autoimmune and 0,6 unit per patient with hematological disease.

P-472

DIAGNOSTICS AND CORRECTION HEMOSTASIS SYSTEM DISORDERS IN PATIENTS WITH ACUTE ULCERATIVE GASTRODUODENAL BLEEDING

B Bakhovadinov[1], MA Kucher[1], DE Pevcov[1], NK Ashyraliev[2] and A Vakhidov[2]

[1]First State Pavlov Medical University, St. Petersburg, Russian Federation [2]The Republican Scientific Blood Center, GCH SMP, Dushanbe, Tajikistan

Background: In acute gastrointestinal bleeding hemostasis disorders may occur, with formation of disseminated intravascular coagulation syndrome (DIC) that initially manifested with signs of hypercoagulation, which can be relieved at timely correction. In severe bleeding prolonged activation of coagulation system can lead to depletion of haemostatic factors, consumption coagulopathy and massive blood loss. Thus the effectiveness of haemostatic therapy of acute massive hemorrhage depends on early pathogenic correction of disorders in hemostasis system.
Aims: To study the relationship between development of coagulopathy in patients with ulcerative gastrointestinal bleeding, depending on the amount of blood loss.
Methods: Hemostasis system in 160 patients with ulcerative gastrointestinal bleeding was investigated (age from 20 to 86 years). Depending on the amount of blood loss, patients were divided into 3 groups: group 1 (n = 50) blood loss 1,04 ± 0,14 l; group 2 - blood loss 1,70 ± 0,17 l (n = 50); 3rd - blood loss 2,68 ± 0,27 l (n = 60). Control group included 20 practically healthy persons. Amount of Hb, Ht, erythrocytes, platelets, bleeding time by Duke, RPT by Bergerhoff-Rocca, APTT, PI by Quick, TT by Sirmai, antithrombin-III, tolerance of plasma to heparin, retraction of blood clot, fibrinogen concentration by Rutberg were evaluated.
Results: Patients in group 1 with moderate bleeding had a decrease of hematological parameters and platelets compared with control group 14,7% ($P > 0.5$). Indicators of coagulation hemostasis were characterized by a moderate increase in haemostatic activity, but rates of anticoagulant and fibrinolytic system were not significantly different from the control group. Patients in group 2 had 1,5 times decrease of red blood indices, antithrombin-III in 1,35 times, platelets in 1,52 times, decrease in PI in 1,3 times ($P < 0, 05$), decreased fibrinogen concentration by 15,15% and antithrombin III by 28% ($P > 0.05$). Patients in 3rd group in comparison with the 2nd group noted a significant decrease in platelet level, depletion of coagulation system and enhancement of anticoagulant and fibrinolytic system, reduction of antithrombin III in 1,54 times ($P < 0.05$). Patients of the 1st group didn't need any correction of hemostatic system. The deficit of blood volume was corrected by i.v. crystalloid solutions at the ratio 1:3 to blood loss. In the second group except crystalloid solutions (30–45 ml/kg), fresh frozen plasma (10–15 ml/kg) was used to prevent the manifestation of DIC. Patients is third group received crystalloid solutions at the ratio 1:3 (20–25 and 60–75 ml/kg respectively) in combination with high doses of antifibrinolytics and 400 ml of 1,5% Reamberin. Due to dynamic control of hemostasis and its correction with new schema mortality from massive ulcerative gastrointestinal bleeding was reduced from 7,5% to 2,5%. Among 55 patients from group 2 and group 3 treated by specialized resuscitation and anesthesia blood transfusions teams there were no lethal outcomes.
Summary: Ulcerative gastrointestinal bleeding is associated with impairment of hemostatic system depending on the amount of blood loss. Early diagnostic of hemostasis disorders, its correction with high doses of FFP in conjunction with i.v. crystalloid solutions, antifibrinolytics, including specialized blood transfusions brigade are an effective measures to stop bleeding and reduce mortality.

P-473

This abstract has been withdrawn.

P-474

COMPARING LINEAR AND BINOMIAL-NORMAL MIXED MODELS FOR META-ANALYSIS OF COUNT DATA ON RARE EVENTS

NH Saadah[1], T Stijnen[2], RA Middelburg[3], S le Cessie[3], MR Schipperus[4] and JG van der Bom[5]

[1]Leiden University Medical Center, Leiden, The Netherlands [2]Department of Medical Statistics, LUMC, Leiden, The Netherlands [3]Department of Clinical Epidemiology, LUMC, Leiden, The Netherlands [4]Haga Teaching Hospital, The Hague, The Netherlands [5]Sanquin CCTR, Leiden, The Netherlands

Introduction: Linear mixed models (e.g. DerSimonian Laird technique) are often used in meta-analysis of count data on rare events - a data type used extensively in analysis of transfusion related adverse events. These models assume a normally distributed within-study likelihood with variance uncorrelated to and equal to the mean and require correction factors for zero-case studies to avoid mathematical singularities. Binomial-normal models more properly model incident count data by assuming a binomially distributed within study likelihood with a normally distributed random effect, both with unknown variance, allowing estimation of pooled incidences (π) and odds ratios (ORs) without the inaccuracies introduced by correction factors and the assumed mean-variance equivalence.
Study Aim: We compared linear mixed and binomial-normal models in prediction of pooled incidence (π) and predictor OR via iterative simulation of meta-analyses on rare incident count data.
Methods: Monte Carlo methods were used to generate random count data describing two treatments with known adverse event incidences. Iterative simulation of 10,000 meta-analyses with 15 studies in each treatment arm were performed. Data with inflated variance, zero cells, and publication bias (characteristics common to transfusion medicine data) were analyzed. Means and 2.5%/97.5% percentiles ($P_{2.5}$, $P_{97.5}$) for the absolute prediction error difference between the two methods (ε_{LIN}-ε_{BN}) were computed for both π_{pred} and OR_{pred} (comparing the two treatment arms).
Results: Binomial-normal [BN] techniques were superior to mixed linear [LIN] techniques in predicting pooled incidence and OR for datasets in which one arm had a high proportion of zero-cells (π: ε_{LIN}-ε_{BN}=0.10 events/1000 exposures [$P_{2.5}$,$P_{97.5}$:-

0.05–0.44]; OR: ε_{LIN}-ε_{BN}=0.17[$P_{2.5}$,$P_{97.5}$:-0.12–0.61]) or exhibited publication bias (π: ε_{LIN}-ε_{BN} =0.06[$P_{2.5}$,$P_{97.5}$:-0.05–0.26]; OR: ε_{LIN}-ε_{BN} =0.11[$P_{2.5}$,$P_{97.5}$:-0.12–0.43]). Absent zero-cells and publication bias, the two methods returned comparable results analyzing data sets with both low variance (π: ε_{LIN}-ε_{BN}=0.02[$P_{2.5}$,$P_{97.5}$:-0.04–0.12]; OR: ε_{LIN}-ε_{BN}=0.05[$P_{2.5}$,$P_{97.5}$:-0.10–0.31]) and extreme variance (π: ε_{LIN}-ε_{BN}=-0.09 [$P_{2.5}$,$P_{97.5}$:-0.39–0.05]; OR: ε_{LIN}-ε_{BN}=0.02[$P_{2.5}$,$P_{97.5}$:-0.14–0.25]).

Conclusions: Binomial-normal models are superior to linear mixed models in meta-analysis of (adverse) incident count data with zero-cells and publication bias. They are at least as good as linear mixed models for count data with and without high variance.

P-475

This abstract has been withdrawn.

P-476

TRANSFUSION THERAPY PATIENTS' EXPERIENCE OF PATIENT-CENTRED COMMUNICATION

M Cankovic Kadijevic[1] and IB Petrovic[2]

[1]National Institute of Blood Transfusion, Belgrade, Serbia [2]Department of Psychology, Faculty of Philosophy, University of Belgrade, Beograd, Serbia

Background: At the National Blood Transfusion Institute of Serbia Department of clinical transfusiology, patients with different primary, mostly oncological and hematological, diagnoses, receive transfusion therapy under the day hospital conditions. When they come for the treatment, majority of patients express prominent need to communicate about the course of their illness and health condition. Thus, in addition to standard transfusion therapy procedures, drawing inspiration from the communications, management, social marketing and service quality literature, doctors at Department of clinical transfusiology introduced innovative approach to interacting with patients. Doctors developed individualized, patient-centred "just-in-time"communication. Patient-centred communication was based on active listening to the patient, openly expressing empathy, giving space to patient to ask questions and responding to patient's communication needs. "Just-in-time"communication was built around specific patient's concerns that change along the course of illness and therapy (questions about the primary diagnosis, other therapies, medication taking regimen and dietary guidelines, and all other issues that affect patient's life, including end-of-life issues).
Aim: To explore patients' perception of innovative, patient-centred "just-in-time"-communication with transfusion therapy specialist.
Method: The sample consisted of 200 transfusion therapy patients (38% women; sample age range 19–92 years, 53% age group 51–70; 49% with high school; receiving blood transfusion 43%, therapeutic phlebotomy 57%). Exploratory, qualitative study was based on semi-structured individual interviews about patients' experiences of transfusion therapy related interaction. Qualitative data were analyzed by identifying and exploring content themes applying Weft QDA software.
Results: All patients' reactions were extremely positive. Key themes in patients' experiences of innovative, as opposed to traditional doctor-patient interaction were related to: 1. perception of doctor's attitude towards the patient and illness, and 2. information sharing. Patients' overall impression was that, contrary to some doctors focusing on illness and completely disregarding the patient as a person, doctor truly cared about their well-being, which made them feel appreciated and esteemed (*Doctor cared for me as a person*; *I felt as a human being; This was beyond my expectations*). Patients were satisfied with the opportunity to discuss with doctors about their illness and/or condition and treatment (*I know what to expect; Everything was clear to me; Doctor talks as teacher at school, I could easily understand everything*). Moreover, patients assessed the overall atmosphere as warm and pleasant (*I felt protected; I am in a good mood when I come for the therapy*). There were no differences in patients' experiences based on their gender, age, educational level and received transfusion therapy.
Conclusion: Patient-centred communication supports patients in overcoming some of the barriers they encounter in the course of their illness and motivate them to adopt behaviors that might be most beneficial to them. Though patient-centred communication should be expected as inherent part of doctor-patient interaction, patients seldom report experiencing it. Effective communication design, guidelines and training would be essential for building lasting and rewarding doctor-patient relationships that could supplement transfusion therapy in a substantial way. Communication should be regarded as a core competency of doctors specialized in transfusion medicine. *Support: Ministry of Science, Project-149018D.*

P-477

ANALYSIS OF PERIOPERATIVE BLOOD USAGE OF VENTRICULAR SEPTAL REPAIR IN DIFFERENT TYPES OF HOSPITALS

Z Qu

Wuhan Blood Center, Wuhan, China

Background: The allocation of blood products in hospitals in a big city is usually difficult, as the clinical demand is beyond the exist blood resources. As a result, blood center have to analyze the use and requirement in various types of hospitals, and then find a scientific method to allocate blood products to hospitals and patients.
Aims: To analyze the actual blood usage in ventricular septal repair operation of different hospitals, providing useful reference for rational clinical use of blood.
Methods: The blood components usage of patients with ventricular septal repair operation in specialized hospital, large general hospital, district general hospital were retrospectively analyzed. We collected infusion rate and quantity of red blood cell suspention, plasma and cryoprecipitate, then making a statistic analysis for the data.
Results: In specialized hospital, RBC transfusion rate (52.5%), infusion volume (1.00 ± 0.99 U) were significantly lower than the large general hospital infusion rate (95.3%), infusion volume (3.25 ± 1.77 U), was also significantly lower than the district general hospital infusion rate (95.8%), infusion volume (3.08 ± 1.63), with both $P < 0.05$. In specialized hospital, the plasma transfusion rate was 1%, significantly lower than the large general hospitals (94.5%) and regional general hospitals (87.3%), $P < 0.01$. The cryoprecipitate infusion rate in Large general hospital for ventricular septal defect surgery was zero, below the specialized hospital (1.5%) and regional general hospitals (2.5%), respectively, both $P < 0.01$.
Conclusions: Specialized cardiological hospital carried on a well-controlled blood usage in ventricular septal repair opreation, with a significant low usage than that of large and regional general hospitals.

P-478

DIAGNOSIS AND MANAGEMENT OF THROMBOTIC THROMBOCYTOPENIC PURPURA: HOSPITAL PRACTICE SURVEY BY THE AUSTRALIAN & NEW ZEALAND THROMBOTIC THROMBOCYTOPENIC PURPURA AND THROMBOTIC MICROANGIOPATHIES REGISTRY

L Kivivali[1], N Waters[1], L Fox[1], E Wood[1], Z McQuilten[1], P Blombery[2] and S Cohney[1]

[1]Monash University, Melbourne, Australia [2]Peter MacCallum Cancer Centre, Melbourne, Australia

Background: Thrombotic thrombocytopenic purpura (TTP) and thrombotic microangiopathies (TMAs) are life-threatening conditions. Few clinical trial data exist to guide diagnosis and management (including plasma exchange [PEx] and adjunctive therapies).
Aims: To describe approaches to diagnosis, treatment, and transfusion management by hospitals contributing to the Australian and New Zealand (ANZ) TTP/TMA Registry.
Methods: The ANZ TTP/TMA Registry was established in 2009 and prospectively collects data on diagnosis, management and clinical outcomes of TTP and other TMAs. Forty-two hospitals across ANZ participate, with 258 patients enrolled to April 2016. A survey was distributed to all participating hospitals in 2009 and again in 2015.
Results: Response: The survey was sent to 25 hospitals in 2009 and 36 hospitals in 2015, with 20 (76%) and 26 (72%) responses respectively.
Clinical team and protocol: Management of patients with TTP via a prescribed protocol was reported by under half of hospitals in both 2009 and 2015 (Table 1). A majority of hospitals reported in both 2009 (70%) and 2015 (54%) that patients were managed by both haematology and nephrology, or by either department depending on the patient's presentation (Table 1.).
Diagnosis and testing: Hospitals reported an increase in requesting ADAMTS13 levels from 50% in 2009 to almost 100% in 2015; however, anti-ADAMTS13 antibody testing only increased slightly (Table 1).
Management: If there was delay in starting PEx, plasma infusion was commonly used as a temporising measure (Table 1). Cryodepleted plasma (CDP) was the favoured PEx fluid in 2009, changing slightly to fresh frozen plasma (FFP) in 2015; no preference was cited by a relatively large number of hospitals (2009: 15%, 2015: 19%; Table 1.). Tapering of PEx before stopping in all patients was reported by just over half the hospitals in 2009; however, in 2015 only a third of hospitals reported

Table 1. Hospital survey on the diagnosis and management of TTP patients from hospitals participating in the Australian and New Zealand TTP/TMA Registry

Question	2009 (n=20)	2015 (n=26)
Protocol for TTP management?	8 (40%)	9 (35%)
ADAMTS13 testing always requested?	10 (50%)	25 (96%)
Anti-ADAMTS13 antibody testing always requested?	3 (16%)	7 (27%)
Patient managed by haematology or nephrology (vs other service)?	14 (70%)	14 (54%)
Plasma infusion if delay in PEx?	12 (60%)	13 (50%)
Preferred PEx fluid?	FFP: 7 (35%) CDP: 9 (45%) No preference: 3 (15%)	FFP: 10 (39%) CDP: 8 (31%) No preference: 5 (19%)
Always taper PEx?	11 (55%)	8 (31%)
Anti-platelet agent used?	Always: 1 (5%) Sometimes: 8 (40%) Never: 8 (40%)	Always: 4 (15%) Sometimes: 8 (31%) Never: 11 (42%)
Rituximab for patients refractory to PEx?	12 (60%)	12 (46%)
Steroids always prescribed?	11 (55%)	15 (58%)

tapering in all patients, with the majority reporting they would only taper PEx sometimes (54%). Always using an anti-platelet agent was reported in 5% of hospitals in 2009 and slightly increased to 15% in 2015; the remaining hospitals either used anti-platelet agents sometimes (2009: 40%, 2015: 31%), or never (2009: 40%, 2015: 42%). Use of rituximab for refractory patients did not increase between survey time points (2009: 60%, 2015: 46%). Other agents reportedly used in refractory patients include cyclophosphamide, pulsed methylprednisolone, N-acetyl cysteine, and bortezomib. Steroid prescription did not change with just over half of hospitals in 2009 and 2015 using these in all patients.

Conclusion: In hospitals managing TTP in ANZ between 2009 and 2015 ADAMTS13 testing at diagnosis increased, however the majority of hospitals currently do not request ADAMTS13 antibody testing. There have been slight changes in PEx fluid preference, with fewer hospitals requesting CDP. The number of hospitals describing no PEx fluid preference remained largely unchanged. Less than half the hospitals participating in the TTP/TMA Registry have institutional treatment protocols for TMA, highlighting an opportunity for practice improvement and a potential role for national guidelines.

P-479

THE RELATIONSHIP OF ABO BLOOD GROUPS AND CERTAIN HEMATO-ONCOLOGICAL DISEASES IN A SERVICE OF PEDIATRIC HEMATO-ONCOLOGY (SHOP)

S Jayche[1], A Loutfi[2], R Alami[3], M Benajiba[1] and AT Ahami[2]

[1]CNTS Rabat Maroc, Rabat, Morocco [2]Unité de Neurosciences et Santé, Département de Biologie, Faculté des sciences, Kenitra, Morocco [3]Institut de Formation aux Carrières de Santé, Rabat, Morocco

Aims: The knowledge of the of ABO blood groups is imperatively important in hematological diseases because of the need for one or more transfusion for a patient. The association between disease and ABO blood group had long been studied and has been subject of many subsequent studies. This study sought to determine the relationship between blood groups ABO / Rhesus and the expected risks being affected with hematological diseases such as: thalassemia, hemophilia, leukemia and sickle cell anemia.

Methods and Results: This is a retrospective study of 630 child patients SHOP (241 girls and 389 boys) who needed blood during 2011. The information is taken from data registered with the Secretariat of the immuno-hematology recipient laboratory. We compared the prevalence of blood in patients suffering from thalassemia (45.36% of group O and 53.63% of non-O groups), hemophilia (34.78% of group O and 65.21% of non-O group), sickle cell disease (40.32% of group O and 59.67% of non-O groups) and leukemia (46.15% of group O and 53.84% of non-O group), differentiation was not significant $P = 0.72$ even for rhesus $P = 0.03$. In addition, it was not significant concerning the gender of patients $P = 0.40$.

Conclusions: The possible correlation between some illnesses and blood groups was studied, the results revealed that there is no impact of positive or negative Rhesus on these diseases and the differences between disease and blood groups seem to have no correlation, even if they are varied, but they follow the natural distribution of blood groups in Morocco. At present, it is impossible to give a plausible explanation for the associations and the differences mentioned above. Further studies must be conducted to clarify the possible relationship.

P-480

This abstract has been withdrawn.

P-481

VALIDATION STUDIES FOR THE THAWING OF A POOLED, SOLVENT/DETERGENT TREATED PLASMA (OCTAPLASLG®) USING DIFFERENT THAWING DEVICES

A Heger, P Wenz, M Karlovits, K Pock and J Römisch

Octapharma PPGmbH, Vienna, Austria

Background: The most common way for the thawing of plasma, such as the pooled, solvent/detergent treated plasma octaplasLG®, is a water bath with good circulation at 37°C. The thawing process takes minimum 30 to maximum 60 min depending on the number of bags thawed in parallel.

Aims: The aim of this study was to perform validation of the thawing process for octaplasLG® using different thawing devices. Optimized settings for temperature and thawing time should be defined based on the results of both temperature measurements and extensive biochemical characterization studies.

Methods: Thawing of octaplasLG® bags was validated for the water bath system Quick Thaw (DH4, Helmer), the dry tempering systems Plasmatherm (Barkey) and SAHARA-III (Transmed Medizintechnik GmbH), as well as the microwave oven Transfusio-therm 2000 (EIC Umwelt- und Medizintechnik Ltd). Product surface temperature was monitored using infrared-sensors. Plasma temperature within the bags was determined after thawing by introduction of a calibrated thermometer. For each device, times to defrost plasma and to reach 37°C were defined. In the second part of the studies, using the selected thawing conditions, 7 batches of octaplasLG® of different blood groups were thawed and tested on product release parameters. Plasma bags thawed with the water bath circulator (MB-13A, Julabo GmbH) were used for comparison.

Results: The fastest thawing was observed for the microwave oven. However, all octaplasLG® bags thawed by different devices and optimized thawing conditions were clear and free of solid and gelatinous particles, indicating no protein denaturation or overheating. In addition, no significant differences were found in the coagulation and inhibition activity and haemostatic potency of octaplasLG® when thawed by the different devices tested.

Summary/Conclusions: Our study confirmed that octaplasLG® can be thawed using all above listed devices without any negative influence on the plasma quality, presupposed that optimized settings defined for this plasma product are used.

P-482

WAYS TO IMPROVE THE PREVENTION OF HEMOLYTIC TRANSFUSION REACTIONS

B Bakhovadinov[1], DE Pevcov[1], MA Kucher[1], A Tretyakova[1] and GS Ashurova[2]

[1]First State Pavlov Medical University, St. Petersburg, Russian Federation [2]Dushanbe city maternity hospital N3, Dushanbe, Tajikistan

Background: Hemolytic transfusion reactions (HTR) are relevant problem in medical practice which remains high: 1 case for 6000–29,000 transfusions. Interaction of antigen with antibody on the membrane of the erythrocyte is able to induce the chain of neuroendocrine reactions, activation of complement, cytokine and coagulation system, which cause clinical manifestations of HTR, especially in AB0 - incompatible transfusions. One of the most important reasons for development of immunological conflict, leading to HTR, is the presence of antibodies in the recipient serum to donor red blood cells antigens. In accordance with current Russian law in transfusion medicine, identification of alloantibodies to antigens of red blood cells prior transfusion is required.

Aims: To analyze HTR incidence.

Methods: A retrospective analysis of HTR was performed from 1989 to 2015, which were registered in Republic of Tajikistan maternity institutions and in 2 intensive care and detoxification centers. During this period 86 cases of HTR were registered - approximately 1 case for 10,116 blood transfusions. Of these complications 49 (56,9%) were in patients with obstetric and gynecological profile.

Results: It was found that the reasons for HTR development were: incompatibility in AB0 blood group system - 9 cases; incompatibility of antigen D - 31 cases, in 7 cases incompatibility of minor antigens Rhesus system (C, C, E, e) and in 16 cases in Kell system. In one case, HTR was due to hemolyzated blood, as a result of improper

storage in refrigeration. In all cases, complications were doctor's mistakes. In 3 cases doctors did not identify AB0 by conducting initial blood testing, taken transfusion data from the previous hospitalizations. As a result of doctor violation and insufficient knowledge of transfusion medicine in 14 of 49 cases, patients were unnecessarily carried out blood transfusion. In 13 cases of 49, HTR had not been diagnosed in time, which led to a delay in implementation of therapeutic interventions for the prevention of acute renal failure, uncontrolled bleeding, shock and impact on patient outcomes: mortality was 51,2% (25 patients).

Summary: To prevent HTR it is needed to train doctors in the field of immunohematology and clinical transfusion, to use modern methods of blood testing, to identify possible alloantigens, to understand the specificity of identified alloantigens in pregnant and postpartum women. To make an individual blood donor selection, use three-step method of testing for compatibility with the use of antiglobulin serum in accordance with W.H.O recommendations. It is also necessary to use W.H.O standards for the rational clinical use of blood transfusion and alternatives to transfusion of donor blood components.

P-483

RESIDUAL RISK OF INFECTIONS TRANSMISSION ASSOCIATED WITH BLOOD TRANSFUSION

B Bakhovadinov[1], NK Ashyraliev[2], MA Kucher[1], YA Serov[1], DE Pevcov[1] and MA Estrina[1]

[1]First State Pavlov Medical University, St. Petersburg, Russian Federation [2]The Republican Scientific Blood Center, GCH SMP, Dushanbe, Tajikistan

Background: The risk of possible transmission of hemotransmissive infections through blood transfusion and blood components still remains an important issue.

Aims: To study the occurrence and specificity of hemotransmissive infections markers among various categories of donors in the Republican scientific blood Center of Tajikistan (RSBC) and to compare the obtained results with analogous indicators in other countries.

Methods: 46,780 blood samples from RSBC donors were tested by ELISA for HBsAg, anti-HCV, anti-HIV 1/2, p24 antigen, antibodies to *Treponema pallidum.* Initially positive blood samples, in accordance with international recommendations were retested in two formulations of the same method. Also 20,906 blood samples from first-time donors were tested by polymerase chain reaction (PCR) in the case of negative ELISA test for DNA, RNA viruses of hepatitis B, C, HIV in a separate trial.

Results: ELISA revealed 798 primary positive tests for HCV and 20 cases in secondary donors, HBV marker was identified in 1462 primary donors and in 6 secondary donors, antibodies to *Treponema pallidum* were detected in 1065 donors and in 13 secondary donors, HIV markers in 140 and 5 donors respectively (Fig. 1).

The residual risks of infections associated with blood transfusion in the United States are 2,03 for HIV; 9,70 for HCV; 15,83 for HBV - for 1 million donations. For the city of Dushanbe 5,0; 69,57 and 20,64 for 1 million donations respectively. Thus the residual risk of transfusion infections for HIV in Dushanbe is higher than in USA 2,46%, for HCV - 7,17%, HBV - 1,30 times. A comparison with the city of Astana (Kazakhstan) shows that the residual risk for HIV in Dushanbe 4,16 times higher, for HCV is less than 1,97 times, HBV is less than 6.07 times. Such results can be explained by presence of additional PCR testing of blood donors and primary shortening of the serological negative infection window period.

Summary: For the first time the prevalence and incidence of hemotransmissive infections among blood donors in the city of Dushanbe was determined. High residual risks of blood transfusion infections such as HIV, HBV and HCV, should stimulate the implementation of measures to improve infection safety of blood by more careful recruitment and selection of donors from populations at lower risk, the validity of PCR testing, implementation of pathogen inactivation in blood components and blood conservation technologies in clinical practice.

Fig.1: «Prevalence and incidence of blood transfusion associated infections among blood donors at the Republican scientific blood Center of Tajikistan »

Infection markers	Primary donors (n=20960)		Secondary donors (n=25 820)	
	Positive	All cases	Positive	All cases
HCV	798	4 524,6	20	430
HBV	1 462	8 289,5	6	129
Anti-pallidium	1 065	6 038,5	13	284,7
HIV	140	793,8	5	107,5
All	3 465	19 646,5	49	1 053,5

P-484

MEASUREMENT FOR STRATEGIC IMPROVEMENT AND CLINICIAN FEEDBACK: PATIENT BLOOD MANAGEMENT IN NEW SOUTH WALES, AUSTRALIA

S Francis, A Thomson and A Dalli

NSW Clinical Excellence Commission, Sydney, Australia

Background: In 2006, the Blood Watch program was established in NSW to support best practice transfusion medicine initiatives in the NSW health system. Over time, there has been a paradigm shift from traditional transfusion medicine approaches, such as appropriate use of blood and blood products and donor blood management, to the management of the patients own blood to improve outcomes and reduce the known risks of anaemia, bleeding and the use allogeneic blood products. This is known as Patient Blood Management (PBM).

In Australia, there are 5 modules, collectively known as the Patient Blood Management Guidelines, progressively published since 2011. Some assessment has been undertaken on the uptake of PBM as best practice across Australia, with results indicating variation in practice, resources, knowledge, and capacity for services to implement PBM strategies. There is scarce evidence on how clinical networks, local departments or individual clinicians have adopted PBM principles at the patient level.

Aims: The end goal of our project is to develop a strategic implementation framework for PBM guidelines in NSW Health facilities. This first required developing a knowledge base of the current system capacity and clinical practices as they relate to PBM.

Our aim was to: Measure health services capacity to provide PBM care. Assess care delivery and compliance with perioperative and medical PBM guidelines.

Methods: A pilot phase medical record audit was undertaken in the cardiothoracic surgical population in 8 tertiary referral hospitals in NSW. The tool was refined following the pilot phase, and 25 hospitals across NSW were invited to participate in an audit of perioperative and medical patient journeys. Individual hospitals were provided with data on high red cell using specialty groups, and selected a specific population for local auditing.

The audit was a retrospective medical record audit, based on principles outlined in the PBM guidelines. Wherever possible, responses were in a yes/no format. Patient selection for audit was random form the previous 12 months, and was not limited to those receiving blood products.

Results: All hospitals provided responses to the survey on leadership, service design and procedures developed for our first aim. All hsopitals had a massive transfusion protocol, and most had executive and committee support for PBM. Key areas for improvement were identified in specific policy and procedures and access to supportive techniques and skills.

Across 25 hospitals, 472 perioperative, and 458 medical patient journeys were assessed for aseessment, and management of anaemia, bleeding risk, blood loss and interventions related PBM. Key areas identified for system wide improvement opportunities were identified and include:

In the surgical population: Fe deficiency assessment and management, intraoperative techniques to maintain red cell mass, and the use single unit red cell transfusion

In the medical population: Fe deficiency assessment and management, and the use of single unit red cell transfusion.

Summary/Conclusion: The audit process and tool has provided a great baseline snapshot on actual patient care as it related to PBM. Information has been fed back to local hospitals and work plans are being developed.

Collated results have been provided at a state level and 4 key areas have been agreed upon for the first phase program; single unit transfusion, tranexamic acid in surgery, appropriate use of FFP for INR <1.8, improving anaemia and Fe deficiency assessment and management. A pilot phase obstetrics audit is also currently underway.

P-485

PRACTICES ASSOCIATED WITH PLATELET PREPARATION AND TRANSFUSION: COUNTRY-WIDE SURVEY

RC Dara, AK Tiwari, D Arora, DP Acharya, G Aggarwal and G Bhardwaj

Medanta- The Medicity, Gurgaon, India

Background: Platelet concentrates (PC) are generally prepared from whole blood either by platelet-rich plasma-method or buffy coat-method. Apheresis platelets are relatively uncommon but growing. Significant disparity exists between the preparation methods, modifications, quality assessments and transfusion of platelets especially with respect to ABO and Rh compatibility. Though several countries have well defined guidelines for preparation and transfusion of platelets few countries still have wide variation in practices. Recently a survey has been published from North America with an objective to determine practices regarding the use of platelets

containing ABO- incompatible plasma. In India, again there is substantial variation in terms of preparation and practice of platelet transfusion with some of the centers moving to even ABO and Rh incompatible platelet transfusions.
Aim: To determine current practices of preparation and usage of platelets in blood transfusion services across India.
Study Design and Methods: An electronic survey was sent to 1676 transfusion services across the country during the study period (Nov 2015-March 2016) requesting information on type of facility, accreditation status, whole blood collection, platelet preparation and modification (leuco-reduction/pooling/volume reduction), quality parameters, ABO and Rh-D matching policies, anti-A and anti-B measurements in platelets and practices regarding ABO and Rh-D incompatible PC transfusions in adult and pediatric patients.
Results: Of the 1676 transfusion services, we received 396 (23.6%) responses from all regions of the country. Most of them were from medium-sized blood banks (68.9%) with the collection between 5000 and 10000 units/annum. Of these respondents, 80.8% (n = 320) prepare platelets while 19.2% (n = 76) of the respondents did not have the facility to prepare platelets (only whole blood is practiced). 63.9% (n = 204) respondents practice PRP method while 36.1% (n = 116) practice platelet preparation using buffy coat method. Only 5.3% did practice pooling of platelets while leuco-reduction is being practiced by only 16.1% respondents. Majority of respondents 63.1% (n = 201) practice whole blood derived platelets and facility of collecting apheresis platelets were not available with 21.3% (n = 69) of respondents. Significant practice variation was reported in context of incompatible platelets transfusion in adults. One or more elements were reported for transfusions: only ABO-compatible platelet transfusion (32.9%); ABO-incompatible platelet transfusion (14.2%), Rh-incompatible (37.4%), notification of treating physician (5.2%); notification of treating and blood bank physician (37.8%), volume reduction of ABO-incompatible plasma (30.2%); screening for critical titer of anti-A or anti-B (11.6%). In contrast in neonate, majority practice ABO-identical platelet transfusions (65.8%) while 14.8% have no separate policy for neonates.
Conclusion: There is significant variation in practice of platelet preparation and transfusion among surveyed transfusion services in our country. This suggests need for additional studies to produce more robust evidence eventually leading to appropriate country specific guidelines.

P-486

STORAGE TIME AFFECTS THE EFFECTIVENESS OF PLATELET TRANSFUSION

EB Protopopova

Pirogov National Medical Surgical Center, Moscow, Russian Federation

Background: Platelet transfusion is an effective method of treatment and prophylaxis of platelet hemostasis deficiency in patients with various pathologies. However this resource is limited due to short shelf life and the risks bound to transfusion.
During the storage functional properties of platelets are changing. It can influence efficiency of transfusions.
Aim: To evaluate efficiency of platelet transfusion with various period of storage.
Material and methods: One 158 transfused platelet concentrates (PC) have been retrospectively studied. Components were collected by apheresis and contained not less 2×10^{11} cells. 73% of units contained 3×10^{11} and more cells.
We performed prophylactic platelet transfusion with the platelet count at or below 10,000/μL.
Body temperature of recipient after transfusion, platelet count prior to and after transfusion, corrected count increment (CCI) in 24 h after transfusion, number of noneffective (CCI < 7500) transfusions were analyzed.
Results: Sixty-five percentage of platelets were transfused on the first 3 days of storage. 24% of units were transfused on the last (5th) day of shelf life.
Febrile non-chemolysis transfusion reactions were identified in 5,1% of transfusions. Quarter of reactions should be differentiated with infections and other reasons of temperature increase. The association of febrile transfusion reactions with storage time and number of cells in the unit was not revealed.
One full-fledged platelet apheresis unit provides CCI on average 16,100.
In platelets transfused on 4–5 day of storage there is a significant (33%) CCI decrease in compare with platelets transfused on 1–3 day of storage.
CCI after transfusion of PC containing less than 3×10^{11} cells is at 18% lower than of PC containing 3×10^{11} or more cells.
16% of platelet transfusions are noneffective. 32% of platelets transfused on 4–5 day of storage are noneffective. 72% of all noneffective transfusions were transfused with 4–5 day stored PC.
The proportion of effective transfusions decreases with increasing PC storage time: from 93% on day 1–3 to 68% on day 4–5.
Part of effective transfusions is not related to the number of cells in unit.
Conclusion: Febrile transfusion reactions are not associated with storage time and number of cells in the unit.
The full-fledged platelet apheresis unit provides CCI on average 16,100.
Efficiency of PC containing less 3×10^{11} cells is lower than of PC containing 3×10^{11} or more cells.
Transfusion of platelets is most effective during the first 3 days of storage.
The proportion of effective transfusions decreases from 93% to 68% with increasing storage time.
Part of effective transfusions is not related to the number of cells in PC.

P-487

THE INDEPENDENT EFFECT OF A SINGLE EARLY PLATELET TRANSFUSION ON BLEEDING AND ADVERSE OUTCOMES IN CARDIAC SURGERY

FMA van Hout[1], EK Hogervorst[1], PMJ Rosseel[2], JG van der Bom[1], M Bentala[2], ELA van Dorp[3], A Brand[1], NJ van der Meer[2] and LMG van de Watering[1]

[1]Sanquin, Leiden, The Netherlands [2]Amphia Hospital, Breda, The Netherlands [3]Leiden University Medical Centre, Leiden, The Netherlands

Background: Conflicting results have been reported concerning the effect of platelet transfusion on bleeding and adverse outcomes in cardiac surgery.
Aim: The aim of this study was to assess the independent effect of a single early intraoperative platelet transfusion, in the absence of concomitant red blood cell or plasma transfusion, on bleeding and adverse outcomes in cardiac surgery patients.
Methods: For this observational study a cohort of 23,860 patients undergoing cardiac surgery was analysed. Patients who received one early (shortly after cardiopulmonary bypass while still in the operating room) platelet transfusion (consisting of 5 pooled buffy coats), and no other blood products, were defined as the intervention group. The intervention group was matched 1:3 by propensity score to identify the reference group. So the reference group consisted of patients who received no early transfusion and were most comparable to the intervention group.
Results: The intervention group comprised 169 patients and the reference group 507 patients. No difference between the groups was observed concerning blood loss, reinterventions for bleeding / tamponade, thromboembolic complications, infections, organ failure and in-hospital mortality. However, patients in the intervention group more often required vasoactive medication 139/169 (82.2%) vs 370/507 (73.0%), (odds ratio 1.72; 95%-confidence interval 1.10–2.67); prolonged mechanical ventilation 92/169 (54.4%) vs 226/507 (44.6%) (odds ratio 1.49;1.05–2.11); prolonged intensive care 95/169 (56.2%) vs 240/507 (47.3%) (odds ratio 1.43;1.01–2.03); red blood cell transfusion 75/169 (44.4%) vs 145/507 (28.6%) (odds ratio 1.99;1.39–2.85), plasma transfusion 29/169 (17.2%) vs 23/507 (4.5%) (odds ratio 4.36;2.44–7.78) and platelet transfusion 72/169 (42.6%) vs 25/507 (4.9%) (odds ratio 14.31;8.64–23.70), in the intensive care unit, than patients in the reference group.
Conclusions: In our study, patients in the intervention group more often required vasoactive medication; prolonged mechanical ventilation; prolonged intensive care and blood products postoperatively. However, an early platelet transfusion was not associated with increased blood loss, more reinterventions for bleeding, thromboembolic complications, infections, organ failure or in-hospital mortality.

P-488

EFFECT OF STORAGE TIME OF PLATELET PRODUCTS ON CLINICAL OUTCOMES AFTER TRANSFUSION: A SYSTEMATIC REVIEW AND META-ANALYSES

AL Kreuger, C Caram-Deelder, J Jacobse, JL Kerkhoffs, JG van der Bom and R Middelburg

Sanquin, Leiden, The Netherlands

Background: Extending storage time improves availability of platelet products, but could also influence safety and efficacy. This systematic review and meta-analyses summarizes and quantifies the evidence of the effect of storage time of transfused platelets on clinical outcomes.
Methods: A systematic search in seven databases was applied to seven databases: MEDLINE (Pubmed), EMBASE, Cochrane, CINAHL, Academic Search Premier,

ScienceDirect, and Web of Science. Reports and meeting abstracts of randomized trials and observational studies, reporting clinical outcomes after transfusion of platelet products of different storage times were selected. The search was updated until February 2016. Outcomes were pooled as mean differences, risk ratios or risk differences, depending on the outcome. Random effects models were used to account for heterogeneity. To quantify heterogeneity, I^2 was calculated, and to assess publication bias, funnel plots were constructed and Egger's bias coefficients were calculated.
Results: The search strategy yielded 4234 papers, of which 32 were included in this systematic review. Twenty-three studies reported safety outcomes and fifteen efficacy outcomes. Twenty one studies were included in the meta-analyses. The relative risk of a transfusion reaction after old platelets compared to fresh platelets was 1.53 (95% confidence interval (CI): 1.04 to 2.25). This was 2.05 (CI 1.47 to 2.85) before and 1.05 (CI 0.60 to 1.84) after implementation of universal leukoreduction.The relative risk of bleeding was 1.13 (CI 0.97 to 1.32) for old platelets compared to fresh. The transfusion interval was 0.25 days (CI: 0.13; 0.38) shorter after transfusion of old platelets. Storage time of platelets was not associated with the number of units of transfused platelets, red blood cells or plasma.
Conclusion: These findings suggest that transfusion of old platelets increase the risk of transfusion reactions in the setting of non-leukoreduction. Prolonged storage might be associated with an increased risk of bleeding and shortened platelet transfusion intervals. Yet, transfusion of old platelets is not associated with increased use of any type of blood products.

P-489

CHANGES IN BLOOD PRODUCT USAGE IN CARDIOTHORACIC SURGERY AFTER SERVICE CONSOLIDATION AT BARTS HEALTH NHS TRUST

DGJ McKechnie[1], A Maddison[1], S Allard[2] and L Green[2]
[1]*Barts Health NHS Trust, London, United Kingdom* [2]*NHS Blood and Transplant, London, United Kingdom*

Background: In May 2015, a major service reconfiguration took place for cardiovascular services in central London. Cardiology & cardiothoracic surgery services from three hospitals were consolidated into one unit, the Barts Heart Centre (BHC). Our transfusion lab supplied blood products to two of the three sites pre-merger, and currently supplies the new BHC.
Aims: To capture the transfusion workload associated with a major service reconfiguration, and any change in transfusion patterns following consolidation of cardiac surgery services onto one site.
Methods: Data was collected retrospectively for all operations occurring in cardiothoracic theatres between July to September 2014 (pre-merger period), and all operations occurring in BHC between July to September 2015 (post-merger period).
Patient details were cross-referenced with transfusion laboratory records to obtain information of blood products transfused between the operation date and up to 28 days post-operatively.
Results: In the pre-merge period there were 267 cardiac operations, compared to 428 operations in the post-merger period, representing a 60% increase in volume: of these 243/267 and 357/428 were coronary artery bypass grafts (CABGs), cardiac valve replacements, or combined CABG and valve replacements.
72% of pre-merger patients and 70% of post-merger patients received at least one blood product transfusion within 28 days of surgery.
The total volume of RBCs, platelets, FFP and cryoprecipitate transfused to cardiac patients increased following the merger, by 53%, 29%, 26% and 188% respectively (see table 1), but the mean number of RBCs, platelets and FFP transfused per patient actually fell, from 4.9 to 4.6, 1.6 to 1.2, and 2.7 to 2.1 respectively. The mean number of cryoprecipitate units transfused per patient increased from 0.3 to 0.5.
Wastage rates of platelets & cryoprecipitate increased slightly post-merger (from 0.05 to 0.06 and 0.01 to 0.03 units per patient respectively), but red cell and FFP wastage rates dropped (from 0.26 to 0.09 and 0.32 to 0.18 units per patient).

Blood product	Number of units transfused across all cardiac surgery patients between operation date and 28 days post-operatively	
	July-September 2014	*July-September 2015*
RBC	889	1364
Platelets	279	360
FFP	502	631
Cryoprecipitate	54	156

Table 1. Total number of blood products transfused pre- and post-merger.

Conclusions: The creation of a major cardiac centre at Barts has produced an increase in workload for the local transfusion services. However, the demand for RBCs, platelets, and FFP per patient have fallen.
Centralization of cardiac services nearer supporting services, including transfusion laboratories, has also lead to reduction in wastage of blood products. It also provides an excellent environment in which to study transfusion practice in the future, given the volume of patients, operations and associated transfusions at this centre.

P-490

TRANEXAMIC ACID IN ORTHOPEDIC SURGERY: OUR EXPERIENCE

L Caser, S Balen, E Ivankovic, N Lukežic, S Buneta-Skorup, T Kovacevic-Cuculic and S Zubovic-Velepic
Clinical Hospital Center, Rijeka, Croatia

Background: Knee and hip replacement surgery is often associated with substantial perioperative blood loss. One of the measures to decrease blood loss is an usage of tranexamic acid (TXA). Recently we started to administer TXA in knee and hip arthroplastic surgery procedures.
Aim: In the study we assessed the effectiveness of TXA to reduce blood loss and therefore to decrease a need for blood transfusion in orthopedic surgery.
Methods: Blood transfusion data were evaluated in two comparative two-month periods during 2014 (no TXA used, group I) and 2015 (TXA administered, group II). Total of 300 patients with transfusion request were included in study: 79 patients undergoing hip and 33 undergoing knee arthroplasty in group I, and 121 patients undergoing hip and 67 knee arthroplasty in group II.
All patients received venous thromboembolism prophylaxis with low-molecular-weight heparin. In group II TXA was administered 1 g IV prior to surgery and 1 g IV after the procedure.
In both groups we analyzed age, gender, preoperative hemoglobin, proportion of transfused patients, number of transfused units and thromboembolic complications - separately for knee and hip arthroplastic surgery.
Results: No difference was observed between groups regarding patients' gender and age. There was a slight predominance of women in both groups (56,9% and 57,4% respectively). Median age was 67 (range 41–86) years. Taking both groups together, mean value of preoperative hemoglobin level was 150 g/l (111–175) in males and 136 g/l (105–157) in females. However, 38 of 52 (73,1%) of men in group I had hemoglobin value >140 g/l, compared to 50 of 75 (66,7%) of men in group II. Similarly, 55 0f 60 (91,7%) of women in group I had hemoglobin value >120 g/l, compared to 97 of 113(85,8%) in group II (n.s.). No patient was admitted to surgery with hemoglobin value <100 g/l.
In the group I, 33 of 112 (29,5%) patients were transfused, compared to 36 of 188 (19,1%) in the group II ($P < 0,05$). Among patients undergoing total hip arthroplasty, 20 of 79 (25,3%) patients in group I and 24 of 121(19,8%) in group II received blood transfusion (n.s.)*. However, for knee arthroplasty, 13 of 33(39,4%) and 12 of 67 (17,9%) patients were transfused in the group I and II, respectively ($P < 0,05$). In both groups, patients were usually transfused with 2 units of packed red cells: only 9(27,2%) and 8(22,2%) patients in group I and II, respectively, received more than 2 units. In both groups there was no evidence of clinical signs of venous thromboembolic events.
Conclusion: Although relatively small number of patients was included in the study, use of TXA seems associated with lower transfusion rate, especially in patients undergoing knee surgery. Further studies with larger number of patients are needed to provide better informations whether introduction of TXA will change our approach and Maximum Surgical Blood Ordering Schedule in orthopedic surgery.

