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Chapter 15

Cord Blood Banking: Operational and Regulatory Aspects

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Chapter Outline

1. INTRODUCTION

The demonstration that umbilical cord blood (UCB) contained cells capable of reproducing hematopoiesis in vitro and that they could be cryopreserved for long periods without significant loss in their function paved the way for the use of this new source of stem cells in the field of clinical hematopoietic stem cell (HSC) transplantation.[1–5] Although the first attempt to transplant UCB was reported in 1972,[6] the first successful related UCB transplant was performed in 1988 in a patient with Fanconi anemia.[7] The first unrelated UCB transplants were then performed in 1993 and the report of a large series of patients was published in 1996.[8]

These results prompted the establishment of large repositories of frozen unrelated UCB units, i.e., cord blood banks (CBBs) to support this clinical development. The first UCB bank was set up in 1991 by P. Rubinstein at the New York Center in the United States,[9] and this was followed by the establishment of CBBs in Dusseldorf, Milan, Paris, and London.[10–13] At present, there are over 130 CBBs all over the world storing over 650,000 unrelated UCB units readily available for transplantation.[14,15] These CBBs have enabled the performance of over 30,000 unrelated UCB transplants in children and adults with both malignant and nonmalignant diseases.[16–21]

With the increased clinical use and exchange of these UCB units it became clear that it was necessary to standardize practices across the various CBBs. In order to contribute to this, a group of experts involved in UCB banking established the NetCord organization in 1998.[22] The initial remit of NetCord was to set up an international registry of UCB and to develop procedures and quality standards for the safe collection, exchange, and clinical use of these banked units. These efforts culminated with the development of the NetCord-FACT (Foundation for the Accreditation of Cellular Therapy) International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection, and Release in 2000 with the last version (5th) published in 2013.[23] These standards form the basis for the NetCord-FACT accreditation program. The American Association of Blood Banks (AABB) has also developed standards and an accreditation

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scheme, and since 2004 have been incorporated into the Standards for Cellular Therapy Services.[24]

Different types of UCB banking programs have been established depending on the genetic relationship of the donated UCB unit with the potential recipient, i.e., allogeneic or autologous, and on the funding sources, i.e., public or private.[25–27]

In the allogeneic setting, an additional distinction needs to be drawn between unrelated (also called altruistic) and related donations. Unrelated allogeneic UCB banking includes the collection, processing, and storage of altruistically donated UCB, in order to create an inventory of HSCs that can be searched for any patient in any part of the world and in need of an unrelated allogeneic HSC donor. These programs are also referred to as public UCB banking.

In the allogeneic-related setting, the UCB is collected from a healthy sibling of a patient with a disease that can potentially be treated with a UCB transplant. These collections are performed at the request of the physician treating the patient, with the agreement of the obstetrician looking after the mother.[28–31]

Although the standards for the collection of unrelated and related UCB are similar, in the banking of related UCB units, no threshold values for minimum volume collected are required and there is no operational need for volume reduction (VR) of these units, although clinically VR may be beneficial (see below). Also, the exclusion from banking due to microbial contamination does not always apply since antibiotic sensitivity tests can be performed if and when the collection is required for transplantation.

An important consideration in related UCB banking is that in approximately 70% of cases, the collected UCB unit is not fully human leukocyte antigen (HLA)-matched with the patient and it is unlikely that it will ever be used for the intended patient. These units will have to be kept frozen indefinitely unless clear policies regarding their disposal are put in place. With the availability of prenatal genetic diagnosis, including HLA typing, it will be possible to collect units selected only from HLA-identical siblings, as has already proved possible.[32]

So far, the majority of the transplanted related UCB units have been fully HLA-matched and in patients with hemoglobinopathies. In fact, in some centers, related UCB transplantation is the first line of treatment for patients with thalassemia major.[33]

In most European countries, these two types of allogeneic UCB banking (unrelated and related) are carried out by government-administered institutions and funded by the national health systems available in each country.

In the autologous or family UCB banking setting, the collections are normally performed by privately funded institutions at the request of the family of the potential donor, and as its name indicates these collections are mainly for autologous use or for a named recipient normally within the family. Worldwide, many private CBBs are collecting and storing UCB for eventual autologous or family use. Although there are more than 1 million of these units stored in private CBBs, a very small number have been transplanted, mostly with unknown outcomes. There are anecdotal cases of autologous UCB transplantation, but in general the scientific and clinical arguments for the banking of these units are not universally accepted.[34] There are also a number of ethical issues associated with this practice, which have been extensively reviewed.[35–39] Furthermore, it appears that quality parameters for privately stored units seem to be inferior to those measured in those stored in public CBB, highlighting the fact that if the clinical value of autologous UCB transplantation is established, the issue of the quality of these units will become even more relevant.[40]

Alternative models of CBB, called hybrid or mixed, have been developed and in these programs the UCB collection is carried out on behalf of (and paid for) the family requesting the collection for either autologous use or for a named person within the family. In one of these models, the collection is split into three and 1/3 is donated to the unrelated public bank and the other 2/3 is stored as a private collection. However, with the increasing evidence on the impact of high total nucleated cell (TNC) and CD34+ cell content on the clinical outcome of UCB transplantation, which is directly correlated with the volume collected, the value of these small volumes of UCB is limited. The other "mixed" model involves the collection and storage of the UCB, for a fee, for autologous or for a named family member, but in this case, the relevant information on the UCB unit is made accessible to unrelated donor registries. Once or if the unit is selected for an unrelated patient, the family is then asked to release the stored unit with the consequent refund. To date, no evidence on the application of this model has been presented.

The majority, if not all of, the UCB banking procedures and standards were initially developed primarily for the collection and banking of allogeneic unrelated cord blood units (CBUs) from altruistic donors. However, in order to ensure the quality and efficacy of all collected units and to safeguard the eventual recipients of these products, NetCord-FACT and AABB have also developed standards that are applicable to the collection and storage and release of allogeneic related and autologous UCB.

The main aspects of an unrelated UCB banking program include the following:

1. Promotion, recruitment, donor selection, informed consent, collection, and transportation to processing facilities and donor follow-up
2. Processing, testing, cryopreservation, and storage
3. Listing, searches, selection, testing, and distribution to a transplant program and posttransplant clinical follow-up

All these procedures and activities of a CBB program are supported by comprehensive inventory and quality management systems covering all the various components of UCB banking as described below (see Figure 1).

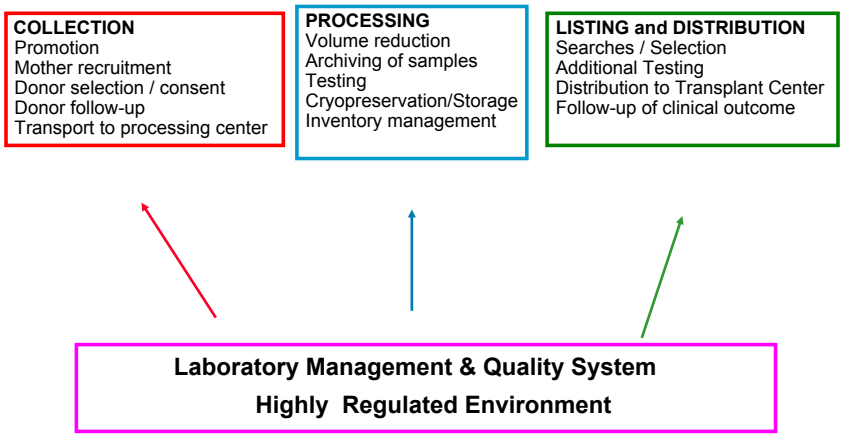

FIGURE 1 Operational aspects of umbilical cord blood banking.

1.1 Promotion, Recruitment, Donor Selection, Consent, Collection, Transportation to Processing Facilities, and Donor Follow-up

1.1.1 Promotion

The promotion and model of collection of UCB vary from country to country and depend largely on the nature of the funding and health-care system available in each country. In CBBs funded with public or government monies, the promotion is generally carried out and restricted to antenatal clinics selected as collection sites. The material used in the promotion includes leaflets, videos, seminars, etc. These should provide as much information as possible about the program including the need for consent, the right to withdraw at any step of the process, the clinical use of the collection, and about its potential use in research if the collected unit is not suitable for clinical banking. The promotional material should be clearly understood by the potential mother donors, and it should be translated into various languages if required. The latter is particularly important when recruiting donors are from an ethnic minority background and are not fluent in the language of the country where the recruitment is taking place.[41]

1.1.2 Recruitment

The majority of the banked units so far have been collected in countries with a population of predominantly European Caucasoid ethnic background and expressing the HLA profile of this ethnic group. There is therefore a need to increase the HLA diversity of the banked UCB and one way of achieving this is to recruit donors in maternity units with high numbers of deliveries from an ethnically diverse population.