P-491

BLOOD FOR WHIPPLE: RISK FACTORS FOR BLOOD TRANSFUSION IN WHIPPLE SURGERY

M Sampaio, C Silva, J Aguiar, J Davide and M Amil
Centro Hospitalar do Porto, Porto, Portugal

Background: Pancreaticoduodenectomy (PD), either classic Whipple operation (CW) or pylorus-preserving Whipple procedure (PPW or Traverso-Longmire), provides the only chance of cure for patients with periampullary and pancreatic carcinoma. Patients undergoing PD, a technically difficult operative procedure, often require perioperative blood transfusion. Surgical blood loss leading to a subsequent need for

blood transfusion could have both short- and long-term negative consequences. Blood management requires risk identification and patient optimization.
Aims: Identify factors associated with blood requirements in patient submitted to PD.
Methods: Retrospective study of patients submitted to PD between January 2012 and June 2015. Patients' data were codified, recording demographics and comorbidities, preoperative blood count, anaesthesia evaluation, surgical procedure, histopathological results, blood components transfusion, length of stay (LOS) and clinical outcome. Analysis was performed using IBM SPSS Statistics 23.
Results: Seventy-five patients were submitted to PD (65 CW, 10 PPW) due to pancreatic neoplasia (52), biliary neoplasia (16), or intestinal neoplasia (4). In three patients malignancy was not confirmed on postoperative histopathological examination. Median age: 67 years (range 32–82), 42 patients were male (56%). Four patients (5.3%) died within 30 days after surgery. Median LOS: 34 days (range 9–98), median time until surgery: 12 days (interquartile range 4–18). Most frequent comorbidities were arterial hypertension (62.7%), dyslipidaemia (37.3%), diabetes mellitus (36.0%), and arrhythmia (16.0%). Ten patients were under antiplatelet or anticoagulant drugs at admission, without increased blood loss. The American Society of Anaesthesiologists (ASA) Physical Status classification was ASA II (71.2%), III (26.1%) and I (2.7%). Preoperative mean haemoglobin: male-13.3 g/dl (range 8.2–16.6) and female −11.7 g/dl (range 9.1–15.5). Mean platelets: $266*10^3/\mu L$ (range 114–519). Sixty-four percent of patients had moderately differentiated lesion and 43 had perilesional tissue invasion. Most common TNM staging was pT_3 for primary tumour (48%), N_0 for regional lymph nodes (34%) and distant metastasis could not be evaluated at the time in most patients (58.7%). Twenty-one patients (28%) stayed in intensive care unit (ICU) for more than 24 h or were readmitted. A total of 181 units of erythrocyte concentrate (EC) were transfused in 42 (56.0%) patients. Four patients received plasma intraoperatively and one of them a pool of platelets. One other patient required a pool of platelets. Twenty-two patients (29.3%) required EC on first 24 h, 17 of them intraoperatively (median 0.86 units/patient, range 0–4). Of transfused patients, 22 (52.3%) were transfused beyond fifth postoperative day during ICU admission (median 2.21 units/patient, range 0–33). Transfusion was significantly associated with need for ICU admission, surgical re-intervention, and development of sepsis. Among female, preoperative haemoglobin was not associated with EC requirements, yet among men it was associated with EC transfusion of at least one unit. Platelet level was not associated with EC requirements. Intraoperative blood loss was another significant risk factor. Other risk factors associated with EC transfusion were increased age, arterial hypertension, ASA III classification, and increased LOS.
Conclusions: Despite the bad conditions of those patients and surgical procedure complexity, only 29.3% of patients required EC in first 24 h, therefore a type and screen policy was implemented, except for anaemic patients. Blood transfusion needs are mostly related to patients' co- morbidities, reflecting relevance of appropriate surgical and anesthetic techniques and appropriate use of blood performed by highly differentiated teams. Postoperative complications, mainly related to sepsis, surgical interventions or ICU admission, are associated with blood transfusion beyond fifth postoperative day.

Haemorrhage and Massive Transfusion

P-492

PLATELET TRANSFUSION EFFECTIVENESS

E Zhiburt[1], EB Protopopova[1], I Paramonov[2], S Madzaev[1], N Filina[3], E Mar'yasova[3], E Kutefa[4], L Davydova[5], M Gubanova[6], T Kopchenko[6], U Sultanbaev[7], K Tankaeva[8] and F Sherstnev[2]

[1]National Medical Surgical Center, Moscow, Russian Federation [2]Kirov Research Institute of Hematology and Blood Transfusion, Kirov, Russian Federation [3]Krasnoyarsk Regional Blood Center 1, Krasnoyarsk, Russian Federation [4]Khanty-Mansiisk Regional Clinical Hospita, Khanty-Mansiisk, Russian Federation [5]Yakutya Republic Blood Bank, Yakutsk, Russian Federation [6]Stavropol Regional Blood Bank, Stavropol, Russian Federation [7]Bashkortostan Republic Blood Bank, Ufa, Russian Federation [8]Kuraev Dagestan Repiblic Children Clinical Hospital, Makhachkala, Russian Federation

Background: Platelets transfusions are an important tool for the prevention and treatment of bleeding in thrombocytopenia and / or thrombocytopathy. Unlike decreasing of red blood cells and plasma transfusions in developed countries the incidence of donor platelets transfusions are increasing. Platelet concentrates are prepared by apheresis on different devices or isolated from whole blood units (with or without pooling) in an additive solution or plasma with or without additional treatment like leukodepletion and pathogen inactivation. Methods for the preparation of platelet concentrates affect the functional state of the cells and their clinical effectiveness.
Aims: To study clinical efficacy of platelets treated by different methods in the Russian Federation.
Methods: The questionnaire 'Platelet transfusions survey' has been sent to members of the Russian Transfusionist Association. We received answers about 520 transfusions from 8 regions.
Results: In the surveyed organizations part of prophylactic platelet transfusions ranged from 10.0% to 91.0%. On average platelet transfusions for the prevention of bleeding in 3.6 times more often than to stop the bleeding. 67.5% of recipients of platelets have oncology diseases. Target platelet concentrations for preventive transfusions seems higher compared to modern evidence-based value - 10×10^9 / L. 25% of prophylactic transfusions included in the study were performed in patients with platelet count of 30×10^9 / L or more. In 76.7% of transfusions used platelet concentrates prepared by apheresis. Single whole blood - derived units are used at 3.7 times more often than pooled platelets.
Three types of platelets have been transfused with CCI24 (mediana; interquartile range) 1) amotosalen-treated: 7,6 (1,9; 13,7); 2) riboflavin-treated: 6,0 (0,6; 21,7); 3) non-treated: 12,4 (3,3; 20,0).
Recipients of platelets treated with riboflavin had significantly more severe haemorrhages (grade 2–4). 8 therapeutic platelet transfusions (42.1%) treated with riboflavin did not stop the bleeding. 13 therapeutic platelet transfusions (21.7%) with not treated plateletes in 11 patients didn't stop bleeding. All therapeutic platelet transfusion treated by amotosalen stopped bleeding.
Organizations using riboflavin pathogen inactivation ensured maximum cells in platelet concentrates. However CCI24 in this group was reduced, and its interquartile range was maximal. Thus 25% of transfusions resulted with CCI24 less than 0.6×10^9 / L. It can be assumed that the riboflavin treatment influence on the activity of certain cells and quality of platelets respectively.
Conclusion: Platelet concentrate preparation method and additional treatment influences on transfusion effectiveness. Amotosalen treated platelets are effective for bleeding treatment. Inactivation platelets using riboflavin needs in further research for clinical efficacy evaluation.
www.transfusion.ru

P-493

ASSESSMENT OF RISK OF BLEDDING IN PATIENTS ON DABIGATRAN

A Antic and Z Stanojkovic

Blood Transfusion Institute, Nis, Serbia

Background: Dabigatran, an anticoagulant drug of a new generation, is a direct thrombin inhibitor, used in a fixed dose and its application does not require monitoring of efficiency, as is the case with vitamin K antagonists. However, there are clinical conditions that require an immediate assessment of the dabigatran efficiency. For this purpose, it is recommended to perform an activated partial thromboplastin time (aPTT).
Methods: The study included 32 patients (20 men, 12 women) with nonvalvular atrial fibrillation who received dabigatran in a single dose of 110 mg or 150 mg. The average age of patients was 52.36 ± 10.14 years. Patients came to transfusion department at the occurrence of bleeding, thrombosis, renal dysfunction or upcoming surgery. aPTT was determined from a sample of the citrate blood on *ACL Elite Pro* (*Instrumentation Laboratory*). The samples were divided into three groups, depending on the time that has elapsed since taking the last dose of dabigatran (4 h, 8 h, 12 h).
Results: In patients taking 150 mg of dabigatran aPTT was extended 1.5 to 1, 8 times after 4 h of last dabigatran dose, 1.3 to 1.6 times after 8 h, while after 12 h was 1.2 to 1.4 times extended in relation to the normal value (reference: 25–35 sec). In patients who received 110 mg of dabigatran aPTT was extended 1.4 to 1.7 times after 4 h, while after 12 h of taking the drug aPTT was almost normalized (1.05 to 1.2 times extended). Only in one patient, who complained on bruising and hematomas aPTT after 12 h of taking a dose of 150 mg dabigatran was significantly prolonged (2.1 fold). The study showed a significant correlation between dabigatran plasma concentration and aPTT-a ($r = 0.89$), which decreases with increasing a dabigatran dose.
Conclusion: aPTT is a useful test for assessing the effect of dabigatran and can be used as a screening test in patients who urgently need to determine the efficiency of the drug.

P-494

THE TIME TO SUPPLY CRYOPRECIPITATE AND CURRENT WASTAGE RATES – ARE THEY ACCEPTABLE?

D Roxby[1] and R Sinha[2]

[1]*SA Pathology, Adelaide, Australia* [2]*SA Health, Adelaide, Australia*

Background: Quantitatively, fibrinogen is the most common coagulation factor and is the first procoagulant to fall to critical levels (<1.5 g/L) in massive transfusion or major haemorrhage. Rapid access to and early replacement of fibrinogen is critical to successful haemostatic resuscitation. With increased use of visco-elastic testing at point of care, results for fibrinogen levels and other clotting factors are known faster than traditional standard laboratory testing, supporting a need for more rapid access to replacement products. Fibrinogen replacement can be achieved using fresh frozen plasma (FFP), batches of pre-pooled whole blood cryoprecipitate (10 units) or apheresis cryoprecipitate (5 units), or fibrinogen concentrate. However significantly, fibrinogen levels are not standardised in FFP or pooled cryoprecipitate leading to variable amounts infused. A clear disadvantage in critical haemorrhage is the time required to thaw FFP and cryoprecipitate prior to use and also the short shelf-life of 6 h of thawed cryoprecipitate which contributes to high wastage rates.
Aim: To examine cryoprecipitate inventory management time, time taken to provide cryoprecipitate and reasons for clinical wastage.
Method: A prospective observational study was carried out at 5 major hospitals (2 trauma centres, 2 general hospitals and a specialised women's and children's hospital) over an 8 week period during 2014–15. Time taken for cryoprecipitate inventory management and product handling was recorded. Parameters included time to count stock, order replacement, receipt stock, processing clinical orders, thawing, labelling and dispatching to patients and discarding unused/expired thawed product. Local State discard reasons and wastage rates were extracted from the State database.
Results: The overall median processing time for 60 episodes which included request registration, thawing, labelling and dispatching was 40 min (IQR 30–44) with 40 (IQR 31–44) minutes for batches of apheresis and 35 (IQR 30–43) minutes for whole blood cryoprecipitate. Transport to the clinical location, bedside checking and pooling added a further 13.5 min before the product could be transfused. Transfusion time was dependent on whether a rapid infuser was used or by gravity alone. The State cryoprecipitate discard rate in 2014–15 was 12.6% (608/4837). The main reasons for avoidable cryoprecipitate wastage included damaged during thawing 60/608 (9.9%), thawed not used 57.6% (350/608), ordered for patient and not used 70/608 (11.5%) and time expired 13.2% (80/608). These potentially avoidable causes of cryoprecipitate wastage accounted for 92.1% (560/608) of the total cryoprecipitate wastage with an equivalent monetary value of $156,330.
Conclusion: In massive haemorrhage early and aggressive replacement of fibrinogen has led to improved patient outcomes. To minimise the time to fibrinogen replacement and decrease wastage, sources other than FFP or cryoprecipitate may be considered more appropriate. When compared with cryoprecipitate and FFP, fibrinogen concentrate offers a virally inactivated product requiring minimal preparation and staff handling time, standardised fibrinogen concentration per vial thus minimising variability in dosing, smaller infusion volumes and decreased avoidable wastage.

P-495

STUDY OF COAGULOPATHY, EFFECTS, COMPLICATIONS AND OUTCOME OF MASSIVE TRANSFUSION

P Fulzele[1], J Sharma[2], C Modi[2], S Bhagwat[2] and S Bankar[2]

[1]*HBT Medical college and Dr R N Cooper Hospital, Mumbai, India* [2]*Seth G S Medical College and KEM Hospital, Mumbai, India*

Background: Hemorrhage remains the major cause of preventable death and in recent years there has been increasing evidence that early and more intensive replacement of FFP and platelets may improve the outcome of massive hemorrhage. The need for study was to evaluate the management, transfusion practices, complication and outcome in patient with massive transfusion.
Aim: The study was undertaken to identify the coagulopathy, complications and clinical outcome of massive transfusion.
Materials and Methods: This was a single centric, prospective, observational study of 60 patients at King Edward VII Memorial Hospital, Mumbai who received massive transfusion(≥10 units of RBC in 24 h). Ratio of blood component transfused, complication associated massive transfusion, outcome and other laboratory parameters were assessed both before and after massive transfusion. Data was collected from patient's laboratory investigation reports, case record files and Blood bank records of patients undergoing massive transfusion. Statistical analysis was performed using SSPS software (Version 17.0) using appropriate test and proportions.
Results: Total sixty patients were studied for analysis. Complication and side effect of massive transfusion observed in present study were Hypothermia 33.33%, Acidosis 40.0%, Alkalosis 8.3%, Hyperkalemia 31.7%, Hypokalemia 16.67%, Hypocalcemia 81.7%, DIC 16.67%, FNHTR 5% and Possible TRALI 1.67%. Coagulopathy occurred in 55% of the nonsurvivors and 15.0% of survivors. Mortality rates were highest with patients who had undergone massive transfusion with PRBC: FFP ratio >1.1 (41.94%) and with PRBC: Platelet ratio <0.9 (50%). Lowest mortality rate were in patients with PRBC: FFP ratio <0.9 (23.53%) and with PRBC: Platelets ratio 0.9–1.1 (28.57%). Overall mortality was 33.33%.
Conclusions: Our study suggest that transfusion with higher FFP (Decreased PRBC: FFP ratio) was associated with increased survival rate and transfusion with higher platelet have not show any survival benefit. In some patients, coagulopathy was not corrected despite identification by serial clinical laboratory analysis. The relationship between coagulopathy and mortality is complex and further more clinical studies are necessary. The study supports the existing literature that outcome of massive transfusion is affected by coagulopathy, hypothermia, electrolyte changes which was statistically associated with mortality and to provide maximum benefit there should be an established policy for massive transfusion.

P-496

HOW USEFUL ARE HOSPITAL DATA REPORTS TO CLINICIANS? EVALUATION OF THE AUSTRALIAN AND NEW ZEALAND MASSIVE TRANSFUSION REGISTRY HOSPITAL DATA REPORTS

R Ruseckaite[1], ZK McQuilten[2], JC Oldroyd[2], TH Richter[2] and EM Wood[2]

[1]*Transfusion Research Unit, Mebourne, Australia* [2]*Transfusion Research Unit, Monash University, Melbourne, Australia on behalf of the Australian and New Zealand Massive Transfusion Registry Steering Committee*

Background: The Australian and New Zealand (ANZ) Massive Transfusion Registry (ANZ-MTR) collects observational data on all patients with critical bleeding (CB) requiring massive transfusion (MT) at participating hospitals. The ANZ-MTR now has data on >4300 patients collected from 22 sites in ANZ. On a biannual basis hospital data reports (HDR) are distributed to local investigators (LI), summarising patient profiles, the frequency of MTs, bleeding contexts in which MT occur, management and clinical outcomes (including survival) at each site.
Aim: To evaluate if HDRs are being used to inform policy, practice, and quality of care in MT events; and to receive structured feedback on the content and presentation of the HDRs.
Methods: An evaluation survey consisting of 16 multiple choice questions on the presentation, content and quality of MTR HDRs was designed using the *SurveyMonkey* platform. An email invitation with the study description and survey link was sent to 46 transfusion specialists, nurses/practitioners, LIs and CEO's at 20 participating sites. Survey results were collected and stored electronically. Results were analyzed using SPSS v.22 statistical package.
Results: A total of 30/46 invited participants from 14 sites responded to the survey, representing an overall response rate of 65%. Of the respondents, 40% identified themselves as transfusion nurses/practitioners, 20% as transfusion laboratory scientists, 13% as anaesthetists, and the remaining were transfusion medicine specialists, risk managers and one hospital CEO. More than half the respondents were happy to receive HDRs on bi-annual basis; two thirds reported that they read the report routinely. Nearly two thirds of all respondents were likely to forward the reports to hospital transfusion committee (HTC) members. Half the respondents rated the reports as excellent or very good, while 27% thought they were average. 57% of the respondents thought that reports were somewhat easy to read, while 6.7% said not so easy. To the question of whether reports made an impact on hospital policy, practice or patient outcomes, 47% were not certain if evaluation has been undertaken, others thought the reports improved understanding of blood product use in different areas, and that they were discussed at HTC meetings. The usefulness of structuring the HDR according to the ANZ patient blood management (PBM) guidelines (https://www.blood.gov.au) to report the provision and timeliness of blood products, benchmarked against other sites was surveyed. The majority (65%) of respondents thought it would be relevant and others did not know. The vast majority (80%) of the respondents agreed that charts and figures were the best way to display the data; however an executive summary was desired.
Conclusions: The study provides insights on how to improve the ANZ-MTR HDRs. Future HDR reports will be designed to reflect the feedback from the survey by reporting the MTR data according to the PBM guidelines and providing benchmarking results. More research is required to formally evaluate the effect of feedback from the registry on policy, practice and outcomes.

P-497

ARE PRE-OPERATIVE ROTATIONAL THROMBOELASTOMETRY (ROTEM) PARAMETERS PREDICTIVE OF POTENTIAL MASSIVE TRANSFUSION IN ORTHOTOPIC LIVER TRANSPLANTATION?

D Roxby[1], JP Boey[1] and R Sinha[2]

[1]SA Pathology, Adelaide, Australia [2]SA Health, Adelaide, Australia

Aim: Conventional coagulation parameters in isolation have not been useful in predicting transfusion requirements in patients undergoing orthotopic liver transplantation (OLT). A retrospective study was undertaken to determine the predictive value of pre-operative ROTEM parameters and massive transfusion in OLT and whether pre-operative values were associated with increased transfusion requirements.

Methods: All adult patients undergoing OLT in our hospital from January 2010 - December 2014 were identified. Patients were divided into those who received massive transfusion (MT) - defined as ≥5 units of red cells within 4 h and those who did not. ROTEM parameters, full blood count, biochemistry, International Normalised Ratio (INR), activated partial thromboplastin time (aPTT), fibrinogen, intraoperative blood product use and baseline clinical characteristics were compared.

Results: Of the 115 OLT patients, 39 (34%) required a massive transfusion .These patients had a higher Model for End-Stage Liver Disease (MELD) score [24 (IQR 16–33) vs 19 (IQR 13–22), P = 0.007] and a lower baseline haemoglobin level [94 g/L (IQR 80–109) vs 114 g/L (IQR 94–124), P = 0.001]. INR, aPTT, fibrinogen level and platelet count were similar in both groups. Pre-operatively, MT patients had abnormal ROTEM parameters that are associated with poor clot quality and massive transfusion as shown in Table 1.

Conclusion: Abnormal ROTEM baseline parameters may be predictive of MT in OLT patients. Further analysis in a larger population to confirm these findings in anticipating increased bleeding risk and blood product use is required.

ROTEM normal parameters	*Massive transfusion (Median, IQR), n=39*	*No massive transfusion (Median, IQR), n=76*	*p-value*
EXTEM CFT: 34-159s	219 (144-303)	145 (110-235)	0.03
EXTEM A10: 43-65mm	34 (27-44)	42 (32 – 47)	0.04
EXTEM MCF: 50-71mm	44 (38-53)	49 (43-57)	0.05
FIBTEM MCF: 9-25mm	7 (4-11)	10 (7-13)	0.04
FIBTEM A10: 7-23mm	7 (5-10)	10 (6-12)	0.03

Caption 1: Rotem Parameters

Adverse Events, Including TRALI

P-498

ASSOCIATION BETWEEN BLOOD TRANSFUSION AND MITOCHONDRIAL DNA CONTENT IN GLIOMA PATIENTS

Q Zhang, J Zhang, X Zhu and R Xia

Huashan Hospital, Fudan University, Shanghai, China

Background: Blood transfusion is lifesaving in many circumstances and is safer than it has ever been, but it still poses significant risks. Cancer progression was suggested to be affected by blood transfusion. Allogeneic blood transfusion is associated with poorer prognosis. Moreover, mediating mechanisms underlying the alleged cancer-promoting effects of blood transfusion are unknown. A growing number of epidemiological studies have demonstrated alterations in mitochondrial DNA (mtDNA) content is closely related to risk of various human cancers and considered a common cancer hallmark. More importantly leukocyte mtDNA copy number could serve as an independent prognostic marker and an indicator of immune functions in glioma patients.

Aims: This study aims to explore the relationship of blood transfusion and mtDNA copy number and immunomodulatory effects in glioma patients, which may benefit for future improvement of treatment.

Methods: We collected data for glioma patients from July 2014 to June 2015. The mtDNA copy number of peripheral blood leukocytes from glioma patients was examined using a real-time PCR-based method. Plasma concentrations of several cytokines (IL-2,4,8 and INF-γ) were detected by enzyme-linked immunosorbent assay (ELISA).

Results: A total of 108 glioma surgery patients were recruited in this study. Overall, 38 patients (35.18 percent) received blood transfusion. Our data showed that patients received blood transfusion had increased mtDNA copy number as compared with the patients without blood transfusion with 0.74 copies (interquartile range = 0.36–1.44 copies) vs 0.17 copies (interquartile range = 0.09–0.67 copies). Immunological analysis indicated that transfusion patients had significantly higher plasma concentrations of IL-4 (65.3 vs 26.34 pg/ml, P = 0.012) and lower plasma concentrations of INF-γ (23.69 vs 59.36 pg/ml, P = 0.027), suggesting an immunosuppression-related mechanism involved in blood transfusion-mediated prognosis. No significant concentration difference of IL-2 and 8 were noted between two patient subgroups.

Conclusions: Our findings indicated that blood transfusion is associated with high mtDNA content and immunosuppression which contribute to poor prognosis in glioma patients, indicating that blood transfusion may affect cancer progression through regulation of mitochondrial function and its mediated immune function. Further efforts to understand the mechanisms of blood transfusion-induced mitochondrial function will help clarify the complexities of blood transfusion-associated poor prognostic factor.

P-499

RETROSPECTIVE ANALYSIS OF TRANSFUSION REACTION REPORTING AT A TERTIARY HOSPITAL IN ABUDHABI

FM AlMazam, HS Alhumaidan and E AlZaabi

Sheikh Khalifa medical city / SEHA, Abu Dhabi, United Arab Emirates

Background: Transfusion reactions range from mild allergic reactions to life threatening complication and its recognition and reporting is of utmost importance, however, the actual incidence of transfusion reactions is often underestimated. Transfusion reactions require immediate recognition, laboratory investigation, and clinical management. Proper reporting of transfusion reactions is a crucial pre-requisite for a national hemovigilance program aiming to improve quality and safety of blood products.

Aim: Identify the challenges in the current transfusion reaction reporting process in our institute.

Method: From the period between 2010 and 2015, the total number of blood products transfused was collected and a retrospective analysis of transfusion reactions was conducted.

Results: There was an increase in the number of blood products transfused over the years, from 17877 transfusions in 2010 to 19350 in 2015. This increase was seen with red blood cells (RBC), cryo-reduced plasma, apheresis platelets (AP) and cryoprecipitate. There was a decrease in the utilization of whole blood derived platelet concentrates and fresh frozen plasma. 353 transfusion reaction reports were reviewed and classified according to the transfusion reaction report findings as depicted in **Table 1**:

1- Febrile reactions. 2- Allergic reactions. 3- Non-hemolytic transfusion reactions (NHTR). 4- Acute Hemolytic transfusion reactions (AHTR). 5- Delayed hemolytic transfusion reaction (DHTR). 6- Non-immune hemolytic transfusion reaction (NIHTR). 7- Transfusion associated circulatory overload (TACO). 8- Transfusion related acute lung injury (TRALI). 9- Acute pain transfusion reaction (APTR). 10- Hypotensive transfusion reaction. 11- Transfusion associated dyspnea (TAD).

The total incident over the years was 0.3%. Most of transfusion reactions are associated with RBC followed by AP and the majority of reported transfusion reaction were allergic, which accounts for 64% of all transfusion reactions. The allergic reactions were not classified before 2014 into mild and severe and the review of cases demonstrated that out of the 227 allergic reactions, two were severe, one reported in 2014 and a second in 2015. There was one case of hemolysis in 2010 which was due to the usage of wrong IV solution during blood administration. The 18 NHTR reported were due to hypotension, chest tightness, vomiting, mild fever, back pain in addition to the unknown/other category of reactions.

Pertaining to delayed transfusion reactions, none were reported by the clinical team. In addition, the incidence of DHTR is falsely low because while it was recognized in the blood bank, reporting it in our transfusion reaction electronic reporting system was not started till mid-2015.

Table:1 Transfusion Reactions 2010-2015 Total number of units transfused during this period is 91814							
Transfusion reaction	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**Total**
Allergic	37	41	38	41	35	35	227
Febrile	18	22	10	20	16	12	98
AHTR	0	0	0	0	0	0	0
NHTR	3	3	5	2	2	3	18
DHTR	0	0	0	0	0	1	1
NIHTR	1	0	0	0	0	0	1
TACO	1	0	0	0	0	0	1
TRALI/ Suspected	0	0	0	1	0	2	3
TAD / Possible	0	0	0	0	0	1	1
Hypotensive	0	0	0	0	0	1	1
APTR	0	0	0	0	1	1	2
TOTAL	60	66	53	64	54	56	353

Caption 1: Reported transfusion reactions from 2010 until 2015

Discussion: Our institute is a tertiary center consisting of 586 bed and 14 outpatient specialty clinics. It was noticed that the number of products transfused has increased over the years, but the number of transfusion reaction reports remains unchanged and in comparison to the international data, our incidence was low. Transfusion reaction report in our institute depends on the health care provider's clinical judgment. The nurse would stop the transfusion, contact the physician, fill out a transfusion reaction report form and an electronic incident report and submit it to the blood bank. In addition, there was obvious variability between pathologist in the reporting process, with apparent allergic and febrile reactions reported as NHTR.
Conclusion: Despite the presence of international guidelines for reporting, efforts shall be made to increase awareness of the reporting process best practice among clinical team, technologists and pathologists with an effort to start a future patient hemovigelance program.

P-500

UNDERFILLED CONTAINERS WITH BLOOD, AS THE ADVERSE EVENT AT THE STAGE COLLECTION OF BLOOD AND ITS COLLECTION IN THE REGIONAL BLOOD CENTER IN POZNAN IN THE YEARS 2011–2014.

E Przybylska, A Skrzypczak and K Olbromski
Blood Center in Poznan, Poznan, Poland

Background: Adverse events - unintended and disadvantageous events connected with donating, testing, preparing, storing, distributing and transporting of blood or blood components, taking place before, during or after collection which could lead to occurrence of adverse reactions. Failure to complete the blood donation successfully is one of such events. This event occurs when the collected whole blood from a donor totals an amount of less than 405 ml which affects the proportions of the collected blood with the preservative solution and as a results leads to the removal of the unit from the blood bank system.
Aim: The aim of the study was to evaluate the disposal of containers of whole blood in the stage of collection from first-time and repeat donors using the criteria of sex of the donor and age in 2011–2014 in Regional Blood Center in Poznań and the determination of the causes for the disposal and methods to prevent them.
Methods: The analysis included donations the volume of which does not meet the quality requirements, namely the total amount of collected blood was less than 405 ml. The analysis involved 350971 donation of whole blood in the Regional Blood Center in Poznań in the years 2011–2014. Three age ranges were included: 18–24, 25–44, 45–65, with division into group of men and women, first-time and repeat donors. The analysis was based on the data from the Regional Blood Center in Poznań recorded in the computer system "Blood Bank"by Asseco.
Results: A total of 5819 donation were removed, i.e. 1.66% of the total number of collected donations in the years 2011–2014. The annual disposal of not fully-filled containers totaled in particular years: 1179 in 2011, 1492 in 2012, 1576 in 2013 and 1572 in 2014. The frequency of obtaining the underfilled containers in 2011–2014 was as follows: the first-time donors averaged 3.78% out of which 4.45% were women, and 3.38% were male. Removed donations from repeat donors accounted for an average of 1.25% out of which 2.09% were women, and 1.05% men.
Conclusions: The spread of the disposals among donors suggests that the most common reason is the fear of the donor before the first donation, which can be confirmed by the increased number of under-filled containers among first-time donors.
In the age group 18–24 the number of removed donations was on average 2.56%, including 4.48% in the first-time donors and 1.81% in repeat, which indicates a lack of sufficient information regarding the process of donation.
It has been observed that the number of removed donations in women was two times higher than in men, respectively 2.72% and 1.35%, which can be caused by: weaker venous system, hypotension, vasovagal reaction, vein punctures during the donation, low blood flow, or reaction at the sight of other donors fainting, which are more common in women and do not allow for complete the procedure successfully.
It's difficult to find suitable corrective action for this adverse event. Probably donor education, adequate hydration, having a light breakfast and the acceptance of time necessary for donation of blood and its components would give appropriate results. In addition, the implementation of donor satisfaction questionnaires with their analysis and also publishing relevant information on websites the Blood Center will reduce the number of removed containers resulting from stress in connection with blood donation.

P-501

MECHANISMS UNDERPINNING TRANSFUSION RELATED IMMUNE MODULATION: ERYTHROPHAGOCYTOSIS AND IMMUNE MODULATION IN DIFFERENT MYELOID CELL SUBSETS

MM Dean[1], K Ki[1], S Henry[2], HM Faddy[3] and RL Flower[1]
[1]Australian Red Cross Blood Service, Kelvin Grove, Australia [2]Biotechnology Research Institute, AUT University, Auckland, New Zealand [3]University of QLD, Brisbane, Australia

Background: Packed red blood cell (PRBC) transfusion is associated with increased rates of infection, prolonged hospital stay and mortality. These adverse transfusion outcomes are collectively referred to as transfusion-related immune modulation (TRIM); however the mechanism(s) remain largely undefined. As a bridge between innate and adaptive immunity, production of cytokines and chemokines by monocytes and dendritic cells (DC) are key to both initiation and regulation of immune responses. We assessed potential mechanisms underpinning TRIM in three important myeloid cell subsets - monocytes, myeloid DC (mDC) and a specialised subset of mDC ($BDCA3^+$) that are involved in cross presentation (presentation of exogenous antigens on to MHC-I).
Aims: This study investigated phagocytosis of PRBC by different myeloid cells subsets and assessed transfusion-mediated modulation of blood mDC and specialised $BDCA3^+$ mDC subset.
Methods: An erythrophagocytosis assay was developed to assess uptake of fresh (day (D)2) and day-of-expiry (D42) PRBC by three myeloid subsets in parallel. PRBC (labelled with FITC conjugated FSL (Kode Biotech Materials Limited, Auckland, New Zealand)) and peripheral blood mononuclear cells were mixed (10:1 ratio), and incubated (30 min, 37°C, 5% CO_2). PRBC that had not been internalised were lysed (FACs lyse, BD biosciences) and uptake of PRBC determined by flow cytometry in gated monocyte ($CD14^+$), mDC ($CD14^-$, DR^+, $CD11c^+$) and $BDCA3^+$ mDC subsets ($CD14^-$, DR^+, $CD11c^{+\cdot}$ $BDCA3^+$). PRBC uptake was assessed using unpaired T-tests (D2-PRBC vs D42-PRBC, 95% CI). An *in-vitro* whole blood culture model of transfusion was then used to characterise mDC and $BDCA3^+$ mDC inflammatory mediators (IL-6, IL-8, IL-10, IL-12, TNF-α) following exposure to D2 and D42 PRBC. In parallel cultures, LPS was used to model inflammation in a transfusion recipient. Changes in inflammatory mediators were assessed compared to matched no transfusion controls (ANOVA with Tukey's post-test (95% CI)).
Results: Both fresh (D2) and date-of-expiry (D42) PRBC were phagocytosed by monocytes, mDC and $BDCA3^+$ mDC, with D42-PRBC phagocytosed at a significantly higher rate by all three myeloid subsets. An *in-vitro* whole blood model of transfusion was then used to assess whether uptake of PRBC impacted on DC immune responses. In the absence of LPS, exposure to D2 and D42 PRBC did not significantly modulate mDC function. However, exposure to both D2 and D42 PRBC attenuated the mDC response to LPS with significantly reduced expression of IL-12 and pro-inflammatory cytokines IL-6 and TNF-α. For the specialised cross-presenting BDCA3 + mDC subset, exposure to both D2 and D42 PRBC alone significantly reduced IL-8 production. When LPS was added to model recipient infection, both D2

and D42 PRBC attenuated the BDCA3$^+$ response to LPS with significantly reduced expression of IL-6 and IL-12.
Summary/Conclusions: These data provide evidence that multiple myeloid cell subsets are involved in clearance of PRBC post-transfusion, and that exposure to PRBC results in alteration of the production of multiple cytokines and chemokines critical for the immune response. We hypothesise that uptake and processing of PRBC impacts on the capacity of these cells to respond appropriately to subsequent immune challenge and this may be an important mechanism underpinning TRIM.

P-502

ANALYSIS OF TRANSFUSION-RELATED ADVERSE REACTIONS AT A MEDICAL CENTER IN SOUTHERN TAIWAN

H Chen, CR Chung and LC Wang
Chi Mei Medical Center, Tainan, Taiwan

Background: Blood transfusion is an important and essential therapy in modern medicine; however, it is also accompanied with complications such as adverse reactions (ARs). Some transfusion adverse reactions are serious and life-threatening, including acute haemolytic transfusion reactions and transfusion-related lung injury (TRALI); therefore, how to recognize adverse events and provide safer blood components are major issues for transfusion-related services. Many studies have demonstrated that patients transfused with leukoreduced components could reduce ARs. Since March 2015, leukoreduced products have been provided more widely than before did at ourinstitution (from 0.3% to 20.8%).
Aims: The aims of this study were to estimate the frequency and types of adverse transfusion reactions at our facility and to analyze the incidence among different blood products, especially pre- and post- leukoreduced blood components.
Materials and Methods: All ARs were reported by nurses through an online reporting system from 1st March 2015 to 31st March 2016. All collecting data were analyzed by computer software (SPSS. Version 19).
Results: Over 13 months, total 95190 units were transfused to 35025 patients, 35002 transfusions reactions (both no transfusion reaction and adverse outcomes) were reported, and the reporting rate was 99.9%. Out of the total 317 ARs were reported to the blood bank, and the frequency of ARs was 0.9% (317 out of 35025) on a per transfused patient basis; on a transfused unit basis, the incidence of ARs was 0.55%. The most common ARs type was chills (32.6%), followed by fever (17.2%), and urticaria (16.7%). The incidence of ARs to RBC, FFP, PLT and cryoprecipitate per patient was 1.29%, 0.48%, 0.29%, and 0.094% respectively. For pre- and post- leukoreduced blood products, the ARs rate was far high for pre-leukoreduced RBC units compared to post-leukoreduced (6.9% vs 0.91%), considered statistically significant (odds ratio=8.08, 95% CI=3.03–21.57, P = 0.00); pre-leukoreduced (RL-PH) and post-leukoreduced PH units (PH with filter) had the same result (2.29% vs 0.39%), and it also displayed a statistically significant (odds ratio=5.95, 95% CI=1.48–20.03, P = 0.00).
Conclusions: In this study, we presented our ARs types and incidence of different blood products. During the study period, neither TRALI nor TTBI (transfusion- transmitted bacterial infection) was reported. Surprisingly, the effectiveness of leukoreduced units of our report differed from other investigations. Research duration and sample size might cause the distinct results, and further study is a need to figure out the transfusion outcomes not only by comparing leukoreduced and not leukoreduced components but collecting patient medical history to improve transfusion practice.

P-503

UNDER-DIAGNOSIS VS MIS-IDENTIFICATION OF SERIOUS ACUTE TRANSFUSION REACTIONS: RETROSPECTIVE ANALYSIS OF 86 REPORTS FROM A SINGLE CENTER OVER THE LAST EIGHT YEARS

M Al Zaabi, S Al Busaidi, S Johnson and M Theresa
Khoula Hospital Directorate of Laboratories, Muscat, Oman

Background: Monitoring of incidence, trends and severity of acute transfusion reactions (ATRs) is fundamental part of haemovigilance schemes. Standardised classification of these reactions is an essential tool that can help identifying root causes and applying necessary corrective and preventive actions.
Aim: To evaluate methods used in classifying ATRs and to study patients' clinical characteristics and laboratory parameters around time of reaction occurrence.
Methods: We retrospectively collected ATRs reported to hospital blood bank from April 2008 to March 2016. All data were obtained from 3 resources (Manual Transfusion Reaction Forms, Laboratory Information Management System and Electronic Patient Notes). We assessed demographic features, direct observations, standard investigations and necessary actions taken whenever a reaction is suspected. We tried to compare our reporting system to ISBT/IHN Classification of ATRs.
Results: A total of 86 ATRs (50 females, 36 males) were documented over the last 8 years with a mean age of 43 year-old. 84/86 were associated with red cell transfusion and 2/86 with FFP. Fever and rash were the most common observations occurring in 63% of cases. Acute febrile non-haemolytic transfusion reactions (FNHTRs) and allergic transfusion reactions collectively accounted for 91% of reported ATRs. No cases of haemolytic transfusion reactions, TRALI, TACO or TA-GVHD were mentioned. However; - after retrospective analysis - we identified 6 patients with typical features of TACO and 4 patients with probable TRALI. Out of these 10 patients, 2 died during first week following transfusion. In about one third of allergic reactions, anaphylaxis was incorrectly added as possible association. After careful evaluation, only 3 patients were found to have typical features of anaphylaxis. No reported cases of ABO discrepancy or septic reactions.
Conclusions: Our analysis clearly demonstrates that serious complications of transfusion (particularly TRALI and TACO were considerably under-diagnosed or mis-identified). Implementation of standard definitions (e.g. ISBT/IHN Classification) is crucial to improove way of reporting ATRs.

P-504

PLATELETS ARE NOT DIRECTLY ACTIVATED BY HUMAN NEUTROPHIL ALLOANTIGEN-3A (HNA-3A) ANTIBODIES

J Wesche[1], T Berthold[2], S Muschter[1], A Reil[3], E Hammer[4], L Steil[4], U Völker[4], J Bux[3] and A Greinacher[1]
[1]Universitätsmedizin Greifswald / Inst. für Immunologie und Transfusionsmedizin, Greifswald, Germany [2]ChromaTec GmbH, Greifswald, Germany [3]Deutsches Rotes Kreuz - Blutspendedienst West, Hagen, Germany [4]Ernst-Moritz-Arndt-Universität Greifswald - Funktionelle Genomforschung, Greifswald, Germany

Background: Severe immune mediated transfusion-related acute lung injury (TRALI) is often caused by antibodies directed against the human neutrophil alloantigen-3a (HNA-3a). Besides neutrophils and endothelial cells, platelets are suspected to play a role in the pathogenesis of TRALI. Direct activation of platelets by HNA-3a antibodies could lead to co-activation of neutrophils, the major effectors of acute inflammation. This process might be an additional aspect in the multicausal pathogenesis of TRALI.
Aims: To assess the impact of HNA-3a antibodies on platelet activation and neutrophil-platelet aggregate formation.
Methods: HNA-3 antibodies were affinity-purified from plasma by adsorption and elution using washed platelets or purified granulocytes of genotyped homozygous donors. Platelet activation was determined by measurement of P-selectin surface expression (flow cytometry) and platelet aggregation (turbidimetry). Formation of neutrophil-platelet (PMN-PLT) aggregates was measured by flow cytometry. The influence of platelets on HNA-3a antibody-induced granulocyte aggregation was investigated using the granulocyte aggregation test (GAT) with or without addition of 50 times higher platelet numbers and increasing HNA-3a antibody concentrations.
Results: HNA-3a antibodies eluted from granulocytes bound to platelets; and HNA-3a antibodies eluted from platelets bound to granulocytes and activated them in the GAT. Incubation of HNA-3a/3a platelets with HNA-3a antibodies did neither lead to increased P-selectin expression nor to platelet-aggregation. Additionally, HNA-3a antibodies did not augment the formation of PMN-PLT aggregates compared to control conditions when purified granulocytes and platelets were used. Besides enhancing TRALI, platelets may bind HNA-3a antibodies competitively "protecting"PMNs from activation. However, addition of platelets to the standard GAT did not affect the HNA-3a-antibody concentration required for granulocyte aggregation.
Summary/Conclusions: The same HNA-3a antibodies, which bind to platelets also activate PMNs. We found no evidence for direct activation of platelets by these antibodies making a primary role of platelets in HNA-3a mediated TRALI unlikely. It is unclear why platelets do not competitively inhibit binding of HNA-3a antibodies to PMNs.

P-505

HYPERHEMOLYSIS SYNDROME IN A SICKLE CELL DISEASE/ THALASSEMIA PATIENT: THE ROLE OF IMMUNOTHERAPY

F ALKaabi, KK Hellstrom, E Aizaabi and HS Alhumaidan
Sheikh Khalifa Medical City / SEHA, Abu Dhabi, United Arab Emirates

Background: Hyperhemolysis syndrome (HHS) is a rare and potentially fatal complication in sickle cell disease (SCD). Multiple treatments have been reported to be beneficial including steroids, IVIG and erythropoietin. In addition, there are few case reports demonstrating the benefit of Eculizumab and Rituximab.
Aim: Present a case of HHS in a SCD/Thalassemia patient who was successfully treated with Eculizumab and Rituximab.
Case report: Patient is a 31 years-old male with SCD/thalassemia on hydroxyurea, has a history of vaso-occlusive crises, splenectomy, acute chest syndrome (ACS), multiple alloantibodies complicated with delayed hemolytic transfusion reaction and HHS. Patient admitted with pneumonia/ACS and was started on IV antibiotics. Upon admission, Hb was 120 g/L, decreased to 100 g/L, additional laboratory parameters performed include percent reticulocytes (8.6%), LDH (495 IU/L) and Haptoglobin (<0.10 g/L). The Hb further decreased over time to 39 g/L and other laboratory values were suggestive of hemolysis. Due to patient critical status, transfusion was requested. One phenotype compatible unit was issued; it was crossmatch compatible by tube technique and incompatible by column agglutination. 50 ml test dose was administered; patient Hb following the test dose was performed and was 73 g/L indicating a questionable pre-transfusion Hb report. Transfusion was recommenced, the full unit was transfused and Hb increased to 88 g/L. On **Day 4 post transfusion**, patient complained of sever back pain, his Hb dropped to 62 g/L which was lower than the pre-transfusion 73 g/L value. Hb continued to drop, lowest was 5.3 g/L and there was a sudden increase in LDH (1764 IU/L) and total bilirubin (60.3 micromol/L). In addition, patient reticulocyte dropped during the event from 26.1% to 12.5%. Patient was started on Dexamethasone and was given a single dose of IVIG.
For blood bank evaluation, refer to **Table 1.**
Eculizumab was administered **Day 4 post transfusions** (900 mg weekly for 2 weeks). Two days later, one dose of darbepoetin was given and the first dose of Rituximab (700 mg) was started. He continued on daily hydroxyurea and Dexamethasone was tapered. Patient improved clinically and his hemolytic laboratory parameters and Hb immediately stabilized and continued to improve. 9 Days following the hyperhemolysis episode, the patient was stable enough to be discharged with an Hb of 82 g/L; he continued to improve in his clinical and laboratory parameters and completed the remaining doses of Rituximab as an outpatient.
Discussion: The pathophysiology of hyperhemolysis is unknown with multiple hypothesis been reported such as activated macrophages. Eculizumab inhibits complement activation and therefore inhibiting intravascular hemolysis. In addition, rituximab has an effect on macrophages implicated in the HHS pathophysiology. Our Patient had a quick recovery, he was treated with IVIG, steroids, erythropoietin, Eculizumab and Rituximab, and whether the resolution was due to one of the treatments provided or due to a combined and synergistic effect is unknown. In addition, the patient absent spleen could have contributed to the milder course and quick recovery.

Table 1: Blood bank evaluation

Patient phenotype:
D+, C+, E-, c variant,e+,K-,k+,Kpa-,Kpb+,Jka+,Jkb-,M+,N-,Fya+,Fyb-,S-,s+
Unit transfused :
E-, K-,Jkb-, Fyb-, S- and c+

Pre-transfusion	Post-transfusion
• Alloantibodies present:	• Alloantibodies present:
- Anti-E, Anti-Jkb, Anti-Fyb, Anti-S	- Anti-E, Anti-Jkb, Anti-Fyb, Anti-S
- Anti-K was not ruled out	- Anti-K was not ruled out
• Warm autoantibody	• Warm autoantibody
• IgG and IgM Positive DAT.	• IgG, IgM, in addition to **C3d positive DAT**
• Negative elution	• Negative elution
• Could not totally rule out the presence of antibody against a high frequency antigen.	• Patient found to be c variant. Anti-c was not identified but the unit patient received was c positive.

Caption 1: Blood bank evaluation on pre transfusion and post transfusion specimens

Conclusion: Eculizumab will rapidly reduce the acute intravascular hemolysis observed; however, due to the parallel treatment with steroid, Rituximab and IVIG, it was difficult to draw any strong conclusions about its efficacy. Nevertheless, due to the life-threatening nature of HHS, it is important to have a high index of suspicion and respond quickly by managing all implicated pathways. **Hence the use of Eculizumab either alone or combined with rituximab merits consideration.**

P-506

ISCHEMIA/REPERFUSION MEDIATED CARDIAC DAMAGE AS A COMPLICATION OF BLOOD TRANSFUSION AMONG BETA-THALASSEMIA PATIENTS

M Bani-Ahmad, G Rahimi and A Abu Awad
Jordan University of Science and Technology, Irbid, Jordan

Background: Blood transfusion is the primary therapeutic approach for thalassemia patients. Several complications to transfusion with accumulative effects were evident including iron overload, hemolytic reactions and infection. However, cardiomyopathy is the most common life threatening complications as a consequence to iron deposition within cardiac tissues as well as the increase in cardiac index to compensate for the reduction in oxygen carrying capacity. Primarily, transfusion aims to restore oxygen carrying capacity (reperfusion) following to tissue hypoxia (ischemia). Ischemic organs are vulnerable to necrotic damage that can be further exaggerated upon reperfusion through several intracellular and extracellular mechanisms.
Aims: Multiple events of blood transfusion expose organs to repetitive cycles of ischemia and reperfusion. This preliminary study aims to investigate the impact of ischemia and reperfusion events on cardiac injury among beta thalassemia patients.
Methods: Twenty one beta thalassemia major patients (Age range is from 7 to 30 years old) were included in the study. All patients are packed red blood cells (pRBC) units recipients every 3–4 weeks. Blood samples were withdrawn from study subjects just prior to blood transfusion and 24-h post transfusion. A control group of apparently healthy ten volunteers with no personal or family history of hematological diseased or cardiac diseases.
Serum levels of Ischemic modified albumin (IMA) and troponin I (TnI) were measured by enzyme linked immunosorbent assay (ELISA) using commercially available kits where manufacturer's instructions were followed. Results were presented in nanogram per milliliter (ng/ml) as Mean ± SEM (standard error mean). Statistical analysis was conducted using Statistical package for social sciences (SPSS) software. Results with P value less than 0.05 were considered to be statistically significant.
Results: Before pRBC administration, serum IMA levels have been shown to significantly higher among thalassemia patients (76.32 ± 19.95 ng/ml) compared the healthy control (11.06 ± 4.87 ng/ml) with a P-value of 0.005. following to transfusion, mean IMA level was normalized (26.09 ± 7.91 ng/ml) and was insignificantly higher compared to control levels (P = 0.12).
With regard to TnI levels, result showed significantly higher levels among study subjects (1.12 ± 0.21 n/ml) compared to control group (0.19 ± 0.06 ng/ml) with a P-value <0.001. Following to transfusion, TnI levels were reduced compared to those before transfusion but still significantly higher compared to control subjects (0.52 ± 0.15 ng/ml) with P-value equals 0.02.
Summary/Conclusions: Serum IMA levels confirm an ischemia/reperfusion status among thalassemia patients before and after transfusion events, respectively. The higher pre-transfusion serum level of troponin is strongly suggestive of an ischemic cardiac damage. That damage was partially reversible as revealed by the reduction in TnI levels post transfusion compared to pre-transfusion levels. However, the significantly elevated TnI levels post-transfusion in comparison to control group may indicate an irreversible cardiac damage that might be referred back to the previous history of repetitive ischemia reperfusion cycles, the extent and duration of the current ischemia/reperfusion status of patients or the additional effect of other factors including iron overload.