A number of CBBs have now been established in countries with an ethnic and HLA profile not historically represented in the international registries of unrelated HSC and these units may contribute to expand the HLA profile of the internationally available pool of unrelated UCB units.

A number of publications have indicated that the volume and TNC content of the units collected are smaller in mothers from Afro-Caribbean and Asian ethnic background compared to those collected from European Caucasoid donors.[42,43] It is therefore crucial to carefully select these donors in order to minimize the waste of these units.

The National Health Service Cord Blood Bank (NHS-CBB), formerly known as the London Cord Blood Bank, was set up in 1996 with the aim of enriching the national and international HSC donor pool with units from ethnic minorities (EM).[44,45] At present, nearly 38% of the banked CBUs are from EM and ethnically mixed genetic backgrounds, expressing unique HLA haplotypes. This is of great benefit to EM patients as reflected by the fact that nearly 36% of the units issued for transplantation by the NHS-CBB are from EM donors.

Looking into the future it will be important to try to enrich the CBBs with units from a mixed genetic background. In fact, the majority of patients who have difficulties in finding an HLA-matched stem cell donor express a combination of a common and a rare HLA haplotype.

1.1.3 Donor Selection

This is one of the most important aspects of CBB and detailed and comprehensive procedures and policies for the selection and acceptance of mothers donating their UCB are crucial to ensure the quality and safety of the collected units. The responsibility for the selection of an UCB donor lies with the Medical Director of the CBB who should ensure that an appropriate medical and social history is obtained from the mother in order to prevent the transmission of microbiological infection and/or genetic, malignant, or degenerative disease. Donors with a family or personal history of genetic disease, particularly relating to the hematopoietic or immune system, should be asked

for details of those suffering from such diseases and their family relationship to the infant donor. Further details may be required from the general physician or other professionals. The communicable disease risk history of a surrogate mother who carries an infant not genetically related to her, and of a sperm, egg, or embryo donor shall also be obtained and documented if applicable. Travel history of potential donors is also important to assess risks. More recently, a number of publications have indicated other factors such as the ethnicity, gender, and age of gestation which may affect the size/volume of the collections.

1.1.4 Consent

Initially and in order to make the best use of resources, consent was only obtained from mothers from whom a successful collection had been obtained. However, in Europe following the implementation of the European Union Tissues and Cells Directive (EUTCD) 2004/23/EC in April 2006, stating that "procurement of human tissues or cells shall be authorised only after all mandatory consent or authorisation requirements have been met," all collected UCB units need to have a signed consent obtained prior to delivery.[46] At present, in most CBB programs, consent is obtained when the mothers attend their hospital at around 30 weeks of pregnancy or via a "mini consent" form completed before the mother is in established labor. The introduction of the "mini consent" form, which allows for the collection of the CBU, has facilitated the implementation of the EUTCD, and it has also increased the efficiency of collections by decreasing the wastage of CBUs discarded due to the lack of consent.[47] Following the initial mini consent, a full more detailed consent is required and this provides the basis for proceeding to the processing and testing of the collected unit.

An important aspect of the consent process is to provide detailed and clear information about the tests required, the intended use of the unit, particularly in relation to the altruistic nature of the donation, and about the potential use of the clinically unsuitable units for research and development. Also, it needs to include the consent to contact relevant health professionals in the event of a positive result relevant to their health, to obtain and store samples for future testing, and to store personal information.

This also means that for mothers who do not speak the language of the country where they are giving birth, all relevant information and the process of obtaining consent should be performed not only in their own language, but also by somebody able to communicate and answer the relevant questions of the mother. Consent should be taken when the mother is able to concentrate on the process and certainly not when the mother is in labor. Importantly, the NetCord-FACT Standards also mention that regardless of whether the unit is collected for unrelated or related use, if this unit may potentially be used for reasons other than the initial clinical intent, not only this should be mentioned in the informed consent but also the donor should have given consent with documents and information related to the potential related or unrelated use of the unit.

1.1.5 Collection

The collection of the UCB is carried out by suspending the placenta, cannulating the vein and allowing the blood to drain by gravity into a specially designed UCB collection bag placed on a shaker in order to avoid the formation of clots.

Collections can be carried out at fixed and nonfixed sites and in either case an agreement between the CBB and the collection site is required.[23]

Fixed sites. In this model, the UCB units are collected by trained staff employed by the CBB or by the maternity units in each hospital. In Europe, the collection of UCB can only be performed in sites that comply with the regulatory requirement of the EUTCD, i.e., in licensed or fixed sites and by trained staff.

Nonfixed sites. In this case, the collection is performed at any maternity unit by either their own staff or by agency staff. The CBB provides the appropriate kit and instructions for the UCB collection. Although this practice facilitates and allows for the collection of altruistically donated units anywhere in the country, it is also associated with a reduced number of units suitable for banking due to an increase in the number of bacterial infections, and low volumes and TNC of the collected units. This may be due to the lack of training or experience of the personnel performing these collections. This practice is not very common in Europe due to the stringent training requirement of the EUTCD. The current revised version of the NetCord-FACT Standards covers the collection at both fixed and nonfixed sites.

When selecting the collection sites, it is important to consider the number of deliveries per year in order to maximize the resources and to maintain the training of the collection staff.

1.1.5.1 In Utero versus Ex Utero Collections

The UCB collection can be performed in utero or ex utero, following full-term normal delivery or cesarean section. The minimum gestation period for collection is 34 weeks.

In utero collections are performed by a trained member of the delivery team during the third stage of labor before the placenta is delivered. Ex utero collections are carried out by CBB trained staff, normally outside the delivery room to avoid interference with the delivery process. In these collections, the risk to the mother or infant is minimal, but the risk of microbial contamination may be higher.

Initial studies had indicated that in utero collections yielded larger volumes (and TNC doses) than ex utero

collections, but more recent studies have shown that, if appropriately trained staff are involved in the collection, there is no significant difference in the volume, or indeed in the contamination rate, with either of these two methods.[47,48]

Since the safety of mother and child are paramount and because of the possible diversion of the attention from the mother and newborn to the UCB collection, both the UK Royal College of Obstetricians and Gynaecologists (RCOG) (Scientific Advisory Committee for the Royal Colleges of Obstetricians and Gynaecologists, 2001) and the Royal College of Midwives in the United Kingdom have recommended that all UCB collections be carried out ex utero.[49] Also, following reports on the effect of delayed clamping on infant development and iron status, the UK RCOG has also issued guidelines regarding the timing of clamping.[50–52]

Once a successful UCB collection has been obtained, blood samples from the mother are taken for communicable disease testing within 7 days before or after collection. A history of the current pregnancy and delivery, and the infant's donor birth data should be obtained and documented including gender, gestational age, and results of any other relevant test. Information about the clinical examination or any finding suggestive of disease potentially transmitted through transplantation should also be recorded.

The collected units and the associated maternal samples are then transported to the CBB processing centers in a temperature-controlled environment as soon as possible following the collection. An agreement between the collection site and the staff responsible for the transport of these units is required, as well as a documented evidence of training the staff involved in this task.

1.1.6 Donor Follow-up

In some CBBs, a follow-up telephone interview is carried out 8–12 weeks postcollection in order to check the health status of the mother and the newborn from whom the UCB was collected. Other CBB programs have a policy of contacting the mother when a unit has been reserved or prior to its release to the transplant program. All CBUs are quarantine frozen in temporary containers until all relevant test results are reviewed and the units are medically released for long-term storage. Counseling resources should be in place to support the donor and family in case of a positive infectious disease marker other than cytomegalovirus.

1.2 Processing, Cryopreservation, Storage, and Testing

An appropriately signed consent authorizing the processing, testing, and storage of the units and associated samples is required before commencing these procedures.

Although the viability and functionality of the collected stem cells seem to be preserved for up to 96 h, the majority of publications have shown the benefits of processing the UCB units as soon as possible and ideally within 48 h of collection.[53–56] The current NetCord-FACT Standards indicate that the cryopreservation of unrelated UCB units should be performed within 48 h of collection in either a closed system or in an environmentally controlled clean room. For related UCB, the cryopreservation should commence within 72 h of collection.[23]

1.2.1 Processing

The TNC and CD34+ cell content per kilogram of patient's weight (as well as HLA matching) are important factors influencing the outcome of UCB transplantation, particularly in improving engraftment rates.[19,25,57,58] As a result, a minimum dose of TNC and CD34+ cell content of the units has been proposed for transplantation into patients with malignant and nonmalignant diseases.[59–61]

Furthermore, World Marrow Donor Association (WMDA) data indicate that the minimum cut-off TNC and CD34+ count of the transplanted units have increased throughout the years.[14]