Haemovigilance and Transfusion Safety

P-507

STEPS TO SUCCESSFUL IMPLEMENTATION OF HAEMOVIGILANCE IN BIRJAND TEACHING HOSPITALS

G Anani Sarab

Birjand University of Medical Sciences, Birjand, Iran

Introduction: Haemovigilance is a national system to detect, gather and analyse unwanted events related to blood transfusion. The first haemovigilance system was estabilished in 1993 in Japan and the first one in Europe was initiated in France in 1994. Although haemovigilance is currently functioning in many countries but implementation of a national haemovigilance system is not a straight forwards task and usually takes long time of planning. Iranian blood transfusion organisation has been put the haemovigilance programme into practice from 2012 and it is now operational. It was implemented nationally and targeted the hospital blood banks. The aim of this study was to assess a newly implemented haemovigilance system in Birjand university hospitals.

Materials and Methods: A set of standard preformatted form for the requesting blood, and notification of an adverse reaction in a transfused patient has been developed by the Iranian blood transfusion organisation. We assessed the Simplicity and workability of these forms relating to capturing transfusion reaction events in real time. Proportion of returned information for each on the forms was determined. Further evaluation was done by means of validated and reliable questionnaire for understanding the viewpoint and expectation of the nurses and blood bank staff involved in blood transfusion regarding the haemovigilance programme.

Results and Discussions: The data showed some imperfections in hemovigilance establishment in Vali-asre hospital. Basically most of these deficiencies are related to the national level of designing haemovigilance programme rather than regional and local limit.

The studied blood request and transfusion incident report forms were found not fully completed and the returned information on the forms partially covered. This observation could be related to the number of issues. In fact hamovigilance is a new type of responsibility in a hospital, in addition to the already existing other vigilances for examples pharmacovigilance. The people who were in charge with transfusion and capturing the event believed in doing the haemovigilance as an additional responsibility without adequate compensation. In addition to that blood request and transfusion incident report forms in some part are far from simplicity, with the request for inadequate or unnecessary information in some sections. This finding brings forward the necessity of modifying the forms according to the lessons of experience.

Insufficiency of educational programmes on haemovigilance and the shortage of motivated and interested people amongst those were in charge of haemovigilance were amongst other obstacles found in this study. Basically, this fact is related to the lack of blood transfusion teaching in the health and medical education in Iran and is needed to be introduced in the education curriculum.

Although, the collaboration between blood centres and hospitals which is contributed to the successful implementation of haemovigilance programme was found to be in the right path but more improvement is needed.

Conclusion: Haemovigilance system is operating from its implementation in Birjand university hospitals, but regular supportive supervision is needed to improve the effectiveness of the system. The data need to be collected and analysed and collaboration at regional level between blood centres and hospitals also required more strengthen.

Keywords: Haemovigilance, Transfusion incident, Blood

P-508

A REPORT OF ADVERSE TRANSFUSION REACTIONS AFTER HEMOVIGILANCE ESTABLISHMENTIN IN SHIRAZ, FARS

L Kasraian and MH Karimi

Blood Transfusion Research center/ High Institute for Research, Shiraz, Iran

Backgrounds: In spite of vital role of blood transfusion in saving lives of many people, but may cause adverse reactions in many patients

Aims: We aim this study to determine the frequency and type of Acute Transfusion Reactions (ATRs) after hemovigilane establishment in Shiraz, Iran.

Methods: This was a retrospective study on all the transfusion reactions that were reported to the transfusion service at Fars,Iran from2013–2014. Due to hemovigilance guideline all transfusion reactions must be reported. The transfusion reactions occurring during or within 24 h after transfusion were evaluated according to demographic characteristics, type of blood products, frequency, and type of transfusion reactions.

Results: The incidence rate of acute transfusion reactions reported during the study period were 0.17%,and0.31%.The most common reactions were allergic reaction (34.4%),and febrile non haemolytic reactions (41.9%).The most common symptoms were pruritus, chills,and rash.58.8%of transfusion reaction was occurred in thalassemia patients.

Conclusion: The frequency of transfusion reactions in our patients was found to be 0.17%, and0.31%. This may be an under reported figure. In spite of establishment of hemovigilance system, There is a need for emphasizing on diagnosing, and reporting of all transfusion reactions.

P-509

PREVALENCE OF ADVERSE REACTIONS DUE TO PLASMA DERIVATIVES TRANSFUSSION

J Vitlarova

Institute of Transfusion Medicine, Regional center Stip, Stip, Macedonia

Background: The use of plasma et plasma derivates has is increased over the past ten years. On the other hand, the transfusion of plasma derivates is associated with a number of pontential adverse reactions such as allergic reactions, hemolytic reactions etc. as well as transmitted infection diseases.

Aim: To determine the prevalence of adverse reactions in patients who received plasma derivatives in our Regional Center (RC) for Transfusion Medicine.

Material and Methods: In the period of 2013–2014 we have collected 7540 whole blood units from voluntary, non-remunerated blood donors from the eastern region of Macedonia. From the total 7540 whole blood units, 7437 units of plasma derivatives were prepared as follows: Fresh Frozen Plasma (FFP), Plasma prepared within 24–48 h, Cryo-poor plasma and Cryoprecipitate. From the total 7437 units of plasma components, 6829 were received by 2771 patients (1341 male and 1430 female. The highest number of patients treated with plasma derivatives were from the Internal medicine Ward 3016 (45.4%). The rest of the patients were from diverse wards alike: Orthopedic Ward 1691 (24.7%), Surgical Ward 942 (13.7%), Gynecology and obstetrics 454 (6.6%), Urological Ward 382 (5.5%), Intensive care unit 203 (2.9%) and the other 54 (0.7%) in the Pediatric Ward.

Results: From the total 2771 patients treated with plasma components, adverse reactions were reported in 46 patients (1,6%). Allergic reaction have shown 24 patients (0,8%), Febrile non-hemolytic reaction was present in 19 patients (0,6%), Transfusion-related acute lung injury (TRALI) in only 2 patients (0,07%), and in 1 out of 2771 patients a severe anaphylactic reaction was reported (the patient received one unit FFP after section re-caesarea). Allergic (urticaria or other rash) and febrile reactions were most common in our patients and their occurrence was at some point during plasma transfusion or up to 3 h after the procedure. Hemolytic reactions as well as transfusion related infectious disease were not reported.

Conclusion: The number of adverse reactions due to plasma transfusion in our RC was reported in 46 out of 2771 patients. To avoid the occurrence of plasma components side effects we recommend an interdisciplinary approach before every application. Namely, regularly consultations between the clinicians and the transfusion medicine physician as well as assistance with rigorous indications are required before every application of plasma derivatives in each patient. Furthermore, with the future higher standardization of plasma fractionation protocol we could increase the quality and safety of the derivatives.

P-510

ADVERSE REACTIONS IN BLOOD AND APHERESIS DONORS IN 2015: LISBON BLOOD CENTRE REPORT

F Pinto, P Gomes, P Borrego, S Marques, MA Escoval and M Santos

Portuguese Institute of Blood and Transplant, Lisboa, Portugal

Background: Haemovigilance's aims, as a set of organized surveillance procedures relating to adverse events or reactions in donors or recipients, is to report, monitor and analyze unexpected and undesirable reactions and events temporally related to blood and apheresis donations, in order to prevent their occurrence and enhance blood donation safety.

Aims: To assess data on whole blood and apheresis donations adverse reactions in order to define appropriate preventive and corrective measures to improve blood donation safety.

Methods: A retrospective analysis of Donor Adverse Reactions (DAR) reported to the Portuguese Haemovigilance System (PHvS) by Lisbon Blood and Transplant Centre (CSTL), during 2015, has been performed.
Data was recorded according to local procedures and classified using the ISBT/IHN Standard for surveillance of donation complications. Only the reactions that required any kind of clinical intervention were reported.
Results: In 2015, 57276 whole blood donations and 451 apheresis procedures were performed in CSTL. A total of 992 (1,72% of all blood donations) adverse reactions and events were recorded but only 202 DAR were reported to the PHvS for an overall rate of 0,35%.
The analysis has shown that the prevalence of DAR is higher in mobile sessions (0,38%) than in the facility site and that the prevalence of reactions in apheresis donations is higher (3,10%) than in whole blood donations.
The imputability of the reported reactions was in 65% of the cases classified as probably/likely related to the event and in 35% of the cases certain. Only 2,48% of DAR were severe with a rate of 0,009%.
These reactions occurred in different stages of the donation process: 44,55% from the introduction to the removal of the needle, 31,19% after needle removal, 16,34% ambulatory donor on site and about 8% when the donor has left the blood collection site.
The generalized complications (vasovagal reactions) represented a rate 0,23% of the overall donations. Immediate vasovagal reactions were the most common complications (55,61% of the total number of reactions), and delayed vasovagal reactions occurred in 6,12% of cases.
The most prevalent reactions with local symptoms (0,12% of overall donations) were haematoma (25,5% of total reactions) and delayed bleeding (6,12%).
DAR were more frequent in females (62,87% of the total DAR), 35.64% of DAR occurred in first-time donors and 40,10% in recent donors (1 to 5 previous donations).
In what concerns the recovery of the blood donors, only about 1,98% of total DAR needed hospital attention (0,007% of the total donations), about 76% had a rapid recovery and 22,28% a slow recovery.
Conclusions: These data is consistent in frequency and severity to the reported in medical literature, underline the safety of blood donation, with a low rate of severe adverse reactions.
These events are mainly preventable by measures of education and training in the use of a validated venipuncture technique, careful donor vigilance and strategies to reduce vasovagal reactions providing donors with better pre and post-donation educational materials with instructions on avoiding vasovagal reactions and a post donation report of any medical problems.

P-511

A STUDY OF PATTERN OF ADVERSE TRANSFUSION REACTIONS TO BLOOD PRODUCTS IN A TERTIARY CARE TEACHING HOSPITAL: A CROSS-SECTIONAL STUDY

M Upadhye[1], S Divhare[1] and P Shidhaye[2]
[1]*B.J.Government Medical College, Pune, India* [2]*L.N.Medical college, Bhopal, India*

Background: Hemovigilance studies the transfusion related adverse reactions so as to increase the safety and efficacy of blood transfusion. Such reactions range from acute hemolytic to non-hemolytic hypersensitivity reactions. Whether there is an association of these reactions with different blood products, blood group & gender of the patient is not known.
Aims& Objectives: To find out association of adverse transfusion reactions (ATRs) with different blood products, gender & blood group of patients.
Material & Methods: A cross-sectional study was conducted in the tertiary care teaching hospital over a period of one month. All patients of all age groups and of either gender were included in the study. The products undertaken for the study were Human Red Blood Cells (HRBC), Saline Washed RBC (SWHRBC) Platelet Rich Plasma (PRP), Fresh Frozen Plasma (FFP) and Cryoprecipitate (Cryo). A total of 892 units of different blood products were transfused to 445 patients over a period of 30 days. Patients showing various adverse reactions were analyzed with a predesigned questionnaire. The prevalence of these ATRs according to the gender and blood group of the patient & the blood product(s) transfused were analyzed separately and also considering the three variables together, to find an association between the variables and the ATRs. Data were analyzed using Statistical Package of Social Sciences (SPSS) 16.0. The statistical significance was set at $P \leq 0.05$.
Results: The overall prevalence of adverse transfusion reactions amongst 892 transfusions is 5.27% whereas 10.56% (47) of the total 445 recipients had ATRs. 70.21% of the affected patients had a past history of blood transfusion. The prevalence of ATRs in female and male patients is 11% & 10.04% respectively. A+, B+, O+ & AB+ are the blood groups frequently showing ATRs, 1 ATR to O- was seen in females. HRBC is the most frequently transfused blood product followed by SWHRBC, FFP, PRP & CRYO in that order. Acute febrile reactions are the most common reactions followed in frequency by dyspnea & orthopnea, with acute hemolytic reactions being a rarity. We also noted a case of Transfusion associated Acute Lung Injury (TRALI) and two cases of skin rashes. We documented certain odd reactions whose references could not be found in the literature.
Conclusion: Though the overall prevalence of ATRs seems to be less, 10.56% of the transfusion recipients are at the risk of having adverse transfusion reactions. Presence of a past history of blood transfusion is a strong predisposing factor for developing ATRs. Both female and male recipients are equally affected. The prevalence of acute hemolytic reactions is negligible. The most prevalent reactions are hypersensitivity reactions. There is no significant association of adverse transfusion reactions with blood products, blood group & gender of the patient.

P-512

INCIDENCE OF ADVERSE TRANSFUSION REACTIONS IN MULTI-TRANSFUSED THALASSAEMIA PATIENTS IN MIRPUR, AZAD JAMMU KASHMIR, PAKISTAN

A Wazeer[1], Z Qasim[1], Z Iqbal[2], U Waheed[3] and HA Zaheer[3]
[1]*Divisional Headquarters Hospital, Mirpur, Pakistan* [2]*Thalassaemia Care Centre, Mirpur, AJK, Pakistan* [3]*Safe Blood Transfusion Programme, Ministry of National Health Services, Islamabad, Pakistan*

Background: The transfusion of blood and blood components is life-saving but can lead to a number of adverse reactions in the recipients. The multi-transfused thalassaemia patients are particularly at risk to develop these reactions due to frequent transfusions. Thalassaemia is a major health care challenge in Pakistan and the biggest consumer of its transfusion services. These patients are not hospitalized yet require regular transfusions. In most of the cases, the reactions in thalassaemics are under-reported due to fear of reprisal or lack of recognition of the signs and symptoms of adverse reactions.
Aims: The current study was carried out to determine the incidence of adverse transfusion reactions occurring in the Thalassaemia Centre of DHQ Hospital, Mirpur.
Material and Methods: This prospective study was conducted from January - March, 2016, at the Thalassaemia Centre, Divisional Headquarters Hospital, Mirpur, Azad Jammu and Kashmir, Pakistan. A standardized patient haemovigiliance reporting form, developed by the Safe Blood Transfusion Programme, was used to collect the adverse reactions data. Of the 235 registered thalassaemics, who regularly receive transfusions in the Thalasaemia Centre of the DHQ Hospital. The results were entered in SPSS software and frequencies calculated.
Results: From January - March 2016, 1003 packed red cells were transfused at the Thalassaemia Centre. 315 patients suffered a total of 451 immediate transfusion reactions in three months and none of the reactions caused death. No haemolytic and delayed transfusion reaction was reported. The reactions included 120 cases (26.6%) of febrile non-haemolytic reaction, 21 cases (4.65%) of allergic rashes, 38 cases (8.42%) of restlessness, 21 cases (4.65%) of cannula blockage, 37 cases (8.2%) of bruising, 10 cases (2.21%) of haematoma, 58 cases (12.86%) of fever, 136 cases (30.1%) of pain at infusion site, 16 cases (3.54%) of hypotension. In 96 cases, multiple pricks were performed to transfuse and in 34 cases premedication (Avil/Solocortef) was given. All transfusions were monitored by the duty nurse.
Conclusion: This is the first time that a serious effort was made to record haemovigilance data and analyze it. The study will help to promote haemovigilance in thalassaemia care set-ups and in hospitals also. The number of immediate adverse events reported in this study are comparatively less compared with anecdotal evidence from other thalassaemia centres in the country.

P-513

HAEMOVIGILANCE – EVALUATION OF REPORTING SERIOUS ADVERSE REACTIONS AFTER TRANSFUSIONS IN THE REGIONAL BLOOD CENTER IN POZNAN IN 2012–2015

B Andrys and K Olbromski

Blood Center in Poznan, Poznan, Poland

Background: Reporting by hospitals serious adverse reactions after transfusions to the blood centre is one of the elements necessary for the safety of blood. Serious adverse complications include reactions that cause death, endanger life, injurie, health disorders or result in prolonged illness or hospitalization. All serious adverse transfusion reactions should be reported to the donation center within 24 h. Reactions that are subject to this condition: AHTR, TRALI, TACO, PTP, TA-GvHD and transfer of infectious factors. All of the above cases are reported to the Institute of Hematology and Transfusiology in Warsaw (IHiT).

Aim: Retrospective analysis of proceedings in case of serious adverse reaction after transfusion reported by the hospital.

Methods: Forms regarding adverse reactions after transfusions reported to the Regional Blood Center in Poznań in 2012–2015 were analyzed for:

Type of reported reactions and their correlation with the transfusion

Clinical condition of the patient, including the effects of transfusion

Research confirming correlation with the transfusion

Consequences of the research for blood donors

Notifications to the IHiT

In every case of serious adverse reaction after transfusion a physician from the Regional Blood Center in Poznań contacted the physician responsible for the transfusion in the hospital.

Assessment of the correlation of complications after transfusion with the transfusion was performed according to a 4 - point scale: 0-Excluded, 1-Questionable, 2-Possible, 3-Sure(proved).

In case of the necessity to perform tests for anti-leucocyte antibodies and/or antiplatelet antibodies the material for analysis was sent together with the medical documentation to the IHiT.

Results: The analysis covered 316 report forms of adverse reactions after transfusions - the ones indicating serious reactions after transfusions were presented in **Table 1**.

In every case of a mistaken transfusion, the Regional Blood Center in Poznań performed an inspection of the entity in which the adverse event occurred.

In every case of an anaphylactic shock and TACO, a clinical transfusion specialist met with the corresponding physician to analyze their clinical situation and to determine its correlation with the transfusion.

In every case of suspected TRALI and PTP the material and medical documentation of the patient were sent for consultation to the IHiT.

Whenever necessary the doctor supervising the safety of blood in the Blood Center intruduced the look back procedure, recorded donor's deferral in the blood bank's computer system, informed the donor in writing about this fact and the reasons for the deferral and made recommendations regarding the potential treatment with blood components in the future.

Conclusions: Haemovigilance should be based on close cooperation and flow of information between medical health care entities, blood centers and the responsible entities such as IHiT. As concluded, the procedure of reporting serious adverse reactions after transfusion by the hospital meets the criteria for haemovigilance and ensures the safety of use of blood components.

Table 1. Summary of cases of serious adverse reactions after transfusions in 2012-2015 reported to the Regional Blood Center in Poznań

No.	Type of reported complications	Clinical condition of the recipient	Type of confirmatory test to the complication	Result	Disqualification donor	Institute of Hematology and Transfusion Medicine in Warsaw notification	Notes
1	TRALI	Critical	Detection of anti-leucocyte antibodies	-	No	Yes	TRALI probable. Leve 1 of causality 3
2	TRALI	Fair	Detection of anti-leucocyte antibodies	-	No	Yes	TRALI probable. Level of causality 3
3	AHTR	Fair - no clinical symptoms	Serology (non compliance between the recipient and the donor. It was two populations of cells in the ABO system)	+	No	Yes	Erroneous transfusion of PRBC Recipient 0 RhD- Giver a RhD + Level of causality 3
4	AHTR	Critical- operation of congenital heart disease	Serology (non compliance between the recipient and the donor. Confirmed the presence of all antibodies anti-Ik a)	+	No	Yes	Transfusion of PRBC antigen to which the recipient antibodies are found. Level of causality 3
5	TRALI	Fair	Detection of anti-leucocyte antibodies	-	No	Yes	TACO Level of causality 2
6	TACO	Fair	EKG, echocardiogram	+	No	Yes	heart attack - contact with the treating doctor. Level causality 0
7	TACO	Fair	Physical examination, laboratory tests on the function of the urinary tract	+	No	Yes	TACO - pulmonary edema with anuria to the patient. Level of causality 3
8	AHTR	Critical	Serological tests, laboratory testing for red blood cell hemolysis	+	No	Yes	Hemolysis - Presence immune antibodies, Level of causality 3
9	PTP	Fair	Detection of antiplatelet	+	No	Yes	Thrombocytopenia caused by the presence of anti-heparin-dependent antibodies directed against the complex heparin/platelet factor 4 (PF4). Level of causality 0
10	Anaphylaxis / hypersensitivity	Good	Physical examination, laboratory tests	+	No	Yes	Anaphylactic shock , Level of causality 3
11	TACO	Critical	Physical examination, ECG, echocardiogram, laboratory tests	+	No	Yes	Cardiogenic pulmonary edema , Level of causality 1
12	PTP	Critical	Detection of anti-leucocyte antibodies Detection of antiplatelet	+		Yes	The cryoprecipitate detected anti-HLA Class 1 Heparin-dependent antiplatelet antibodies detected directed against the complex heparin/platelet factor 4(PF4). Level of causality 3
13	TRALI 2x in this patient	Fair	Detection of anti-leucocyte antibodies	-	No	Yes	TRALI probable on a child with heart failure. Level of causality 3
14	TRALI	Fair	Detection of anti-leucocyte antibodies	+		Yes	Detected Anti-HLA Class 2 antibodies nonbinding of the complement. Pulmonary edema could be caused by circulatory overload and/or TRALI. Level of causality 3 **Notification after 4 days**
15	AHTR	Critical	Serological	+	No	Yes	Mismatch found in the ABO system, between the recipient and the donor. Patient group B received FFP group 0. Level of causality 3
16	TRALI	Critical	Detection of anti-leucocyte antibodies	-	No	Yes	TACO Level of causality 3

Caption 1: Table 1

P-514

ALLOIMMUNIZATION OF THALASSEMIA PATIENTS IN JORDAN

S Bohisi

King Hussein Cancer Center, Amman, Jordan

Background: Treatment of Beta-thalassemia (β-thalassemia) involves a regular transfusion program, which despites being a life-saving process, is associated with inherent risks of alloimmunization against red cells antigens, this occurs due to genetic disparity between donor and recipient red cells antigens. The development of anti-RBC antibodies (alloantibodies and/or autoantibodies) can significantly complicate transfusion therapy and awareness of such alloantibodies is essential for selecting appropriate RBC products for transfusion[5].

Although thalassemia is most prevalent in Asia; worldwide, data discussing the incidence of RBC alloimmunization in thalassemia patients from this region has been scarce.

Aim: This study aims to investigate the frequency of alloimmunization, and most common alloantibodies of thalassemia patients in Jordan. It will also shed a light on the possible relationship between ABO blood groups distribution and thalassemia disorder by reviewing the by ABO blood groups data of our thalassemia patients.

Materials and Methods: This was a retrospective study that was carried out at King Hussein Cancer Center (KHCC) in Jordan over the period from January 2013 to December 2015. Records of 63 pre-diagnosed thalassemia patients undergoing a regular transfusion program (every 2–4 weeks) were reviewed and categorized into different age and ABO blood type groups.

Results: During the study, 63 thalassemia patients were reviewed. Their ABO blood groups were: 22 (34.92%) A patients, 6 (9.52%) AB patients, 10 (15.87%) B patients, and 26 (41.27%) O patients this data was used to investigate the possibility of a relationship between ABO blood groups distribution and thalassemia disorder and as indicated by the above percentages, the distribution of ABO blood groups did not differ significantly with respect to the normal (source: Jordanian) population[6] (P value= 0.984)(table 1).

Those 63 patients were categorized according to age into four groups; group one comprised a total of 18 patients between 3 and 10 years of age, of those 1 (5.56%) were alloimmunized. Group two consisted 34 patients between 11 and 20 years old, 2 (5.88%) of which had allo antibodies. Group three had 9 patients between 21 and 30 years of age, none were alloimmunized (0%). Group four comprised two patients beyond 30 years of age; both of these patients were alloimmunized (100%) (Table 2). A total 5 of 63 thalassemia patients (7.64%) had allo-antibodies (3 males and 2 females) of those one had 2 irregular antibodies; alloimmunization against K antibody had the highest incident rate (3/6; 50.00%) followed by one incident (16.67%) for each of Le^a antibody, E antibody and c antibody.

Conclusion: Several factors affect alloimmunization: The recipient immune status, RBC antigenic difference between donor and recipient (homogeneity) and the immunomodulatory effect of the allogenic blood transfusion on the recipient immune system[3].

In Jordan, the frequency of alloimmunization and of alloantibodies in thalassemia patients; and the relationship between ABO blood groups and thalassemia disorders has never been studied. This study indicated a 7.64% of alloimmunization in the Jordanian population, this is considered relatively low when compared to results of neighboring countries in the region.

The relatively low incidence of RBC alloimmunization in this study can be attributed to two main factors:

Homogeneity of RBC antigens between blood donors and receiving patients [4, 19]

Throughout their treatment at KHCC, patients received leukodepleted blood. This suggests a lower alloimmunization rate when compared to non-depleted blood [3, 4].

P-515

STUDY ON ADVERSE REACTIONS FOLLOWING TRANSFUSIONS IN DISTRICT GENERAL HOSPITAL HAMBANTHOTA – SRI LANKA

M Adikarama, KI Madarasinghe and PGA Iroshini

Blood Bank, Hambantota, Sri Lanka

Background: Haemovigilance is a set of surveillance procedures covering the whole transfusion chain from the collection of blood components to the follow up of its recipients. The ultimate purpose of haemovigilance is surveillance of adverse reactions and to prevent their occurrence or recurrence. That will ultimately lead to the improvement of patients' safety. In Sri Lanka all blood banks report transfusion adverse reactions monthly to the national haemovigilance unit.

Product	No of total transfusions	No of reactions	Percentage
RCC	3467	61	1.7
LRRC	493	7	1.4
FFP	1197	7	0.6
PLT	361	4	1.1
CSP	21	1	4.7

Table 1. The reactions pattern.

Aims: To evaluate the occurrence of transfusion reactions among transfused patients in District General Hospital Hambanthota for the period of one year from January 2015 to December 2015.
Method: Retrospective data taken from Haemovigilance Monthly statistics Register in DGH Hambanthota from 1st Jan 2015 to 31st Dec 2015 was analysed.All the reported transfusion reactions occurred following a transfusion of packed red cell concentrates (RCC), leucoreduced red cell concentrates (LRRC), FFP, PLT, and CSP were included.
Results: Total number of reactions reported in the said period was 80. Total number of RCC, LRRC, FFP, PLT and CSP transfused was 3467, 493, 1197, 361 and 21 respectively. The reactions pattern showed in Table-1.
Number of total Febrile Non Hemolytic Reactions (FNHTR) was 38 (47.5%). All of them were reported for red cell products [RCC- 35 (92%), LRRC -3 (8%)]. This value as a percentage to total red cell transfusion is 0.9%. This finding is comparable (0.84%) with study; Haemovigilance -Trends in Transfusion reactions among transfused patients in Ampara cluster done by Perera K.M.R.S, Adikarama B.M.G.M.P.Y in 2014.
Number of total minor allergic reactions was 29 (36.3%). Out of them 20 cases were following transfusion of red cell products [RCC- 19 (65%), LRRC -1(3%)], as a percentage to total red cell transfusion is 0.5%. Rest of minor allergic reactions were following transfusion of FFP 5 (17%) and PLT 4 (14%).
Number of severe allergic reactions was 2 (2.5%). Both were following transfusion of FFP, and this is a percentage to total FFP transfusion is 0.16%.
Out of 80, all 4 (5%) major transfusion reactions reported were transfusion associated circulatory overload (TACO) following transfusion of RCC. This value as a percentage to total RCC transfusion is 0.1%. There were no reported other major reactions such as ABO incompatibility,Bacterial contamination and TRALI.
54 female patients (67.5%) had developed reactions to blood and blood products and remaining 26 (32.5%) were male. This is comparable with the study; Haemovigilance: Investigation of adverse reactions following blood transfusion in regional blood center North Central Province Sri Lanka done by Senavirathne KCD in 2008–2010.
Conclusion: Although 4 cases of TACO were reported, there were no other major transfusion reactions such as ABO incompatibility, TRALI, or Bacterial contamination were reported in DGH Hambanthota during the study period. Out of the reported transfusion reactions, FNHTR is the commonest and the 2nd commonest is minor allergic reactions. Female patients are more prone to develop transfusion reactions.No near miss events were reported during this study period due to clinical staff awareness regarding better blood transfusion practices.

P-516

CLINICAL SAFETY OF AMOTOSALEN/UVA PATHOGEN-INACTIVATED APHERESIS PLATELET COMPONENTS IN ROUTINE USE

T Gaponova[1], M Azimova[1], A Rahmani[1] and E Zhiburt[2]
[1]Hematological Scientific Center, Moscow, Russian Federation [2]National Medical Surgical Center, Moscow, Russian Federation

Background: Photochemical treatment (PCT) utilizing Amotosalen (AS) and UVA light allows the inactivation of viruses, bacteria, parasites and leucocytes that can contaminate platelet and plasma components. The method is approved and routinely used at over 100 centres in Europe, the Middle East, the Americas and in several Asian countries.
Aims: The objective of this single-site post-market surveillance study is to collect haemovigilance safety data for patients transfused with AS pathogen-inactivated platelet components in routine therapeutic use to further characterize the Amotosalen PCT safety profile.
Methods: This post-market active haemovigilance study is a prospective, open label, non-controlled, non-randomized, observational study of patients transfused with AS pathogen-inactivated platelet components in 100% plasma (prepared from apheresis). Primary goal is the assessment of transfusion safety by collection and analysis of adverse events followed by classification in transfusion-reaction sub-categories (TRALI/TACO/TRS).
Results: Five hundered and two transfusions of IBS PCs were administered to 169 adult patients as part of their treatment for haematology-oncology disease (99.4%) or surgery (0.6%). Most PCs were $\leq$ 5 days old (99.4%). 52.7% of patients received multiple transfusions during the study. The mean ($\pm$SD) number of PCs administered per patient was 3.0 $\pm$ 3.2. AEs were observed following transfusion of 5 PC (1.0%) in 4 patients (2.4%). No AEs were classified as "serious"(SAE). The AEs were classified as a febrile non-haemolytic transfusion reaction and HLA antibody/lower back pain. The patients recovered from the AE/TRs on the same day, no TRALI, TRS or TA-GVHD was reported.
Conclusions: The types of AEs were consistent with published data, national haemovigilance programs and historical data for AS pathogen-inactivated PCs and comparable to data reported for conventional non-treated PCs. No transfusions were associated with TRALI, TRS, haemorrhage, TA-GVHD, or transfusion related death. Transfusion of AS-pathogen-inactivated PCs was safe and well tolerated in this patient group suffering from severe haematological diseases.

P-517

SET UP OF A LARGE EX VIVO INVESTIGATION ON TRANSFUSED PLATELET COMPONENTS: PLA_TRIP (PLATELET TRANSFUSION RELATED INFMAMMATION AND PATHOLOGY)

O Garraud[1], F Cognasse[2], C Sut[3], C Aloui[3], S Tariket[3], J Fagan[2], CA Arthaud[2], MA Eyraud[2], V Bost[2], S Leyerloup[2], M Sebban[4], E Fromont[4] and H Hamzeh Cognasse[3]
[1]INTS, Paris, France [2]EFS Rhone-Alpes-Auvergne, Saint-Etienne, France [3]University of Lyon-Saint-Etienne, Saint-Etienne, France [4]Université de Lyon, Laboratoire Hubert Curien - UMR CNRS 5516, Saint-Etienne, France

Background: Platelet component (PC) transfusion is not unlikely to lead to adverse events (AEs), of which inflammatory reactions; near half of the AEs recorded by the French national hemovigilance system can be attributed to PC transfusion, while this component accounts to only 10% of the blood components. As yet, no predictive marker is usable in clinical practice to monitor patients needing PC transfusion and to limit AE occurrence.
Aims: Considering that certain pro-inflammatory molecules highly to very high significantly associate with declared AEs (such as soluble CD40-Ligand and half a dozen of others, all secreted by platelets during shelf-life), we aimed at deciphering the most relevant parameters that can predict the occurrence of an inflammatory AE in patients, considering blood donor, blood component and recipient data.
Methods: We sought to design a protocol that allows the collection of samples"'without altering the final quality of the issued PC"'at D0 of production referred to as D_{prod} and at the Day of delivery (from D0 to D5: referred to as D_{del}). All PCs were processed in the regional facility in Saint-Etienne and then shipped to several health care units, of which two university hospitals in Saint-Etienne and Clermont-Ferrand. According to the French organization, PCs are shipped daily from the Production Unit to the Hospital Blood Bank (which is overviewed the National Blood System); PCs are thus issued to patients in need within a regulatory timeframe. Whenever possible, a sample was made from the PC pipes at D0 in the production

unit (D_{prod}) and another sample from the same PC was shipped to the Research Department at D_{del}. In case an AE occurred, supervisors were assigned to the recovery of a third sample from the PC and"'any time this was possible"'from the patient. All AEs were shipped back ASAP to the Research Department for immediate processing. A dozen of specific platelet factors were tested *ex vivo* from the samples (not all 12 for each sample, however); some biological material was kept frozen to test for other parameters later on, along with DNA from leukocyte extracts (when the University Hospital Ethics Committee allows testing).

Results: Thus, from March 2013 to April 2016, we collected 10,042 samples: 4120 (41%) from Single Donor aphaereris"'SDA"'PCs; and 5922 Whole-Blood Buffy-Coat Pools of 5, referred to as Pooled PCs or PPCs). Recovered samples originate from for D_{prod}: n = 6,909, and D_{del}: n = 3,133). A total of 141 samples were recovered in the main 2 clinics: 78 after SDA PC transfusion (55.3%) and 63 after PPC transfusion (44.7%).

Conclusion: Pla_TRIP stands for a large *ex vivo* investigation. Data are now underway to determine whether one given PC presenting with high load of inflammatory marker such as sCD40L is (almost) systematically not associated with AE or whether some recipients afford being infused with elevated levels of platelet-originating cytokines and biological response modifiers. This will allow the design of strategies to protect at particular risk patients from additional inflammatory states.

P-518

ANALYSIS OF CD62P EXPRESSION AFTER A PLATELET CONCENTRATES (PCS) TRANSFUSION

T Triyono[1], B Mulyono[2], S Sutaryo[2] and AS Sofro[3]

[1]Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia [2]Sardjito Hospital/ Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia [3]Faculty of Medicine, Universitas YARSI, Jakarta, Indonesia

Background: There is an increasing platelet transfusion for treatment and prophylaxis of bleeding in patients with hematologic disorders and malignancies. Because of limited resources, leukoreduced platelet concentrates is not yet implemented in most Indonesian hospitals. The high frequent of using platelet transfusions are followed by increased risk of transfusion reactions. *In vitro* platelet activation may cause morphology, functional, and ultrastructure changes. Those changes will reduce the platelet viability, *in vivo* functions, and clinical efficacy. High CD62P expression is the cause of faster platelet destruction in the reticuloendothelial systems. Post-transfusion *in vivo* Hemostatic efficacy can be determined by the measurement of *corrected count increment* (CCI), *recovery*, and CD62P expression.

Aim: To analyze the expression of CD62P of non-leukodepleted compared to pre-storage leukodepleted PC transfusion.

Methods: This was a prospective cohort designed study. Subjects were children aged 1–18 years with indication of platelet transfusions in the children's ward of Sardjito Hospital and met the inclusion and exclusion criteria. The patient samples were collected 1 h post-transfusion, and the expression of CD62P was determined by flowcytometry method. Statistical analysis was performed for the relative risk by determining the significant limit of P value <0.05 with 95% confidence interval.

Results: There were 102 patients who were divided into two groups. Fifty-one subjects recieved non-leukodepleted PCs and the other fifty-one transfused by pre storage leukodepleted PCs. The CD62P was measured both pre- and post-transfusion. The mean pre-transfusion CD62P for non-leukodepleted and leukodepleted groups were 26.2 and 27.7 respectively, and the post-transfusion CD62P for non-leukodepleted and leukodepleted groups were 36.1 and 24.4 respectively. It was shown the increased of post-transfusion CD62P for non-leukodepleted group, and it was significantly ($P < 0.05$) higher than in the leukodepleted groups.

Conclusion: There was a higher post-transfusion CD62P expression in non-leukodepleted than those in leukodepleted PC transfusions.

P-519

BACTERIAL SCREENING OF PLATELET CONCENTRATES; HOW SAFE IS THE PROCEDURE?

SE Spelmink

Sanquin, Nijmegen, The Netherlands

Background: Bacterial contamination of platelets still remains an important microbiological risk of blood transfusion. In the Netherlands all platelet concentrates (PCs) are tested for bacterial contamination by culturing a sample in the BacT/Alert system for 7 days. PCs are issued as 'negative to date' implying that transfusion of a possible contaminated PC is inherent to this system. Because of the potential for false negative results and the time frame between the final BacT/Alert results and the start of transfusion, some countries have introduced an additional rapid screening test for bacterial contamination pre-transfusion. Within this context we want to determine whether our detection system meets the current requirements for microbiological safety.

Aims: 1) To determine the rate of transfusion reactions in patients who received an afterwards confirmed positive PC. 2) To investigate how many cases of reported Transfusion Transmitted Bacterial Infection (TTBI) may be related to a false negative BacT/Alert result.

Methods: During a 3-year period (2013–2015) data from the BacT/Alert system were analyzed and related to clinical data collected from patients who received PCs that were released as 'negative to date' but afterwards became positive in the BacT/Alert system. In case of an afterward positive BacT/Alert result, hospitals are always informed. In case the PC already had been transfused, they were asked whether any transfusion reaction had occurred. Over the same period we evaluated all from hospital to blood bank reported TTBI cases in relation to BacT/Alert results.

Results: In 2013–2015 a total of 190,773 PCs were tested in the BacT/Alert system. Of them 620 (0,32%; 95% CI 0,30–0,35) tested positive. Of the 562 units labeled as confirmed positive 196 units were already issued as 'negative to date' and transfused. From 8 of 196 transfused PCs a transfusion reaction was reported to Sanquin Blood Bank (4,1%: 95% CI 1,9–8,2). The following bacteria were involved in these transfusion reactions: *Propioni species* (n = 7) and *Staphylococcus epidermidis* (n = 1). Four of these 8 patients reported fever without clinical consequences; one of these reactions was possibly related to the PC transfusion. Four other patients had serious underlying diseases and died shortly after PC transfusion, yet these deaths were unlikely to be related to the PC transfusion. Ten cases of TTBI after PC transfusion were reported; the results of these patients are described in table 1. Six cases of TTBI showed no relation between results of bacterial screening and culture of blood from the patient and/or PC bag and imputability was considered unlikely. Three cases were possibly and one case probably related to transfusion of a PC. None of these transfused PCs had a positive BacT/Alert screening.

Table 1: TTBI cases after PC transfusion reported to Sanquin in 2013-2015

	Medical grade	Imputability	Result of bacterial screening	Culture from patient	Culture from PC bag
Case 1	4	Probably	negative	*Staphylococcus aureus*	*Staphylococcus aureus*
Case 2	3	Possibly	negative	*Enterococcus faecalis*	*Enterococcus faecalis*
Case 3	2	Possibly	negative	*Staphylococcus hominus*	*Staphylococcus hominus*
Case 4	2	Possibly	negative	negative	*Staphylococcus hominus*
Case 5	4	Unlikely	*Propioni acnes*	not tested	not tested
Case 6	4	Unlikely	*Propioni species*	*Morganella species*	not tested
Case 7	4	Unlikely	*Propioni acnes*	not tested	not tested
Case 8	2	Unlikely	*Staphylococcus epidermidis*	*Enterococcus faecalis*	not tested
Case 9	2	Unlikely	negative	*Staphylococcus aureus*	not tested
Case 10	1	Unlikely	*Propioni acnes*	negative	not tested

Conclusion: We found a 4.1% rate of transfusion reactions related to (afterwards) bacterial contaminated PCs with a low imputability. Over a period of 3 years only 4 cases of TTBI (0,0001%) were reported for which the results in the BacT/Alert system could have been false negative. Therefore, we consider the BacT/Alert system as robust without need to change the current screening methods.

P-520

METHODOLOGY COMPARISON AND ANALYSIS OF IRREGULAR ANTIBODY DETECTION

X Liu and D Xiang

Shanghai Blood Center, Shanghai, China

Background: In recent years, there are many reports about the IgG antibody detection method sensitivity, many of them think that the microcolumn gel test is the most sensitive.

	Anti-Ec(E、c)	Anti-D(G)	Anti-Jk$^{a/b}$
IAT more sensitive	6	1	6
MPT more sensitive	18	4	4
MGT more sensitive	8	3	0
No difference	25	12	5
X^2	0.889	1.441	6.923
P	>0.05	>0.05	<0.05

Table 1. Compare the otherness of different testing methods in various irregular antibody detection

Aims: Compare the different testing methods in various irregular antibody detection, and find any relationship between the methods sensitivity with antibody specificity.
Methods: Retrospective comparison and analysis of 293 cases of irregular antibodies (including Rh, Kidd, MNS, Lewis system antibodies) detected in our laboratory by saline method, the Indirect antiglobulin test (IAT), the munual Polybrene test (MPT) and the microcolumn gel test (MGT) are used.
Results: There was no significant difference in Rh system antibody detected by different methods except saline method, Kidd system antibodies are most likely to be detected in the indirect antiglobulin test, as shown in Table 1. And some of IgM antibodies which are negative in room temperature saline method, can be more likely detected by microcolumn gel test.
Conclusion: Using a variety of detection methods, can increase the ability of irregular antibody detection and identification, and should be increasing the safety and effectiveness of clinical blood use.

P-521

IMPLEMENTATION OF STRATEGIES TO MINIMIZE ABO MISMATCH RED CELL TRANSFUSIONS: BEDSIDE ABO GROUPING AND TIME OUT

F Karim and B Moiz
Aga Khan University Hospital, Karachi, Pakistan

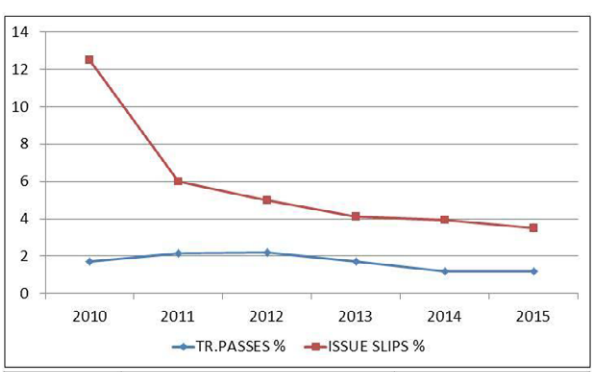
Background: Wrong transfusions are the most feared serious hazards of blood transfusion worldwide. The risks are more than the risks of transfusion transmitted infections. ABO mismatch red cell transfusions can lead to serious consequences in the patient and at times these prove to be fatal. In order to enhance transfusion safety at our institution, we started a quality project to develop and implement strategies which could help in minimizing ABO mismatch red cell transfusions.
Aim: To implement strategies to minimize ABO mismatch red cell transfusions and improve transfusion safety.
Methods: This quality project was initiated by the blood bank at Aga Khan University Hospital, Karachi, Pakistan. PDSA (plan, do, study and act) tool was used as methodology.
In the planning (P) phase, all cases of wrong transfusions from 1st January 2010 to 31st December 2015 and their root causes were analyzed. Various strategies to minimize wrong transfusions were proposed in hospital transfusion committee meeting held in September 2014. The two strategies which were finally agreed upon and approved by the transfusion committee and hospital leadership included implementation of bedside ABO grouping and introduction of "Time out"before red cell transfusions.
In the Do (D) phase, all stakeholders were taken in the loop. Bedside ABO cards [Serafol ABO, Bio-rad] were selected and ordered for ABO typing. "Time out"form was designed by transfusion medicine consultant. Training workshops were conducted for nurses. Bedside ABO grouping and "Time out"were implemented from 1st October 2015.
In the Study (S) phase, total number of bedside ABO groups done from 1st October 2015 to 29th February 2016 was calculated. Concordance between blood bank results and bedside grouping results was assessed. Incidence of wrong transfusion during this period was calculated. In Act (A), these strategies are continued for improving transfusion safety.
Results: During last six years, 133888 red cells were transfused. Eight wrong transfusions and three deaths were observed. The computed incidence of ABO-mismatch transfusion was 1 in 16000 with a mortality rate of 1 in 67000 red cell units transfused. Final bedside error was identified as the main reason [n = 5].
Training workshops were conducted for nurses in March and April 2015 in which 100 master trainers were trained and certified for bedside ABO grouping and "Time out". From May to September 2015, the remaining 1300 nurses working in hospital were trained by the master trainers. Time out form was approved by Health Information Management Services (HIMS) committee in May, 2015.
From 1st October 2015 to 29th February 2016, 2664 bedside ABO groups were performed. We observed 100% concordance between bedside and laboratory ABO grouping in all these cases. In these five months, no wrong transfusion was reported.
Conclusion: Proper planning and team work led to successful implementation of bedside ABO grouping and "Time out"before transfusion. We found these measures to be effective interventions for minimizing wrong red cell transfusions and improving transfusion safety.