Thus, in order to ensure that the stored units can meet the requirements of transplant center acceptable values for volume, TNC and CD34+ cell content of the UCB units collected (i.e., preprocessing) are established by each CBB program in order to compensate for the cell loss occurring during the processing of the units at various stages of the procedure (approximately 10%). Therefore, in order to improve the quality of the units banked and to minimize the costs of CBB, most UCB banks evaluate the collected units for a number of parameters before processing a unit, and these include volume, TNC, CD34, and colony forming unit (CFU) content.[62–68]

The volume of the collected unit was the original parameter used to select those collections that should proceed to processing and subsequent banking and it remains a simple, fast, and cost-effective surrogate marker to be used in the assessment of the collected units. Although the collected volume has a strong correlation with the TNC content, most CBBs use the TNC parameter to inform decisions throughout the CBB process and for the selection of the UCB unit for transplantation.

The benefits of measuring the CD34+ cell content of the units has been highlighted by studies showing that this marker was a better correlate for engraftment than the TNC dose.[19,69] However, in the past, not all banked units had CD34 counts performed at banking, and there was no standardized test for the identification and measurement of CD34+ cells. Today, this test has become more standardized and it is now also possible to assess simultaneously both the percentage of CD34+ cells and viability.[70]

It has been suggested that the potency of the UCB measured by the number of CFUs is strongly positively associated with engraftment rates in children.[71] Page et al. have shown that the potency of the units (measured by the CFU content) was a strong predictor of engraftment.[72] Current methods for assessing the number of CFUs are complex and time-consuming, and unless these assays are performed on every single unit banked at a considerable cost bearing in mind that only about 1–2% of banked units are issued for transplantation. Also, the results may not be available when the unit is requested (they can take up to 14 days). Alternative methods to assess the function or potency of the UCB units have been recently described and are currently undergoing a more extensive clinical evaluation. Our own studies have shown a very good correlation between the amount of volume collected with the TNC, CD34, and CFU content of the units (see Figure 2).

Because the processing procedures can impact on the recovery of the TNC and CD34 (and CFUs), all these parameters have to be measured pre- and postprocessing (or before freezing), and also on the finally selected units released for transplantation.

Other obstetric factors including birth weight also seem to affect the characteristics of the collected units.[73–75]

More recently, Kurtzberg and colleagues have developed a scoring system called the Cord Blood Apgar to optimize UCB unit selection for transplantation.[76] This system considers a number of characteristics of the UCB unit such as volume, TNC, CD34, and CFUs before and after processing, and it also includes thawing techniques and other donor or patient variables.

1.2.1.1 Volume Reduction

Initially, all UCB units were frozen without any manipulation but it soon became clear that the long-term storage of large numbers of frozen units would create a space issue. These considerations led to the development of protocols to reduce the volume of the collected UCB units prior to storage. A number of VR methods are currently being used, the majority of which deplete the unit of red cells and plasma, leaving the buffy coats in a standard volume.[77,78] An important consideration with any VR method is the preservation of a maximum number of viable TNCs and CD34+ cells in the stored buffy coat layer.

The first semiautomated system for VR, the OptiPress, was introduced in 1999. At present, most CBUs are reduced to a standard volume of 21 ml prior to freezing, using automated systems such as SEPAX 540 or the AutoXpress. Through the introduction of two new filters, one for the hydroxyethyl starch/anticoagulant (complete with a small sample bulb to allow for resampling) and a second to add the dimethyl sulfoxide (DMSO), the SEPAX 540 system remains sterile and is referred to as "closed."[79–81] This means that processing of these units can be undertaken in a grade C room, under a laminar flow cabinet.

An additional clinical benefit of VR is that it reduces the amount of DMSO contained in the unit, which is particularly beneficial for units that will be infused to small children. Initially, due to the large volume of DMSO, CB cells had to be washed prior to infusion, especially in the case of small children. Nowadays, washing is not essential for volume-reduced units.