P-522

ENSURING SYSTEMATIC TRACEABILITY OF BLOOD COMPONENTS IN GENERAL HOSPITAL CELJE

J Pajk
General Hospital Celje, Celje, Slovenia

Background: In the General Hospital Celje (GHC) special care was dedicated to collect data about donors and recipients of blood components from the very beginning of the activities in the Transfusion Department established in 1949 and later in the Transfusion Centre. (TC).
Aims: The main aim was to promote high standards on reporting transfusions following the EU Directives, the Guidelines of the Council of Europe and national legislation. Since the Slovenian law in 2006 was passed, it has been mandatory to fulfil all the requirements concerning traceability of transfusion components to the patients, i. e. systematically collect, report, analyse and regularly monitor this data.
Methods: In 2006 the documentation - the so called "Transfusion passes"were introduced in paper form, for every transfused blood component to the patient to all departments in GHC. Recently transfusion specialists have been giving workshops for doctors and nurses at different departments to educate them about the appropriate care of the patient and the documentation, when the blood component is transfused or not. Till 2010 the data was collected only on an "Issue slip"; later when TIS was updated and the information from passes corresponding to issued and transfused blood component was returned to TC monthly and recorded manually into TIS.
Results: The use of blood components and reporting about the outcome of transfusions in General Hospital Celje is presented and analysed. It was very difficult to switch to the new system, from sending Transfusion passes, to Issue slips in the transition period; so we used both. The understanding for users was better when they fulfil Transfusion passes than Issue slips and the percentage of undetectable units



Year	% of missed Transfusion passes	% of missed Issue slips
2010	1,72	12,5
2011	2,14	5,99
2012	2,21	4,98
2013	1,7	4,11
2014	1,21	3,93
2015	1,2	3,52

Caption 1: % of missed Transfusion passes and Issue slips in General Hospital Celje from 2010 to 2015

decreased from 12.5 to 3,52% in six years period. The percentage of undetectable units in GHC from Transfusion passes was lower in total in the beginning, from 1,72 to 2.21 the highest, to 1,20 through years, but at that time, after six years of training the users were used to report this data regularly to TC and it was much easier for them. Every month, reports for every department is analysed by a different responsible nurse for each department. We compare and analyse the data from Transfusion information system Datec about realised blood components in TC to the patients with the information we get about transfused or not transfused units from the departments.

Conclusions: Our main success is that our users understand the philosophy of traceability of every single unit, transfused or not, and send this data back to TC. After this period we got almost all the information which we put into TIS and calculated the percentage of those about which we could not find the clear data, if they were really transfused or discharged. We always try to find all the missing units through collaboration with many different departments involved in patient treatment. In the near future we hope all this steps will be performed in real time of the transfusion monitoring electronically at the patient's bedside.

Month/yr	% compliance
Dec 15	99.23
Nov-15	98.17
Oct-15	98.15
Sep-15	98.37
Aug-15	97.53
Jul-15	97.63
Jun-15	98.75
May-15	98.8
Apr-15	98.9
Mar-15	99.0
Feb-15	97.5
Jan-15	99.3
Dec-14	98.6
Nov-14	97.75
Oct-14	97.5
Sep-14	95.25
Aug-14	97.25
Jul-14	95.0
Jun-14	89.25
May-14	88.93
Apr-14	91.92

P-523

INVESTIGATING PLATELET CD36-DEFICIENCY AMONG THE PLATELET-APHERESIS DONORS

LY Wang, IS Hong, LF Tsai and KH Lin

Taipei Blood Center, TBSF, Taipei, Taiwan

Background: CD36, also known as platelet (PLT) glycoprotein IV (GPIV), is a class B scavenger receptor on platelets. Previously, prevalence of platelet CD36-deficiency in Taiwanese was reported to be 1.6–4%. There are two types of CD36 deficiency. Type I is characterized as no expression on platelets and monocytes, while Type II has expression of CD36 on monocytes but not platelets. CD36 deficiency subjects might be immunized by transfusion and induce anti-CD36 antibody, which could result in platelet transfusion refractoriness, post-transfusion purpura, and other types of immune-related thrombocytopenia.

Aim: The aim of this study was to investigate the frequency of platelet CD36 deficiency among the platelet-apheresis donors.

Methods: Whole blood samples were collected from platelet apheresis donors. CD36 expression on the platelets and monocytes were analyzed using flow cytometry.

Results: Of the 2,116 healthy blood donors analyzed, 38 donors failed to express CD36 on platelets were identified. Nine of 38 are classified as type I, the rest are classified as type II (29/38). In this study, our results demonstrated that the frequencies of type I and type II CD36 deficiency were 0.4% and 1.4% respectively. Among the 38 CD36-deficiency individuals, 33/38 (86.8%) are males and 18/38 (47.4%) are type O blood group.

Conclusion: Here we were able to identify and further classify CD36-deficiency individuals among platelet apheresis donors, which allows us to establish a donor registry for supplying CD36-negative platelets for patients in need.

P-524

EFFECTIVENESS OF CONCURRENT AUDITS AND REGULAR TRAINING IN TRANSFUSION PRACTICE IN A TERTIARY CARE HOSPITAL

A Verma

Max Super Speciality Hospital, Ghaziabad, India

Background: Auditing the use of blood components is necessary for all transfusion services and is required by NABH, AABB and the Joint Commission. The main purpose of auditing blood utilization is to assess appropriate use of blood components and help to optimize their use. Different audits (prospective, concurrent or retrospective) formats may be used depending on the objectives and resources available. Adequate knowledge of various aspects of blood transfusion by all clinical staff, including nurses is essential for safe transfusion practice. In all cases providing feedback and using the resulting interventions are both necessary to optimize transfusion practice.

Aims: The main aim of the study was to find whether concurrent audits combined with regular training programmes help in improving transfusion practices in our hospital.

Methods: A total of 6544 concurrent blood transfusion audits were done in various clinical departments including the emergency department of the hospital over a total period of 21 months (April 2014 to December 2015). Regular training classes on blood transfusion protocols of one hour duration were organized twice a month for nursing department over a period of 16 months (July 2014 to December 2015).The main focus of concurrent blood transfusion audits was documentation of all blood transfusion details in the patient's medical records and included:

Transfusion order
Recipient consent
Component name
Donation identification number
Date and time of transfusion
Name and signature of doctor and nurse
Pre and post transfusion vital signs
Volume transfused
Transfusion related adverse event
Total transfusion time lapsed
Transfusion indication

During the audits individual education on blood transfusion was also provided to the nurses and other clinical staff. All documentation was monitored in the 12–24 h following transfusion and reviewed.

Results: Transfusion details that were not meeting the criteria were marked as non-compliance. % compliance was calculated by dividing the compliance audits per month with the total number of audits done in that month. It was found that % compliance increased gradually during this period with increasing training programmes and concurrent audits.

% compliance in our audits for over a period of 21 months are summarized in Table below.

After performing the concurrent audits, all transfusion staff was contacted and guided appropriately and asked to complete the lacking transfusion information.

Conclusions: We found that Concurrent audits combined with regular training programmes greatly helped in evaluating transfusion practices in our hospital. Also, they permitted identification of suboptimal transfusion practices. Although further studies are required to determine which interventions are most effective or to compare different interventions including their cost effectiveness. Nonetheless, audits and regular training programmes for transfusion staff remain critical to evaluate blood utilization and improve transfusion practices.

P-525

IDENTIFICATION VALUE OF ANTIERYTHROCYTE ALLOANTIGEN TO INCREASE THE IMMUNOLOGICAL SAFETY OF BLOOD TRANSFUSIONS

B Bakhovadinov[1], MA Kucher[1], DA Sattorova[2], A Tretyakova[1] and DE Pevcov[1]

[1]*First State Pavlov Medical University, St. Petersburg, Russian Federation* [2]*National Research Center for Transplantation Medicine, Dushanbe, Tajikistan*

Background: According to various foreign authors allosensitized persons among general population varies from 0,1 to 1,0% and among pregnant women and individuals who received multiple blood transfusions - 1,64% and 12,4%, respectively. In order to prevent transfusion hemolytic complications and alloimmunization it's

recommended to identify antierythrocyte antibodies in donors and blood recipients. Screening of antierythrocytes alloantibodies has important clinical value for identifying patients which are allosensitized and prevent hemolysis of donor red blood cells, and also to prevent hemolytic disease of the newborn. The risk of sensitization in immunesuppressed cancer and blood recipients is much lower - 5% (among D^- recipients who received D^+ platelets).

Aims: To reveal incidence and prevalence of detection of antierythrocyte alloantibodies in blood donors, pregnant women and patients who received multiply transfusions, to establish the specificity of antibodies.

Methods: Search of alloantibodies was performed by indirect antiglobulin test using gel cards ID Card Liss/Coombs (DiaMed, Switzerland) and by using 10% gelatin (manufactured by FGUP 'Moshimpharmpreparaty' named after N.A.Semashko, Moscow) with microscopy. Antibody screening was performed with test-erythrocytes Seraskan Diana GRIFOLS I+II+III+IV (phenotypes: CCDee; ccDEE; ccdee; CwCDee). Identification of antibodies was performed in gel cards Liss/Coombs using 11 samples of red blood cells, phenotypically for 36 antigens (DiaMed). To the study were included 9900 samples of donor's blood from 18 to 65 years old, 1275 pregnant women, 450 outpatients and 450 serum samples of patients taken for the selection of compatible blood and 300 patients who received blood transfusion more than 5 times. All blood transfusions were carried out without phenotype identifying.

Results: Antierythrocyte antibodies were detected in 49 donors (0,49%), among them - 44 women donors who have had more them 1 children. In 45 cases antierythrocyte antibodies were revealed by indirect Coombs or gel method. Only in 16 donors with gelatin conglutination method antibodies were detected. In 400 outpatients, antierythrocyte antibodies were detected in 12 cases (3,0%) and in 99 cases (12,4%) of 800 Rhesus-negative pregnant women. When selecting a compatible blood for transfusion, antierythrocyte antibodies were detected in 23 cases out of 450 (5,1%). In 41 (13,7%) patients with multiple blood transfusions alloantibodies were identified. Only in 60 cases out of 224 gel method was used to identify antibodies. As a result, in 30 cases were identified anti-D antibodies, in 7 - anti-C, in 9 - anti-C, 6 - anti-E, 4 - anti-K, 2 - anti-Jka, 2 - anti-Fyb. Thus, the identification of antierythrocytes antibodies, their specificity in combination with erythrocyte antigens typing allows to make correct selection of compatible blood for transfusion, and thereby to prevent the development of adverse post-transfusion reactions and complications.

Summary: Gel micromethod as a part of Coombs method is an effective option for search of antierythrocyte antibodies. It is necessary to supply immunoserological laboratories with advanced panels of red blood cells for identification of antibodies using the gel test in order to prevent the development of adverse post-transfusion reactions and complications. To ensure immunological safety of blood transfusions it's economically feasible to test patient's blood for AB0 and Rhesus with alloantibodies search.

P-526

FREQUENCY OF REACTIVE BLOOD DONORS IN A TERTIARY CARE HOSPITAL, KARACHI, PAKISTAN

B Nepal, S Kalwar, R Nazar and N Mughal

Dr. Ishrat Ul Ebad Khan Institute of Blood Diseases, Kathmandu, Nepal

Background: Human Immunodeficiency Virus (HIV), hepatitis C virus (HCV), and hepatitis B virus (HBV) are blood-borne viruses and share transmission routes among at-risk populations, specifically injection drug use and remote blood transfusions before modern donor screening for these pathogens, making co-infection common. Morbidity and mortality from infection with HCV in HIV-infected patients are increasing and have become a major challenge in the management of such patients. In recent years, number of patients infected with HBV or HCV or HIV or co-infected with either of the two viruses, has increased tremendously in Karachi population. IDUs (intravenous drug users), MSM (Men who have Sex with Men) and individuals having unsafe sex are among the people who are identified as groups at higher risk of contracting these infections than others. But these studies does not give an exact picture of prevalence and frequency of these infection in Karachi's population as focus of most of these studies were individuals already involved in behaviors (intravenous drug use and unsafe sex) regarded as high risk behaviors.

Aims: To find the frequency of different types of reactive healthy blood donors at a tertiary care hospital, Karachi, Pakistan.

Methods: The retrospective observational study carried out on both male and female healthy blood donors. Data from complete blood screening from January 2013 to December 2014 were collected and frequency of various types of reactive blood donors was sorted out to get an actual picture. All the blood products were screened for HBV and HIV Using enzyme linkedimmunosorbent assay (Elisa plate washer version 3 and Elisa plate reader stat fax 3200). HCV screening was performed on Architect 2000 SR Chemiluminescent micro plate immune assay (CMIA). Malarial parasite tested by making thick and thin smear seen under microscope. Syphilis was tested by ICT method.

Results: A total number of 6996 healthy donors were received and about 624 were found to have blood screening positive in various combination. The highest numbers of isolates was HbsAg reactive 214, HCV 213, VDRL 170, HIV 26 and 1 case of malarial parasite. More prevalent in male population. In this study there were seven donors found with HCV -VDRL co infection and five co infected with HCV and HbsAg two donors with HIV and HbsAg infected and two donors were HbsAg and VDRL reactive.

Summary/Conclusion: This study supports that HBV, HIV and syphilis prevalence is high and HIV prevalence is low in healthy blood donors.

P-527

A RETROSPECTIVE ANALYSIS OF THE 'LOOK BACK' PROCEDURE IN SZPITAL WOJEWÓDZKI (REGIONAL HOSPITAL) IN POZNAN UNDER THE SURVEILLANCE OF REGIONAL BLOOD CENTER IN POZNAN

M Sobisz-Blachowiak and K Olbromski

Blood Center in Poznan, Poznan, Poland

Background: Carrying out the procedure 'Look back' is one of the forms of ensuring the safety of blood and blood components used in therapy. The procedure of tracing the process of blood distribution and identification of its recipients is carried out according to the Regulation of the Ministry of Health from December 11, 2012 in case when the current test of repeat donor confirms the presence of markers of HBV, HCV or HIV infection, and its previous donation was used for treatment in the window period.

In the Szpital Wojewódzki in Poznań all activities relating to transfusion of blood components are carried out on the basis of the standard operating procedures (ISO) which allows full traceability of blood and its components i.e. the possibility to trace the course of each individual unit of blood or its component.

Aim: The aim of the study was to analyze activities resulting from the implementation of the 'Look back' procedure in relation to the patients in the Szpital Wojewódzki in Poznań in years 2008–2016 (till February 2016).

Methods: Analysis covered the documentation and the protocols of notification 'Look back' procedures in 2008- 2016 i.e. 16 cases of possible transmission of infection that the hospital was notified about by the Regional Blood Donor Centre in Poznań. Also medical files of patients who were the recipients of units analyzed in the procedure were reviewed.

Results: Among the 16 analyzed donations 2 detections of HBV, 3 detections of HCV and 9 detections of HIV were the factors initiating the 'look back' procedure. In case of one donor, blood components were transfused to two patients that needed to be checked.

In 10 cases, the transfused component of blood was RBCs Concentrate, in 4 cases pooled Platelet Concentrate, one case of Platelet Concentrate from apheresis and FFP.

Among the analyzed donations all virus tests performed using serological and PCR methods were negative.

In 15 cases, the individual PCR tests from the archive sample also gave negative results, in one case - HBV DNA was detected in quantities below the sensitivity of the test.

The longest time between donation subjected to the 'Look back' procedure and the donation in which there were markers of virus infection was 8 years, the shortest - 78 days.

None of the recipients that were tested using serological and PCR control tests in Blood Center in Poznań showed transmission of viral infections.

In case of the kidney-transplant patient - viras tests performed in the Nephrology-Transplantation Clinic in her place of residence were also negative.

Conclusions: In the Szpital Wojewódzki in Poznań, all cases reported by Regional Blood Center in Poznań' in years 2008–2016 regarding the possibility of transmission of infection were investigated in accordance with the' Look back' procedure.

In the years 2008- 2016 in the Szpital Wojewódzki w Poznań there was no recorded infection of recipient of blood component from a donor who was later testes positively for presence of markers of HBV, HCV, HIV infection (in the window period).

The suggested solution i.e. testing in Blood Center in Poznań - is positively welcomed by patients who were the recipients of blood components.

P-528

TO 'D' OR NOT TO 'D': RHD INCIDENT REPORTING

L Bielby[1], C Akers[1], B Glazebrook[1], A Harper[1], P Beard[1] and C Hogan[2]

[1]Blood Matters, West Melbourne, Australia [2]Australian Red Cross Blood Service, Melbourne, Australia

Background: Blood Matters Serious Transfusion Incident reporting (STIR) system has been in place since 2007. This voluntary system collects de-identified data regarding transfusion incidents and adverse events. STIR has standard definitions and case report forms, with all cases reviewed by a multidisciplinary expert group.
STIR provides a reporting mechanism for serious transfusion incidents. This provides local information on the number and type of serious reactions that occur. Data collected by STIR is collated, aggregated and reported with recommendations for improvements for transfusion practice.
In January 2015, STIR focus on fresh blood components expanded to collect reports related to cell salvage and Rhesus D immunoglobulin (RhD Ig) administration.
Aim: RhD reporting includes incidents related to prescribing, dispensing, and administration errors, adverse reactions and failure of prophylaxis for RhD negative women of child bearing potential, including following transfusion of RhD mismatched red cells or platelets.
Method: Participating health services report via an electronic notification form which triggers the STIR secretariat to send the reporter a detailed investigation form for completion. Data received is reviewed by members of STIR expert group for validation.
Results: To date (1/1/15 to 30/3/16) seventeen RhD Ig notifications have been submitted with 13 investigation forms returned. Of the returned detailed investigation forms, all occurred in hospital, with the majority detected by ward staff. The types of incidents reported include:
5 prescription/ordering errors where the problem was generally found prior to administration. This includes 1 incident where infant details were used to order from the laboratory, but the product was administered to the woman, without changing the identification.
6 inappropriate administrations: 4 to RhD positive women, and 2 postnatal to RhD negative women, with RhD negative infants.
2 reactions to intravenous product.
Common issues relate to confirmation of the maternal blood group prior to ordering/dispensing RhD Ig. Where a shared care model, within and between different health services/providers/practitioners, is in place the pathology provider may not have the maternal blood group on record, however may be under pressure to dispense product. This compounds as clinical staff fail to check the blood group, or miss-interpret the report prior to requesting product.
The use of incorrect patient identification details to order RhD Ig from the pathology provider, compounded with the lack of available maternal blood group for checking, removes the opportunity to identify a problem prior to issuing.
Summary/Conclusions: Since STIR commenced capturing RhD Ig incidents in January 2015 all reports to date relate to use in maternal settings. The reporting of reactions related to intravenous preparations has resulted in changes to the investigation form to improve the information gathered. Availability of, and careful attention to maternal and/or infant blood group, prior to ordering and administration will enhance appropriate dosing and prevent inappropriate administration of RhD Ig. Health services need to review arrangements including shared care to ensure blood groups are accessible to all staff for checking the appropriateness of RhD Ig use.
STIR will continue to monitor and report on these incidents to help health services improve patient care.

P-529

ESTIMATION OF PREVALENCE OF ACUTE TRANSFUSION REACTIONS IN TAIWAN: A PRELIMINARY STEP TOWARDS TO PATIENT-FOCUS HEMOVIGILANCE

J HChien[1], T LZheng[1], ZY Hsu[1], HF Liu[1], CY Lin[2], SC Liu[3], MY Kan[4], CP Li[5] and TF Ho[6]

[1]Taichung Tzu-Chi Hospital, Buddhist Tzu Chi Medical Foundation, Tanzih Town, Taichung, Taiwan [2]Hualien Tzu Chi Medical Center,Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan [3]Taipei Tzu Chi General Hospital, Buddhist Tzu Chi Medical Foundation, Taipei, Taiwan [4]Dalin Tzu Chi General Hospital, Buddhist Tzu Chi Medical Foundation, Chiayi, Taiwan [5]Department of Biochemistry, School of Medicine, Tzu Chi University, Hualien, Taiwan [6]Department of Medical Laboratory Science and Biotechnology, Taichung, Taiwan

Background: The haemovigilance reporting systems have been established in Tzu Chi Hospital, Taiwan. It is an electronic document management system with Patient-Focused monitoring of transfusion-related adverse events and also for patient safety.
Although blood transfusion is life saving for patients, it is responsible for a series of complications and exposes the patients to a variety of risks. Therefore knowing different adverse effects of blood transfusion represents a great issue in managing recipient patients.
Aim: The aim of this study is to evaluate the different types of transfusion reaction and blood components transfusion by using information technology-based system to record the recipient transfusion responses especially on patient vital sign monitoring.
Methods: A retrospective data were collected from three branch hospitals of Tzu-Chi medical foundation. We collected the data of blood transfusion and transfusion reactions from 2011 to 2015. All data included gender, age, blood types, and vital signs including temperature, heart rate, respiratory rate (TPR report system), and symptoms of suspected transfusion reactions in our hemovigilance reporting system. In our hospital hemovigilance system, all transfusion patients required to report any signs and symptoms within 24 h. According to transfusion guideline, body temperature raising over 1°C is classified as febrile non-hemolytic transfusion reactions.

Table 1. Comparison of single vs. multiple transfusion reactions according to different blood component

Blood component	Feedback report no.	Single event		Recurrence event		Overall ATR	
		Patients no.(%)		Patients no.(%)		Patients no.(%)	
PRBC	132108	1779	1.35%	805	0.61%	2584	1.96%
LP-RBC	3455	25	0.72%	41	1.19%	66	1.91%
WRBC	1230	5	0.41%	15	1.22%	20	1.63%
WB	1856	17	0.92%	11	0.59%	28	1.51%
LRP	5718	33	0.58%	138	2.41%	171	2.99%
PL conc.	5642	51	0.90%	63	1.12%	114	2.02%
PL ph.	18048	158	0.88%	170	0.94%	328	1.82%
Cryo.	2768	9	0.33%	5	0.18%	14	0.51%
FFP	40386	234	0.58%	192	0.48%	426	1.05%
FP	7167	49	0.68%	34	0.47%	83	1.16%
All	218378	2360	1.08%	1474	0.67%	3834	1.76%

Single event: reported transfusion reaction once
Recurrence event: reported transfusion reaction more than once

Table 2. Frequency of signs and symptoms of all transfusion reactions

Signs and symptoms	Total no.	Frequency
Body temp raising>1-2℃	1638	39.52%
Chill/Rigor	903	21.79%
Pruritus	414	9.99%
Fever	387	9.34%
Body temp raising>2℃	317	7.65%
Urticaria	258	6.22%
Dyspnea	84	2.03%
Blush/Red	30	0.72%
Tachypnea	22	0.53%
Nausea/Vomiting	20	0.48%
Shock	17	0.41%
Chest pain	11	0.27%
Other transfusion reaction	10	0.24%
Rapid hearbeat	9	0.22%
Reaction over 30 min	8	0.19%
Anxious	6	0.14%
Headack	5	0.12%
Chest tightness	1	0.02%
Wheezing	1	0.02%
Back pain	1	0.02%
Hemoglobinuria	1	0.02%
Purpura	1	0.02%
Delirium	1	0.02%
Total	4145	100.00%

Likewise, body temperature rise over 1 is recoded as acute transfusion reaction (ATR).
Results: Total of 218,378 feedback reports and 221933 units of blood components had been transfused, which feedback report rate is 98.4% in hospital haemovigilance report system. There are 3834 patients (1.76%) were recorded as transfusion reactions and total of 4145 signs and symptoms were analyzed. The increase body temperature between 1 and 2°C (39.52%), the most common signs and follow by the chill 21.79%. Noteworthy, the rate of ATRs to allogenic red blood cell components after the implementation of prestorage-leukoreduction (LR-RBC) was 1.19% for recurrence events compared to 0.72% for single events. And, the rate of ATRs to prestorage- leukoreduction platelet (LRP) was 2.41% for recurrence events compared to 0.58% for single events.
Conclusion: Although the prestorage leukoreduction blood components could not reduce transfusion reaction in those who had transfusion reaction, we still consider it is important to use the prestorage blood component as the first choice in blood transfusion. Furthermore, we used the auto-reported TPR system; the overall of transfusion reaction is 1.76% in this study, which is higher than other studies. Monitoring the body temperature is very important in transfusion safety.

P-530

GATEKEEPING QUALITY IN THE DEVELOPING WORLD – HEMOVIGILANCE: A CHICKEN AND EGG PROBLEM?

CTh Smit Sibinga[1], I Kajja[2] and M Makhmudova[1]
[1]*IQM Consulting, Zuidhorn, The Netherlands* [2]*Makerere University, Kampala, Uganda*

Background: Hemovigilance is being advocated worldwide. However, there is distinct difference between advocating the principles as part of quality culture and management (*first the pudding, then the tasting*), and stimulating the set-up and implementation as a seemingly stand alone operation.Performance of the blood supply and consumption in general follows the 5 key elements of Quality Management -1. Organization/(infra-)Structure;2. Standards/ References;3. Documentation (quality pyramid);4. Education (teaching and training);5. Assessment - monitoring and evaluation (M&E).Hemovigilance as a quality gatekeeping surveillance tool is part of the assessment, based on M&E of what has been done, and whether that matches standards of performance. Hence, hemovigilance is an integral part of the blood transfusion chain, a gate keeping tool to sustain overall quality.
Aim: Analysis of hemovigilance systems in developing countries.
Methods: Hemovigilance systems and management were evaluated for 18 developing countries (2001–2014) in 5 WHO Regions (table) as part of projects focused on strengthening existing blood transfusion structures. Projects were based on a step-by-step introduction of concept and principles of quality. Beneficiaries were solicited to indicate their development priorities.
Results: WHO Regions involved (table):Europe (Newly Independent States/Central Asia) - 7;Africa (Sub-Sahara)- 4;Eastern Mediterranean - 3;South-East Asia - 3;Western Pacific - 1.Countries belong to low and medium Human Development Index (L- and M-HDI) groups and range from a highly fragmented to regionalized national blood transfusion organization. At the beginning of their project none of them had a quality system and management in place, no current national standards and a grossly underdeveloped non-structured documentation system. Staff competence showed major knowledge gaps, particularly among clinicians. Hemovigilance is virtually non-developed, albeit listed high priority.Countres listed hemovigilance a top priotity - 12/18, and 2nd or 3rd priority - 3/18. Only 3 more advanced countries understood the importance to first have the managerial and operational framework developed.

Table - WHO Regions and Countries involved

WHO Region	Countries involved
Europe	Estonia, Kazakhstan, Kyrgyzstan, Montenegro, Slovenia,Tajikistan, Uzbekistan
Africa	Cameroun, Rwanda, Tanzania, Uganda
Eastern Mediterranean	Jordan, Pakistan, Sudan
Southeast Asia	Bangladesh, Cambodia, India
Western Pacific	Mongolia

Conclusion: Hemovigilance is virtually non-developed, albeit listed a high priority in the majority of L- and M-HDI countries: a chicken and egg problem.Fundamental to hemovigilance as a quality gatekeeping surveillance and overall assessment tool, is a well-developed quality-based vein-to-vein blood transfusion organization and related quality culture with proper understanding of M&E values.

P-531

RESPIRATORY TRANSFUSION REACTIONS IN SLOVENIA IN THE LAST 10 YEARS

I Bricl and I Maric
Blood Transfusion Centre of Slovenia, Ljubljana, Slovenia

Background: Respiratory transfusion reactions are all the reactions that affects lungs and are a consequence of blood transfusion. Primary reactions include TACO (transfusion-associated circulatory overload), TRALI (transfusion-related acute lung injury) and TAD (transfusion-associated dyspnea). The secondary reactions are all those that have respiratory symptoms but are not focused on lungs (anaphylactic reaction, haemolytic reaction, etc.)
Aims: Our aim was to observe what was the trend of respiratory transfusion reactions in past 10 years and their impact on patient management.
Methods: Every report on respiratory transfusion reaction was investigated by transfusion medicine specialist by interviewing the patient and checking the patient's medical history. Additional information was provided by patient's physician. Where the respiratory reaction was suspected it was essential to perform chest x-ray and obtain laboratory results (NT-proBNP) from before and after the reaction. It was important to distinguish transfusion reactions from patient's ongoing conditions from before. Whenever TRALI was suspected we checked for anti-HLA and anti-HNA antibodies. If the antibody tests were negative and all the signs and symptoms were in favour of TRALI, the reaction was characterised as a non-immunological TRALI.
We reviewed transfusion reactions reports for the last 10 years by checking the type of reaction, the causes, severity, relation to the transfusion and outcomes. We checked all primary respiratory transfusion reactions reported since 2005.
Results: Between 2005 and 2015 there were 1724 transfusion reactions reported. The average number of reactions per 1000 issued units in the last 10 years was 1,44. From those 1724 reactions, 135 were respiratory transfusion reactions (about 8%). Vast majority of respiratory reactions were TACO (112 reactions or 83%). Second and third most common reactions were TAD, and TRALI (14 and 9 reactions, respectively). 57 respiratory reactions (42%) were reported as life threatening, while the remaining 78 reactions(58%) were non-life threatening. Almost all the cases of TRALI were reported as life threatening. In all 135 cases of respiratory reactions no death or long term illness due to transfusion was observed.
Summary: Heamovigilance is an important system of detection and prevention of transfusion reactions. By detecting the problems we can apply the solution. To reduce TRALI cases in 2007, we applied the rule of not using plasma from female donors for clinical use and in 2014 we stopped using apheresis platelets from female donors. To reduce TACO cases we organised many seminars and workshops to educate physicians to recognize patients in risk of TACO and measurements to prevent it. Reporting on transfusion reaction in general is not satisfactory. Our assessment is that the majority of respiratory transfusion reactions, especially cases of TACO, stay undetected. The reason behind this is the fact that it is hard to connect circulatory overload with transfusion in patients that received a lot of intravenous fluids. In many cases there is no before x-ray and no laboratory results for comparison. Because of this many physicians don't even consider the possibility of respiratory transfusion reaction. We strongly believe that education and team approach can reduce severity of respiratory transfusion reactions.

Year	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Absolut no. of reactions	146	191	190	204	173	170	141	162	113	115	119
No. of reactions / 1000 issued units	1,27	1,69	1,66	1,70	1,35	1,40	1,08	1,24	0,93	0,99	1,06
TRALI	0	1	0	1	2	0	1	1	2	1	0
TACO	3	12	14	11	15	12	10	13	9	8	5
TAD	0	0	0	2	1	2	4	3	0	1	1

Caption 1: Number of reactions per year since 2005

P-532

POTENTIAL USE OF THE ISBT 128 PRODUCT DESCRIPTION CODE DATABASE FOR HAEMOVIGILANCE REPORTING AND ANALYSIS

M Freire, P Distler and P Ashford

ICCBBA, San Bernardino, United States of America

Background: The ISBT 128 labelling standard was created in 1994 as an international strategy for improving transfusion safety. ISBT 128 ensures unique identification and traceability of blood products using a rigorous coding system based on internationally agreed terminology. The World Health Organization has recognized the ISBT 128 Standard as the sole global standard for identification and coding of medical products of human origin.

Reporting and analysing denominator data for haemovigilance can be labour-intensive. Because of this, the potential use of ISBT 128 product codes and their supporting database to automate this process was explored.

Aims: Evaluate the potential use of ISBT 128 product codes and supporting Product Description Code Database for haemovigilance reporting and analysis of denominator data.

Methods: Internationally standardized terminology is used to uniquely describe products that are then given ISBT 128 product codes. For example, the product "Apheresis PLATELETS/ACD-A/XX/20–24C|Irradiated|Residual Leukocytes <5E6"has been given the code "E3046."Each characteristic within a product description is given a code and these codes are combined to create a unique formula (see Table 1). Such formulae support sorting, filtering, and enumeration of data by characteristics of interest. For example, the number of irradiated products in denominator data can be determined by filtering for "irradiated"("V0013002"within the formula). Furthermore, the search can be narrowed to identify all apheresis platelets that were irradiated and leukocyte reduced.

The ISBT 128 Product Description Code Database contains over 8000 blood products that reflect different combinations of >250 characteristics. The high level of granularity within the ISBT 128 product description permits detailed analysis. The ISBT 128 database is regularly updated and expanded as new technologies emerge and standards evolve.

Results: The information in the ISBT 128 Product Description Code Database can support haemovigilance efforts. Transfusion facilities may report denominator data using ISBT 128 product codes and haemovigilance organizations can link the product codes to the descriptions and formulae in the ISBT 128 database. Data can then be sorted, filtered, and analysed.

Summary/Conclusions: The use of ISBT 128 product codes and the supporting database can support national and international haemovigilance efforts by providing product descriptions in computer-friendly formulae for analysis of denominator data.

Each year, over 42 million blood products are labelled with ISBT 128 product codes and this could provide a rich source of denominator data. The reporting and analysis of this data can potentially be standardized and automated. In the US, the Centers for Disease Control are incorporating ISBT 128 formulae into their haemovigilance reporting and analysis system.

While the ISBT 128 Product Description Code Database was originally developed for blood products, it has been expanded to include other products"'such as tissues, cells, organs, and milk"'allowing it to be used in surveillance and vigilance for other products.

Table 1 Example of ISBT 128 Product Description Code Formula

CHARACTERISTIC	CODE
Platelets	C0006
Apheresis	M0007
ACD-A/XX/20-24C	V0001073
Irradiated	V0013002
Residual Leukocytes: <5E6	V0014004
Formula for this product: C0006-M0007-V0001073-V0013002-V0014004	

P-533

HOW TECHNOLOGY CONTRIBUTES TO REDUCE ERRORS IN TRANSFUSION PROCEDURES OF A TERTIARY-CARE HOSPITAL

N Carpio, Pmd Solves, Imd Gómez, A Saus, M Guinot, J Osorio and MA Sanz

University Hospital La Fe, Valencia, Spain

Background: Since the beginning of the 20th century when the first tests for blood compatibility were performed, there have been many important developments in the transfusion area. Probably one of the most significant is related to advances in technology. Technology has enabled to automate manual procedures to avoid the human error and probably trasfusional management software is the first step.

Aims: We want to analyse the impact of the electronic management of transfusion on errors reported in our Haemovigilance program.

Methods: Transfusion procedures at our hospital included: patient identification with a hand badge carrying a transfusion security code before sample collection, registration of samples in a transfusion software, component selection, automatic compatibility tests, and checking blood component label and patient hand badge before transfusion at bedside. The Blood Bank Software, implemented since 1996 to the present, in web technology (e-Delphyn Hemasoft Company), permits the electronic cross match, establish conditions for blood components liberation (Irradiation, etc), connection in bidirectional way with autoanalysers and other devices for electronic control of all transfusion steps. Events in any phase of transfusion procedure during the period from 2000 to 2015 were reviewed from our Haemovigilance database and classified in errors in blood component administration (BAE) and events that was detected before administration (NME).

Results: Between 2000 and 2015 a total of 727.301 blood components were transfused. Of them, 469.255 corresponded to red blood cell concentrates. Overall, 162 events were detected in blood component administration (BAE) and 437 episodes of near miss event (NME) but in some episodes more than one error occurred. Finally 506 errors were detected before transfusion and 176 finished in EAC resulting in a prevalence of 1 in 1066 blood components administrated. Regarding the causes of NME, the mistakes were most commonly seen during component prescription (41%), sample collection (28%) and sample registration (16%). The incorrect use of patient's identification pre-printed adhesive labels was the commonest reason for error. Other NME were 2% in selection phase, 7% in compatibility testing and 4% en transfusion procedures at bedside. By contrast, the BAE more common occurred in prescription phase (25%), selection of blood components (38%) and transfusion (29%), due to use pre-printed adhesive labels for patient identification, use of blood components without irradiation and miss identification at bedside respectively. The other errors were minor and corresponded to sample extraction (1%), registries (2%) and compatibility testing (4%).

Summary: A significant number of mistakes can occur during the transfusion procedure. In our experience, the BAE due to errors in compatibility testing, sample extraction and registries are infrequent, suggesting that the use of a specific hand badge, analytical test automation and electronic management of transfusions is effective to reduce the errors. The most frequent errors were detected in those phases of transfusion in which electronic control was not implemented. In our opinion, most errors due to the use of adhesive pre-printed labels and identification of patient before transfusion could be avoid by electronic prescriptions y electronic check at bedside.

P-534

ANALYZING NEAR MISS ERRORS: A STEP TOWARDS TRANSFUSION SAFETY

A Khetarpal and B Arora

Artemis Hospitals, Gurgaon, India

Background: Blood transfusion subjects a patient to various risks, ranging in severity from febrile nonhemolytic reactions to disease transmission and death from transfusion of grossly incompatible blood. Safety and reliability in blood transfusion are not static, but are dynamic occurrences. Strict adherence to standard operating procedures mandated at an institutional level and use of dedicated phlebotomy service personnel to collect pre-transfusion samples can decrease the incidence of wrong blood in tube (WBIT), however, performance deviations continually occur in complex systems, their detection and correction must be accomplished at the earliest. Our institution encourages the reporting of near misses as it reflects directly on our quality assurance program enabling us to recognize and hence correct any deficiencies recognized thus.

Aim: To evaluate the near miss events occurring in our department with a view to bring about an improvement in quality and safety.

Near Miss Category	Near Miss Subcategory	Near Miss type	No. of cases	Location	Total Near Miss Events in 2015	Percentage of Total Near Miss Events out of total samples received
Pre transfusion Sample collection	Mislabeled	• Discrepency between information on sample label and request form	04	Wards	13	92.86%
		• Wrong registration number	0	-		
		• Wrong name	2	Wards		
		• Wrong blood group mentioned	2	ICU		
		• Lack of clinical information & laboratory data	5	Wards		
	Miscollection (WBIT)	• Difference in blood group in sample tube from patient records	1	Emergency Department		7.14%
Lab testing	Wrong component issued/ Documentation Error on issue form	• Another component issued than what was asked	0	-		0%
		• Error in Unit ID/ Patient GNID on issue form	0	-		
		• Error in group & cross match	0	-		
Pre transfusion patient identification	Misidentification of patient Pre-transfusion	• Error in patient identification	0	-		0%

Methods: Any event recognized as a near miss is recorded by the blood bank. They are broadly divided into three categories according to the nature of the problem as those related to 'pre transfusion sample collection', 'lab testing' and 'pre transfusion patient identification'. Further categorization is detailed in Table 1. Once identified and categorized, each near miss event initiates a root cause analysis to identify crux of the problem and to take immediate corrective action. If any deficiency in system is recognized, highlighting human and system failure associated with transfusion, necessary actions are taken to rectify them including formulation of new policies and procedures whenever required. This study analyzed all the near miss events recorded in the blood bank, Artemis Hospitals for a period of one year from 1st January - 31st December '15.
Results: The results are tabulated in table 1. A total of 14 near miss events were recorded in year 2015, at a rate of 0.84 (14/16472) per 1000 units transfused. All the 14 near misses were categorized as errors in pre-transfusion sample collection, with 92.86% (1 in every 631 sample) further categorized as mislabeled and 7.14% (1 in every 8207 sample) categorized as WBIT. Of all errors reported 78.5% (11/14) were from wards, 14.2% (2/14) were from ICU and 7.14% (1/14) were from emergency department. All near-misses were identified at the time of receiving the request form for blood components in the blood bank or during the compatibility testing.
Summary/Conclusion: Most near-misses were a result from human actions and thus preventable. All the recorded events occurred outside the blood bank; with the bedside of the patient as the main location. The rate of near misses observed by us was far lower as compared to others. Education of the staff responsible for transfusion to strictly adhere to the existing guidelines, play a robust role in improving performance, quality and safety. We conclude that use of hemovigilance and near-miss information, by augmenting barriers supporting error prevention, their recognition and mitigation, increases our capacity to get the right blood to the right patient at the right time.

P-535

TWO CASES OF DENGUE INFECTION WERE FOUND IN BLOOD DONORS DURING GUANGZHOU DENGUE OUTBREAK IN 2014

Q Liao and Y Fu

Guangzhou Blood Center, Guangzhou, China

Background: The 2014 dengue outbreak in Guangdong, one of the southern provinces in China, led to 45171 dengue virus (DENV) clinical diagnosed cases. However, this outbreak brought potential risk to transfusion safety by donors who were asymptomatically infected with DENV.
Aim: To evaluat the impact of Dengue virus (DENV) on blood safety in Guangzhou during an epidemic outbreak.
Methods: This study collected 4500 serum samples from healthy blood donors. These donors were recruited in Guangzhou blood center, which located in Guangzhou, the provincial city of Guangdong province. The DENV RNAs were detected by quantitative polymerase chain reaction(Q-PCR) and anti-DENV IgM/IgG antibodies were detected by enzyme-linked immunosorbent assay (ELISA). The partial fragments of the NS5 gene were sequenced and further applied for the phylogenetic analysis.
Results: Anti-DENV IgG antibody was detected in 3.4% (51/1500) of the donors. We also examined another 3000 serum samples and only 2 individuals were found DENV RNA positive. These two samples were DENV antibody IgM(+)/IgG(-). One sample belongs to DENV-1 and the virus loads was 9.44×10^2 copies/ml,while the other sample belongs to DENV-2 and the virus loads was 1.65×10^3 copies/ml.
Conclusion: Blood donors with asymptomatic DENV infection can cause the risk of blood transfusion. It may provide crucial implications that the government need to introduce DENV RNA or serological detection methods to prevent infection through blood transfusion in high dengue epidemic areas such as southern China during the outbreaks.

P-536

HBV VACCINATION PROGRAM IN NORTH-ITALIAN BLOOD DONORS: EFFECTIVENESS AND POTENTIAL THREAT OF VACCINATION FAILURES TO THE SAFETY OF BLOOD SUPPLY

M Spreafico[1], F Fumagalli Maldini[1], L Raffaele[1], B Foglieni[1], A Berzuini[1], I Guarnori[1], P Lanzini[2], L La Rosa[3], F Rossi[4] and D Prati[1]

[1]Azienda Socio-Sanitaria Territoriale (ASST), Lecco, Italy [2]Department of Transfusion Medicine and Haematology, ASST-Valtellina, Sondrio, Italy [3]Servizio Immuno-trasfusionale, ASST-Vimercate, Vimercate, Italy [4]Department of Transfusion Medicine and Haematology, ASST-Monza, Monza, Italy

Background: In an attempt to reduce the global impact of Hepatitis B Virus (HBV) infection, universal vaccination of newborns against HBV became mandatory in Italy in 1991 and was extended to 12-year-old children.
Recombinant HBsAg vaccines of A2 genotype, suggested to protect also against infection by non-A2 HBV genotypes, are used. However, reported rare infections with non-A2 HBV genotype in vaccinated blood donors presenting with occult HBV infection (OBI: HBsAg negative, HBV DNA positive) raised concerns about the broad genotype efficacy of vaccines as well as to the safety of blood supply. OBI is a potential risk factor for post-transfusion hepatitis, hepatocellular carcinoma, cirrhosis and HBV reactivation.
Aim: The aim of the study was to investigate the efficacy of HBV vaccination in Italy, where HBV D genotype is prevalent.
Methods: In March 2015 we started 12 month project among Italian blood donors vaccinated at 12 years of age (born from 1983 to 1990: group A) or in infancy (born from 1991: group B). Repetitive donors attending the blood transfusion centres afferring to the Macroarea of Lecco (North of Italy), were enrolled and tested for HBsAg, anti-HBc, anti-HBe, anti-HBs titre and HBV DNA. Dilution and avidity tests were performed on anti-HBc positive samples to confirm true positive results. The persistence of anti-HBs according to the time elapsed from vaccination was also evaluated.
Results: Out of 1531 enrolled blood donors, 687 were vaccinated in infancy (45%) and 844 (55%) at 12 years. One donor in group B (0.15%), and 5 donors (0,7%) in group A were anti-HBc positive. All anti-HBc positive donors were HBsAg and none of them have detectable circulating HBV DNA (P = 0.3). Avidity testing confirmed the anti-HBc positivity in 4/6 donors (anti-HBc specificity: 99.74%), all with high avidity. One of these donors, vaccinated at 13 years of age, was also anti-HBe positive. Anti-HBs titres were <10 IU/ml in 617 (40.3%) subjects, corresponding to 66.3% of donors vaccinated in infancy (n = 409) and 33.7% of vaccinated at 12 years of age (n = 208). Age at vaccination was an independent predictor of low anti-HBs titer (R2: 0.048; 95% CI: 12.28–17.82)by logistic regression analysis.
Conclusions: In Italy, HBV vaccination program in newborns seems to be effective to ensure protection against HBV infection. The prevalence of anti-HBc positivity

among donors vaccinated in infancy or at 12 years of age was low, but further studies are needed to clarify whether vaccinated anti-HBc positive donors are threat for transfusion safety. Vaccination in adolescence results in more prolonged immunogenicity than vaccination in infancy, reflecting a more developed immune system.

Alternatives to Blood Transfusion

P-537

EFFECTIVENESS OF REINFUSION SYSTEM AFTER TOTAL KNEE REPLACEMENT. EVALUATION AT A SINGLE CENTRE DURING TWO YEARS

B Delgado, C Monteiro, H Gomes, A Leite, L Gonçalves, M Carvalho and K Carmo

Centro Hospitalar São João, Porto, Portugal

Background: Although total knee arthroplasty (TKA) can result in substantial blood loss, current transfusion approach in elective orthopedic surgeries aims to avoid allogeneic blood transfusion. Preoperative autologous blood donation, intraoperative and postoperative blood salvage, as administration of drugs which reduce blood loss or stimulate the production of red blood cells have been increasingly used.

Aims: Evaluation of the effectiveness of reinfusion systems in reducing allogeneic blood transfusion in TKA and the impact in length of hospital stay.

Methods: All patients (n = 546) who underwent a TKR between January 2014 and January 2016 were consecutively reviewed retrospectively. Data was collected using the electronic database of our institution.

Patient demographics, pre and postoperative hemoglobin levels and length of hospital stay were evaluated.

Statistical analyses were performed using IBM SPSS® statistics 23. Significance was assumed if p was <0.05.

Results: The normality of population, samples and controls, was assessed by the Shapiro-Wilk test, showing normal distribution for age, pre and postoperatively hemoglobin (Hb) level.

Autologous reinfusion was used in 88 patients (16%). This group of patients had a pre-operative Hb average of 13,1 g/dl and post-operative Hb average of 11 g/dl. None of these patients needed transfusion support.

In the control group the pre and post-operative Hb average was respectively 13,4 g/dl and 10,35 g/dl. Forty-seven patients (8.6%) received allogeneic blood transfusion, in a total of 78 red blood cells units.

No statistical difference was established between groups concerning length of stay in hospital.

Conclusion: Although in our institution the transfusion rate in TKA is low comparing to what is described in literature (20–50%), the use of autologous reinfusion systems has allowed a further reduction.

Contradictory evidence exists with respect to whether low or high preoperative hemoglobin affects the protective effect of cell salvage. It may be necessary to establish a hemoglobin range predicting the use of autologous reinfusion systems, in order to optimize costs associated with transfusion management.