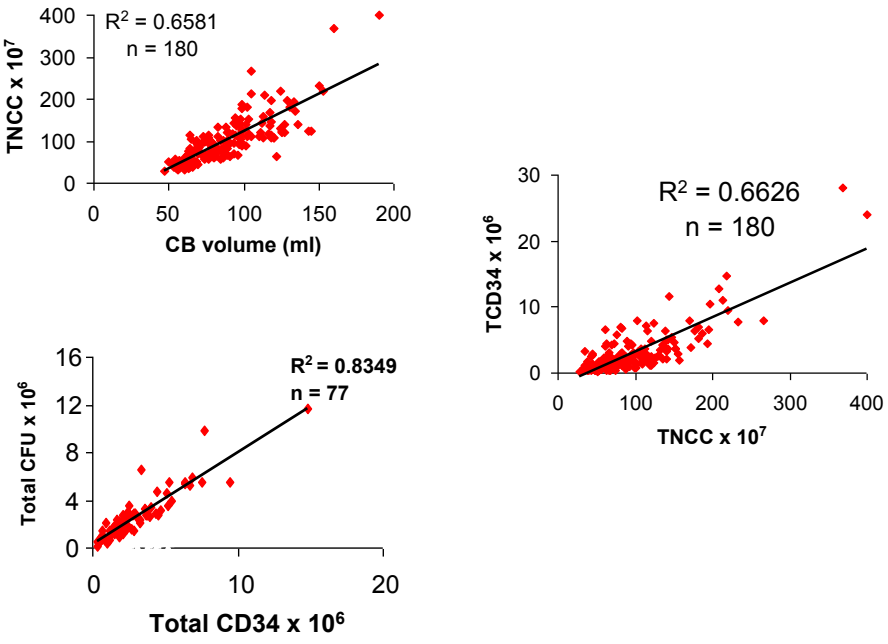

FIGURE 2 Correlation between volume, TNC, CD34, and CFU content of UCB units.

With the introduction of VR, it is now necessary to perform full blood count, TNC, nucleated red blood cell, and CD34 (and CFUs) counts before and after processing, in order to assess the effect of the manipulation on the viability and quality of the unit prior to its long-term storage.

1.2.2 Cryopreservation and Storage

The cryopreservation of buffy coats containing the HSC can be performed using manually or automated (BioarchiveSystem) controlled rate freezer equipment. The automated system provides a platform to freeze and store cells in the same place minimizing exposure to temperature changes and also allowing the electronic identification of the archived units. Thus, when a unit is required for issue, it can be automatically retrieved, through a periscope, without exposing the other units to temperature changes. The VR UCB units are resuspended in 10% DMSO cryoprotectant (50% DMSO diluted in dextran 40) in a freezing bag with two compartments, which is placed in a metal container for cryopreservation and long-term storage However, both the automated and the manual systems are perfectly adequate, provided the temperatures are regularly monitored and the process is fully evaluated and quality controlled. Long-term viability of the frozen cells was also of concern but it is now known that the standard cryopreservation protocols of freezing the cells in 10% DMSO in controlled rate freezers and storage below −135 °C give an average of 80% recovery of nucleated cells and >90% recovery of progenitor cells, as measured by stem cell surrogate markers, CD34+ cells, and CFU assays.[82–85]

1.2.2.1 Archiving of Samples

In order to maximize the amount of cells stored, all the "waste" components produced during the processing of the units are utilized for testing and archiving. Archiving of samples is crucial, in order to be able to perform additional tests in future when a unit is selected for transplantation and, if required, to test for any new marker that may affect the use of the units. At the NHS-CBB, a blood film is prepared from the fresh CB to perform an initial hematological screening of the unit. In addition, a small piece of cord tissue is collected and frozen as a source of DNA for future testing, if required.

1.2.3 Testing

The algorithm for testing the UCB collection is complex. Some tests need to be performed upfront before banking (pre- and postprocessing) and others are carried out when the units are listed for searches. Some tests are performed on the mothers, others in the UCB unit, and others on both. Other tests can (and some must) be performed once the unit has either been reserved or selected for transplantation (see Figure 3).

Among the tests required at banking are those performed on the mother's blood, which in the United Kingdom at least, is the same as those required for blood donors. With the shortening of the window period of infectivity by the introduction of nucleic acid testing for human immunodeficiency virus/hepatitis B virus/hepatitis C virus, it should be possible to eliminate the need for a second 6-month follow-up sample from the mother to retest for infectious disease markers. This requirement was one of the important reasons why significant numbers of units had to be discarded, in spite of their compliance with the banking requirements. Also, mostly depending on the country of origin of the mother-donors, additional screening, such as tests for malaria, Chagas disease, and, more recently, West Nile virus and severe