P-538

RARE RH PHENOTYPE WITH PAN-REACTIVE ALLOANTIBODY: WHERE AUTOLOGOUS TRANSFUSION IS PROBABLY THE ONLY MOST EFFECTIVE READILY AVAILABLE OPTION

M Al Zaabi, L Al Quraini, M Theresa and S Johnson

Khoula Hospital Directorate of Laboratories, Muscat, Oman

Background: Presence of pan-reactive antibody can be associated with a difficulty in finding crossmatch compatible red cell units. In such cases, alternatives to allogenic blood transfusion should be considered.

Aim: We present challenges in peri-operative blood management of a patient with very rare Rh phenotype developing antibody against a high incidence antigen.

Methods: A forty-eight-year old expatriate man presented to our trauma centre with a fracture of proximal femur. Pre-transfusion testing revealed blood group A, RhD positive with an allo-antibody reacting with all cells in antibody identification panels (see figure). No crossmatch compatible red cell units could be obtained and an assistance from reference transfusion laboratory was sought.

Figure 2a. Antibody Identification

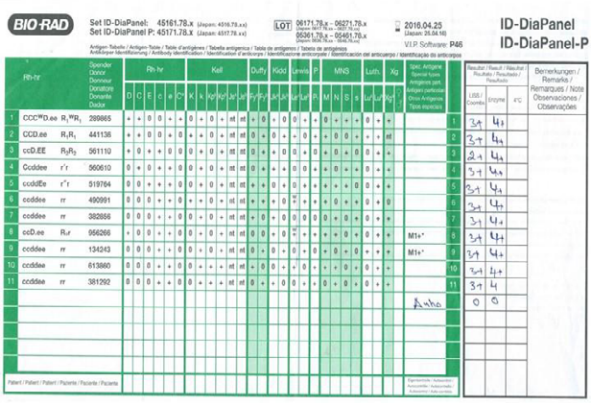


Figure 2b. Rh Phenotype

Table 1. Options available for managing perioperative blood loss

Option	Pros	Cons
Least incompatible allogenic transfusion	Readily available from blood bank stock	Risk of haemolytic transfusion reaction
Erythropoietin and intravenous iron	Readily available from hospital pharmacy	Delayed onset of action and of less proven to raise haemoglobin level
Intraoperative Cell Salvage (ICS)	Effective modality to manage intraoperative haemorrhage	Requires special equipment and trained personnel
Acute normovolumic haemodilution (ANH)	Effective modality to manage intraoperative haemorrhage	Does no necessarily obviate need for allogenic transfusion
Pre-operative autologous transfusion	Effective modality to manage perioperative haemorrhage	Requires at least baseline haemoglobin of 12 g/dL
Full Rh phenotype matched donor	Best therapy	Difficult to find donor

Results: A rare Rh phenotype was identified as patient's red cells lack all antigens [C,c,E,e] apart from RhD. Despite crossmatching more than 70 units at both hospital and reference laboratory, no compatible units could be found. Rh genotyping was considered but not readily accessible. Antibody was reported as possibly 'anti-Rh17' after literature review. Options to optimise patient blood management during surgery -though limited- were discussed in multidisciplinary team meeting (see table) and acute normovolemic haemodilution was suggested. However, patient opted out and preferred to travel to his home country where similar Rh phenotype might be more common.

Conclusions: Where allogenic blood transfusion is inappropriate, autologous transfusion can be tried. However, it is not always feasible or successful in clinical practice. This highlights importance of establishing rare blood group registries for provision of blood in timely manner.

P-539

IMPLEMENTATION OF PATIENT BLOOD MANAGEMENT (PBM) IN LIVER TRANSPLANTATION: EXPERIENCE FROM A SINGLE CENTER

S van der Allanki

Global Hospital, Hyderabad, India

Background: Patient blood management(PBM) refers to implementation of evidence-based practices to minimize transfusion of blood and blood products and improve patient outcomes. It is multidisciplinary ie, it involves transfusion medicine specialists, surgeons, anaesthesiologists and critical care specialists. It is a new standard of care in medicine and a patient safety issue. PBM principles are based on three pillars: Recognition of anaemia and optimization of RBC mass, minimization of blood loss, and improved tolerance to anaemia.
Aim: To examine the outcomes of liver transplantation with maximal conservation of blood products and to analyze the potential benefits of blood conservation. This is a descriptive study.
Methods: Four hundred and eleven patients underwent liver transplantation(LT) from February 2003 to April 2016 in this center. Preoperatively, intravenous iron was given to patients with anaemia. Intra-operatively cold ischemia time was kept to minimum, blood loss was minimized by surgical piggyback techniques, anaesthetic low central venous pressure and hemodilution strategies, use of autologous cell salvage, point of care monitoring and targeted correction of coagulopathy, adopting evidence-based transfusion protocols and going for transfusion alternatives wherever possible.
Results: Of 411 LTs,123(30%) patients underwent DDLT and 288(70%) LDLT. Out of 411 LTs, 221(53.7%) patients received <4 units of PRBC peri-operatively, 117(28.4%) received >4 units of PRBC, 39(9.5%) patients received >10 units of PRBC, and 34 (8.2%) patients did not receive blood transfusions. In transfusion-free LT patients, ICU stay was shortened, with faster recovery and better patient outcome with reduced in-hospital charges.
Conclusion: Evidence for the negative impact of transfusion upon outcomes has driven the reduction in transfusions, alongside refinement of surgical and anaesthetic techniques, and use of point of care coagulation monitoring with goal directed haemostatic interventions.

P-540

PATIENT BLOOD MANAGEMENT IN ELECTIVE ORTHOPAEDIC HIP-AND KNEE REPLACEMENT SURGERY – A REPORT ON CLINICAL OUTCOME

C So-Osman[1], J Balradj[1], AWMM Koopman-van Gemert[2], R Onstenk[3], WB van den Hout[4], R Brand[4], JA van Hilten[1], A Brand[1] and RGHH Nelissen[4]

[1]Sanquin Blood Bank, Leiden, The Netherlands [2]Albert Schweitzer Hospital, Dordrecht, The Netherlands [3]Groene Hart Hospital, Gouda, The Netherlands [4]Leiden University Medical Centre, Leiden, The Netherlands

Background/Aims: In elective hip-and knee replacement surgery, recent studies report that cell salvage and erythropoietin are no longer cost-effective transfusion alternatives. However, how these transfusion alternatives are related to the patient's clinical outcome is still not clear. With this study we aim to provide more evidence regarding the association of these patient blood management interventions and the patients' postoperative outcome.
Methods: Data were extracted from a randomised, multicentre controlled study (n = 2442). Patients with Hb values from 10 to 13 g/dl (n = 683) were randomised for erythropoietin and received oral iron suppletion. All patients were also randomised for autologous blood reinfusion by cell saver (intra-and postoperative collection of shed blood, washed and reinfused) or DRAIN (post-operative collection of shed blood, not-washed but filtered blood reinfused). Clinical outcome parameters were postoperative composite complications (categorized as respiratory, cardiovascular, neurological, mental, bleeding, infections and drug-related complications) which were here reported as composite complications and infections separately, length of hospital stay (LOHS) and mortality after 14 days and 3 months. Data were analysed by logistic regression analysis, corrected for possible confounders. A p- value of < 0,05 was considered statistically significant.
Results: In 746 patients (30,5%), postoperative complications were found with an infection rate of 6,4% (n = 156). Fourteen patients (0.57%) died during follow up. Most complications (77%) occurred within the early postoperative period up to 14 days: the group that randomised for both Epo and cell saver had the highest complication rate (adjusted OR 2,8; 95%CI [1,4 to 5,4]) and the highest infection rate (adjusted OR of 12,5; 95% CI [1,4 to 111,1]). LOHS was significantly increased in the group who randomised for Epo and DRAIN (adjusted OR 1,8; 95% CI [1,1 to 3,0; P = 0,03), however, without an increased complication or infection rate. Use of only Epo resulted in a significant lower infection risk (adjusted OR 0,4, 95% CI 0,1 to 0,9; P = 0,03). In the period after 14 days and in the normal Hb group, differences between the autologous intervention groups and controls were not found.
Summary/Conclusions: This study showed that patients who randomised for Epo resulted in significantly more postoperative complications when combined with cell saver and in an increased LOHS when combined with DRAIN, respectively. These findings support a de-implementation strategy of the use of cell saver and DRAIN devices in these elective hip and knee arthroplasty patients, especially when combined with Epo. However, the patients who received only Epo had a reduced infection risk, which should be further elucidated.

P-541

MEGAKARYOPOIESIS FROM INDUCED PLURIPOTENT STEM CELLS: GRAY PLATELET SYNDROME IPS CELL DIFFERENTIATION UNCOVERS A ROLE FOR GFI1B IN MEGAKARYOPOIESIS AND ENDOTHELIAL SPECIFICATION

M Hansen[1], A Marneth[2], C van der Zwaan[1], AJM Koornneef[1], R van Oorschot[2], T Wust[1], M von Lindern[1], S Meijer[1], B van der Reijden[2] and E van den Akker[1]

[1]Sanquin, Amsterdam, The Netherlands [2]Roudboud UMC, Nijmegen, The Netherlands

Background: Limited platelet shelf-life, risk of infections and allo-immunization are problems in platelet transfusions. These problems could potentially be solved by using Induced pluripotent stem (IPS) cells, a promising (autologous) renewable source to initiate *ex-vivo* production. However, megakaryoid differentiation and yield from IPS is ill-defined and in need of optimization. We and others have shown that megakaryopoiesis in gray platelet syndrome (GPS) caused by GFI1B mutations is characterized by increased megakaryoblast frequency in bone marrow, inhibition of differentiation, reduced alpha granule content and maintained CD34 expression on platelets. Unravelling the molecular mechanism fundament to the increased megakaryopoiesis, may potentially be exploited to increase IPS-derived megakaryoid yield.
Aims: We aim to optimize the expansion and differentiation of IPS lines to the megakaryoid lineage by studying the molecular requirements that define megakaryopoiesis.
Methods: IPS differentiation to megakaryoid cells was induced through colony differentiation. Megakaryocyte commitment and differentiation was assessed by morphology, flow cytometry, confocal microscopy, CFU-MK potential and Mass spectrometry.
Results: Differentiation resulted in a wave of megakaryopoiesis between days 14–17. Multi-nucleated CD41, CD42a/b and CD61 megakaryocytes, pro-platelet formation and CFU-MK were observed. Yields were 3 logs increased compared to CD34 + cell megakaryopoiesis. Interestingly, GPS-IPS differentiation showed maintained megakaryoblast specification leading to 10 times increased megakaryoid yield compared to control IPS. Mass spectrometry confirmed specific molecular hallmarks of GPS. In addition, terminal megakaryoblast differentiation was inhibited. Surprisingly, endothelial precursor yield was elevated compared to controls, suggesting a role for GFI1B in endothelial specification.
Summary/Conclusion: In conclusion, IPS lines were generated from megakaryoblasts and re-differentiated to megakaryocytes. GFI1B mutations result in a selective growth advantage of megakaryoblasts and an endothelial population. These data confirm the usefulness of patient specific IPS-lines to study pathologies. The pathways resulting in this growth advantage may be exploited to increase hematopoietic output and specifically platelet yield.

Cellular Therapies: Stem Cell and Tissue Banking, including Cord Blood

P-542

RETROSPECTIVE VALIDATION OF PROCESSES IN ROUTINE USE FOR AUTOLOGOUS PERIPHERAL BLOOD STEM CELL PREPARATION AND STORAGE

J Antoniewicz-Papis, E Lachert, A Rosiek, A Ejduk, K Pienko and M Letowska

Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Background: The basic condition for autologous peripheral blood stem cells (PBSC) transplantation is their long term storage following special preparation and use of cryoprotectant to retain quality parameters. Although there exist common guidelines on preparation of transplant material each laboratory should develop its own methods for PBSC preparation and storage subjected to regular validation. The validation criteria accepted at our Laboratory include at least 80% mononuclear cells recovery and 90% viable cells in transplantation material.
Aim: The aim was to evaluate the quality of peripheral blood stem cells (PBSC) prepared in our cell bank and stored for up to 3 years.
Methods: In the consecutive years of the 2010- 2015 period the process used for preparation of 44 PBSC units dedicated for transplantation was subjected to validation. Validation tests were performed before and after freezing in units stored up to 3 years to confirm the effectiveness of preparation and storage processes. Autologous PBSC separation was performed using COBE® Spectra and Spectra Optia® (Terumo BCT; Japan). PBSC were frozen in DMSO and 5% albumin mixture. The final concentration of DMSO was 10%. PBSC were frozen in control temperature using Ice Cube (SY-LAB; Austria) and stored in vapor nitrogen then thawed in control conditions with Sahara TSC until liquidized. Tests were performed immediately before freezing and after thawing. The parameters evaluated included: leukocyte count (WBC), mononuclear (MNC) and CD34 + cells counts, MNC and WBC viability.
Results: The following results were obtained (Table).
Conclusions: Our results indicate that the method of PBSC preparation developed and put to routine use in our Laboratory as well as storage conditions in place, ensure adequate quality of transplant material in accordance with acceptance criteria.

Parameter	Prior to preparation and freezing	Recovery after thawing (mean ± SD)
MNC	2,70 x 10^9 - 17,00 x 10^9,	94,3 ± 26,91%
CD34+	36,60 x 10^6 - 332,50 x 10^6	83, 34 ± 31,84%
WBC viability	99,34 ± 0,61%	79,43 ± 19,95%
MNC viability	99,71 ± 0,47%	94,30 ± 26,91%

P-543

ANALYSIS OF FACTORS AFFECTING STEM CELL YIELD IN CORD BLOOD COLLECTION

S Prakash, A Jain, RR Sharma, JK Kalra and N Marwaha

Post Graduate Institute of Medical Education and Research, Chandigarh, India

Background: Umbilical cord blood become a valuable source for allogeneic hematopoietic stem cell transplantation. Ease of collection, ready availability and relatively lower graft-versus-host disease compared to peripheral blood stem cell favored umbilical cord blood stem cell transplantation.
Aims: To assess the factors affecting stem cell yield in cord blood collections.
Material and Method: A total of 200 Cord blood units were collected (ex-utero) in CPDA-1 bag having anticoagulant:cord blood ratio of 1:7 under aseptic condition. Before processing units were stored at 4°C. Volume reduction were done by 6% hydroxyethyl starch (HES) with ratio of HES: Cord blood were 1:5. Upright centrifugation of CBUs at 30 g for 10 min were done to collect leucocyte rich plasma followed by upright centrifugation at 450 g to obtain cellular pellet. Differential cell counts were done through sysmex cell counter, viability testing by trypan blue exclusion test and CD34 and CD45 estimation were flow cytometry. Two sample were found to be HBsAg positive and not included in the study.
Result: Maternal factors like age, gravida, gestational age and method of delivery were not significant in relation to TNC, MNC and CD34 + counts. However gestational age of mother showed significant correlation with Placental weight, birth weight and volume of cord blood units collected.
Among neonatal factors, higher birth weight of new borns was yielded larger volume of CBU collected and higher absolute TNC, MNC and CD34 + cell counts. Gender of the new born was of no significance in relation to all quality control parameters.
Placental weight also showed positive correlation with volume of collection (P = .000), birth weight (P = .000), TNC (P = 0.019) and MNC (P = 0.018) but not with absolute CD34 + counts (P = 0.288).
Among collection related variables, CBUs collected from normal vaginal deliveries and deliveries by caesarean section did not shown any significant correlation in terms of any quality control parameters. However volume of cord blood collected ex-utero was significantly greater in normal vaginal delivery as compared to delivery by LSCS (P value 0.001).
Conclusion: Volume of CBUs collected were the single most important factor of prognostic significance in relation to stem cell(CD34 + cell counts) yield however CBUs collected from pre-term deliveries should not be discarded as these CBUs were shown to have approximately equal amount of CD34 + cells.

P-544

HYPOXIA PRECONDITIONING IMPROVE THE THERAPEUTIC EFFECT OF HUMAN UMBILICAL MESENCHYMAL STEM CELLS IN EAE MODEL

M Kheirandish[1], Z Asad Pour[1] and A Pourghadiri[2]

[1]Research CenterHigh institute for Research and Education in Transfusion Medicine, Tehran, Iran [2]Department of Surgery, University of British Columbia, Vancouver, Canada

Objective: Multiple sclerosis (MS) is a chronic, autoimmune, inflammatory demyelinating disorder of the central nervous system. Remarkable progress in experimental and clinical applications of mesenchymal stem cell (MSC)-based therapy has known as a potential candidate for MS therapy. Hypoxic preconditioning is supposed to improve the therapeutic effectiveness and period of persistence of engrafted MSCs.
Aims: In this study, we expected to prolong the duration of survival of engrafted MSCs and to increase the effectiveness of UCB-MSC transplantation therapy by the use of hypoxia-preconditioned MSCs.
Methods: Mesenchymal stem cell from human umbilical cord blood (UCB-MSCS) were exposed to hypoxic condition (2.5% O_2 for 48 h) in cell culture. In order to evaluate the therapeutic efficacy of HPC-UCB-MSC, experimental autoimmune encephalitis (EAE) was induced using myelin oligodendrocyte glycoprotein (MOG, 35–55 peptides) in C57BL/6 mice.
Results: Our results showed that HPC increased cell survival rate of UCB-MSCs. The transplantation of HPC-UCB-MSCs leads to considerable functional improvement and ameliorates pathological disease outcomes such as reduced immune cell infiltration, increased MAP-2 expression and reduced GFAP expression in EAE model.
Conclusion: This study showed that the transplantation of hypoxia preconditioned umbilical cord blood mesenchymal stem cells to prevent tissue damage and/or augment remyelination and axonal regeneration would be more effective in comparison with normoxia condition.

P-545

EFFECT OF ANDOSAN™ ON CD34+ HUMAN HEMATOPOIETIC STEM CELLS AND LEUKEMIC CELL LINES

MR Mirlashari[1], JM Tangen[2] and G Hetland[1]

[1]Department of Immunology and Transfusion Medicine, Oslo University Hospital, Oslo, Norway [2]Deparment of Haematology, Oslo University Hospital, Oslo, Norway

Background: AndoSan™ is a water extract prepared from medicinal *Basidiomycetes* mushrooms, mainly *Agaricus blazei* Murill (82%), but it also contains *Hericeum erinaceus* (15%) and *Grifola frondosa* (3%). In recent years, many studies have reported

that different extracts of *Agaricus blazei* Murill have various anticancer effects, ranging from the improvement of immunomodulatory activity to the inhibition of tumor growth via direct inhibition of tumor-induced angiogenesis in mice.

Aim: The present study was carried out to investigate the effect of AndoSan on $CD34^+$ human hematopoietic stem cells, human acute myelogenous leukemia cell line (KG1a), human promyelocytic leukemia cell line (HL-60) and human megakaryocytic leukemia cell line (Meg-01) *in vitro*.

Methods: $CD34^+$ hematopoietic stem cells and leukemic cells lines were exposed to various concentrations of AndoSan (5, 10 and 20%) and maintained in a humidified atmosphere with 5% CO_2 at 37°C for 96 hr in 24-well plates. The total number and percent viable cells were counted by NucleoCounter using the NucleoCassette kit (Chemometec, Allerød, Denmark) according to the manufacture's manual. The induction of apoptosis after exposure to AndoSan was examined by flow cytometry with the Annexin V-FITC Apoptosis Detection kit (BD PharMingen).

Results: Treatment with AndoSan has no effect on viability of $CD34^+$ hematopoietic stem cells but AndoSan significantly reduced cell viability and increased apoptosis of KG1a, HL-60 and Meg-01 in a dose-dependent manner.

Conclusion: Our data suggest that AndoSan can reduce leukemic cell lines viability via induction of apoptosis without harming vulnerable normal cells such as $CD34^+$ hematopoietic stem cells.

P-546

LABORATORY DIAGNOSIS AND INCIDENCE OF POSTTRANSPLANT PURE RED CELL APLASTIC ANEMIA AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

M Jiang

Wuhan Blood Center, Wuhan, China

Background: Allogeneic haematopoietic stem cell transplantation (HSCT) is a commonly used standard method for the treatment of malignant hematological diseases and some refractory hematological diseases, tumors, immunodeficiency disease. But post transplant pure red cell aplastic anemia (post-transplant PRCA) is a common serious complication after HSCT.

Aims: We observed the occurrence and incidence of post-transplant prca by continuous monitoring of the relative number of transplanted red blood cells in 225 patients.

Methods: We reviewed the records of 225 patients who underwent allogeneic hsct at the Tongji Hospital and divided them into 4 groups: a major ABO-mismatched, a minor ABO-mismatched, a bidirectional abo-mismatched and ABO-matched. Besides there was incompatibility in other red blood group systems, such as MNS, Rh. When patients with 5% donor red blood cell, blood samples were collected every 20 days for a continuous monitoring of the relative amount of red blood cell growth. The day of red cell engraftment is when the number of donor red blood cell growth to 100%, and two consecutive monitoring (40 days) maintained 100%. In this study, the growth period of transplanted red blood cell (donor RBC) growth less than 5% was defined as the reflection period. And the relative number of transplanted RBC from 5% to 100% was the logarithmic growth phase. Prca was diagnosed when reticulocytopenia (reticulocyte count < 1%) and the proportion of nuclear red blood cells to nuclear cells in bone marrow was less than 0.5% persisted 7–20 days after reticulocyte count <1% and the proportion of nuclear red blood cells to nuclear cells in bone marrow was less than 5%.

Results: In Table 1, there is significant statistics diference in one-factor analysis between patients with prca and patients without prca of sex, plasma SHLA-G1, incidence of hemolysis and reflection period ($P < 0.05$). While the multi-factor analysis shows the unique features of prca were decrease of plasma SHLA-G1 (correlation coefficient 0.045, wald 6.241, P value 0.012) and the prolong of reflection period (correlation coefficient 0.015, wald 7.897, P value 0.005). Table 2 shows characteristics of 21 patients with prca.

Summary: Post transplant pure red cell aplastic anemia (post-transplant prca) is a common serious complication after HSCT. The reports of post-transplant prca always focus on patients of ABO incompatibility[3]. It's rare reported post-transplant prca of abo-matched. In this study, we considered other RBC blood group system to separate donor RBC and recipient RBC. That made us more research about post-transplant PRCA. During 7 years, we reviewed the records of 225 patients who underwent allogeneic hsct and observed their RBC growing. We have observed 21 patients with post-transplant prca which divided into two groups (group a and b). Two of them have completely different performance on RBC growing which has not been reported before. But we could not do further study due to incomplete data. In the future,we hope to collect more data of hsct.

Table 1 Patient characteristics

Characteristics		Total,n	Patients with PRCA, n(%)	Patients without PRCA, n(%))	odd ratio	P value	95%IC
Total		225	21(9.33)	204(90.67)			
Sex	Male	117	16(13.68)	101(86.32)	5.43	<0.05	
	Female	108	5(4.63)	103(95.37)			
Average age		55	58	52	1.77	>0.05	
Average CD34+ cell docs (×10⁶/KG)		4.27	4.58	4.27	2.035	0.338	
Diagnosis	AML	72	3(4.17)	69(95.87)			
	CML	41	5(12.20)	36(87.80)			
	ALL	51	6(11.76)	45(88.24)	1.117	0.567	0.765-1.630
	AA	26	3(11.54)	23(88.46)			
	MDS	21	2(9.32)	19(90.48)			
ABO blood groups	ABO-matched	119	13(10.92)	106(89.08)			
	Major ABO-mismatched	46	5(10.87)	41(89.13)			
	Minor ABO-mismatched	43	2(4.65)	41(95.35)	0.831	0.563	0.444-1.557
	bidirectional ABO-mismatched	17	1(5.88)	16(94.12)			
aGVHD	0-I	129	15(11.63)	114(88.37)	0.527	0.110	0.241 1.155
	II-IV	96	6(6.25)	90(93.75)			
Plasma sHLA-G1 (average±standard deviation)			20.73±14.81	53.35±39.42	1.045	0.018	1.007 1.083
Plasma sHLA-G5 (average±standard deviation)			138.6±61.34	185.3±88.09	1.008	0.167	0.997 1.018
Incidence of hemolysis		123	17(13.82)	106(86.18)	8.80	<0.01	
Reflection period (days)			106±117	32±21	0.986	<0.015	0.975-0.997

Table 2 Characteristics of Patients with PRCA

No.	Sex	Age	ANC> 0.5×10⁹/L (day)	Plt> 20×10⁹/L (day)	Reflection period (day)	Logarithmic-decay phase (day)	Diagnosis	ABO Blood Groups	Peripheral blood: Hb (g/L)	WBC (×10⁹/L)	Plt (×10⁹/L)	Retic (%)	Bone marrow: Nuclear RBC:nuclear cells (%)
1	Male	22	12	15	18	100	ALL	B→B	74	6.7	255	0.8→0.2	1:30→1:200(0.5)
2	Male	44	14	18	350	-	CML	A→O	90	5.2	156	0.0→0.0	0.200→0.200(0)
3	Male	31	13	16	64	140	ALL	A→O	44	9.5	210	0.5→0.0	1:26→1:200(0.5)
4	Male	4	12	14	21	80	ALL	A→O	34	10.2	256	0.8→0.1	1:8→1:100(1.0)
5	Male	27	12	18	22	160	ALL	B→B	82	5.9	190	0.7→0.1	1:20→1:200(0.5)
6	Male	42	20	26	31	180	ALL	O→O	91	8.7	216	0.9→0.1	1:24→1:200(0.5)
7	Male	57	18	29	48	160	MDS	A→B	26	10.7	190	0.9→0.0	1:34→1:200(0.5)
8	Male	51	14	22	240	200	MDS	B→O	33	4.1	116	0.5→0.0	1:64→0:200(0)
9	Male	40	12	18	180	200	CML	AB→O	43	4.4	196	0.7→0.0	1:42→1:200(0.5)
10	Female	69	13	17	42	140	CML	B→AB	69	5.1	211	1.1→0.1	1:20→1:200(0.5)
11	Male	17	12	16	44	180	AA	O→AB	93	4.5	200	0.7→0.0	1:26→1:200(0.5)
12	Female	27	13	12	24	160	CML	O→O	87	9.5	228	0.7→0.2	1:16→1:200(0.5)
13	Female	12	13	13	27	180	AML	A→A	84	7.8	198	1.3→0.1	1:18→1:200(0.5)
14	Male	54	19	35	31	180	ALL	B→B	75	6.5	300	1.1→0.1	1:45→1:200(0.5)
15	Male	47	14	28	350	-	AML	B→B	65	4.3	210	0.0→0.0	0.200→0.200(0)
16	Female	13	12	16	120	160	AA	O→O	81	4.6	188	0.6→0.0	1:26→1:200(0.5)
17	Male	12	11	15	28	180	AA	O→O	74	8.8	226	1.0→0.2	1:19→1:200(0.5)
18	Male	41	15	19	120	160	CML	A→A	86	7.0	199	0.5→0.0	1:90→0:200(0)
19	Female	15	12	18	87	180	AA	B→B	66	6.1	203	0.9→0.0	1:18→1:200(0.5)
20	Male	26	15	18	28	160	AML	O→O	54	5.4	130	1.1→0.1	1:19→1:200(0.5)
21	Male	48	20	26	350	-	AA	A→A	63	5.1	195	0.0→0.0	0.200→0.200(0)
NP	-	-	9-22	11-29	-	-	-	-	110-150	4.3-11	100-300	0.5→1-2	1:6-2:5(17-40)
Normal	-	-	-	-	-	-	-	-	123-165	5.5-8.8	130-300	1-2	1:4-2:5(25-40)

ANC:the absolute of neutrophil cell; Plt: the platelet; Hb: the hemoglobin; WBC: the white blood cell; Retic: the reticulocytes; NP: datas of Patients without PRCA, in this study

P-547

FIBRINOGEN-DEPLETED POOLED HUMAN PLATELET LYSATE FOR EFFICIENT EXPANSION OF MESENCHYMAL STROMAL CELLS

M Öller[1], S Laner-Plamberger[2], M Feichtner[3], ZA Dunai[3], A Raninger[4], M Gimona[3], A Hartl[5], C Hauser-Kronberger[6], E Russe[7], K Bieback[8], E Rohde[2], G Brachtl[4], C Scharler[4], D Strunk[4] and K Schallmoser[2]

[1]Paracelsus Medical University, Salzburg, Austria [2]SCI-TReCS,Transfusion Medicine, Paracelsus Medical University, Salzburg, Austria [3]SCI-TReCS, Paracelsus Medical University, Salzburg, Austria [4]SCI-TReCS, Experimental & Clinical Cell Therapy, Paracelsus Medical University, Salzburg, Austria [5]Physiology & Pathophysiology, Paracelsus Medical University, Salzburg, Austria [6]Department of Pathology, Paracelsus Medical University, Salzburg, Austria [7]Department of Plastic Surgery, Hospital of the Barmherzigen Brüder, Salzburg, Austria [8]Transfusion Medicine and Immunology, Heidelberg University, Mannheim, Germany

Efficient animal serum-free propagation of mesenchymal stem/progenitor cells (MSPC) could be established by the use of pooled human platelet lysate (pHPL) as alternative for fetal bovine serum (FBS) and HPL manufacturing is widely implemented. In this study we asked whether porcine heparin can be avoided during MSPC propagation under completely xeno-free conditions. As additional technical variations of the procedure depending on fibrinogen content may impede comparative studies, the influence of fibrinogen depleted modifications of pHPL on MSPC biology was analyzed.

According to our recently developed protocol (J Transl Med. 2015;13:354) we prepared medium-clotted pHPL (mcpHPL) by adding pHPL to cell culture medium without heparin and pHPL serum (pHPLS) by adding $CaCl_2$ during pHPL preparation (12 mM), both enabling porcine heparin avoidance. All pHPL preparations were tested for fibrinogen by ELISA. Concentration of growth factors was analyzed in differentially supplemented media (culture d0 and d5). White adipose tissue (WAT; n = 3) and umbilical cord (UC; n = 2) derived MSPC were isolated and cultured in medium supplemented with pHPL, mcpHPL, pHPLS, or FBS for up to 4 passages. Cell proliferation, clonogenicity and trilineage differentiation capacity were analyzed. MSPC surface markers were tested by flow cytometry.

During 4 passages cumulative population doublings in all media supplemented with pHPL variations (WAT-MSC median 22; range 18–26; UC-MSC 30; 27–32) were significantly higher than in FBS supplemented media (AT-MSC 16; 10–16; UC-MSC 15; 13–16). In FBS medium clonogenicity remained constant until passage 4 whereas in all pHPL media clonogenicity declined continuously.

MSPCs of all medium conditions revealed characteristic cell surface marker patterns and typical trilineage differentiation potential. Fibrinogen decreased from mean 776 µg/ml in pHPL to <1 µg/ml in mcpHPL and pHPLS. PDGF-BB (median 285; range 217–317 pg/ml), EGF (127; 118–154 pg/ml) and BDNF (828; 640–985 pg/ml) were similar in all pHPL media on d0 and were exhausted until d5. In pHPL media VEGFa increased until d5 up to 18,448 pg/ml compared to 12,741 pg/ml in FBS media.

In conclusion, MSPC culture was feasible in a completely animal-component free system without fibrinogen and heparin. Our results therefore may contribute to the optimization of clinical-grade MSPC expansion enabling applications in advanced somatic cell therapy and tissue engineering.

P-548

IDENTIFICATION OF KEY REGULATORS OF SYMMETRIC VERSUS ASYMMETRIC CELL DIVISIONS DURING HUMAN HEMATOPOIETIC LINEAGE SPECIFICATION

A Görgens, S Radtke, S Vitoriano, F Murke, M Möllmann, J Dürig, H Hanenberg, PA Horn and B Giebel

University Hospital Essen, Essen, Germany

Hematopoietic stem cells (HSCs) contain lifelong potentials to self-renew and to create progenitors of all mature blood cells. According to the current view, HSC homeostasis is controlled by both, HSC-niches as well as asymmetric cell divisions. Our previous studies linked the process of asymmetric cell division of human hematopoietic stem and progenitor cells (HSPCs) to the expression kinetics of the stem cell surrogate antigen Prominin-1/CD133 (Beckmann et al, Blood 2007). Furthermore, by characterizing human HSPCs subpopulations by means of their CD133 surface expression we gained evidence that $CD133^+$ multipotent progenitors (MPPs) create $CD133^+$ lymphomyeloid (LMPP) and $CD133^{low}$ erythromyeloid (EMP) daughter cells. The LMPP lineage was shown to contain lymphoid and neutrophil potentials, while the EMP lineage mainly creates eosinophils and basophils as well as erythrocytes and megakaryocytes (Görgens et al, Cell Reports 2013). Regarding lineage specification, we showed for the first time that under conventional culture conditions almost all MPPs divide asymmetrically to create a set of LMPP and EMP daughter cells, resulting in a loss of MPPs after the first cell division (Görgens et al, Stem Cell Reports 2014). Thus, our data suggest that under conventional culture conditions asymmetric cell divisions are rather lineage instructive than self-renewing (Görgens et al, Cell Cycle 2013). Now, aiming to identify key factors regulating the MPP division mode, we study whether conditions reported to promote HSC/MPP expansion interfere with the outcome and symmetry of the HSC/MPP cell division. In this context we co-cultured human MPPs with murine and primary human stromal lines (human *bona fide* MSCs) and surprisingly observed that LMPPs are maintained and expanded but not MPPs. This contrary finding can be attributed to the former experimental definition of multipotent cells based on the *classical* model of hematopoiesis, according to which cells with dual lymphocyte and granulocyte (conventionally neutrophil) potentials can insufficiently be considered as multipotent. Currently, we test other culture conditions reported to expand human HSCs/MPPs *in vitro*. After confirming any of these conditions as HSC/MPP expansion condition, we will analyze its impact on the division mode of HSCs/MPPs using multi-parametric flow cytometry, live-cell imaging and functional differentiation assays at the single cell level.

Collection, Processing, Storage and Release

P-549

THE RECOVERY OF WBC AND CD34+ IN DEFROSTED CONCENTRATES OF HEMATOPOIETIC STEM CELLS FROM UMBILICAL CORD BLOOD

B Janowska-Stuchlak, A Skrzypczak, K Olbromski and H Skalisz

Blood Center, Poznan, Poland

Background: Cord blood (CB) is a source of stem cells having many unique and valuable advantages in comparison to the stem cells from bone marrow or peripheral blood. This is mainly due to the immaturity and immunological naiveness of these cells:

They convert into another cell types very easily have a 10 times greater proliferative potential, have less alloreactivity of lymphocytes and consequently lower risk of occurrence of GvHD after transplantation, allow transplantation with less compliance of HLA antigens.

Obtaining CB is a simple, short-lasting procedure. It's safe for the mother and the newborn and does not require the use of invasive methods in the donor.

The small volume of blood is a limitation and consequently low baseline fraction of mononuclear cells. That is why the methods of collecting, processing and storage should be selected in order to ensure maximum recovery of these cells.

Aim: The assessment of recovery of WBC and CD34+ in defrosted concentrates of hematopoietic stem cells from CB on the basis of their own experience in the Bank of Stem Cells in Regional Blood Center in Poznan.

Methods: Twelve of cord blood units (CBU) were selected for testing.

Obtained CBU were centrifuged in a Beckman centrifuge (2400 x g for 10 min, 22°C) in order to separate into: plasma, erythrocyte concentrate and buffy coat containing the stem cells and subsequently subjected to separation using manual press in order to reduce the amount of plasma and red blood cells.

A cryoprotective agent (55% DMSO +5% Dextran, pre-filled syringe in a tube, allowing for closed method of preparation) was added to the obtained buffy coat in a such amount that the final DMSO content in frozen portions totaled 10%.

Portions were frozen in a preprogrammed way (device: Ice-Cube) at a rate 1°C/min to −80°C, then placed and stored in the vapor of liquid nitrogen at a temperature below −150°C for 14 months.

They were defrosted at +37°C in a water bath and tested immediately after thawing. Each testing was performer twice.

Capacity CBU ml	Before freezing			After defrosting			Recovery	Recovery WBC
	WBC x 10^8/j	CD 34+ x 10^6/j	Vitality CD34+ (%)	WBC x 10^8/j	CD 34+ x 10^6/j	Vitality CD34+ (%)	CD34+ (%)	(%)
73 ± 16	7,0 ± 2,1	2,4 ± 1,1	95 ± 5	4,5 ± 1,5	1,3 ± 0,4	80 ± 17	59 ± 15	65 ± 9

The number of WBC, CD34+ and vitality of CD34+ were tested in a buffy coat before freezing and after defrosting using flow cytometry i.e. Beckman Coulter FC500 flow cytometer with Stein -Kit Reagents (IM 3630) and with Stem-Trol Control Cells (IM3632). The vitality was determined using the dye 7-AAD.
Results: Table 1.
Summary/Conclusions: The number and vitality of CD34+ cells after defrosting indicate that cord blood can be a valuable source of stem cells for transplantation.
The average recovery of CD34+ and WBC compared with the values before freezing indicate the need for taking relevant actions to improve the quality of the obtained material, eg.: obtaining larger volumes of CB, conducting tests on larger amounts of defrosted portions, optimizing the method of preparation and freezing (eg. time from collecting to freezing, the speed of adding the cryoprotectant, the final concentration of DMSO in the frozen portions), modification of the preparation method of material for hematopoietic stem cells after defrosting (eg. defrosting temperature, removing DMSO before test).

P-550
CD34 COLLECTIONS IN 4 HOSPITALS

H Vrielink and J Drent - van der Kooi
Sanquin Blood Supply, Amsterdam, The Netherlands

Background: In 2015, the JACIE accredited collection facilities of Sanquin Blood Supply Facility performed in 219 patients 357 autologous stem cell apheresis procedures to collect CD34+ve cells. Procedures were performed in 4 different hospitals (2 university and 2 general hospitals) in the Netherlands. All collection facilities are hierarchically one team with the use of the same Quality manual, SOPs, one brand of apheresis machines, software version and disposables.
Aim of the Study: To analyze data in the various patient groups in order to prepare a policy to customize procedures for every patient.
Materials and Methods: From all procedures, relevant data concerning patient (gender, disease, number of procedures needed to collect 3–10 × 10^6 CD34+ve cells/kg) and apheresis procedure (processed volume, collection efficiency) are registered. Data were analyzed in excel with the unpaired T-test. P-values <0.05 are considered significant.
Results: See table.
Conclusion: Independent from the requested number of CD34+ve cells/kg, no differences between hospitals were found in the collection efficiency. The collection efficiency is depending on the background disease in the patient. Surprisingly also a difference was observed between male and female patients in multiple myeloma patients.

	Patients		Procedures					P-value[1]
				Male		Female		
	#	M/F	#	Mean/M	CE	Mean/F	CE	M vs F
Hospital 1[2]	32	21/11	39	1.2	54.2%	1.2	55.5%	ns
Hospital 2	37	25/12	77	2.0	59.8%	2.2	52.2%	ns
Hospital 3	83	55/28	162	1.8	57.4%	2.2	56.4%	ns
Hospital 4	67	46/21	79	1.2	55.6%	1.2	49.0%	ns
AML[3]	25	13/12	41	1.2	61.6%	2.2	54.1%	ns
Lymphoma[4]	75	48/27	104	1.3	53.8%	1.6	59.4%	ns
MM[5]	103	77/26	190	1.9	61.6%	1.8	50.0%	0.0003
Others	16	9/7	22	1.2	39,7%	1.6	51,6%	ns

[1] T-test; ns = non significant
[2] No differences between hospitals in unpaired T-test
[3] AML vs Lymphoma (in male and female patients): ns
[4] AML vs MM (in male and female patients): ns
[5] Lymphoma vs MM (in male and female patients): <0.05

Caption 1: Results of CD34 collections

P-551
SAFETY OF PBSC COLLECTION IN CHILDREN WEIGHTING LESS THAN 20 KG

I Kumukova, E Kurnikova, P Trakhtman, I Guz and E Boyakova
Federal Research Center for Pediatric Hematology,Oncology and Immunology, Moscow, Russian Federation

Background: Peripheral blood progenitor cell collection (PBPC) has become the main source of hematopoietic cells for autologous and for allogeneic transplantation. This procedure can be complicated in the children with low body weight. We report the results of the retrospective study to evaluate the incidence and severity of adverse events during PBPC collection in the smallest children (<20 kg b.w.).
Aim: Assessement safety and efficiency of PBSC in children with low body weight.
Patients and Methods: Between March 2012 and December 2015, we performed 124 apheresis for 118 patients: 57 boys and 61 girls, median age was 2.9 years (5 months-7 years) and median body weight was 13.05 kg (range 5.8–19.0 kg). Primary diagnosis were: neuroblastoma - 97 pts., central nervous system tumors - 6 pts., germ cell tumor - 5 pts., and 10 pts. had other malignancies. G-CSF was used for stem cells mobilization at a dose of 10–20 μg/kg per day in all patients, and additional mobilization with plerixafor was required in 16 cases. Leukapheresis using the Spectra Optia or Cobe Spectra apheresis machines (Caridian BCT Inc, Lakewood, CO, USA), was performed via double-lumen central venosus catheter (CVC) of 5–7 FR, or two single-lumen CVC of 18G each.
Results: The median CD34+ cells precount in peripheral blood was 89,06 cell/ μL (range 14–714 cell/ μL). Irradiated and leukoreduced red blood cell (RBC) units was used to prime the circuit in 97 patients weighting 15 kg or less. For 10 children under 10 months and 9 kg body weight (bw) or less, apheresis were performed in the intensive care unit, under mild sedation with propofol, using continuous monitoring with ECG, pulse oximetry and non-invasive blood pressure, also a blood warmer was used. For others 108 patients apheresis was performed in the pediatric apheresis unit without monitoring, only under physician clinical examination. The whole blood count, electrolytes levels was taken before and after each apheresis. Maximal ACD-A infusion rates were 1.4 ml/min/L BW, calcium gluconate was not prophylactically used. The median total blood volume 2,9 L (range 1,4–5,4 L) was processed in a separation time of 293 min (range 160–591 min). The median CD34+ cells yield was 13,76 × 106/kg bw (range 2,68–74 × 106/kg bw) and median number of collected nucleated cells was 12,04 × 108/kg (range 2,18–30,87 × 108/kg). The median of collection efficiancy (CE2) was 62,87% (range 29,13–111,37%). All apheresis were tolerated well by the patients; only one case of mild citrate toxicity and one case of allergic reaction were encountered. No severe adverse events, such as hypotonia, hypovolemia were observed. None of patients had severe thrombocytopenia or anemia after apheresis. We observed 3 cases of catheter-associated complications; all of them were related to catheter placement, and did not interfere with PBSC collection.
Summary: In conclusion, our data show that collection of PBPC in low weight patients is a safe and efficient procedure. We did not observe any severe adverse events related to apheresis, and few complications, we have seen, were mild and easily resolved. The most significant complications were related due to catheter placement. However, extensive experience in the field of pediatric apheresis is required for safe collection in low-weight patients.

P-552
SAFETY AND EFFICACY OF HEMATOPOIETIC STEM CELL APHERESIS COLLECTION FROM PERIPHERAL BLOOD IN HEALTY DONORS

R Grubovic
Institute for Transfusion Medicine, Medical Faculty, Skopje, Macedonia

Background: Mobilized hematopoietic peripheral blood stem cell (PBSC) has been widely used for allogeneic transplantation in different hematologic malignancies. Optimal donor and recipient outcomes require maximized stem cell collection efficiency.
Aims: The aim of our study is to present our experience of 15 years in collecting of PBSC in healthy donors.
Methods: This is a retrospective study performed in the Institute for Transfusion Medicine of Republic of Macedonia and University Hematology Hospital for period from January 2001 till December 2015. All donors were HLA typed and matched; they were fully informed on the donation procedure and signed an informed consent for donation. Minimum dose required to ensure successful and sustained engraftment was 2 × 10^6/kg CD34+ cells and 2 × 10^8/kg mono-nucleated cells (MNC). PBSC harvesting was performed with continuous flow cell separator Baxter C53000 and COBE Spectra using conventional-volume apheresis processing the 2–2.5 total blood volumes per apheresis. A femoral catheter was used for harvesting and Acid Citrate Dextrose formula A is used for anticoagulation. Recombinant human granulocyte colony-stimulating factor (G-CSF) is used to mobilize PBPC for collection. Harvesting of PBSC is usually performed after 4 to 5 days of G-CSF subcutaneous administration at a dose of 10 μg/kg body weight.
Results: All the donors were siblings of the patients treated at the University Hematology Hospital. There were 126 apheresis procedures performed in 74 healthy sibling donors. There were 48 males and 26 females, aged 19–55. This year we had for a first time unrelated donor who voluntarily donated blood for unknown receiver as

requested by the Bone Marrow Donors Worldwide registry. The single procedure usually took 3–4 h and the volume of collected stem cells was 50–220 ml. The needed number of MNC and CD34+ cells was successfully collected by 1.7 apheresis (range 1–2). There were 9 ABO incompatible donors. Procedures for mobilization and collection of PBPC from healthy donors are generally well tolerated. The only adverse effects of the apheresis procedure were bone pain as reaction of G-CSF and numbness of the extremities as reaction of ACD-A (hypocalcemia), which occur rarely and were very mild. The collected PBSC were used in allogeneic stem cell transplantation in patients with: acute myeloid leukemia - 61.3%, acute lymphoblastic leukemia - 17.7%, chronic myeloid leukemia - 9%, myeloproliferative disorders - 4.1%, severe aplastic anemia - 2.7%, non-Hodgkin lymphoma - 2.7%, chronic lymphoblastic leukemia - 1.3%, Hodgkin disease - 1.3% and multiple myeloma - 1.3%.
Summary/Conclusion: The apheresis collection of PBSC in healthy donors is an effective and safe procedure. We should continue on developing our National Stem Cell Donors Registry as a part of Bone Marrow Donors Worldwide. In that way we hope we will help widen the world network of stem cell donors and enlarge the possibility for each patient to find the right match.