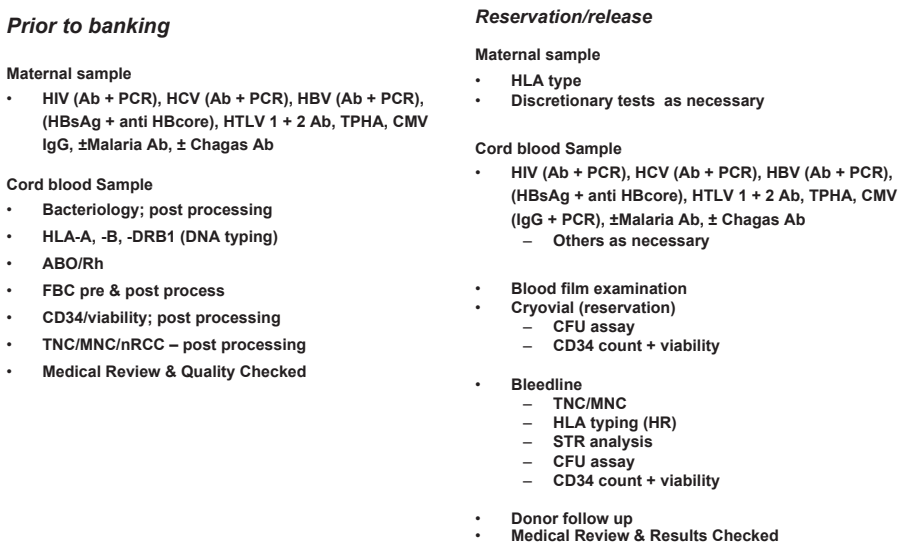

FIGURE 3 Cord blood unit and maternal sample testing.

acute respiratory syndrome, are required to comply with regulations in each country. The UCB unit is also tested for these markers, once selected for transplantation.

The finally processed unit is also tested for both aerobic and anaerobic cultures prior to freezing to assess the presence of bacterial and/or fungal cross-contamination from the birth canal or systemic sepsis in the donor-mother or infant. Initially, lightly contaminated units were kept in the bank provided an antibiotic sensitivity test was performed and the results communicated to the transplant center if required. However, the current NetCord-FACT Standards mandate that bacterially contaminated unrelated units should be discarded. UCB units collected for directed use, either related or autologous, can still be banked provided the above-mentioned tests are performed.

All UCB units are tested for ABO/Rh, TNC, and CD34, and some CBBs also perform CFU on all units post processing. The HLA typing of the units is also carried out at the time of banking. Current standards indicate that all HLA typing should be carried out using DNA-based molecular techniques. Low-resolution HLA–ABC and high-resolution HLA-DRB1 typing should be performed prior to the listing of the units with the relevant UCB or adult unrelated HSC donor registries. A number of recent publications have indicated that if the transplanted UCB unit is a mismatch at one of the noninherited maternal HLA alleles, there is an improvement in the outcome of this transplant.[86,87] As a result, a number of UCB banks are now typing and reporting the maternal HLA type of the listed units. The role of the KIR receptor/ligand mismatching is still controversial;[88,89] therefore, the majority of CBBs do not routinely test their units for these markers before listing them in the national or international HSC registries.

1.3 Listing, Searches, Selection, Testing, Distribution, and Follow-up

1.3.1 Listing, Searches, Additional Testing, and Selection of UCB Units

On completion of the processing and testing, all the information regarding the mother and the CBU must be reviewed by the Medical Director of the CBB (or a designee) to assess the suitability of the unit for inclusion into the bank. Once the units are medically released, they can be listed for searches with both the national and international registries. All units are listed under a unique identifier with the following information: HLA type, volume of collected UCB, and TNC (and CD34+, CFUs, and maternal HLA typing if available) of the final product. The issue as to whether the CD34 count should be included at registration is currently under discussion. Some CBBs are also registering the HLA typing of the mother of the CBU in order to provide the option of selecting mismatched CBU based on the noninherited maternal antigens (NIMAs.)

At present, there are several international registries, NetCord listing only UCB units and AsiaCord and Bone Marrow Donors Worldwide (BMDW), which contain both adult HSC donors and CBUs (http://www.bmdw.org). The National Marrow Donor Program (NMDP) also lists units of its associated partners for searches.[90] There are approximately 200,000 CBUs in NetCord, and over 600,000 registered with BMDW.[15] Most units registered with NetCord are also in BMDW (see Figure 4). In future, with the implementation of the European Marrow Donor Information System (EMDIS) Cord, an electronic system designed for the rapid exchange of information and requests between transplant centers and donor registries, this system should speed up the whole process of donor searches and selection, since all relevant information about a UCB unit will be readily available.

Some of the first CBBs that were established operate as independent registries. However, today, the vast majority of CBBs work through their national registries due to the fact that most transplant centers prefer a combined search report, listing all available, suitably matched adult donors and CBUs at the same time.