P-553

PERIPHERAL BLOOD STEM CELL HARVEST BY CELL SEPARATOR: EVALUATION OF TWO SYSTEMS

S Ojha[1], P Nagaraju[1], M Poojary[1], SB Rajadhyaksha[2] and P Rane[1]
[1]Tata Memorial Centre - ACTREC, Navi Mumbai, India [2]Tata Memorial Hospital, Mumbai, India

Background: The majority of hematopoietic stem cell transplantations are being done with Granulocyte-colony-stimulating factor (GCSF) mobilized peripheral blood stem cells (PBSC) harvest using cell separators, to optimise the recipient's transplant outcomes. The quality of the harvest product is not only dependent on variables pertaining to cell separators but also on the physiognomic variables of individual subject (patient or donor). Therefore, in our study we have chosen identical subjects for comparison to minimize such variability.
Aim: To evaluate peripheral blood stem cell harvest in identical subjects using two cell separator systems for collection efficiency(CE), product yield, total nucleated count (TNC), mono nuclear cell (MNC) percent and viability.
Methods: Retrospective analysis of 92 PBSC harvest on 42 GCSF mobilized subjects [Autologous patients (n = 28) and allogeneic donors (n = 14)] was done. All PBSC harvest was performed on two systems, namely, COBE Spectra (n = 48) and Fresenius COM.TEC (n = 44). CD34+ PBSC were enumerated by FACS Calibur Flow cytometer (BD) as per International Society of Hemotherapy and Graft Engineering(ISHAGE) protocol. TNC and MNC% was calculated by using ADVIA 2120i hematology analyzer. Viability was done using trypan blue dye exclusion method. American Association of Blood Banks (AABB) acceptance criteria for Hematopoietic stem cell (Apheresis) was used to assess TNC, MNC% and viability. CE was calculated based on number of cells collected divided by number of cells processed and multiplied by hundred. Comparison for both systems was done on individual subject only to minimize intra-individual variability. Data was analyzed using Student's t-test by IBM SPSS software (version 21). All values were given in mean and range. $P < 0.05$ was considered significant.
Results: The mean CE of CD34+ PBSC by COM.TEC (58.75%) was significantly higher ($P = 0.001$) than COBE spectra (52.63%). The mean CE of COM.TEC was also significantly higher in autologous collections compared to COBE Spectra ($P = 0.001$). However no statistical difference was observed in mean CE of the two systems in allogeneic collections ($P = 0.106$). Mean CE amongst autologous and allogeneic collections was significant in COM.TEC ($P = 0.019$) but not in COBE Spectra ($P = 0.092$). There was no statistical significant difference between two systems vis-à-vis product yield, TNC, MNC% and viability.
Conclusions: Overall collection efficiency of CD34+ PBSC was better with COM.TEC than COBE Spectra, especially in autologous peripheral blood stem cells collection. However there is no significant difference in overall PBSC product quality. Thus both cell separators are safe and efficient PBSC harvest.

P-554

EX VIVO EXPANSION INDUCES SUBSTANTIAL ALTERATIONS IN CELL SIZE AND CYTOSKELETAL PROTEINS IN T LYMPHOCYTES AND MESENCHYMAL STROMAL CELLS (MSCS)

R Henschler[1], K Kollar[2] and I Weber[2]
[1]Blood Service Zürich, Zürich-Schlieren, Switzerland [2]Institute for Transfusion Medicine and Immune Hematology, Frankfurt, Germany

Background: Immune cells used as cellular therapeutics in many cases undergo ex vivo expansion protocols. This includes the selection and clonal amplification of antigen-specific T cells, the generation of chimeric antigen receptor-transduced (CAR) T cells, or the generation of therapeutic MSCs. Recent data have indicated that during ex vivo expansion, MSCs or immune cells succumb to impairments in migration or antigen recognition that may affect their therapeutic potential. So far, the impact of culture expansion on proteins which are crucial for these functionalities such as actin-binding proteins are not known.
Methods: Transcription and protein content of the following actin-binding proteins or modifiers cofilin and profilin, alpha-actinin, filamin A, and the proteins linking actin and integrin signalling complexes, paxillin, vinculin and talin were analyzed. Transcription was quantified by qRT-PCR. Protein levels were quantified by flow cytometry of permeabilized cells using fluorescence-labelled antibodies pre-titrated to exceed the concentration of the analyzed antigens. Cell size was determined by flow cytometry using calibrated microbeads.
Results: Analysis of cell size indicated that during anti-CD3/antiCD28-induced ex vivo expansion in RPMI/10% FCS 30U/ml Interleukin-2, immune-magnetically isolated murine CD3+ T lymphocytes increased in cell diameter from 7 to 14 mm and from approximately 179 to 1436 mm^3 cell volume during a culture period of 7 days. In contrast, protein levels of all seven actin binding molecules remained constant on a per cell basis. Transcript mRNA levels of all seven actin-binding molecules, relative to the housekeeping gene GAPDH, were reduced between 5 and 50 fold. Expanded MSCs displayed still higher cell diameters and >10 fold cell volumes than expanded T lymphocytes, but on a cell basis a further reduced mRNA expression levels of the investigated actin binding molecules compared to expanded T lymphocytes.
Conclusion: Ex vivo expansion can induce substantial increases in size and intracellular volume of T lymphocytes and MSCs. In parallel, functionally relevant actin-binding proteins such as profilin, cofilin, alpha actinin, filamin A, paxillin, vinculin and talin do not undergo any parallel increase, and their transcription is strongly reduced. Our findings indicate that cell culture skews proteins with key functions in the migration and function of T lymphocytes and MSCs.

P-555

OUTCOME OF 18 AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTS AFTER UNCONTROLLED-RATE FREEZING ('DUMP FREEZING') USING −80°C MECHANICAL FREEZER

S Arora, R Setia, A Handoo, T Dadu, D Choudhary, SK Sharma, G Kharya and M Kapoor
BLK Super Speciality Hospital, New Delhi, India

Background: Controlled-rate freezing (CRF) is a complicated, expensive and time consuming procedure hence there is a growing interest in uncontrolled-rate freezing (URF) with −80°C mechanical freezers, also known as "dump freezing"which is more economical option for cryopreservation (up to 6 months) in resource constrain settings.
Aim: This study is a retrospective analysis of efficiency of UCF and study of outcome of autologous peripheral hematopoietic stem cells (PBSC) transplants at our centre from March 2015 to March 2016.
Material and Method: Indications of PBSC transplants were both haematological and non-haematological. Granulocyte-colony-stimulating factor was used as the stem cell mobilization and Plerixafor was added in case of poor mobilization. Cobe Spectra and Amicus cell separators were used for leukapheresis. Cryoprotectant solutions included 5% DMSO, 5% Albumin and 2% HES, mixed at 4°C using laminar hood and stored with cryostore freezing bags. −80°C mechanical freezers were used for cryofreezing and storage till transplant.
Evaluation of cryopreservation was studied by analysing the variation in cellularity, viability and CD34+ stem cell recovery and clinical follow up with engraftment. Blood cell counts were done using LH750 Beckman Coulter (Florida, Miami, USA). Viability (7-AAD), absolute mononuclear cell count, CD45+ & CD34+ cell count was done using BD FACS Canto-II Flow-Cytometer. Enumeration of CD34+ cells was

Parameter	Before Cryopreservation	After Cryopreservation	Recovery (%)	P Value
Absolute Nucleated cell count (ANC; x 10^3)	55.1±26.7	53.3±26.3	96.2±10	0.83
Absolute CD 45+/ CD34+ cells (x10^6)	329±290.8	272.7±263.3	78.9±14.7	0.54
Viability (7-AAD; %)	95.8±3.29	82.5±10	86.4±9.9	<0.05

Caption 1: Evaluation of cryopreservation using UCF

Parameter	Before apheresis	After apheresis
WBC (10^3/L)	6.6 ±1.6	6.6 ± 1.7
RBC (10^{12}/L)	4.92 ± 0.29	5.23 ± 0.27
Hb (g/dL)	14.8 ± 1.0	15.7 ± 1.0
Hct (%)	43 ± 0.2	46 ± 0.2
PLT (10^3/L)	232 ± 55	234 ± 58
MPV (fL)	9.6 ± 1.1	9.7 ± 1.0
TP (g/dL)	6.83 ± 0.33	6.03 ± 0.28
IgG (mg/dL)	817.4 ± 185.9	720.0 ± 162.4

Caption 1: Peripheral blood counts and protein before and after plasmapheresis

done by flow cytomtery as described by International Society of Hematology and Graft Engineering (ISHAGE) guidelines.
Results: A total of 18 patients (8 female, 10 male) underwent autologous PBSC transplantations with median age of 24.3 years (range: 3 to 59 years). In six out of 18 were poor mobilizations, plerixifor was added in the mobilizing regimen. 7 out of 18 patients required additional day of harvest. In total 25 PBSC harvest, COBE Spectra was used in 11 patients (15 procedures) and Amicus in other 7 patients (10 procedures). Mean 3.9 times (range: 3–5.5 times) of blood volume was processed for each harvest.
Volume of leukapheresis product harvested was 309.4 ± 133.6 ml. Plasma volume reduction of 181.1 ± 124.4 ml was done to achieve final hematopoietic stem cell volume to 127.33 ± 22.4 ml. Cryoprotectant solutions (CS) added was 129.56 ± 22.4 ml to cryopreserve the product. The final volume of the mixture to be cryopreserved achieved was 256.56 ± 43.9 ml. Mean recovery and effect of cryopreservation by UCF at −80°C is discussed in Table 1. Out of 18 patients under study 3 expired (due to infections). Rest 15 patients, median days to neutrophil engraftment was 10 (range 8–11 days) and platelets engraftment was 15 (range 10–41 days). The cryopreserved product was in storage at −80°C for median 6 days (range 5–8 day) before transplant.
Conclusion/ Summary: Our analysis shows that PBSC can be successfully cryopreserved with mechanical uncontrolled rate freezing. This is a cheap and simple method to freeze the stem cells for short periods in resource constrain setting.

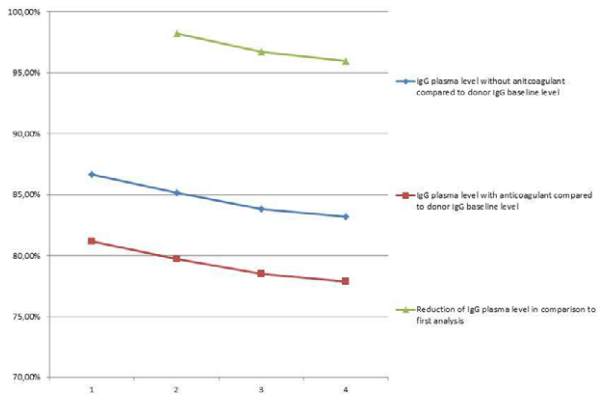
P-556

QUALITY OF SOURCE PLASMA OBTAINED WITH THE AURORA PLASMAPHERESIS SYSTEM

R Moog, T Burkhardt, R Rothe and A Karl

German Red Cross Blood Donor Service North-East, Cottbus, Germany

Background: The quality of plasma for fractionation is of utmost importance.
Aim: To investigate total protein (TP), IgG and citrate levels before, during and after plasmapheresis. Furthermore, parameters of product quality were analysed.
Material and Methods: Fifty-four plasmapheresis donors (39 male, 15 female) fulfilling current national and European eligibility criteria underwent



Caption 2: IgG level during plasmapheresis

plasmapheresis using the Aurora Plasmapheresis System (Fresenius, Lake Zurich, IL, USA).
Donors' peripheral blood counts were analysed berfore and after plasmapheresis using an electronic counter (Sysmex, Sysmex Corporation, Kobe, Japan). Platelets and red blood cells in the plasma products were determined by means of a Neubauer hemacytometer.Total protein, IgG and citrate were measured before, during and after apheresis. Samples during apheresis were drawn every 200 ml of collected plasma. TP an IgG were measured turbidimetrically (AU 640, Beckman Coulter, Krefeld, Germany) and citrate was measured photometrically (Dr. Lange, Berlin, Germany).
Results: Donor characteristics and vital signs are shown in Table 1. An average of 2751 ± 247 ml blood was processed in 47 ± 6 min. Citrate consumption was 177 ± 15 ml and the collected plasma volume was 850 ± 1 ml. Blood counts, total protein und IgG before and after apheresis are shown in Table 1. IgG during plasmapheresis is depicted in Figure 1.
Conclusions: IgG levels during apheresis showed only a slight decrease allowing for the collection of a plasma with good quality.

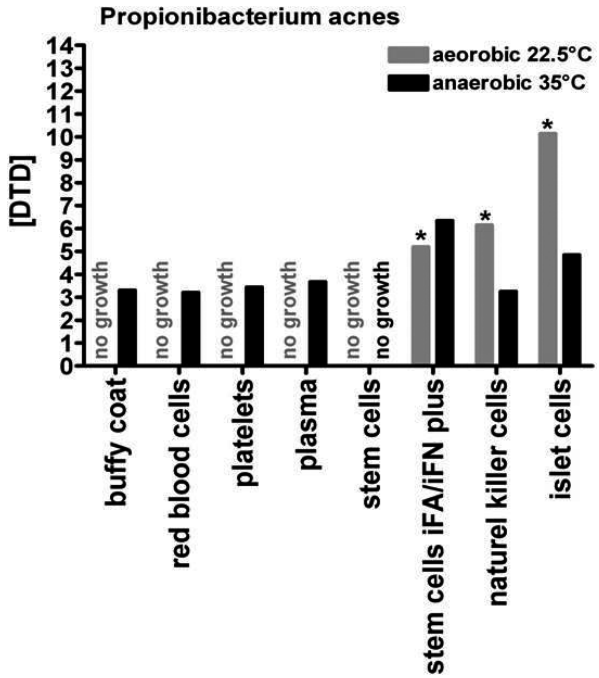
P-557

VALIDATION OF 7 MATRICES OF BLOOD COMPONENTS AND ATMPS FOR STERILITY RELEASE TESTING USING PROPRIONIBACTERIUM ACNES

R Moog[1], N Arlt[1], R Rothe[1], T Juretzek[2], H Peltroche[2] and T Tonn[1]

[1]*German Red Cross Blood Donor Service North-East, Cottbus, Germany* [2]*Carl-Thiem Clinic, Cottbus, Germany*

Background: Slow-growing bacteria like *Propionibacterium acnes* represent a challenge for quality control investigations in sterility release testing of ATMPs.
Methods: A convenient validation with 7 matrices was performed using: buffy coat, stem cells, islet cells, natural killer cells, red blood cells, platelets and plasma in the microbial detection system Bact/Alert®3D incubator with Dual-T- module. All matrices samples were spiked with microbes recommended by the Paul-Ehrlich-Institute with respect to Ph. Eur., General Chapter 2.6.1/2.6.27. *Propionibacterium acnes* with 55 colonies forming units (CFU) were spiked twofold in iAST and iNST culture bottles for 14 days using multishot bioballs from Biomeriéux. Additionally, the stem cell preparation was also incubated in iFA*plus* and iFN*plus* culture bottles, which include neutralizing polymers. Aerobic bottles were incubated at 22.5°C in the Bact/Alert®3D low temperature module (including a refrigeration unit to maintain the incubation temperature) and anaerobic bottles at 35°C in a Bact/Alert®3D 240 incubator.
Results: The Bact/Alert®3D-System detected *Propionibacterium acnes* as shown in Fig. 1 in anaerobic culture bottles in buffy coat [3.3 d (=positive signal day to detection as mean value)], red blood cells [3.21 d], platelets [3.3 d], plasma [3.67 d], natural killer cells [3.255 d] and islet cells [4.845 d]. No growth of the bacterium was found in stem cells using iAST and iNST culure bottles compared to iFA*plus* and iFN*plus* culture bottles where Propioni could be detected in stem cell matrix [6.34]. A successful media validation was performed. All negative controls were conformed as negative and all results were reproducible.
Summary/Conclusions: Our study shows that Bact/Alert®3D-System safely detects the slow-growing bacterium Propionibacterium acnes in different matrices in a practical way except stem cells, which can be explained by antibiotic prophylaxis of the patient and can be overcome using iFA/iFN*plus* culture bottles.



Caption 1: Day to microbial detection of blood components and ATMPs

P-558

CROSS CONTAMINATION EVALUATION OF COBAS P 612 PRE-ANALYTICAL SYSTEM IN USE WITH COBAS® 6800/8800 SYSTEMS

C Volejnicek, S Fusco, LF Kuehler, D Abeln and NM Iordanoaia

Roche Diagnostics International Ltd., Rotkreuz, Switzerland

Background: Customers of PCR based testing systems requests both high sensitivity of the assays and cross contamination-free systems that process samples with high titers in a fast, effective and safe mode.

Aims: In this study we aimed at demonstrating that the **cobas** p 612 pre-analytical system, including the **cobas**® connection module (CCM) or as a standalone, is designed to prevent sample to sample cross-contamination, and therefore suitable for laboratories performing PCR-based nucleic acid testing, e.g. in combination with the **cobas**® 6800/8800 systems.

Methods: For this reason, HBV high titer samples (>1E08 IU/ml) and HBV-negative samples were loaded on the **cobas** p 612 in an alternating pattern, and tested in standard workflows as described below:

Decapping of primary tubes, aliquoting in secondary tube (performed with filtered tips) followed by transfer of open tubes to the sorting area (OutSort Area)

Decapping of primary tubes followed directly by sorting and transfer of the open tubes using the cobas® connection module (CCM) to the cobas® 6800.

Decapping of primary tubes followed directly by transfer of the open tubes to the sorting area (Outsort Area)

Decapping of primary tubes, aliquoting in secondary tube (performed with unfiltered tips) followed by transfer of the open tubes to the sorting area (OutSort Area)

Samples have been prepared in tubes typically used for blood donations representing with a size range from 13 × 75 mm to 16 × 100 mm including different fill volumes and lid variants. For each workflow 832 samples (624 negative, 208 positive) have been processed, for a total of 3328 samples running through the system. After processing or transporting of positive and negative samples, only the negative samples were tested on the **cobas**® 6800/8800 Systems using the **cobas**® MPX test.

Results and Summary: The cross contamination rate in all tested workflows was determined to be 0%, demonstrating that the **cobas** p 612 pre-analytical system, as a standalone, or including the **cobas**® connection module (CCM) can be used in laboratories performing PCR based nucleic acid testing, e.g. in combination with the **cobas**®6800/8800 systems.

P-559

VALIDATING THE TEMPORARY STORAGE AND TRANSPORT OF WHOLE BLOOD UNITS FROM MOBILE CLINICS TO CENTRAL BLOOD BANK TO ASSURE THE COLD CHAIN MAINTENANCE OF BLOOD, ETHIOPIAN BLOOD BANK EXPERIENCE

AB Ambaye, MK Geremew and HD Kassa

National Blood Bank Service, Addis Ababa, Ethiopia

Background: Blood and blood products are very sensitive to different conditions in the environment one of the most important being temperature. Thus it is a must for them to be transported and stored in the appropriate temperature. The NBBS of Ethiopia collected more than 127,000 units of blood in 2015 .Out of those blood units the majority were collected during mobile blood collection clinics/sessions and transported to the blood banks for testing and distribution. This critical step of temporary storage and transport of blood will have a critical effect if not done properly.

Aims: This validation experiment was done, from February to March 2016, to assure that the temporary storage and transport of blood units from mobile clinics to the central blood bank is within international and nationally accepted standards for cold chain maintenance of blood. And also to set a standard for the amount of blood units and frozen ice pack to be transported in the standard cold boxes being used by the blood bank. This validation was done for 350 ml blood units collected at mobile clinics and expected transport temperature is 1–10°C for whole blood units not used for component preparation.

Methods: A cold box was set up with ice pack, digital mini -max thermometer and card board (pieces of carton) and left closed for 2 hr. The temperature of cold box, whole blood and room temperature were recorded using a Non-contact forehead Infrared Thermometer and min- max thermometer in each step. Thirty 350 ml whole blood units were packed in two layers, with 7 icepacks around and one on the top of the blood units, and insulated from direct contact with the units by card board (pieces of carton) and the cold box was sealed. The whole procedure was repeated for different scenarios. The ambient room temperature for all the validation experiments were between 20 and 30°C. The temporary storage time of transport of blood from mobile clinics to the blood center was selected to be 16, 18 and 3 h based on currently available data in the blood bank.

Discussion: In validation one 29 (96.67%) of 30 whole blood were under the required transportation temperature and 1(3.33%) of 30 whole blood unit was very slightly above the normal transportation temperature. We have found this to be acceptable given the accuracy range of the Infrared thermometer used (Accuracy of +/- 0.3 ^{o} C on a given temperature reading). In validation two 28 (93.33%) of 30 whole blood were within the required transportation temperature and 2(6.67%) of 30 whole blood were slightly above the normal transportation temperature. In validation three one whole blood was the sample and fulfills the required transportation temperature i.e 7.2°C after a storage time of three and half hours in the cold box.

Conclusion: Based on the validation results, the current whole blood transport cold chain system is within the acceptable standards of the NBBS, WHO and AfSBT standards The next step will be performing cold chain validation system on the blood transport system to hospitals. Blood services with limited resources can follow this easy cold chain validation experiment suggested by the WHO to validate their blood transport system.

Clinical Applications

P-560

PLATELET GEL IN ALVEOLAR CLEFT SURGERY

M Sampaio, M Bini Antunes, F Dias, P Pina, B Leitão, J Coutinho and M Amil

Centro Hospitalar do Porto, Porto, Portugal

Background: Topical application of platelet gel has been increasingly used to accelerate and improve tissue repair processes, particularly in the treatment of wounds but also in orthopaedic and maxillofacial surgery.

Aims: To evaluate paediatric patients treated with autologous or allogeneic platelet gel for alveolar cleft correction.

Methods: Prospective evaluation of paediatric patients proposed for alveolar cleft correction surgery using autologous bone graft and topical application of autologous or allogeneic platelet gel. Platelet gel was produced from platelet concentrate (PC) obtained from volunteer whole blood donors(allogeneic gel) or from an autologous whole blood collection. Each aliquot of PC was activated with calcium gluconate and allogenic or autologous human thrombin. Autologous blood collection, with

maximum volume set according to child's weight (10 ml/kg), was performed under supervision of a paediatric anaesthesiologist. Exclusion criteria were children younger than eight years old, weight under 25 kg, haemoglobin less than 11 g/dl, platelets less than 200.000/μL, presence of active infectious disease, cardiovascular or severe neurological disorders. Platelet gel, mixed with autologous bone graft from iliac crest, was applied in alveolar cleft during surgical procedure. Efficacy evaluation and follow-up were performed postoperatively on day eight (+8) and twenty-one (+21) searching for signs of mucosal healing, local infection, and assessment of graft volume. Maxillofacial tomography was performed at 12 weeks for documentation of radiological graft performance.

Results: Eight patients were included, all male, median age 10 years (range 8–13). Six patients underwent autologous blood collection for platelet gel, one patient had allogenic platelet gel application during the first surgical procedure and an autologous platelet gel during a second surgery performed one year later. One patient (with severe neurological disease) had an allogenic platelet gel application. All of the six patients with autologous gel application showed a great evolution at day +21 with mucosal closure and good bone volume which was confirmed with radiological assessment at 12 weeks. The patient with allogenic gel showed a slower evolution, keeping alveolar notch present on day +21. However, slit was closed at 12 weeks observation. Patient submitted to two surgeries lost bone graft after first operation and was re-operated an year later with autologous gel application and autologous bone graft having a favourable outcome, with integral mucosa and good bone volume at day +21. No adverse reactions were observed after blood collection or after platelet gel application.

Conclusion: Autologous platelet gel is an effective adjuvant therapeutic, free from complications, in alveolar cleft correction surgery improving its outcome.

P-561

RED BLOOD CELL TRANSFUSION IN TOTAL HIP AND KNEE REPLACEMENT

ND Grechanyuk[1], AV Ovsyankin[1], AV Zverkov[1] and EB Zhiburt[2]

[1]Federal Center of Traumatology, Orthopedics and Endoprosthesis, Smolensk, Russian Federation [2]Pirogov National Medical & Surgical Center, Moscow, Russian Federation

Background: The number of total hip and knee replacement in Russia is dynamically increasing over the last decade and this operative intervention is often accompanied by transfusion therapy.

The aim of the study was to evaluate the aspects of red blood cell transfusion upon primary total hip or knee replacement. Therefore, we monitored the intraoperative blood loss, the volume of drainage discharge on day 1 after surgery, hemoglobin levels before red blood cell transfusion, the volume and time of transfusion therapy.

Materials and Methods: A clinical prospective observational cohort study was carried out at the Federal Center of Traumatology, Orthopedics and Endoprosthesis from January to December, 2014. A total of 1632 patients were included in the study, of which 632 patients underwent total hip replacement (THR group) and 1000 total knee replacement (TKR group). In the THR group, the mean age of patients was 63 years (range 18–82) and the gender ratio was 64.1% female. The mean age of patients within the TKR group was 64 years (range 26–81) and the ratio was 84.7% female. The rate of neuraxial anesthesia was 98% in the THR group and 99% in the TKR group, and the rate of general anesthesia was 2% and 1%, respectively. Operative time amounted 55 (45–65) minutes in both groups.

Each patient received 10–15 mg/kg Tranexamic acid 30 min before surgery, which was repeated at the same dose after 6 h. Anticoagulants were not applied in the study groups during the preoperative period, the first dose of anticoagulant was administered not earlier than 6 h after surgery. All patients with total knee replacement were operated without a tourniquet. Transfusion of autologous blood was not used.

Results: The median intraoperative blood loss in both groups was 150 ml (interquartile range 100–200 ml). The median volume of drainage discharge in both groups was 250 ml, however the inter-quartile ranges varied with 150–300 ml in THR and 200–350 ml in the TKR group.

A total of 21 patients received red blood cell transfusion, 7 of 632 patients (1.1%) in the THR group and 14 of 1000 (1.4%) in the TKR group.

The mean hemoglobin level before red blood cell transfusion was 68.3 g/l (min 56 g/l, max 77 g/l) in the THR group and 70.8 g/l (min 54 g/l, max 79 g/l) in the TKR group.

Transfusion of a single dose of red blood cells was required for 9 patients, 11 patients received two doses of red blood cells and only one patient got more than two doses of red blood cells. Transfusions were carried out from 1 to 8 day after the operation.

Summary: The intra- and postoperative blood loss in patients after total hip and knee replacement in a specialized orthopedic hospital is low. Therefore it should be considered whether blood transfusion is needed.

P-562

PREDEPOSIT AUTOLOGOUS DONATION: IS IT BENEFICIAL IN SCOLIOSIS SURGERY?

A Spínola[1], A Ramoa[2], S Faria[1], J Condeço[3] and S Sousa[1]

[1]Unidade Local de Saúde de Matosinhos, Matosinhos, Portugal [2]Hemovigilância, Cento do Saúde e da Transplantação do Porto, Porto, Portugal [3]Hemovigilância e Direção Técnica, Cento do Saúde e da Transplantação do Porto, Porto, Portugal

Background: Spinal fusion with instrumentation is among the most common surgical procedures that are often associated with substantial blood loss and the necessity of blood transfusion. We believe that it is important for patients to avoid peri-operative allogeneic blood transfusions (AlloBT). Toward this aim, our hospital regularly uses predeposit autologous donation (PAD).

Aim: Examine AlloBT, autologous blood transfusion (ABT) and intraoperative cell salvage (ICS) rates in scoliosis surgery. Determine the benefit of PAD in diminishing the need for AlloBT.

Methods: Inclusion criteria: patients who underwent scoliosis surgery between 2009 and 2015 and had PAD with or without ICS. All patients had oral iron therapy to prevent anaemia. The following parameters were evaluated: clinical data; haemoglobin concentration pre-donation, pre and post-operative; amount of blood returned to the patient by ICS; transfusion trends. Descriptive analysis was performed using SPSS v.20.

Results: Twenty-four patients, 23(95.8%) female, median age of 20 (min. 14-max.43) y.o. underwent scoliosis surgery. Twenty-three patients had lumbar scoliosis and one thoraco-lumbar scoliosis. All patients were proposed to complete two PAD however one patient could not carried out one donation due to hypotension and other recused to donate a second unit. The median pre first collection heamoglobin was 13.6 (min. 11.5-max.16.5)g/dl and pre second donation 12.3 (min. 10.6-max.14.7) g/dl. Pre-operative anaemia was seen in 8 (33.3%) patients. Twenty-two (91.7%) patients transfused PAD intra-operatively or within 24 h after surgery. Of them, 14 (58.3%) required other transfusions, AlloBT and/or ICS, as shown in Table 1. The median number of autologous and allogeneic blood units transfused were 2 (min. 1-max.2) and 2 (min. 1-max. 8) respectively. Overall, median cross-match to transfusion ratio was 2.0 (min. 1-max.4) and transfusion index was 0.5 (min. 0.2-max.1.0). The median ICS volume returned to the patient was 200 (min. 20-max. 750)ml.

Two patients did not transfuse, neither autologous nor allogeneic blood, and in one ICS was used, volume returned to him of 20 ml (Table 1.).

Regarding patients with pre-operative anaemia: 4 only transfused PAD; 2 required ABT, AlloBT and ICS; 1 needed ABT and ICS; 1 carried out surgery without blood requirement.

Post-operative haemoglobin concentration varied between 5.8 and 12.6 g/dl with a median of 8.9 g/dl. Patients in whom all transfusion modalities were used had a median post-operative haemoglobin level of 7.8 (min. 5.8-max.10.2) g/dl and those who only had ABT had 10.0 (min. 7.8-max.11.1) g/dl.

Conclusions: Although PAD may reduce exposure to donor blood, it does not reduce overall exposure to transfusion procedures, as it did not prevent AlloBT in 10 (41.7%) patients. According to these findings, we can question if PAD is necessary in scoliosis surgery as it is of our knowledge that others centers do not performed it

	Allogeneic blood transfusion	Intraoperative cell salvage used	Allogeneic blood transfusion and intraoperative cell salvage used	No allogeneic blood transfusion nor intraoperative cell salvage used	TOTAL
Autologous blood transfused	2	4	8	8	22 (91.7%)
No autologous blood transfused	0	1	0	1	2 (8.3%)
TOTAL	2 (8.3%)	5 (20.8%)	8 (33.3%)	9 (37.5%)	24

Caption 1: Summary of transfusion trends in scoliosis surgery

and do not have a greater increase in blood needs. Indeed, the availability of PAD may increase the risk of unnecessary transfusion and it can also cause pre-operative anaemia with subsequent increase in morbidity and mortality rates. Moreover it is time and resource consuming as it is, sometimes, inconvenient for the patient.

P-563

MOBILIZED HEMATOPOIETIC STEM CELLS FROM PERIFERAL BLOOD AS PREFERED SOURCE FOR AUTOLOGOUS AND ALLOGENEIC TRANSPLANTATION IN HEMATOLOGICAL PATIENTS – 15 YEARS OF EXPERIENCE

R Grubovic[1], B Georgievski[2], L Cevreska[2], S Useini[1], S Genadieva Stavric[2], A Stojanovic[2], E Petkovic[1] and M Grubovic[1]

[1]Institute for Transfusion Medicine, Medical Faculty, Skopje, Macedonia [2]University Hematology Hospital, Skopje, Macedonia

Background: Hematopoietic peripheral blood stem cells (PBSC) are preferred stem cell source for autologous and allogeneic hematopoietic stem cell transplantation (HSCT). An adequate hematopoietic stem cell collection is fundamental for the success of the stem cell transplantation.
Aims: The aim of this study is to present our experience in autologous and allogeneic mobilized PBSC apheresis collection in hematological patients and healthy donors for the last 15 years.
Methods: This is a retrospective study performed in the Institute for Transfusion Medicine of Republic of Macedonia and University Hematology Hospital between 2001 and 2015 in hematological patients and healthy donors. PBSC harvesting was performed with continuous flow cell separator Baxter C53000 and COBE Spectra using conventional-volume apheresis processing. Mobilization regimens included granulocyte colony-stimulating factor (G-CSF) alone in healthy donors, and G-CSF alone or combination of G-CSF and disease-specific chemotherapy in patients. Minimum dose required to ensure successful and sustained engraftment was 2×10^6/kg CD34+ cells and 2×10^8/kg mono-nucleated cells (MNC).
Results: There were 653 apheresis collections in total, of which 527 performed (80.1%) in 255 hematologic patients (156 males and 99 females, aged 18–65), and 126 procedures (19.9%) in 74 healthy sibling donors (48 males and 26 females, aged 19–55). Sufficient number of PBSC was collected with 2.1 apheresis in patients (range 1–5), and 1.7 apheresis in donors (range 1–3). The single procedure usually took 3–4 h and the volume of collected stem cells was 50–220 ml. The tolerance of collection procedures in our patients and donors was good. The only adverse effects of the collection procedure were bone pain as reaction of G-CSF and numbness of the extremities as reaction of anticoagulant (hypocalcemia), which occur rarely and were very mild. The main indications for autologous SCT in our patients were: multiple myeloma (53.1%), lymphomas (25%) and acute myeloid leukemia (21.9%). The main indications for allogeneic SCT were: and acute myeloid leukemia (61.3%), acute lymphoblastic leukemia (17.7%) and chronic myeloid leukemia (9.7%).
Summary/Conclusion: The needed number of MNC and CD34+ cells was efficiently collected by ~ 2 apheresis, and the autologous and allogeneic stem cell transplantations were successfully performed.

P-564

DONOR LYMPHOCYTE INFUSION FOR TREATMENT OF FALLING CHIMERISM IN A POST ALLOGENIC STEM CELL TRANSPLANT

A Navkudkar, S Ojha, N Khattry, P Desai and S Rajadhyaksha

Tata Memorial Hospital, Mumbai, India

Background: Chimerism is the presence of two or more cell populations in an individual. Chimerism studies are important in post transplant period for early recognition of disease relapse. Donor Lymphocyte infusion (DLI) can be used in management of falling chimerism in post allogenic hematopoietic stem cell transplant (HSCT).
Aims: Case report on Donor lymphocyte infusion for treatment of falling chimerism in a post allogenic stem cell transplant
Case Details: A 35 years old female diagnosed with Primary Myelofibrosis with positive JAK2V617 mutation in November 2014 was started on Chemotherapy. In February 2015, HSCT was planned with 6/6 HLA match with brother, having same blood group. After appropriate conditioning, 291 ml of Peripheral Blood Stem Cells (PBSC) containing 19.4×10^6 CD34 cells/ kg body weight and 21.95×10^7 CD3 cells/kg body weight were collected. 111 ml of 7.8×10^6 CD34 cells/kg body weight was infused in September 2015. Remaining 180 ml of stem cells were stored as 5 aliquots of 36 ml each for DLI purpose as per institutional policy. Platelet and neutrophil engraftment occurred on day+14 and day+19 post transplant respectively. Short tandem repeats (STR) analysis showed 100% donor status day+25 which remained stable with weekly follow up.
On day+75 platelet counts dropped to 5000 with poor increment with Single donor platelets (SDP) transfusions. STR analysis showed 90% donor cells and Fluorescence *in situ* hybridization (FISH) showed 95% XY cells. Bone marrow biopsy showed presence of megakaryocyte with no excess blasts. Immune Thrombocytopenia was diagnosed and intravenous immunoglobulin therapy was started which showed improvement in platelet counts. Subsequent STR analysis showed 100% donor status. However, in December 2015, STR analysis showed 95% donor cells with FISH analysis as 87% XY cells. In view of disease relapse, DLI was planned and 30 ml of 1.3×10^6 CD3 cells were infused over a period of 8 min. After 3 weeks of DLI infusion patient showed 100% donor status on STR and FISH analysis. Subsequent follow up with chimerism studies, patient continued to have 100% donor cells.
Conclusion: In case of ABO incompatible transplant, RBC serology needs special attention. Also falling platelet counts unresponsive to platelet transfusions with mixed chimerism can direct towards disease relapse. In such cases, donor lymphocyte infusions (DLI) can exhibit a graft vs autoimmunity effect and promote engraftment of donor cells.

P-565

HEMOTHERAPY COMPLICATIONS IN PATIENTS AFTER BONE MARROW/PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

J Šianská, I Holusková, M Vodicková and D Galuszková

University Hospital Olomouc, Olomouc, Czech Republic

Background: Routine testing of patients after bone marrow/ peripheral blood stem cell transplantation (HSCT) has been provided on Department of Transfusion Medicine, University Hospital Olomouc since 2000. We test these patients before blood transfusion or as a part of complete imunohematological assay; sometimes we provide testing for blood group in AB0 system and RhD only.
Material: Blood sample collected in K_3EDTA.
Methods: The first information about planned HSCT and indication for the procedure comes to our department from physicians of Department of Hematooncology. Head of the immunohematological laboratories then, after collection of all results, provides a special Card for Patient after HSCT, including recommended hemotherapy, which depends on the type of incompatibility between patient and donor (major, minor, mixed).
Laboratory assessment of patient after HSCT includes testing of blood group in AB0 system and RhD antigen, for which we use two analyzers (Techno TwinStation or Swing TwinSampler, both provided by BioRad, Switzerland) or manual method. We also examine Rh and Kell phenotype in all new hematooncological patients, who may be potentionally recommended for HSCT. For these tests we use gel column agglutination system (Grifols, Spain).
In case of related donor we also examine the blood group of the donor.
Results: Between years 2000 to 2015 we examined totally 341 patients after allogenic HSCT. In 41% of cases (138/341) the donor was related to the patient, in 59% (203/341) the donor was unrelated. There were 35% (118/341) of AB0 identical transplantations in the study group. Positive immunohematological results (i.e. detection of anti-erythrocyte antibodies) were found in 27% (93/341) of patients. In total 4% of patients were suspected to have a post-transfusion reaction and their hemotherapy needed to be modified.
Conclusion: The management of hemotherapy for patients after HSCT is proposed by an immunohematologist and it is based on the type of incompatibility between recipient and donor. Adequate and rationalized hemotherapy minimizes risks of alloimunization and other complications in these patients.

P-566

TANDEM DONOR LYMPHOCYTE INFUSION AND HAPLOID STEM CELL TRANSPLANTATION DURING HEMATOLOGICAL RELAPSE IN ACUTE MYELOID LEUKEMIA

R Sembiring and G Tann

Columbia Asia Hospital, Medan North of Sumatera, Indonesia

Background: Post chemotherapy and stem cells transplantation hematological relapse in AML is a condition that is difficult to overcome because of the high mortality rate.

Aims: Case Report of patient with tandem donor lymphocyte infusion and haploid stem cell transplantation during hematological relapse in acute myeloid leukemia.

Methods: A 27 year old female presented with her first hematological relapse of acute myeloblastic leukemia (AML). This patient had been diagnosed with acute myeloblastic leukemia type II eight month prior to this episode. Remission induction with standard doses of Daunorubicin 45 mg/m^2 and Cytosine Arabinoside 100 mg/m^2 was reinstated the '3–7 regime' and a second remission was achieved. During the periode of aplasia a haploid reduced intensity peripheral blood stem cell transplantation was performed with her mother as donor. The donor was prepared with five days of stimulation with G-CSF. Both donor and recipient were ABO mis-matched. Erytrocyte in the stem cell collection were minimised to less than 20 ml. Donor lymphocyte Infusion (DLI) extraction was subsequently performed on the mother and administered to the patient on day +30 with no anti-GvHD regimen. This was due to previous experience that our mother to child haploid transplants all showed minimal GvHD. The total number of lymphocytes infused was 1.6×10^{10}.

Results: Treatment with tandem donor lymphocyte infusion and stem cells transplant will reduce recurrence due to graft vs leukemia effect. The patient is doing well for fourth months.

Conclusion: Tandem DLI and haploid stem cell transplantation is alternative treatment in hematological relapse of AML.

P-567

PREGNANCY COMPLICATIONS IN WOMEN WITH TROMBOPHILIA

J Hamidovic[1], N Nikolic[2] and L Hamidovic[1]

[1]*General Hospital Novi Pazar, Novi Pazar, Serbia* [2]*Clinical Centre of Serbia, Belgrade, Serbia*

Background: Thrombophilia can be congenital or acquired. Congenital thrombophilia refers to inherited conditions that increase the tendency to develop thrombosis. Acquired thrombophilia refers to conditions that augment the risk of thrombosis such is antiphospholipid syndrome. Persons with blood groups other than type O are at a two to fourfold relative risk of thrombosis. Hypercoagulability is common condition in pregnancy. It is characterized with some haematological changes: elevated levels of Factor II, VII VIII, X, PAI, and decreased level of protein C, and decreased fibrinolytic activity. Thrombophilia has been linked to recurrent miscarriage and possibly various complications of pregnancy such as abruptio placentae, severe pre-eclampsia, intrauterine growth restriction (IUGR), and stillbirth.

Aim: The aim of this study was to evaluate pregnancy complications and pregnancy outcome in women with thrombophilia.

Material and Methods: This study included 36 pregnant women (age 20–38 years) with thrombophilia referred to General Hospital Novi Pazar in period of five years. Data collected from patient medical records. Homozygous Factor V Leiden or prothrombin gene mutation, antiphospholipid syndrome or combination of ≥2 disorders were considered a strong thrombophilia.

Results: Out of 36 patients 28 (77.8%) were diagnosed with inherited thrombophilia and 8 (22.22%) with antipfospholipid syndrome. Factor V Leiden G1691A was detected in in 4 (11.1%), prothrombin G20210A mutation in 19 (52.8%), MTHFR mutation C677T in 22(61.1%), PAI 675 5G/4G in 15 (41.7%). There were 7 (19.4%) women with one mutation, 25 (69.5%) with two mutations, 2 (5.6%) with thre mutations, and 2(5.6%) with four mutations. Eleven women (30.5%) met criteria for strong thrombophilia, and 17(47.2%) for mild thrombophilia.delivery route: vaginal delivery in 16 (44.5%) and caesarean section in 20 (55.5%) cases. Complications during pregnancy and at birth: IUGR 5(13.9%), preeclampsia 15(41.7%), thrombocytopenia 4(11.1%), preterm birth 2(5.6%), abruptio placentae 4(11.1%), sillbirth 4 (11.1%). APGAR SCOR was <7 in 4(11.1%), and 8–10 in 28(77.8%) live newborns.

Conclusions: Thrombophilia in pregnancy is hard to detect, but is a common and important risk factor for pregnancy complications. For appropriate screening and management of thrombophilia in pregnancy multidisciplinary approach is needed. The best results can be achieved in in the tertiary health care institutions engaging the team of experts in gynenecology, haematology and haemostasys.

P-568

This abstract has been withdrawn.

P-569

PERIOPERATIVE PROPHYLACTIC TRANSFUSIONS IN UNCOMPLICATED CARDIAC SURGERIES – IS IT REALLY NECESSARY?

DT Fernando and AM Abeywardane

Sri Jayawardenepura General Hospital, Moratuwa, Sri Lanka

Background: Transfusion of blood and blood components although are beneficial, unnecessary use can have deleterious effects on patients. Therefore, transfusions should be considered only if there is a clear clinical indication.

Aims: To assess the relationship between transfusion of blood components and the clinical outcome of patients who underwent otherwise uncomplicated cardiac surgeries at Sri Jayawardenepura General Hospital (SJGH).

Methods: Retrospective analysis of the Intercostal tube (IC tube) drainage with relation to the volume of blood components transfused prophylactically within the first 12 h of the post operative period. Analysis was done on patients who underwent cardiac surgeries during the three months from 01/08/2015 to 31/10/2015. Thromboelastometry done on random patients was also taken in to consideration when assessing the clinical outcome of the patients.

Results: The clinical outcome of 88 patients who underwent otherwise uncomplicated cardiac surgeries was evaluated. 38.6% of patients (34 out of 88) were transfused with blood components in the perioperative period out of which 25 had a post op IC tube drainage of <400 ml in the first 12 h. Nine patients who were given perioperative transfusions had an IC tube drainage of >400 ml in the first 12 h. 61.4% of patients (54 out of 88) were not given perioperative transfusions out of which, forty nine patients had an IC tube drainage of <400 ml. Out of the five patients who had a drainage of >400 ml, 3 had undergone CABG, while a patient each had undergone double valve replacement and MID CAB surgery. Out of the 54 patients who did not receive perioperative transfusions, 8 had Thromboelastometry analysis where only one had undergone Aortic valve replacement, while the rest were following CABG.

Conclusion: Based on the above evidence, there is no significant benefit in perioperative prophylactic transfusion of blood components in patients undergoing uncomplicated cardiac surgeries, but they have a clear place when they are clinically indicated. Thromboelastometry, when required leads to a better outcome in patients and can help prevent consequences of unnecessary transfusions.

P-570

FACTORS RELATED TO BLOOD PRODUCTS TRANSFUSIONS IN CARDIAC SURGERY PATIENTS OF A LOW VOLUME CARDIAC CENTER

T Tzoka, E Zervou, A Petrou and E Apostolakis

University Hospital of Ioannina, Ioannina, Greece

Background: Considerably more blood products are consumed in cardiac surgery than in most other surgeries. The overall blood demand may increase in the coming years because of the increasing total number of cardiac interventions together with their level of complexity and also due to demographic ageing. While standardization of indications for transfusions could safely become stricter, it is useful to recognize factors that affect transfusion in order to prepare and implement programs for patient blood management (PBM) aiming at improving patients' overall outcomes.

Aim: To identify factors that predispose and affect transfusions in patients undergoing cardiac surgery in a university hospital before the implementation of clinical protocols of perioperative blood management in cardiac surgery in a low volume cardiac center.

Methods: Our cohort consists of 565 patients that were subjected to cardiac surgery with or without extracorporeal circulation during a period of 4 years before the

implementation of the PBM protocols. Multiple linear regression statistical analysis was performed in order to identify risk factors that significantly affect blood products consumption during cardiac surgery.
Results: 99.5% of patients were transfused with any blood product perioperatively. Specifically, 95.6% of the patients were transfused with red blood cells (RBC), 91.7% with fresh frozen plasma (FFP) and 67.4% with platelets (PLT), receiving an average of 5.8 RBC units, 5 FFP units and 8 units of platelets per patient. Most transfusions were performed intraoperatively. Factors associated with increased RBC transfusions were: the duration and type of surgery, the use of extracorporeal circulation, the value of preoperative hematocrit, the age, the body mass index(BMI) and the gender. Moreover, it was confirmed that preoperative anemia significantly affects the final hematocrit value at hospital discharge. The statistical analysis of our data revealed that there is a statistically significant correlation between the increased plasma transfusion and the duration of aortic cross-clamping, the use of extracorporeal circulation, the increased preoperative value of hematocrit and blood types A and O compared with type AB. In addition, increasing numbers of FFP units transfused had a negative impact on hematocrit (Hct) and hemoglobin (Hb), measured immediately after the end of surgery and admission in the ICU.On the contrary, the increased preoperativecount of PLT was a statistically significant factor for lower amounts of FFP units transfused and preserved the platelets countat patient's discharge.The presence of comorbidities was a statistically significant factor in reducing platelets count immediately after surgery. Increasing patient's age, increasing duration of surgery and the use of extracorporeal circulation are statistically significant factors for increased PLT units transfused perioperatively.
Conclusions: The perioperative transfusions in cardiac surgery found to be in discordance with the current literature. Thus having identified the factors that significantly affect them we opt at evaluating our perioperative patient blood management protocols, investigate its effectiveness in terms of safety and transfusions reduction and determine if the application of blood preservation techniques (intraoperative salvage) and bedside coagulation monitoring (thromboelastography) could contribute in reducing perioperative transfusions in our institution.

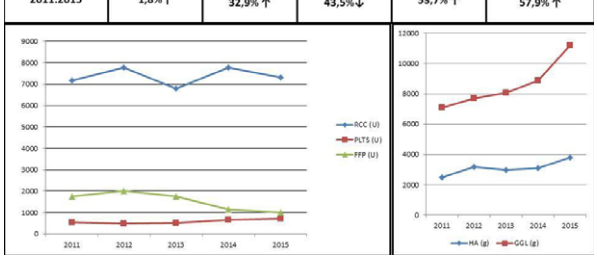
P-571

WAS THE USE OF BLOOD COMPONENTS, HUMAN ALBUMINS AND GAMMAGLOBULINS IN GENERAL HOSPITAL CELJE IN THE PERIOD 2011–2015 RATIONAL?

J Pajk
General Hospital Celje, Celje, Slovenia

Background: In the near past blood transfusion services were introducing the system of quality assurance with the aim to provide optimal quality and safety of blood components and drugs from fresh frozen plasma (FFP). In the past, our hospital constituted Hospital Transfusion Committee (HTC) to help the blood transfusion service to introduce: the guidelines and recommendations of rational use blood components and drugs, autologous blood transfusion and collecting and evaluating data of untoward reactions in patients and blood donors - haemovigilance.
Aims: During the last five years many changes were performed on the field of preoperative orthopaedic surgery, extensive haemato-oncology and neurology treatment. We are interesting the influence of these changes on the use blood components and drugs from FFP.

YEAR	RCC (units)	PLTS (units)	FFP (units)	HA (g)	GGL (g)
2011	7180	544	1764	2475	7085
2012	7781	480	2018	3173	7710
2013	6797	515	1763	2964	8055
2014	7778	649	1138	3093	8875
2015	7310	728	996	3806	11190
2011:2015	1,8%↑	32,9% ↑	43,5%↓	53,7% ↑	57,9% ↑



Methods: The use of blood components and drugs from FFP in the last five years period from 2011 to 2015 at General Hospital Celje (GHC) with 743 beds, is presented. The data were collected from information system Datec and analysed in Excel.
Results: In last five years, the use of red cells concentrates (RCC), platelets (PLTS), human albumins (HA) and gammaglobulins (GGL) in the period 2011–2015 increased; the use of FFP decreased.
There were some peaks of usage RCC in 2012 (7781 units) and 2014 (7778 units); PLTS in 2015 (723 units) and FFP in 2012 (2018 units); HA (3806 g) and GGL (11190 g) in 2015. The results are presented in table and in graphs.
Conclusions: A large number of patients treated in GHC due to serious internal diseases (haemato-oncology from 2013) and operations, the use of blood components increased; RCC for 1,8% and PLTS for 32,9%. The use of FFP was diminished for 43,5% and was more rational in the analysed period. On the other side, the use of HA and GGL increased; HA for 53,7% and GGL for 57,9%.With better cooperation between transfusiologists and clinicians, and good activity of HTC by consideration of guidelines and recommendations for optimal use blood components and drugs from FFP is to be expected in more rational use in the future.

P-572

This abstract has been withdrawn.

P-573

MULTIFACETED APPROACH TO THALASSEMIA CARE & MANAGEMENT – OUR EXPERIENCE & LESSONS LEARNED

S Patil, V Kale, N Tated, A Jain, R Kasodkar, V Ugaonkar and S Nikam
Arpan Blood Bank, Nashik, India

Background: If managed properly with adequate overall medical care and good quality & safe blood transfusion support, the quality of life of thalassemia major patients can be improved significantly.
Aim: To implement a comprehensive, multifaceted thalassemia care and management programme under one roof.
Methods: Thalassemia major patients within the expanse of north Maharashtra were registered with our organisation. A programme for medical management including iron chelation therapy, haematologic, cardiologic, paediatric, orthopedic, pathological, radiological evaluation was defined. A monitoring programme was formulated using guidelines given by Indian Academy of Paediatrics. All patients were offered ID-NAT tested, inline-leucodepleted blood free of cost with the help of corporate support. A dedicated donor programme was implemented for the transfusion support of the study group. All the patients were followed up for febrile non-hemolytic reactions, sero-conversion for TTI, alloimmunization, growth-disorders, iron overload for a period of 3 years.
Results: Total 181 patients with age ranging from 2 years to 30 years were enrolled in this programme. 4662 transfusions have been given over the period of 3 years to 181 patients. 3 patients (1.6%) have developed alloimunization. 1 patient (0.5%) had experienced febrile non-hemolytic transfusion reaction. Severe iron overload (according to T2* MRI) was seen in 2 out of 12 patients (16.66%). Growth disorders/stunted growth have been observed in 25 (13.81%) patients. None of the above patients were found to have sero-conversion for HIV, HBV or HCV.
Conclusion: Implementing a comprehensive transfusion therapy and health monitoring programme for thalassemia patients under one roof is feasible with support from corporate & society. Careful periodic monitoring of these patients & implementation of NAT tested, leucodepleted & phenotype matched blood transfusion can go a long way to improve the quality of life and overall life expectancy of thalassemia major patients.

P-574

THALASSAEMIA, HAEMOGLOBINOPATHIES AND THEIR EFFECT ON GRADE POINT AVERAGE GPA (COGNITIVE FUNCTIONS) AMONG UNIVERSITY STUDENTS, KHON KAEN PROVINCE, THAILAND

L Borhan Ragheb
National Blood Transfusion Centre, Cairo, Egypt

Background: The association between thalassaemia and cognition has been investigated before, but this relationship has not been tackled extensively in South East Asia and previous studies have focussed on patients rather than healthy carriers.

Aims: A quantitative study to detect if there is a relationship between thalassaemia and, University GPA amongst healthy university students enrolled at the Faculty of Associated Medical Sciences, Khon Kaen, Thailand

Methods: Secondary data was collected from 410 university students enrolled at the Faculty of Associated Medical Sciences, Khon Kaen, Thailand. The students come from regions all over Thailand and are divided into two main groups of "Regular"undergraduates and older students from the "Special"Continuing Adult Education Programme. This data was made up of demographical elements; gender, place of birth, type of programme and type of thalassaemia. As well as laboratory data as Haemoglobin, Haematocrit, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, Red Cell Distribution Width-CV(Coefficient of Variation) .GPA (Graduating Grade Point Average) was used as a proxy for Academic achievement by extension and cognitive ability.

Data was tabulated and described then analysed using Excel ®, SPSS 17®for Windows and Minitab®, cross tabulations were performed. Analysis was done between the dependent variable GPA and various independent variables.

Results: We found that the greatest proportion of cases are in the non thalassaemic group (65.3%), followed by Hb E (28.6%). Females had a higher GPA than males, (2.92 vs 2.77, P = 0.001) Regular students more than Special (2.96 vs 2.78, P = 0.00). The Northeast has the highest percentage of cases (67.1%), also the Northeast in relation to the other regions combined (North, Central& South), had the highest GPA (2.92 vs 2.79, P = 0.002), since the majority of regular students come from the Northeast. In terms of laboratory parameters Heterozygous Hb E have generally higher counts in comparison to homozygous Hb E; males also mostly have higher readings than females. GPA among anaemic thalassaemics was higher than among non -anaemic non- thalassaemics, 2.92 vs 2.88.

Conclusion: There was a significant relationship between GPA and place of birth (P = 0.009), which can be attributed to certain regions especially in the North East being more rural than urban, and education being better in some areas. This would be reflected in GPA, but would not necessarily be a true measure of cognitive abilities. GPA in itself is used as a proxy for cognitive ability,but has many drawbacks.

However there was no statistically significant relationship found between GPA and thalassaemia type (P = 0.584), or degrees of anaemia (P = 0.263).This could be attributed to the relatively small sample size and other factors that act as confounders e.g lab quality techniques, and other inaccuracies etc,

This study shows good external validity in that it could serve as a jumping board for many other studies that could possibly discover a link between thalassaemia and cognition, if measured correctly, or with other haemoglobinopathies not as common to the Far East but equally as prevalent, as Beta Thalassaemia in the Mediterranean and Middle east, and Sickle in African countries.

Comparing means of GPA within "thalassaemia type" group and the "anaemia severity" group & "place of birth"

	N	Mean	Std. Deviation	95% CI for mean Lower bound	Upper bound	p-value
Thalassaemia * type						
Non thalassaemic	263	2.88	0.4	1.78	3.86	0.584
Hb E	115	2.85	0.4	1.65	3.60	
α^0- thalassaemia	17	3.01	0.3	2.17	3.56	
β -thalassaemia	4	2.98	0.2	2.73	3.30	
αβ -thalassaemia	7	3.01	0.3	1.65	3.86	
Total	406					
Severity of anaemia*(in g/dl)						
<7.9(severe)	1	3.10	N.A.	N.A.	N.A.	
8-9.4 (moderate)	2	3.00	0.1	2.24	3.77	0.263
9.5-11.9(mild)	84	2.91	0.4	2.83	2.99	
12-12.9(N females)	109	2.94	0.4	2.86	3.02	
>13 (N males)	210	2.83	0.4	2.78	2.90	
Total	406	2.88	0.4	2.84	2.92	
Place of birth**						
North	36	2.77	0.3	2.7	2.9	0.009
Central	44	2.80	0.4	2.7	2.9	
Northeast	280	2.92	0.4	2.9	3.0	
South	46	2.78	0.4	2.7	2.9	
Total	406	2.88	0.4	2.9	2.9	

* performed by *one way ANOVA*
**performed by *Kruskal Wallis nonparametric test*
p value<0.05 is significant

General characteristics of the study population

Characteristics		Frequency	Percentage (%)
Gender	Female	292	71.2
	Male	118	28.8
	Total	410	100
Study programme	Regular	234	57.1
	Special	176	42.9
	Total	410	100
Place of Birth	North	36	8.8
	Central	44	10.7
	Northeast	281	68.5
	South	46	11.2
	Laos	3	0.7
	Total	410*	100.0

*Including the 3 students born in Laos and the single case of Hb Pyrgos

P-575

BLOOD TRANSFUSION IN ORTHOPAEDIC SURGERY DURING THE PERIOD OF 2010–2015

V Jovanoska[1] and I Jovanoski[2]

[1]Institute of Transfusion Medicine, Prilep, Macedonia [2]General Hospital, Borka Taleski,, , Prilep, Macedonia

Background: Approximately over 95%of the hip fractures are managed surgically. The amount of blood loss during surgery varies between patient and given surgical procedure, which limits standardization of preoperative blood orders. Mainly older patients, often with co-morbidities, suffer from hip fractures.

Aims: Identification and analysis of blood transfusion practice at the Department of orthopaedic surgery and traumatology in GH Prilep

Improvement of the actual transfusion practice (order/use)

Identification of the post transfusion reactions.

Material and Methods: We retrospectively analyzed patient's records with hip fracture surgery from 2010 to 2015. Those data are compared with the data in the Department of transfusion medicine Prilep. Our study excludes uncompleted data.

We divided operative procedures in four groups:

Partial hip replacement

Open reduction of fracture with internal fixation, femur (DHS)

Total hip replacement

Revision of hip replacement

Chi square test is used for differences between categorical variables, while numerical variables are analyzed by Student t test. Study has observational nature. Statistical significance is shown for informational purposes.

Results: Total number of patients from 2010 to 2015 is 489 patients with average age of 69,2 years (59–91y); 67% are female, 33% male. Red cell transfusions (Er SAG-M) are given to 270 (55%) patients 1,7 blood units (BU) per transfused patient (from 1 to 7 BU). For the rest 219 patients' pre, intra and postoperative treatment have been without blood transfusion. Mainly, Hgb level under 10 g/dl is taken as indication for transfusion. Total amount of ordered blood units is 1400 BU (2,86 per patient), from that, used are 460 BU (32%), or 0,94 BU per total No of patients. FFP is used rarely, only 32 units, mainly for patients with coagulation disorders or OAT. During this period 7 febrile post transfusion reactions, (3 female: 4 male), are observed - (1,2%).

Conclusion: Blood losses are usually controlled by use of appropriate surgical techniques. Study provides us with the useful information about transfusion practices and contribution to optimize blood usage.

Type of surgery	No patients	Female	Male	ordered BU	used BU	No patients transfused	No patients without transf.
Partial hip rep.	134 27%	103 31,3%	31 19,4%	385 27,5%	89 19,3%	57 1,56BU	77
DHS	306 62,5%	202 61,4%	104 65%	879 63%	350 76,1%	197 1,77BU	109
Total hip rep.	39 8%	19 5,8%	20 12,5%	111 8%	15 3,3%	11 1,36BU	28
Revis. of hip	10 2,5%	5	5	25	6 1,3%	5 1,2BU	5
Total	489	329	160	1400	460	270	219
	100%	**67%**	**33%**	**2,86** BU/patient	**32%** **0,94** BU Patient	**55%** **1,7** BU per transfused patient	**45%**

Table1. Red cell transfusion in orthopaedic surgery 2010–2015

P-576

DOES THE USE OF CELL SALVAGE SYSTEMS PREVENTS RED BLOOD CELL TRANSFUSIONS IN SCOLIOSIS SURGERY?

A Spínola[1], F Mota[2], S Casimiro[2], C Alves[2], A Ramoa[3], S Faria[1], J Condeço[4] and S Sousa[1]

[1]Unidade Local de Saúde de Matosinhos, Matosinhos, Portugal [2]Serviço de Imunohemoterapia, Unidade Local de Saúde de Matosinhos, Matosinhos, Portugal [3]Hemovigilância, Cento do Saúde e da Transplantação do Porto, Porto, Portugal [4]Hemovigilância e Direção Técnica, Cento do Saúde e da Transplantação do Porto, Porto, Portugal

Background: Numerous approaches are used to avoid transfusion of allogeneic blood. Although cell salvage systems (CSS) are widely used in scoliosis surgery, it is not clear whether their use decreases the need for other transfusions. The blood collected by CSS intraoperatively is not always returned to the patient, and there are no current guidelines addressing the amount of blood that is likely to be returned.
Aim: To review the use of CSS in a group of patients undergoing spinal fusion with instrumentation due to scoliosis and determine CSS efficacy in reducing the need for transfusions.
Methods: The study population consisted of 24 patients who underwent scoliosis surgery between 2009 and 2015, and had pre-donated autologous blood consequently receiving oral iron therapy to prevent preoperative anemia. Two groups were formed: Group 1–13 patients who underwent surgery with the use of CSS; Group 2–11 patients who underwent the same procedure without the use of CSS. Descriptive analysis was performed using SPSS v.20, P value < 0.05 was considered significant.
Results: Twenty-four patients, (23 female - 95.8%), were studied with a median age at the surgery of 16 (min. 13-max. 41) years. The patient's average weight was 55 ± 11 kg, 23 patients had lumbar scoliosis and one thoraco-lumbar scoliosis.
The median pre-operative hemoglobin was 12.3 g/dl (Group 1 median 12.5 g/dl; Group 2 median 11.3 g/dl; P = 0.230). The median postoperative hemoglobin was 8.9 g/dl (Group 1 median 8.2 g/dl; Group 2 median 9.6 g/dl; P = 0.072). Twelve patients (50%), 8 from Group 2, used pre-donated autologous blood; 10 patients, 8 from Group 1, were transfused with autologous and allogeneic blood; two patients, one from each group, did not use packed red cells. The median number of units transfused was 2.0 (Group 1 median 3.0; Group 2 median 3.0; P = 0.141) with a median cross-match to transfusion (C/T) ratio of 2.0 (Group 1 median 1.4; Group 2 median 2.0; P = 0.266) and a median transfusion index (TI) of 0.5 (Group 1 median 0.7; Group 2 median 0.5; P = 0.156). In Group 1 the mean CSS recovered volume was 205 ± 116 ml.
Overall, median hospital lengths of stay were 9 (min. 6-max. 15) days (Group 1 median 9 days; Group 2 mean 9 days; P = 0.516).
Conclusion: During this study, we found no statistical differences between groups regarding all parameters analyzed. The use of CSS did not reduce the need for other transfusions in scoliosis surgery. Therefore it seems that there is no benefit in the use of cell salvage systems and pre-donated autologous blood in our patient population.

P-577

BLOOD ORDERING PRACTICES IN ELECTIVE UROLOGIC SURGERY

A Spínola, M Ponte, C Soares, R Lopes, S Faria and S Sousa

Unidade Local de Saúde de Matosinhos, Matosinhos, Portugal

Background: The amount of blood requested and cross-matched is usually determined based on routine, habit and previous experience. It is essential to adjust blood ordering to the type of surgery and patient's comorbidities for an efficient blood usage, which can be translated in transfusion indexes such as Cross-match to Transfusion (C/T) ratio, Transfusion Probability (TP) and Transfusion index (TI). The C/T ratio ≤1.5 is a significant indicator of the need for blood transfusion during surgery. A TP ≥0.30 indicates considerable requirement of blood and TI ≤0.5 cross-match before surgery is not necessary and it is sufficient to determine the patient's ABO and Rh groups and perform an antibody screen.
Aims: Assess blood ordering for various types of elective urological surgery. The purpose was to have a better understanding of which surgical procedures required more blood in order to decrease unnecessary requests, implement institutional protocol and minimize costs.
Methods: A retrospective study, through 2015, of blood-ordering patterns and blood use for elective urologic procedures was done. The following parameters were calculated: C/T ratio (number of units cross-matched/number of units transfused), TP (number of transfused patients/number of cross-matched patients), TI (number of transfused RBC units/number of cross-matched RBC units). Descriptive analysis was performed using SPSS v.20.
Results: Of the 204 patients studied, 174 (85.3%) were male, median age was 67 (Min. 26- Max. 92) y.o and the median age at the surgery was 66 y.o. 135 (66.2%) of the patients had ASA score of 2 and 107 (52.5%) had surgery due to benign cause. Median pre-operative haemoglobin concentration was 13.7 (min. 7.1-max.17.0) g/dl and post-operative haemoglobin was 11.2 (min. 6.8-max.15.2) g/dl. A total of 237 blood requests were made for 215 procedures, median of 1.1 requests per surgery. Each transfusion order requested a median of 2 (min. 0- max.4) blood units. The median number of units transfused was 0 (min. 0- max.7). Overall, the C/T ratio was 7.7, the TI was 0.30 and the TP was 0.07. Only 18 (8.8%) of the patients needed blood transfusion both previous to surgery or in the operating theatre and 20 (9.8%) required transfusion within the 96 h after surgery.
The 215 procedures were grouped in 6 major categories: prostatectomy (118; 55.9%), nephrectomy (39; 18.1%), nephrostomy (24; 11.2%), cystectomy (11; 5.1%), orchiectomy (2; 0.9%) and other minor procedures (21; 9.8%). The median number of units of blood requested and transfused as well as the C/T ratio, TP and TI for each category are in Table 1.
Conclusions: The sum of blood requested and cross-matched for elective urological surgery is greater than the real level of consumption, demonstrated by C/T ratio >1.5, TP <0.3 and TI <0.5. Only in a few surgical procedures, such as cystectomies, do the patients commonly receive blood transfusions. Blood is rarely used for a large number of other procedures. In such cases routine cross-matching of blood can be safely substituted by 'type and screen.' An appropriate, standard blood order protocol would improve blood utilization without jeopardizing patient care, reduce costs and staff workload.

Elective urologic procedures	Number of procedures	Number of blood requests	C/T ratio	Transfusion index	Transfusion probability
Prostatectomy	118	122	22	0.10	0.02
Nephrectomy	39	46	6.5	0.36	0.15
Nephrostomy	24	24	48	0.04	0.04
Cystectomy	11	21	1.75	2.67	0.67
Orchiectomy	2	3	6	0.50	0.50
Other minor procedures	21	21	4.3	0.59	0.41

Table 1. Summary of transfusion trends

P-578

ASSESSMENT OF IMMATURE PLATELET FRACTION IN THROMBOCYTOPENIC PATIENTS: IS THERE A PATTERN ACROSS ETIOLOGIES?

N Mohanan and J Innah

Jubilee Mission Medical College, Thrissur, India

Background: Two major mechanisms implicated in the pathogenesis of thrombocytopenia are increased peripheral platelet destruction or decreased bone marrow production.Reticulated platelets (RP) are the youngest circulating platelet population, analysis of which provides a good estimate of the rate of platelet production in bone marrow, a quantifiable representative of which referred to as Immature Platelet Fraction (IPF). This could be a sensitive and non-invasive test, capable of evaluating thrombocytopoietic activity and would be of substantial clinical value in deciding upon transfusion practices on serial monitoring.

Aim: To determine the IPF range among healthy volunteers and analyse its pattern of variation in patients with thrombocytopenia across varied etiologies.

Methodology: Prospective 4 month,observational study of thrombocytopenic patients(platelet count <1.5 lakhs) grouped into three-

Group A: Central Thrombocytopenias

Group B: Peripheral consumption

Group C: Infectious Etiologies

100 random healthy blood donor samples as controls were analysed forplatelet count and immature platelet fraction (IPF) using Sysmex XN 1000 automated haematology analyser within 6 h of collection. Patients' clinical history along with diagnosis were compared with this parameter.

Results: The median platelet count among control was 269×10^9/L ranging from 171×10^9 to 450×10^9 /L; mean IPF was 2.13%(SD 1.05) and ranged from 0.5 to 5.5% (median: 1.9%). No significant difference noted among mean IPF or their range among males and females.

Patients Sample:

There were128patients.14 in Group-A(AML,Aplasticanemia,Megaloblasticanemia),60 in group-B(ITP,HELPP syndrome,CLD) and 54 inGroup-C (Dengue fever,Malaria, Fever with thrombocytopenia,Sepsis) respectively.

AmongAML patients,mean IPF was 4.17%, and in aplastic anemia, 5.8% indicating a failure in platelet production by the marrow.

Autoimmune thrombocytopenic purpura(ITP)patients demonstrated the highest IPF% (19.1%). In Immune thrombocytopenias, an inverse correlation was found between platelet count and IPF% .ITP cases were correlated with bone marrow findings.

In HELLP syndrome, average IPF was 16.1% (SD: 8.77%), serial IPF monitoring could be capable of screening normal pregnancy progressing for PIH, preeclampsia

Sepsis, dengue, malaria and fever average IPF were 8.5%, 13%, 10.75% and 10.96% respectively,which showed there could also be a mild bone marrow depression even though the values are above healthy reference range.

Conclusion: IPF determination could be non-invasive test for differential diagnosis between thrombocytopenia with increased thrombopoietic activity and thrombocytopenia with normal or decreased thrombopoieticactivity.

IPF could be a parameter to monitor infectious disease eg Dengue, malaria and accordingly to advice on platelet transfusions by serial monitoring.

According toBCSH, Guideline 2003 ITP diagnosis a bone marrow examination is unnecessary in adults unless there are atypical features.So in cases with typical features, IPF can aid the diagnosis without an invasive bone marrow study.

Chronic Liver Diseases patients could have alcohol induced bone marrow supression as well as peripheral destruction due to splenic sequestration, the results of which may be the mixed IPF values.

P-579

ASSESSMENT OF PLATELET TRANSFUSION IN BCT IN ACUTE LEUKEMIA

I Frigaa[1], A Bastandji[2], H Mahnaoui[3] and H Ouelaa[1]

[1]Blood Bank Center of CHU Annaba/Faculty of Medecine, Annaba, Algeria [2]Clinique pédiatrique St Thérèse, Annaba, Algeria [3]Service d'Hématologie-Dorban Hospital, Annaba, Algeria

Background: The evaluation of yield platelet transfusion is a way of testing the various factors influencing the efficiency of transfusion. Generally this assessment is made on the clinical and biological response but it's inadequate.

Aims: This study aims to calculate the yield platelet transfusion, and detecting the reasons of inefficiency transfusion.

Methods: This is a prospective study, during 6 months, evaluating the transfusion efficiency of 111 blood platelet transfusions performed in 64 thrombocytopenic patients followed for acute leukemia.The CCI (plt $\times$ m^2/ μL) for all transfusions was calculated.

Results: Half of the patients have an initial platelet count $<10 \times 10^9$ .Bleeding evaluation comprehended petechiae, haematomas, nose and mouse bleeds, bloody stools and haematuria. 62.50% of transfused patients present a hemorrhagic syndrome (level 02), while 24.66% are clinically stable (no bleeding). The results found an early platelet yield effective of 80, 71%, and ineffective in 27 cases with a percentage of 19, 28%. The study of factors influencing on ineffective yield platelet transfusion showed that fever, antibiotic therapy, hemorrhage, insufficient dose platelet and splenomegaly are responsible for the reduction of the yield of platelet transfusion.

Conclusions: The evaluation of this parameter is a very useful for the clinician and the transfusing physician to adopt better conditions for platelet transfusion.

P-580

COMBINED USE OF CHLORTETRACYCLINE AND PLATELET RICH PLASMA FOR THE TREATMENT OF INFECTED DIABETIC FOOT ULCERS

G Di Domenico, GM Leonardi, G Vaccaro and C Nocera

Immunohaemathology and Transfusion Medicine, Hospital "S.G.Bosco" ASL NA1Center, Naples, Italy

Background: Nonhealing diabetic foot ulcers are a major complication in patients with diabetes and are a major cause of lower limb amputation. The bacterial infection of the diabetic foot is the most important cause of treatment failure. The Chlortetracycline is an antibiotic that can be used for topical use in the treatment of cutaneous infections caused by bacteria Gram + and Gram-. The enhancement of soft tissue healing by the application of platelet rich plasma gel is supported by basic science and numerous clinical studies.

Aims: The aim of our study was to evaluate the efficacy of treatment by combination of the Chlortetracycline applied topically and homologous platelet rich plasma (PRP) gel on infected diabetic foot ulcers.

Methods: A total of 50 diabetic patients (30M/20W; age range: 42–78), with infected and inveterate skin ulcers of feet at different localization (heels, toes), was analyzed. The ulcers of the majority of patients occur with well-defined edges, shapes, surrounded by thin skin, atrophic, often inflamed. The operating clinical protocol included medical history, physical examination (with measuring and photography ulcer), blood tests and instrumental tests (echo-color-doppler arteries and veins of the lower limbs). The homologous PRP gel was produced by units of platelets derived by pool of buffy-coat or plateletpheresis. In order to produce the gelatinous material, the PRP gel was activated with thrombin 1/10 and 10% calcium chloride ($CaCl_2$). The clinical treatment of the foot ulcers was as follows: cleansing with Cadexomer iodine and eventual surgical debridement of the lesion, topical application of Chlortetracycline ointment 30 mg (3%) covered by a layer PRP gel combined by gauze Argentinian Sulfadiazine/ hyaluronic acid sodium salt and subsequent occlusion. The dressing was repeated every 3 days, until complete healing of the ulcer. The therapy had an average term of one month for each patient.

Results: The association of Chlortetracycline ointment and PRP gel has shown since the early days of treatment a rapid improvement of the treated ulcers characterized by reduction in pain, exudate and the lesion diameter, with rapid onset of granulation tissue. At a distance of 30–40 days from the beginning of the treatment all ulcers (100%) came to healing with the appearance of 'healing crust' and subsequent re-epithelialization of lesions.

Conclusions: The complexity and the poor outcome associated with healing chronic diabetic foot ulcers necessitate the need for a multi-disciplinary approach to treating these groups of patients. The data of this study demonstrate for the first time in the literature, that the combination of a topical ointment-antibiotic, Chlortetracycline, and autologous PRP gel, induce a rapid recovery of infected diabetic foot ulcers, trough an antimicrobial therapeutic effect combined with the release of growth factors and cytokines produced by PRP gel. Although, this combined treatment appear promising, more properly structured clinical randomized controlled trials will be required to confirm these results and to establish under which conditions the application of platelet-rich plasma has merit.

P-581

HEALING CHRONIC WOUNDS WITH PLATELET GEL

M Bini Antunes, M Sampaio, R Oliveira, F Dias, M Lopes, R Carvalho, H Neto, R Almeida, I Silva, J Tavares, L Loureiro, P Almeida, A Matos, J Coutinho and M Amil

Centro Hospitalar do Porto, Porto, Portugal

Background: Topical application of platelet gel has been increasingly used to accelerate and improve tissue repair processes, particularly in wounds difficult to heal.
Aims: Prospective assessment of patients with chronic wounds treated with allogenic platelet gel.
Materials and Methods: Platelet gel was produced from platelet concentrate (PC) obtained from volunteer whole blood donors. Each aliquot of PC was activated with allogenic thrombin and calcium gluconate. All patients included in this study had chronic wounds (over six months of evolution) refractory to conventional treatments. Patients were followed prospectively with weekly follow-up registering demographics, comorbidities, wound aetiology (diabetic, neuropathic, venous ulcers, arterial ulcers, mixed ulcers), characteristic of the wound (size and tissue present: granular, fibrin, necrosis, presence of exudate, inflammatory signs associated), pain intensity if present, number and time interval between applications, adverse reactions, and efficacy evaluation: complete healing, partial improvement (50% reduction in the wound area and/or amelioration of pain assessed on a scale from zero to ten) or wound without evolution.
Results: Study lasted between July 1st, 2015 and March 15th, 2016, and included 26 patients: 12 with one or more wounds associated with diabetes, 5 with ulcers associated to peripheral arterial disease, 3 with mixed ulcers, and 6 with venous ulcers. Diabetic patients (10 men and 2 women, median age of 71 years old, range 52–86) had mainly plantar ulcers (median area 1,75 cm^2, range 1–24 cm^2). Comorbidities among diabetic patients were: hypertension (8), dyslipidaemia (6), neuropathy (5), peripheral arterial disease (4), obesity (4), ischemic heart disease (3), retinopathy (3), and nephropathy (1). Seven of the 12 patients had at least three comorbidities simultaneously and also seven out of 12 had wounds for over 12 months of evolution. The median number of applications was 13 (range 5–26) for 8 weeks (range 2–15) with complete healing in 4 patients, partial response in 4 patients, wound without evolution in 2 patients and treatment interruption in 2 (1 with uncontrolled glycaemia and 1 with poor compliance). The 14 patients without diabetes (9 females and 5 males, median age of 70 years old, range 42–89) had generally exudative wounds (median area 6,21 cm2, range 1–19 cm2) with significant associated pain. They were submitted to a median of 9 treatments in 6 weeks with no evolution in 6 patients, interruption because of infection in 4 patients, partial improvement in three patients (two with venous and one with arterial disease) and 1 patient with peripheral arterial disease without reduction in wound area but with clear improvement in pain.
Conclusion: We consider that platelet gel is an adequate therapeutic alternative for chronic wounds related to diabetes and refractory to conventional therapies. We observed a good evolution, particularly in neuropathic diabetic wounds without significant arterial disease. Although non-diabetic patients had less comorbidities, when compared to diabetic patients, we observed a worse outcome in these. One possible explanation may be the fact that these patients presented with larger ulcers areas and with a significant ischemic disease.

P-582

PLATELET GEL APPLICATION IN SURGERY

M Sampaio, M Bini Antunes, F Dias, P Pina, B Leitão, J Coutinho and M Amil

Centro Hospitalar do Porto, Porto, Portugal

Background: Platelet gel has been applied in surgical field due to its haemostatic properties and capability for promoting adhesion and tissue regeneration.
Aims: Prospective evaluation of surgical patients proposed for treatment with platelet gel.
Materials and Methods: Patients were evaluated by assistant surgeon and proposed for therapy with allogenic platelet gel. Platelet gel was produced from platelet concentrate (PC) obtained from volunteer whole blood donors. Each aliquot of PC was activated with calcium gluconate and allogenic human thrombin. Exclusion criteria for applying platelet gel intraoperatively or postoperatively included presence of infection or uncontrolled malignancy. Patients follow-up included registration of demographics, underlying diagnosis, type of application (intraoperative or postoperative), lesion location and characteristics (surgical hollow, fistula), number of gel applications and time interval, and efficacy evaluation: complete healing, partial improvement (reduction of hollow volume, fistula extension, granulation tissue onset, amelioration of pain), or wound without evolution.
Results: Of the six patients treated, three had anal fistula in the context of inflammatory bowel disease: patient one, 45 years old, male, had three platelet gel applications with complete closure of fistula; patient two, a male 56 years old, with multiple anal fistulas and history of squamous cell carcinoma within one of them, with complete distortion of perineal anatomy, had 12 platelet gel application over three weeks with good evolution being proposed for retail placement skin surgery; patient three, 70 years old, male, with anal fistula had four platelet gel applications over our weeks with partial improvement but without complete resolution of the fistula. Patient four, 34 years old, male, with an oesophageal cutaneous fistula after surgery for Zencke diverticulum, had six platelet gel applications over three weeks and complete closure of the fistula. Patient five, 92 years old, female, with history of rectal carcinoma and an extensive rectal hollow after rectal biopsy had four platelet gel applications over two weeks with significant hollow reduction and quality of life improvement. Patient six, 53 years old,female, with history of osteosarcoma of the jaw three years before, had reconstructive surgery of the oral cavity with autologous bone graft from the iliac crest mixed with allogenic platelet gel, placement of cadaveric allograft and titanium condyle prosthesis. This patient had a good outcome with good volume of bone reconstruction and was discharged three weeks later.
Conclusion: Platelet gel application is an effective therapeutic option to facilitate tissue regeneration in surgical patients with fistulous tracks, surgical complications or as a base for intraoperative use mixing autologous bone to facilitate its graft.

P-583

This abstract has been withdrawn.

P-584

THE PREDICTIVE RISK FACTORS OF BLOOD TRANSFUSION REACTION

JH Chien[1], HF Liu[1], TL Zheng[1], CC Kuo[1]and HY Chuang[2]

[1]Taichung Tzu-Chi Hospital, Buddhist Tzu Chi Medical Foundation, Tanzih Town, Taichung, Taiwan [2]Taichung Tzu Chi Hospital, Taiwan

Background: Transfusion reaction is not a rare complication of blood transfusion. To avoid relevant side effects, we try to clarify the probable risk factors of blood transfusion reaction. In this study, certain pre-transfusion basic data including age, gender, body temperature and white blood cell counts, suggests to correlate with the consequent blood transfusion reaction.
Aims: The aim of the study was to focus on the potential risk factors that could lead to common transfusion reaction, e.g. hypertension, fever or infection. We conducted a retrospective analysis of the blood transfusion recipient vital signs and laboratory data before and after blood transfusion in our hospital between 2010 and 2014.
Methods: A retrospective analysis of 19035 patients was included in this study. All symptoms and signs were recorded during the transfusion procedures; including body temperature, respiratory rate, systolic and diastolic blood pressure, and white blood cell count (WBC). The patients were divided into two groups: patient with

Table 1. Baseline characteristics of all transfusion patients

Characteristics	All	TRs	No TRs	p-value
Patient no.	19305			
Mean age, years (±SD)	64±15	60±16	64±15	<0.001
Respiratory rate	18±5	19±4	18±5	0.007
Body temperature(℃)	36.5±0.7	36.8±0.6	36.5±0.7	<0.001
Pulse rate	93±27	92±20	93±27	0.583
Gender				0.003
Female	8190(42.4%)	233(49.1%)	7957(42.3%)	
Male	11115(57.6%)	242(50.9%)	10873(57.7%)	
WBC				<0.001
Normal(5000-15000/ul)	11882(61.5%)	243(51.2%)	11639(61.8%)	
Abnormal(out of normal range)	7423(38.5%)	232(48.8%)	7191(38.2%)	
Systolic pressure				0.014
Normal(90-110 mmHg)	14697(76.1%)	339(71.4%)	14358(76.3%)	
Abnormal(out of normal range)	4608(23.9%)	136(28.6%)	4472(23.7%)	
Diastolic pressure				0.125
Normal(70-90 mmHg)	17978(93.1%)	434(91.4%)	17544(93.2%)	
Abnormal(out of normal range)	1327(6.9%)	41(8.6%)	1286(6.8%)	

TRs: Transfusion reactions, No TRs: No Transfusion reactions
P value were calculated with Chi-Square by comparison of transfusion and no transfusion reaction.

Table 2 The cumulative rate of transfusion reaction events in difference risk factors

Characteristics	n	Events(%)	p-value
Gender			0.003
Female	8190	233(2.8)	
Male	11115	242(2.2)	
WBC			<0.001
Normal(5000-15000/ul)	11882	243(2.0)	
Abnormal(out of normal range)	7423	232(3.1)	
Systolic pressure			0.014
Normal(90-110 mmHg)	14697	339(2.3)	
Abnormal(out of normal range)	4608	136(3.0)	
Diastolic pressure			0.125
Normal(70-90 mmHg)	17978	434(2.4)	
Abnormal(out of normal range)	1327	41(3.1)	

P value were calculated with Chi-Square.

transfusion reaction and non-transfusion reaction. Group characteristics were compared using X^2 test. Multivariable logistic regression model was applied to estimate odds ratios and 95% confidence intervals (95% CI) for the incidence of transfusion reaction.

Results: There were 475 patients were reported with transfusion reaction. The overall incidence of transfusion reaction was 2.5%. The patient with relatively higher body temperature and abnormal systolic blood pressure during pre-transfusion period could develop transfusion reaction (OR 1.92; 95%CI; 1.66–2.22; $P < 0.001$ and OR 1.26; 95%CI; 1.26–1.58; $P = 0.040$). Besides the patient vital signs, WBC was considered as another important risk factor of transfusion reaction.

Conclusions: The present guideline suggests monitoring the vital signs while processing blood transfusion. During pre-transfusion period, not only body temperature but blood pressure, respiratory rate and white blood cell count could be the predictive factors of the blood transfusion reaction.

P-585

DECREASED INTRA-OPERATIVE BLOOD LOSS WITH NON-SCRAPING TECHNIQUE OF SPLIT SKIN GRAFTING FOR GRANULATING BURNWOUNDS

D Gupta, D Krishna, S Kumar and U Sharma

Safdarjung Hospital and Vardhaman Mahavir Medical College, Delhi, India

Background: Chronic burn wounds with a granulating surface require skin grafting for coverage. This is usually achieved by split thickness skin grafting (SSG) after preparation of the wound bed. The preparation involves debridement of granulation tissue, the overall goal being creation of an optimal wound healing environment by producing a well-vascularized, stable wound bed with little or no exudates. Though these objectives are achieved through debridement, it is associated with increased blood and protein loss, requirement of blood component therapy, time intensive hemostasis, and physiological insult to the patient. Thus, we studied the non-scraping technique of SSG to reduce the detrimental consequences of debridement.

Aims: Comparison of amount of blood loss and the outcome of split skin grafting in patients with chronic burn wounds treated with or without scraping of the granulation tissue.

Methods: Prospective randomized trial conducted in 30 patients between 15 and 60 years of age, without known comorbidities, presenting with post burn raw area $\geq$200 cm^2 in dimension and wounds of 4–10 weeks duration. Intraoperative calculation of wound area was done using a graph paper, followed by scraping of half of wound (Group A) and leaving the granulation tissue intact in the remaining half (Group B). Intraoperative blood loss was calculated using gravimetric method and meshed SSG was done followed by dressing and splintage. Dressings were done at post-operative days 3, 5, 7, 10 and as required thereafter, to assess for graft take and requirement of re-grafting.

Results: Area of wound (161.67 $\pm$ 53.92 in Group A Vs 163.48 $\pm$ 49.48 in Group B, P value= 0.73) and mean graft uptake (87.2 $\pm$ 9.48 in Group A Vs 86.58 $\pm$ 11.18 in Group B, P value = 0.55) were similar in the two groups. Average intraoperative blood loss was significantly lesser in Group B (7.2 $\pm$ 1.5 ml/100 cm^2) in comparison to group A (54.58 $\pm$ 9.4 ml/100 cm^2). There was no requirement of re-grafting in both the groups. Duration of complete wound healing was significantly increased in group B (14.66 $\pm$ 2.79 days in Group A vs 15.73 $\pm$ 3.13 days in Group B).

Conclusion: Our study showed that the non-scraping technique,is associated with a lesser intraoperative blood loss, decreased oozing of tissue fluids and reduced need for time intensive hemostasis without affecting the overall graft take. This technique can therefore bring about a decrease in the requirement of perioperative blood and blood component therapy during SSG of granulating burn wounds.

P-586

EFFECTS OF THERAPEUTIC PLASMA EXCHANGE ON COAGULOPATHY AND LIVER FUNCTION TESTS IN CRITICALLY ILL LIVER DISEASE PATIENTS

M Bajpai, A Sharma and P Jain

Institute of Liver and Biliary Sciences, New Delhi, India

Background: In the setting of acute liver failure (ALF), extracorporeal liver support systems (ECLS) provide a potential therapeutic option to temporarily support hepatic function, and thereby create a window of opportunity for intrinsic hepatic regeneration and recovery, or as a bridge for liver transplantation. There is evidence base in critically ill liver disease patients that Therapeutic Plasma Exchange (TPE) may play a role in the therapeutic strategy.

Aims: This study was conducted to analyse the effects of Therapeutic plasma exchange on coagulopathy and liver function test in critically ill liver disease patients.

Methods: It is a retrospective study conducted at Institute of Liver and Biliary Science, New Delhi. It included 60 patients of critical liver disease who underwent 144 cycles of TPE (median session 2.6) since January 2014 to March 2016. Out of these 60 patients, 10 were of paediatric age group which were analysed separately. We analysed the pre and post-TPE laboratory data of the patients which included complete blood count, Coagulation parameters, Liver function tests, Renal function tests and serum electrolytes. The procedural details including estimated total plasma volume exchanged, number of repeated cycles or any complications associated with TPE were documented. The data was analysed statistically using SPSS software.

Results: There were statistically significant changes seen in pre and post TPE Hemoglobin(Hb) ($P < 0.001$), Platelet counts(PC) ($P = 0.005$), Prothrombin time(PT) and International Normalised Ratio(INR) ($P < 0.001$), Serum Bilirubin including both direct and Indirect ($P < 0.001$), Serum Alanine transaminase(ALT) ($P < 0.001$), Aspartate transaminase(AST) ($P < 0.001$), Alkaline phosphatase(ALP) ($P < 0.001$), Gamma Glutamyl transferase (γGGT) ($P = 0.002$), Serum total proteins with globulin fraction($P < 0.001$) and serum potassium ($P = 0.01$). On comparing the two groups of patients, based on the plasma volume exchanged (1–1.5 vs 2) times, the laboratory parameters in the group with Plasma exchange with 1–1.5 times plasma volume yielded equal or even slightly better results than 2 volume exchange.

We also tried to analyse the effect of TPE in paediatrics population separately. There were 10 children who underwent 28 sessions (average 2.8) of TPE. On statistical analysis, no significant difference was found between the pre and post-TPE values in these paediatric populations.

Conclusion: In view of this study and the current lack of evidence supporting the efficacy of other extracorporeal devices, TPE should be considered as an effective and safe option in cases with hyperbilirubinemia. Further randomised studies are required to assess their efficacy in ALF with attention to non-survival endpoints such as bridging to liver transplantation.

P-587

FREQUENCY OF ABO, RH PHENOTYPE AND PROBABLE GENOTYPE IN PATIENTS WITH BLOOD TRANSFUSION DEPENDENT DISORDERS

N Anwar, M Borhany, SH Ansari, S Khurram, M Nadeem and T Shamsi

National Institute of Blood Disease and BMT, Karachi, Pakistan

Background: ABO and Rhesus blood group systems are the most important blood group systems in blood transfusion being implicated most importantly in delayed hemolytic transfusion reactions and Hemolytic disease of new born (HDN).

Aim: The main objective of the study was to determine the frequency of ABO and Rh phenotype and thus reduce the rate of alloimmunization in patients with blood diseases requiring frequent blood transfusions especially Thalassemia Major.

Methods: This observational study was conducted at the Blood Bank Department of National Institute of Blood Disease and Bone Marrow Transplantation (NIBD) Karachi, Pakistan from August 2012 to October 2014. A total of 242 blood disorders

patients requiring frequent blood transfusion were enrolled in the study and ABO and Rh typing were done through tube and gel micro typing system.
Results: Out of a total of 242 patients, 146(60.4%) were males and 96(39.6%) were females. Frequency of ABO and Rh D phenotype among the patients was as follows: O +ve (38.8%), O-ve (2.5%), B+ve (32.2%), A+ve (17.4%), A-ve (1.7%),AB+ve (7.4%).232(95.8%) were Rh D positive while 10(4.2%) patients were Rh D negative. The most frequent Rh antigen was found to be e(97%), followed by D(95%),C(89.6%) then c(62.8%) and finally E(22.6%). The overall frequency of Rh phenotypes was: R_1R_1 (37.2%), R1r (33.1%), R_1R_2(19.0%), R_2r(5.0%), rr(4.1%) whereas in females the most frequent Rh phenotype was R_1r(38%). In all the Rh D negative patients the Rh phenotype was rr.
Conclusion: In our study, the highest frequency of ABO phenotype among the patients was O. The most frequent Rh antigen was e. The overall frequency of Rh phenotypes in order of frequency was: $R_1R_1 > R_1r > R_1R_2 > R_2r$ and rr. Thus, Rhesus antigenic phenotyping and genotyping along with antibody screening and identification prior to transfusion of patients requiring multiple transfusions should be done to prevent and reduce the rate of alloimmunization.

P-588

DETECTION OF PNH RED CELL POPULATION IN A COHORT OF PATIENTS BY USING THE GEL TEST

N Fareed, M Borhany, I Naseer, M Nadeem and T Shamsi
National Institute of Blood Disease and BMT, Karachi, Pakistan

Background: Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal stem cell disorder. The pathogenic mechanism of PNH is a deficiency of glycosylphosphatidylinositol (GPI) anchored proteins, which results in an abnormal sensitivity of red cells to complement. An increase of blood cells with a paroxysmal nocturnal hemoglobinuria (PNH) phenotype is often detected in patients with acquired aplastic anemia (AA). The gel card test is a useful screening tool to detect red cell antigen-antibody reactions.
Aim: The objective of study was the detection of PNH red cell population (CD55-ve & CD59-ve) using the PNH gel card for the investigation of a PNH clone where flow cytometry test was not available.
Methods: Thirty consecutive patients with suspected PNH were included in this cross sectional study, conducted at NIBD, 2013–2014. All patients' peripheral blood samples were collected in EDTA and evaluated through ID PNH gel card (BIO RED) screening test. Clinico-pathological parameters were used for statistical analysis.
Results: A total of 30 patients were enrolled including 19(63.4%) males & 11 (36.6%) females. Median age was 36.5 years (range 09–64 yrs). Out of 30, 25 (83%) patients were diagnosed as Aplastic Anemia (AA), 4 (13%) patients were diagnosed as Hemolytic Anemia & 1(3%) patient of Non-Hodgkin lymphoma. Only 03(10%) patients showed significant population of PNH red cells 2 suffering from Aplastic Anaemia & 1 from hemolytic anaemia. Double cell populations (positive reactions) were demonstrated by the PNH gel test in all 3 blood samples.The presence of PNH clone could not be confirmed in these patients due to non availability of flowcytometry for PNH.
Conclusion: In our center only 10% patients showed PNH cells. The gel test appears to be useful as a screening test for PNH because of its simplicity and increased ability to diagnose PNH. To further validate the usefulness of the PNH gel test, larger sample size without blood transfusion is needed. We missed the PNH patients by gel card due to prior multiple blood transfusions. This technique would be especially easy to introduce in laboratories that are already using this system for blood grouping and cross matching.

P-589

IMPORTANCE OF DISTINCTION BETWEEN WEAK D AND PARTIAL D STATUS IN ALLOGENIC BONE MARROW TRANSPLANT PATIENT – A CASE REPORT

P Parikh, S Ojha, M Poojary, A Tirlotkar, S Sumathi, S Kulkarni and S Rajdhakysha
Tata Memorial Centre, navi mumbai, India

Background: Transfusion support is very important in bone marrow transplant patients. Extended partial D and weak D typing in suspected RhD incompatible allogenic bone marrow transplants is essential to prevent post transplant complications like hemolysis, delayed engraftment and red cell aplasia and it helps in transfusion of appropriate blood components.
A 48 yr old male patient of Acute Lymphoblastic Leukemia with blood group O RhD positive posted for allogenic BMT with a 44 yr old sibling of patient with blood group O suspected weak RhD positive as BMT donor. Proper approach is needed to resolve such cases. Partial D and Weak D are most commonly found D variants which should be considered significant if there is any discrepancy in serological testing obtained with different anti-D reagents.
Specific panels of 12 monoclonal anti-D reagents have the potential to characterize most common 15 Partial D variants and Weak D Type 1 & amp 2 depending on the reactivity pattern.
Aim: To emphasize the importance of extended partial -D typing in suspected RhD incompatible allogenic BMT patients.
Material and Methods: Manufacturer's instructions were followed and RhD typing was done by monoclonal and polyclonal anti-D. Weak D/ Partial D analysis was done using Indirect Antiglobulin Test (IAT) procedure by conventional tube and gel card methods and phenotyping by monoclonal anti- D epitope panel.
Results: In this case no discrepant result was observed in RhD typing in patient but Donor's RhD typing showed a weak reaction with monoclonal anti-D and negative reaction with polyclonal anti- D. IAT procedure showed presence of Weak D and phenotyping by monoclonal anti-D epitope panel showed reactivity pattern of Weak D ruling out presence of Partial D.
Conclusion: After extended RhD typing, this patient received RhD positive blood components which helped in conserving rare RhD negative blood components. Decisiveness of Partial D or Weak D status is major decisive point as different transfusion protocols would be pursed to prevent complications like hemolysis, delayed engraftment and red cell apalsia in allogenic BMT patients due to alloimmunization.

P-590

DETERMINATION OF SERA NEUTRALIZATION ANTIBODIES AGAINST H1N1 AND H3N2 IN BLOOD DONORS IN GUANGZHOU

X Rong, H Wang and Y Fu
Guangzhou Blood, Guangzhou, China

Background: Influenza is the most common but deadly respiratory illness caused by influenza virus infection. Humoral virus neutralizing activity is crucial in the protection against influenza virus infection. However, the status of the influenza neutralizing activity of serum in the general population remains unclear. In this study, we conducted a serological survey using samples from healthy blood donors in Guangzhou, China, a city neighboring HongKong and Macau. These cities represent the central cities of the Pearl River Delta region where many different subtypes of influenza A virus broke out and serological survey in this region should be of great relevance in epidemiological.
Aims: To estimate the positive rate and titer of neutralizing antibody against H1N1 and H3N2 influenza virus in blood donations in Guangzhou.
Methods: 498 sera samples were collected in December 2015 from Guangzhou, China to further estimate Flu A neutralizing activity in healthy individuals who were divide into 3 group according to age points of 18–20, 21–30 and 31–60. Titer of neutralizing antibodies of H1N1 and H3N2 influenza virus were measured by microneutralization (MN) assay. In particular, the specific antibody against hemagglutinin was measured (hemagglutination inhibition assay, HI). To compare the neutralizing antibodies against different subtypes of Flu A viruses, MN and HI were both performed against 2009 pandemic H1N1 (NIBRG-121), H3N2 (A/Victoria/361/2011). and test results were statistically analyzed.
Results: The positive rates of H1N1 and H3N2 were 3.61% (18/480) and 4.62% (23/498) respectively, and no statistical difference were found between these two influenza virus subtype ($P > 0.05$). 6 of 156 male samples displayed neutralizing activity against H1N1(3.85%) and 7 against H3N2 ;while in 342 female donors,11(3.22%) showed H1N1 neutralizing activity and 15(4.39%) of H3N2, The P-value for gender-associated was of no statistically differences ($P > 0.05$). The positive antibody rate and Geometric titer(GMT) of H1N1 for each group were as follows: 4.00% and 1:1.38 for the 18–20 years old group, 5.24% and 1:1.48 for the 21–30 years old group, 0.72% and 1:1.05 for the 31–60 years old group. The positive antibody rate and GMT of H3N2 in three groups were 6.00% and 1:1.62, 5.24% and 1:1.51, 2.17% and 1:1.19 respectively. There were no significant differences in the antibody positive rate of H1N1 and H3N2 in each age groups ($P = 0.087$), neither were titer($P = 0.259$).
Conclusions: The neutralizing antibody level of influenza virus were low in healthy blood donors in Guangzhou area. There was no significant difference in the positive rates between H1N1 and H3N2 in different genders and different age groups. There were no significant differences in the antibody titers of H1N1 and H3N2 in each age groups, which may suggest a generally susceptible to influenza virus in Guangzhou city.

P-591

PREVALENCE OF ABH SECRETOR AND NON -SECRETOR STATUS: A CROSS SECTIONAL STUDY IN A TERTIARY CARE HOSPITAL OF BANGLADESH

A Islam

BSMMU, Dhaka, Bangladesh

Background: The prevalence rate of ABH secretor and non-secretor status has a varied degree of association with certain diseases in clinical and forensic medicine along with geographical and racial differences.

Objective: The main objective of the study was to find out the prevalence rate of secretor and non-secretors status among the random healthy blood donors in a tertiary care hospital of Bangladesh.

Materials and Methods: During a six month period from January 2011 to June 2011, a total of 351 populations- Voluntary healthy blood donors, relatives of patient who willingly come to donate blood for their patients and post graduate medical students & Healthy staff of BSMMU attended in the Department of Transfusion Medicine, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh were enrolled as study sample. Between the ages range of 15 to 45 years, blood and saliva was collected from each healthy individual. For both ABO and Rhesus blood grouping, the conventional tube method was applied, whereas hemagglutination inhibition method of saliva was used to ascertain the secretor status of the studied population.

Results: Among 351 study population, 296 (84.33%) of healthy adults were male and 55 (15.67%) were female and the mean (±SD) age was 26.46 ± 7.78 years. Prevalence of secretor status and non-secretors status among all blood donors were 47.9% (168) and 52.1% (183) respectively. Secretor status was common in respondents with A (39- (23.2)) and AB (9- (5.4)) blood group and non-secretors status was common in B (114- (62.3)) and O (69- (37.7)) blood groups. In consideration of gender, non- secretor status had highly significant association with male gender ($P < 0.01$ (male 164 (89.6) and female 19 (10.4).

Conclusion: Prevalence of ABH non-secretor status is slightly higher than secretor status (52.1% vs 47.9%) in male donors. Secretor status is common in male (78.6%) than female (21.4%). Blood group B has the highest non-secretor frequency (114 (62.3)) and blood group 0 has the lowest 69 (37.7)).

Keywords: ABH, Secretors, Non-secretors, Gender, Bangladesh.

P-592

RETROSPECTIVE STUDY ON THE CURRENT STATUS OF BLOOD PRODUCT TRANSFUSIONS IN PATIENTS WITH DIFFERENT EXTENT BURNS

L Yao

Wuhan Blood Center, Wuhan, China

Background: A severe burn will significantly alter haematologic parameters, and manifest as anaemia, which is commonly found in patients with greater than 10% total body surface area (TBSA) involvement. Burn anemia represents a common complication following a burn injury. Burn anemia etiology carries distinct features occurring at each stage of the post-injury and treatment periods resulting from different causes. While there is no consensus on when to transfuse, an increasing number of authors have expressed that less blood products should be transfused. We also focus on the optimize blood products in patients with burned different extent, including packed red blood cells, platelets, fresh frozen plasma, cryoprecipitate, when transfusion is appropriate.

Aims: The aim of this study was to analyse the current status of blood product transfusions in the treatment of different extent burned patients, and address new information regarding burn and blood transfusion management.

Methods: This was a retrospective study of burn patient data on blood products utilization. We used data collected from the registry of the optimal use of blood, developed through co-operation between National Burn Care Centre and Wuhan Blood Center. Burn patients from 2 to 90 years treated at Burn Care Center of Wuhan University affiliated Tongren Hospital between 2014 and 2015 with an in-hospital stay 1 day to 120 days who received at least one transfusion during their hospital stay were included in this study. Initial assessment and diagnosis was made by thorough history, physical examination and necessary investigations. Patients with major burns, high voltage electric burns and those needing any surgical interventions were admitted for indoor management. Patients with minor burns were discharged home after necessary emergency management, home medication and follow-up advice. The sociodemographic profile of the patients, site of sustaining burn injury, type and extent (total body surface area (TBSA), skin thickness involved and associated inhalational injury) of burn and outcome in terms of survival or mortality, etc., were all recorded on a proforma. The data were subjected to statistical analysis.

TABLE 1

Epidemiologic characteristics of burn patients from the registry and stratified by transfusion.

Classification	All	Non-transfused	transfused	P-Value	
Number of patients	552	100	452		
Male(%)	365 (66.1)	74 (74)	291 (64.4)	0.266	
Female	187 (33.9)	26 (26)	161 (35.6)	0.396	
Mean age in years (range)					
All	27.2 (2-90)	25.2 (2-90)	28.8 (2-90)	0.000*	
Male	25.4 (2-90)	24.8 (2-90)	27.5 (2-90)	0.009*	
Female	29.3 (2-90)	28.6 (2-90)	30.2 (2-90)	0.005*	
Length of hospital stay, mean days (range)					
All	15.2 (1-120)	9.0 (1-120)	28.2 (1-120)	0.000*	
Male	15.8 (1-120)	9.6 (1-120)	30.5 (1-120)	0.000*	
Female	14.2 (1-120)	8.8 (1-120)	25.6 (1-120)	0.000*	
Location of burn, n(%)					
Head and neck, T20	71 (12.9)	13 (13.0)	58 (12.8)	0.158	
Trunk, T21	98 (17.8)	17 (17.0)	81 (17.9)	0.167	
Upper extremity, T22	52 (9.4)	5 (5.0)	47 (10.4)	0.005*	
Hand and wrist, T23	54 (9.8)	10 (10.0)	44 (9.7)	0.101	
Lower extremity, T24	91 (16.5)	13 (13.0)	78 (17.3)	0.050*	
Ankle and foot, T25	37 (6.7)	4 (4.0)	33 (7.3)	0.010*	
Inhalation injury, T27	5 (0.9)	3 (3.0)	2 (0.4)	0.881	
Esophagus, larynx, T28	12 (2.2)	1 (1.0)	11 (2.4)	0.084	
Multiple location, T29	102 (18.5)	28 (28.0)	74 (16.4)	0.000*	
Not specified, T30	5 (0.9)	2 (2.0)	3 (0.7)	0.661	
Not specified, T31	25 (2.7)	4 (4.0)	21 (4.6)	0.198	
External cause of injury (%)					
Scald	267 (48.4)	44 (44.0)	223 (49.3)	0.000*	
Fire flame	214 (38.8)	38 (38.0)	176 (38.9)	0.000*	
Chemical	4 (0.7)	1 (1.0)	3 (0.7)	0.611	
Electric	32 (5.8)	5 (5.0)	27 (6.0)	0.205	
Other	35 (6.3)	12(12.00	23 (5.1)	0.181	
Burned extent[a] (%)					
Minor	10 (1.8)	10 (10.0)	0 (0.00)	0.000*	
Moderate	274 (49.6)	0 (0.00)	274 (60.6)	0.000*	
Severe	97 (17.6)	0 (0.00)	97 (21.5)	0.000*	
Particularly severe	81 (14.7)	0 (0.00)	81 (17.9)	0.000*	
Unrecorded	90 (16.3)	90 (90.0)	0 (0.00)	0.000*	

* Values of $p < 0.05$ as statistically significant. [a] Total body surface area (TBSA), skin thickness involved and associated inhalational injury.

TABLE 2

The use of blood products stratified by burned extent groups. All products were leukoreduced

Burned extent	Packed red blood cells				Platelets			Fresh frozen plasma			Cryoprecipitate	
	Number of patients	Units (Mean±SD)	Hb, g/L Mean±SD, Pre-[a]	Hb, g/L Mean±SD, Post-[a]	Number of patients	Clinical dose[b] (Mean±SD)	Plt, $\times 10^9$/L, Mean±SD, Pre[a]	Plt, $\times 10^9$/L, Mean±SD, Post[a]	Number of patients	Units (Mean±SD)[a]	Hct Mean±SD, Pre[a]	Hct Mean±SD, Post[a]	Number of patients	Units (Mean±SD)[a]
Moderate	274	5.51±3.03[a]	101.28±13.38	137.04±25.38	35	18.3±11.3	338.52±162.98	360.06±163.41	274	18.7±9.9	0.41±0.07	0.31±0.04	26	9.9±1.8
Severe	97	4.59±3.90	104.76±13.18	147.25±24.85	29	33.9±28.1	302.82±156.70	396.28±179.15	97	16.54±15.6	0.43±0.18	0.32±0.03	22	15.6±11.5
Particularly severe	81	13.45±13.4	93.85±1	160.59±1	22	23.4±23.1	282.06±172.66	353.63±172.79	81	39.8±32.6	0.48±0.12	0.29±0.06	18	32.6±23.9
Sum	452	3756			86	2093			452	10518			66	1206

[a]One clinical dose of platelets equals four units of whole blood. [b] Pre-,pre-transfusion, Post-, post-transfusion.

TABLE 3

Comparison of blood coagulation function and renal function between pre-transfusion and post-transfusion by burned extent groups.

Burned extent[a]		PT	APTT	Urine-Crea	Blood-Crea
Moderate	Pre-transfusion	12.04±1.84	33.08±9.68	8.92±14.59	53.17±51.68
	Post-transfusion	12.42±2.97	31.84±6.74	4.72±5.40	83.28±22.76
Severe	Pre-transfusion	12.75±3.06	30.02±12.72	14.79±37.20	62.64±45.31
	Post-transfusion	11.68±2.88	29.64±6.38	10.11±27.69	43.37±46.8
Particularly severe	Pre-transfusion	11.48±2.21	29.79±13.96	12.04±15.31	82.82±73.25
	Post-transfusion	12.06±3.46	42.09±32.52	6.87±3.67	59.72±43.10

[a]No statistical differences among burned groups, $p > 0.05$.

Results: Out of a total of 552 patients, there were 365 (66.1%) males and 187 (33.9%) females. The mean age for patients was 27.2 ± 25.2 years. Among all age groups and both genders, scalds were the commonest burns (48.4%), followed by flame burns (38.8%) and electrical burns (5.8%). Among all 552 burn patients, 452 (81.9%) received blood products during their hospital stay. The transfused cohort comprised 452 burn patients. The study cohort received a total of 17573 units of blood components, including 3756 units of packed red blood cells, 2093 units of platelets, 10518 units of single-donor fresh frozen plasma, and 1206 units of cryoprecipitate. All two types of blood components were administered to 81.9% of patients, whereas 15.6% received platelets and 12% received cryoprecipitate. Transfused patients were significantly severer ($P < 0.001$), experienced scald and fire-/flame-related accidents, and burns to multiple locations ($P < 0.001$), and their in-hospital days exceeded that for non-transfused burn patients.

Summary/Conlusions: We show that burn patients received ample transfusions. The number of blood components transfused varied according to the burned severity and extent. The utilization of blood products in the treatment of major burn injury should be reserved for patients with a physiologic need.

P-593

IMMUNOHISTOCHEMICAL AND GENETIC EXPLORATION OF ABH LOSS EXPRESSION AND INCOMPATIBLE A EXPRESSION IN BREAST CANCER PATIENTS

S Zouine[1], Z Orfi[2], F Marnissi[3], F Yann[4] and N Habti[5]

[1]Casablanca, Morocco [2]Department of Experimental Medicine- University Mc Gill, Montreal, Canada [3]Pathology Department, University Hospital Ibn Rochd Casablanca, Casablanca, Morocco [4]French Establishment of Blood, Brest, France [5]Laboratory of Hematology, Cellular and Genetic engineering- Faculty of Medicine, Casablanca, Morocco

Background and Aims: ABH antigens Expression loss and Incompatible A expression is often observed during the malignant transformation in several types of cancer. Our aim is to study the aberrant ABH antigens expression and the molecular mechanisms involved in expression loss and incompatible expression named "A-like"in breast cancer.
Material and Methods: Expression of ABH antigens in 109 patients with breast cancer was studied by Immunohistochemistry using Envision kit. Genomic DNA was genotyped by using PCR-SSP and sequenced by the Next generation sequencing method. Mutation was confirmed by Sanger sequencing method.
Results: Expression Loss of ABH antigens was observed in 26/109 of type B blood patients; 43/109 of type O blood. A antigen was maintained in the majority of cases. 11/109 of type O blood patients express incompatible A antigen in tumor tissue. Patients with loss expression show O/O or B/O genotypes and there is no mutation associated with the loss of ABH antigens. While, the DNA of patients with incompatible A was genotyped O/O in normal tissue and A/O in the tumor. This A-like incompatible expression is due to a glycosyltransferases A encoded by an allele nearly the same reference A101allele.
Conclusion: ABH antigens expression loss play an important role in mammary carcinogenesis. While, Incompatible A antigen expression could play an important role in immune surveillance triggering against cancer cells and could be destined to targeted therapy.

P-594

TRANSFUSION TRENDS IN THREE TYPES OF PROSTATETOMIES

A Spínola[1], C Alves[2], C Martins[2], F Mota[2], C Brás[2], P Nogueira[2], S Faria[1] and S Sousa[1]

[1]Unidade Local de Saúde de Matosinhos, Matosinhos, Portugal [2]Serviço de Imunohemoterapia, Unidade Local de Saúde de Matosinhos, Matosinhos, Portugal

Background: Open radical prostatectomy has been associated with considerable blood loss. Average blood loss of over 1000 ml has been reported even from high volume centers of excellence. The treatment of choice has been moving in favor of the minimally invasive alternatives such as laparoscopic, transurethral and robotically assisted radical prostatectomy. These procedures take about 3–4 h.
Aims: Evaluate transfusion rates and trends at open (O-), laparoscopic (L-) and transurethral (T-) radical prostatectomy (RP).
Methods: We retrospectively evaluated the transfusion outcome of radical prostatectomy performed by open (retropubic or suprapubic), transurethral and laparoscopic approaches, in 2015. The following transfusion parameters were calculated: Cross-match to Transfusion (C/T) ratio (number of units cross-matched/number of units transfused), Transfusion Probability (TP) (number of transfused patients/number of cross-matched patients) and Transfusion index (TI) (number of transfused RBC units/number of cross-matched RBC units). C/T ratio $\leq$1.5, TP $\geq$0.30 and TI > 0.5 were considered as indicators of blood transfusion requirement during surgery. Descriptive analysis was performed using SPSS v.20.
Results: Of 119 radical prostatectomies, ORP (68), TRP (32) and LRP (19) were included into study. 59.5% (69) of the patients had benign prostate hyperplasia and 38.8% (45) prostate cancer with median ages at the surgery of 67, 72 and 66 years for ORP, TRP and LRP respectively. Median American Society of Anesthesiologists (ASA) class for all radical prostatectomies was II (range I to IV). Mean operation times and length of stay were 03:05 $\pm$ 01:23, 02:24 $\pm$ 01:00, 03:14 $\pm$ 01:17 h, and 7 $\pm$ 2, 6 $\pm$ 3, 7 $\pm$ 2 days for ORP, TRP and LRP, respectively. Median pre and post-operative (pre/post) heamoglobin concentration were 14.4/11.6, 13.5/12.8 and 14.1/11.0 g/dl for ORP, TRP and LRP, correspondingly.
Overall transfusion requests were performed preoperatively as Type and Cross-match with a median order of 2 units per request, C/T ratio was 12.2, TI 0.17 and TP 0.02. For ORP C/T ratio was 13, TI 0.17 and TP 0.03. For procedures such as TRP and LRP the transfusion parameters could not be calculated as no blood units were transfused.
Conclusions: Patients undergoing an elective radical prostatectomy usually do not required blood as demonstrated by low transfusion rates C/T ratio >1.5, TP <0.3 and TI <0.5 and no differences were obtained regarding each treatment choice. For all radical prostatectomies a routine cross-matching of blood can be safely substituted by 'type and screen.' Tailored and rationalized blood ordering is the key for best transfusion practices in prostatectomies.

P-595

PROTECTIVE ROLE OF SOLUBLE ABH ANTIGENS IN SEMINAL PLASMA AND SPERM-CERVICAL MUCUS INTERACTION

RS Pusillico and AS Brufman

National University of Rosario, Rosario, Santa Fe, Argentina

Backgrounds: Fucosyltransferase locus 2 (FUT2) is an autosomal gene that controls the presence or absence of blood group substances (A, B, H) in the saliva and other body secretions such as seminal plasma. ABO and FUT2 contribute to build up oligosaccharide structures of the cell surface. In addition importance of ABO blood groups in blood transfusion, the ABO blood groups has been associated with several metabolic and infectious diseases and it is possible that there is a relationship between blood group and reproductive faillures. Infertility affects nearly 15% of couples in the reproductive age. Although is not life threatening, it is socially a traumatic condition. Incidence of infertility related of both male and female factors continues to rise despite many advances in reproductive technologies. It is well known that ABO antigens are expressed on spermatic membrane and in seminal fluid of secretors as well as ABO antibodies are present in cervical mucus.
Aim: The objective of the present study is to examine the ABH antigens in men for assessing the relationship to male infertility and secretor status. METHODS: Genomic DNA was obtained from 69 infertile semen samples and 61 controls. Two sets of oligonucleotides were designed in order to amplify two different exons of *ABO* genes by PCR. Secretor phenotype was determined in seminal plasma by laboratory standard procedure of inhibition of haemagglutination.
Results: We observed significant loss in progressive motility of spermatozoa of non secretors in relation to secretor ones caused by cervical mucus specific antibodies in ABO incompatible couples. Seminal soluble antigens block mucus antibodies diminishing antibody attack to sperm cells. The results obtained by PCR in sperm cells correlated 100% with the red cells genotypes. In the infertile group, the frequency of non-secretor phenotype (75.8%) was significantly higher than those from fertile ones (21.8%) ($P < 0.03$).
Conclusion: The incidence of infertility continues to rise despite many advances in reproductive technologies. Secretor phenotype of the male partner could facilitate reproductive success blocking cervical ABO antibodies. We proposed to evaluate ABH antigen expression on sperm membrane and seminal plasma to contribute to diagnosis and treatment of human infertility.

P-596

OZONE INDUCES GENERATION OF MICROPARTICLES FROM ERYTHROCYTES, LEUKOCYTES, PLATELETS AND ENDOTHELIAL CELLS

P Radziwon[1], A Lipska[1], M Bujno[1] and P Olbromski[2]

[1]Regional Centre for Transfusion Medicine in Bialystok, Bialystok, Poland
[2]Department of Obstetrics and Gynecology, Healthcare Centre for Mother and Child, Poznan, Poland

Background: Ozone for therapeutic purposes has been applicated in a number of pathologies, such as vascular diseases, ulcers, and acute and chronic viral diseases. Ozone performance arises from generation of highly reactive oxygen species and subsequent activation of antioxidant enzymes. Despite long experience of ozonized autohaemotherapy the putative molecular mechanisms underlying some reported clinical effects of this gas as well adverse effects included myocardial infarction remain mainly unknown.

Aims: We studied the effect of blood ozonation on the generation of microparticles derived from blood cells and endothelium which may be responsible for some clinical effects of ozone.
Methods: Sixty ml of venous blood was taken from 20 healthy volunteers and anticoagulated with citrate phosphate dextrose solution (0.12% final concentration). Each sample of blood was divided into 4 equal volumes and transferred to separate containers. The first part was not further processed, the second part was aerated, the third and the fourth parts were subjected to ozone in a dose of 15 μg/ml and 30 μg/ml respectively. The oxygen-ozone gas mixture was freshly prepared from medical-grade oxygen using ATO-3 MINI ozone generator (CryoFlex, Poland). Blood samples were taken from the blood bag before and after ozonation/aeration. Microparticles derived from erythrocytes (RMP), leukocytes (LMP), platelets (PMP) and endothelial cells (EMP) were quantitatively measured using flow cytometry (FACSCalibur, Becton Dickinson) and murine monoclonal antibodies: anti-CD235 for RMP, anti-CD42 for PMP, CD45 for LMP, anti-CD105 for EMP. For quantitative measurement of microparticles the flow cytometer was calibrated using microspheres size from 1 μm to 1.5 μm (Microbead NIST Traceable Particle Size Standard, Polysciences) and microbeds (TruCount, Becton Dickinson).
Results: We observed statistically significant larger amounts of microparticles derived from erythrocytes, leukocytes, platelets as well as endothelial cells generated in either aerated or ozonized blood compare to control (blood untreated with any gas). Moreover in ozonized blood were statistically significant more microparticles generated compare to blood subjected to air. The generation of microparticles induced by ozone was dose dependent.
Conclusions: Aeration or ozonizing of blood induce generation of microparticles from erythrocytes, leukocytes, platelets and endothelial cells. Microparticles may be involved in mechanism of some adverse effects observed after ozonized autohaemotherapy.

Clinical Immunogenetics: HLA in Transfusion Medicine

P-597

HLA EXPRESSION IN PSORIASIS – AN INDIAN EXPEREINCE

A Anandan, K Radhakrishnan, R Prasad and VK Panicker
Sri Ramachandra University, Chennai, India

Background: Psoriasis is an immune mediated genetically determined skin disorder affecting 0.5–3.0% of the Indian population. Psoriasis is a multi-factorial disease and has been associated with certain HLA expressions. Studies showing HLA association in Psoriasis are mostly from the West with very little information about HLA association in Psoriasis in the Indian Population.
Aim: To screen Psoriasis patients for HLA – Class I (A & B) and determine its association.
Methodology: The study was conducted in the Department of Transfusion Medicine at a teritary care hospital in South India. Fifty Psoriasis patients (cases) and 50 controls (healthy blood donors) were enrolled in the study. Samples from the Psoriasis patients were collected from Dermatology Out Patient Department and the samples from the controls were taken from voluntary blood donors in the Department of Tranfusion Medicine. HLA typing for class I (A & B) was done using PCR – SSP method. The HLA results were analysed and its association with Psoriasis was determined.
Results: The study includes 50 Psoriasis patients of which 8 patients had Psoriasis with arthropathy. The alleles which were found in higher frequency in the cases were HLA A*02 (18 out of 50 cases), HLA A*11 (16 out of 50 cases), HLA A*03(15 out of 50 cases) and HLA A*24 (15 out of 50 cases). HLA B*35 (17 out of 50 cases), HLA B*15 (13 out of 50 cases) and HLA B*40 (13 out of 50 cases) were observed to be in higher frequency among the cases. HLA B*27 was positive in 5 out of 8 Psoriatic Arthropathy patients.
Summary/Conclusion: Certain HLA alleles are present in higher frequency in the disease population than the controls implying that individuals expressing these alleles may have a higher relative risk of developing Psoriasis.

P-598

DOES PATIENT HLA TYPE PREDICT THE RISK OF RED BLOOD CELL ALLOIMMUNIZATION?

G Mawad, ZA Aiyafei, E Aizaabi and HS Alhumaidan
Sheikh Khalifa Medical City / SEHA, Abu Dhabi, United Arab Emirates

Background: Red blood cell (RBC) alloimmunization is the recipient immune response to foreign RBC antigens acquired via transfusion, transplantation or pregnancies. RBC antigens except RhD are considered weakly immunogenic when compared to the 100% response that is usually observed with other exogenous antigens. Alloimmunization is unpredictable and occurs in approximately 3% of transfused patients. The mechanism of RBC antigens immunogenicity is multifactorial and both donor/product factors and recipient factors have been implicated. Recipient factors includes genetic predisposition such as the recipients human leukocyte antigen (HLA) type. The HLA system is a gene complex encoding the major histocompatibility complex (MHC) proteins in humans and is known to regulate antigen recognition by the immune system. It has been reported to have a role in alloimmunization against RBC antigens.
Aim: The aim is to demonstrate that the patient HLA type is a useful genetic predictor of RBC alloimmunization.
Method: The alloimmunization events that occurred in an 83 years old female patient have been looked at retrospectively and the products implicated have been identified. In addition, a literature review was performed to identify the reported HLA-DRB1 alleles associated with increased risk of Red blood cell alloimmunization and results were compared with the patient HLA-DRB1 type.
Case Report: Patient is an 83 yo female with multiple comorbidities who had multiple admissions due to cerebral vascular accident, chronic kidney disease, peptic ulcer disease, hypertension, diabetes, chronic obstructive pulmonary disease, tracheostomy, acute respiratory failure and septic shock. Patient specimen was first sent to the blood bank in November 2013, the antibody screen was found to be negative and she was transfused with O Rh D, C and E negative units. She was subsequently seen in 2015, her phenotype, blood bank results, alloimmunization history and the products transfused are presented chronologically in **Table 1**. Patient demonstrated an alloimmunization event with one RBC unit in the case of Anti-K and Anti-Fya and it was noticed that the identification of Anti-D and Anti-E in patient plasma occurred after the transfusion of whole blood derived platelet concentrates (WBPC), whether it was a primary or a secondary immune response, the identification of Anti-E following a platelet transfusion is uncommon.
In addition, patient HLA-DRB1 typing was performed and results are presented in **Table 2.**
Conclusion: There are multiple factors that could have contributed to the patient alloimmunization events. The patient age, in addition to the low quantity of antigen the patient was exposed to in platelets should have caused a diminished immune response; however, our observation demonstrates the importance of patients HLA-type as a predicting factor. The patient was found to be **HLA-DRB1*15,** a phenotype

Table 1

Patient phenotype:
O Rh-D negative, C-, c+, E-, e+, K-, Jka+, Jkb-, Fya-, Fyb+, M+, N+, S+, s+

Date	Product Transfused	Product Phenotype	Blood bank Results
19-20/6/2015	2 RBCs*	1 was **K positive**	Negative screen
8/2/2016		No transfusion	Anti-K in plasma
14/2/2016	1 Apheresis platelet	Rh and K phenotype matched	Anti-K in plasma
15/2/2016	6 WBPC¥	2 were **E positive**	
15/2/2016	3 WBPC	1 was **Rh D, E positive** 2 were **Rh D,C positive**	
18-20/2/2016	12 WBPC	2 were **E positive**	
9- 29 /2/2016	11 RBCs	All Rh D, C, E and K negative unit **1 Fya positive unit**	
19/3/2016		No transfusion	Anti-K, **Anti-D Anti-E and Anti-Fya**

***RBC: Red blood cells**
¥WBPC: Whole blood derived platelet concentrate

Caption 1: Chronological presentation of products transfused and the patient alloimmunization events

Table 2	
Patient HLA type	**Previously documented association**
HLA-DRB1*07	Associated with formation of: Anti-S[₸] Anti-Dia[&]
HLA-DRB1*15	Associated with formation of: Anti-Fya[₸ ¥] Anti-D[β] Multiple alloantibodies[₸ ®]
₸ Schonewille, Transfusion, 2014	
& Baleotti, Transfusion, 2014	
¥ Picard, Transfusion, 2009	
β Urbaniak, Blood, 2002 (abstract)	
®Hoppe, Am J Hematol, 2009	

Caption 2: Patient HLA DRB1 type and previously reported associations

that has been previously reported as a high-responding phenotype in patients with multiple alloantibodies, Anti-Fya and Anti-D. Therefore, for a group of multiply transfused patients, the recipient HLA type as a genetic predictor for RBC alloimmunization and the need to utilize phenotypically matched blood merit consideration.

P-599

IDENTIFICATION AND MANAGEMENT OF HLA ANTIBODIES IN A PLATELET REFRACTORINESS PATIENT CANDIDATE TO HAPLOIDENTICAL HEMATOPOIETIC STEM CELL TRANSPLANTATION: A CASE REPORT

D Londero, V Miotti and V de Angelis

Azienda Ospedaliero-Universitaria SMM Udine, Udine, Italy

Background: Hematopoietic stem cell transplantation (HSCT) with HLA mismatched donors is for many patients the only curative option. The importance of Donor specific HLA antibodies (DSA-HLA) in HSCT outcome is under debate. The transfusion support management of a patient highly refractory to platelets and HSCT-candidate led us to plan an immunological study and to include the assessment of HLA antibodies in HSCT protocols.

Aims: The aim of this study was to define a protocol to prevent negative outcome transplant in the presence of HLA-immunization.

Methods: A 56-year old female affected by acute myeloid leukemia and refractory to PLTs was studied before transplantation. The cross match incompatibility screening test and the screening of HPA/HLA antibodies were performed with a solid phase system for the detection of IgG antibodies to PLTs (Capture P and Capture P-Ready Screen, Immucor). The identification of HPA antibodies was performed using an ELISA technique (Immucor GTI-PAK Auto). The screening and identification of HLA antibodies were performed with Luminex-based commercial kits (Labscreen Mixed and Single antigen beads class I and II, One Lambda).

Results: HLA/HPA antibodies screening test was positive, but HPA identification didn't detect any specific HPA antibody. Routine testing for anti-HLA performed before HSCT found out only DSA-HLA with the strength of antibodies (900 MFI) below manufacturer's cut off (1000 MFI) and 85% PRA (panel reactive antibodies), while a positive platelet cross-matched between donor and recipient was found. The HSCT was performed and a week after a graft failure was diagnosed. Anti-HLA testing was repeated and a significant increase of DSA-HLA was detected (3000 MFI). Therefore the patient was treated with plasma-exchange and immunosuppressive treatment before a second HSCT with the evidence of donor-recipient cross-match and DSA-HLA negative.

Conclusions: In this case-report, positive platelet cross-match predicted graft failure, in the presence of DSA-HLA independently of MFI value. Although the relationship between clinical outcome and antibody results is becoming acknowledged, the assessment of cross-match incompatibility screening test concurrently with the presence of HLA antibodies should be included in HSCT management protocols.

P-600

DETECTING HLA-DRB1*15:03 AS A GENETIC MARKER FOR DETERMINING RESPONDER THALASSEMIA PATIENTS

P Darvishi[1], AA Pourfathollah[2], Z Sharifi[3] and A Azarkeivan[4]

[1]Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran [2]Iranian Blood Transfusion Organization (IBTO), Tehran, Iran [3]Iranian Blood Transfusion Organization, Research Center, Tehran, Iran [4]Pediatric Hematology Oncology. Associated Professor. Transfusion Research Center, Tehran, Iran

Background: Recently Thalassemia has been introduced as a chronic disease. Prolonging life in these patients has obviously been accomplished. However, a longer life is not always a better life. One of the challenges that make their quality of life poor is alloimmunization. This immune response causes several complications to patients by restricting their options. Some individuals are more susceptible to developing alloantibody than others. They are called responders and non-responders respectively. Determining responders before the first transfusion, allows transfusion services to provide compatible blood and prevent alloimmunization.

Aims: The present study was investigate to determine the relationship between HLA-DRB1*15:03 allele in Iranian Thalassemia patients and alloimmunization.

Methods: Antibody screening tests were performed by gel method and HLA-DRB1*15:03 genotyping was determined by SSP-PCR in 53 alloimmunized and 103 non-alloimmunized patients. HLA-DRB1*15:03 allele frequencies were compared between alloantibody positive and negative groups by chi-squared test.

Results: DRB1*15:03 was more observed among alloantibody positive compared to alloantibody negative subjects (67.9% vs 38.8%) and according to our results the HLA-DRB1*15:03 allele frequencies were significantly different between groups ($P = 0.001$, $OR = 3.335$). Also this allele was seen more among hyper-responders (81.2%) than responders (62.2%) and non-responders (38.8%) and there was correlation between responding and DRB1*15:03 ($P = 0.001$).

Conclusion: According to our results, detecting this allele as a genetic marker in the pre-transfusion test could determine responder patients and improve transfusion safety.

Histocompatibility in Stem Cell Transplantation

P-601

This abstract has been withdrawn.

Histocompatibility in Organ Transplantation

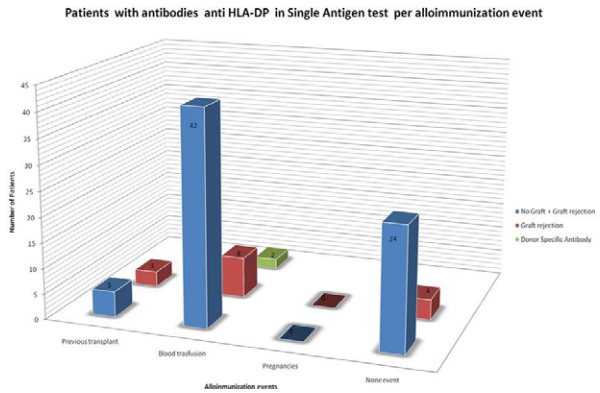
P-602

TYPING OF THE HLA-DP TO ESTABLISH THE CLINICAL IMPORTANCE IN THE DEVELOPMENT OF ANTIBODIES ANTI-DP IN PATIENTS IN KIDNEY TRANSPLANT PROTOCOL FROM THE NATIONAL MEDICAL CENTER SXXI, IMSS FROM 2010 TO 2015

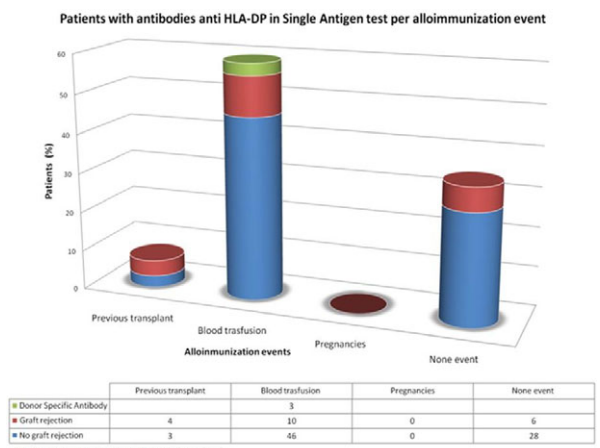
JC Martínez Álvarez, MA Gómez Martínez, MA Arrazola García, RA Fuentes Landa, V Juarez Barreto, MA Chavez Durán, SY Vargas Madrid and G Benítez Arvizu

IMSS, Mexico City, Mexico

Background: In 2005 Chronic Kidney Disease was the 10th cause of dead in Mexico giving place to 10 thousand people dead in this year, with the current conditions it's expected about 221 thousand cases of Chronic Kidney Disease and about 160 thousand dead related to this one for 2025. Kidney transplantation is one of the therapies to this disease as a definitive treatment. Involved in the procedure there exist some bound tests like Human Leukocyte Antigen (HLA) typing due to the unquestionable linking between their compatibility and kidney reject. It's well known that the graft survival decreases as much as the number of HLA incompatibilities. After the kidney transplant, appearance of an antibody anti-HLA against a specific donor antigen is



Caption 1: Patients with antibodies anti HLA-DP in Single Antigen test per alloimmunization event



Caption 2: Patients with antibodies anti HLA-DP in Single Antigen test per alloimmunization event

one of the criteria to diagnose humoral rejection. Antibodies against HLA-DP have been implied in the discussion about their deleterious impact in Antibody Mediated Rejection in kidney transplant. Several studies have elucidated the pathogenic roll of these antibodies in order to evaluate the importance or not of typing HLA-DP antigen as a routine test in the process of kidney transplant to predict graft outcome.

Aims: The aim of this study was analyzing data from patients included in kidney transplant protocol to establish the importance of typing HLA-DP molecule for preventing the Antibody Mediate Rejection in kidney transplant, from National Medical Center SXXI, IMSS.

Methods: For this retrospective study were used files since January 2010 to November 2015 from patients with anti-HLA DP antibodies, detected by Single Antigen Test (Luminex®) and, without the presence of any Donor Specific Antibody against other Class I or Class II HLA antigen. Any other comorbidities was considered. The typing of HLA-DP molecule was made by molecular method, a reverse SSO-DNA typing LABType®. Results were just analyzed with descriptive statistics because of their characteristics.

Results: The result sample of patients with anti HLA-DP antibodies detected were 71 patients with the next alloimmunization events: 59% blood transfusion, 7% previous transplant, and 34% none alloimmunization event. Just 21% presented acute rejection, for separately we got that the 19% of blood transfusion presented rejection, 60% (3/5) of previous transplant and approximately 17% of none alloimmunization event. Only 2/15 rejected cases had Donor Specific Antibody with the next Mean Fluorescent Intensity (MFI) reported, AbMFI1 = 2400, 96 and AbMFI2 = 1000, 19.

Summary/Conclusions: In an initial conclusion we observed that in our hospital population, the routine typing of HLA-DP is not as necessary as the others alleles. Just two cases were openly attributable to the presence of HLA-DP antibody with a not high MFI registered. Such that these antibodies do not represent a high risk in the graft rejection, and it is not justifiable the cost of the test with the profit in the graft outcome, nevertheless it's important to notice that the antibody can have an important pathogenicity due to the not very high MFI reported and the relation with the reject. Also it's interesting that the presence of these antibodies anti HLA-DP is predominant and has more relation with graft rejection when there is a previous transplant than with others alloimmunization events. Finally we concluded that typing HLA-DP isn't necessary as a routine in our patients in their first transplant protocol, but it is highly recommended to make the typing when second or third transplant will be, however further studies are needed.

P-603

ASSOCIATION BETWEEN HUMAN LEUKOCYTE ANTIGENS (HLA-A,-B AND DR) AND THE RISK OF END-STAGE RENAL DISEASE IN TAIWAN PATIENTS

LP Lee, H Tsai, L Wu and H Chen
Chi Mei Medical Center, Tainan, Taiwan

Background: Chronic renal disease (CRD) is one of the most important healthcare issues in Taiwan due to its high incidence, prevalence, and medical expenses. According to the report of United States Renal Data System (USRDS) in 2015, both prevalence and incidence of *end-stage renal disease* (ESRD) in Taiwan ranked the first in the world. Human leukocyte antigens (HLAs) are relevant to the expression of many immunological diseases. The occurrence of ESRD is the final outcome of several disorders processes resulting in renal damage. Various kidney disorders could lead to end-stage renal disease (ESRD), and these disorders are associated with certain frequencies of human leukocyte antigen (HLA) genes. Studies had been demonstrated the association between certain HLA alleles and renal diseases such as diabetic nephropathy and idiopathic membranous nephropathy.

Aims: The aim of this study was to investigate the association among HLA-A, HLA-B and HLA-DR antigens and ESRD in Taiwan patients.

Methods: HLA-A, -B and -DR typing was performed by PCR-sequence-specific primers (PCR-SSP) technique. HLA types of patients with ESRD were recorded between 2000~2015 from Chi-Mei medical center. Besides, we also compared our data to a control group composed of 190 healthy unrelated Taiwanese individuals.

Results: 301 patients were enrolled in our study, 166 male and 135 female with mean age 48.1 ± 12 y/o. The frequencies of HLA-A antigens were similar between patients and control group, indicated there were no significant differences between two groups. For the frequencies of HLA-B antigens, we noted the frequencies of HLA-B38 and HLA-B60 antigens in ESRD patients were higher than control group (patients: 12.62% vs control: 8.42% and patients: 41.53% vs control: 34.74% respectively); however, it showed no significant differences between two groups in HLA-B antigens as well. For HLA class II analysis, there was a significant difference of HLA-DR13 (odds ratio=0.386, 95%CI=0.177–0.843, P = 0.017), the other HLA-DR antigens were not significant differences,such as HLA-DR3(odds ratio=1.125, 95% CI=0.679–1.863, P > 0.05), HLA-DR4(odds ratio=1.349, 95%CI=0.909–2.002, P > 0.05) and HLA-DR8 (odds ratio=1.339, 95%CI=0.846–2.119, P > 0.05).

Summary/Conclusions: Many studies have mentioned that HLA-DR3 was significantly associated with membranous nephropathy and HLA-DR4 was about immune complex-mediated rapidly progressive glomerulonephritis. But in the present study, these two alleles showed no significant differences. On the other hand, HLA-DR13 had a significant difference means it might be a protective factor for preventing the development of ESRD among Taiwan patients. The results of our investigation are not coincident with previous studies might be attributed to a few possible reasons including small populations of participants and different region's study objective groups. To sum up, HLA allele frequencies are highly related to ESDR; therefore, HLA typing could be a useful clinical tool for screening patient with high risk of ESRD.

P-604

C4D-FCXM: AN ANSWER TO COMPLEMENT/NON COMPLEMENT ANTIBODIES IN TRANSPLANT

D Jain, J Choudhuri, S Mehra, P Dorwal and AK Tiwari

Medanta the Medicity, Gurgaon, India

Background: Complement-fixing alloantibodies play a major role in transplant rejection. Methods have been reported to detect HLA/non HLA, complement/non-complement-fixing, donor T/B cells specific alloantibodies. C4d deposition in kidney biopsy is a marker of antibody-mediated rejection (AMR).

Aim: To use C4d-FCXM in differentiating patient of various risk categories.

Method: 47 year old male being worked-up for renal transplant. He showed positivity on B cell FCXM and found negative for all other tests viz. NIH-CDC, Flow-PRA and single antigen bead for HLA class-I/II antibodies. As positive for B-cell (pronase treated/untreated) FCXM, he was initially categorized as "high risk". Subsequently tested for C4d-FCXM; a new approach (developed and validated at author's institution) that additionally identifies complement fixing alloantibodies.

Result: As C4d-FCXM was negative, patient was categorized as "low risk"since the alloantibodies were donor specific but non-complement-fixing. An isolated positive B cell FCXM in present case could be due to non-HLA/non-complement binding antibodies or lack of representation of all HLA antigens in bead based assays.

Summary & Conclusion: We propose C4d-FCXM as a method to limit unwanted deferral of donors, and may be helpful in guiding clinical decisions (Fig-1) and prediction of AMR. This faster, objective and sensitive method may be especially relevant in live-related transplant/s.