The current NetCord-FACT Standards indicate that all registry aspects of the CBB programs need to operate under the guidelines of the WMDA,[91,92] and that these registries should be WMDA accredited or in the process of obtaining accreditation. Transplant centers initiate a search for CBUs in the same way as for adult stem cell donors and once a transplant center receives a match report, it contacts the relevant UCB bank directly to request further information and/or additional tests such as high-resolution HLA data, CFU content, or microbiological markers.

The CBB program should have a fully validated electronic record system to enable the listing, searches, and distribution of UCB to the transplant program.

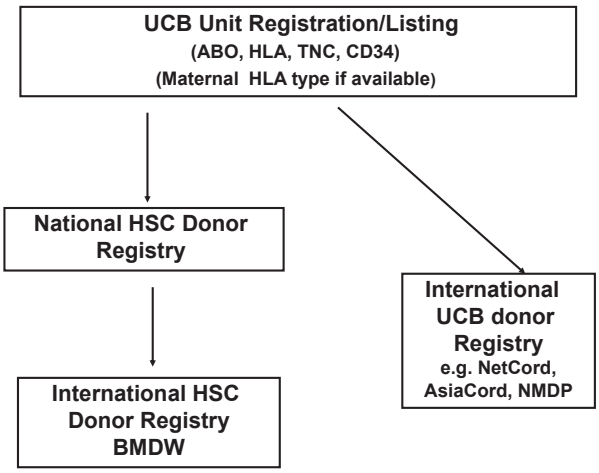

FIGURE 4 Description on listing of banked CB units nationally and internationally.

1.3.2 Additional Testing

When a UCB unit is reserved or released for transplantation, a number of additional tests are performed at the request of the transplant centers. The type and resolution of tests required at this point have changed with the years as a result of the clinical outcome analyses. For instance, the range of required tests for infectious disease markers is expanding and now includes Epstein Barr virus, human herpesvirus 6, 7, and 8, and toxoplasmosis.

The request for CFU assays to assess the functionality or potency of the UCB cells is not consistent and many transplant centers are prepared to go ahead with the procedure in the absence of these results. Due to the high cost of CFU assays and since these results can take up to 14 days, most CBBs perform this test at the stage of reservation of the unit. High-resolution HLA-A, B, C, and DRB1 typing is also performed prior to the release of the unit and on a contiguous segment, if this is not available then another method needs to be used to confirm the identity of the UCB unit. Screening of the selected UCB unit for abnormal hemoglobins has also become an additional requirement prior to their release.

1.3.3 Distribution to a Clinical Program and Follow-up of the Transplanted UCB Units

Clear documentation and procedure for the transport of the frozen selected UCB units to the transplant center should be in place. The units are transported in shipment containers by accredited and trained carriers.

The clinical follow-up of the released units is an important quality aspect of the operation of CBB. This is normally carried out by a national registry and/or by Eurocord[93] and the Center for International Blood and Marrow Transplant Research (CIBMTR).[94] Eurocord was established in 1999 and is responsible for collecting and analyzing all clinical outcome data on CB transplantation on behalf of the European Blood and Marrow Transplant Group.[95] CIBMTR fulfills a similar role for the transplant activity in the United States and other North and South American countries. Eurocord and CIBMTR have recently agreed to share information and analyses in order to avoid duplication of the reported data.

As mentioned above, all the activities of a CBB program need to be supported by robust electronic inventory and quality management systems and programs. All policies and procedures should be documented and updated regularly to incorporate changes in the relevant standards and/or to the outcome of internal or external audits.

The label of the UCB and all associated samples including maternal samples produced throughout the various stages of the process have to be International Society of Blood Transfusion 128 compatible for traceability.

1.3.3.1 Accreditation/Licensing and Regulations

Since CBB and UCB transplantation activities involve the import and export of a cellular product across different countries, they need to operate within a highly regulated environment in order to ensure that the donations released are safe and of high quality. NetCord-FACT and the AABB have developed standards and accreditation schemes to support this activity. These standards also state that all laboratories supporting CBB activities need to have the relevant additional accreditations in place, e.g., European Federation for Immunogenetics or American Society of Histocompatibility and Immunogenetics for the HLA aspects and WMDA for the registry aspects.[23,92,96] Internationally, all aspects related to the clinical transplantation of UCB cells are covered by the FACT-JACIE (Joint Accreditation Committee ISCT & EBMT) Standards and not by the NetCord-FACT or AABB Standards.

The regulatory aspects covering the activity of CBB have also increased significantly in the past years. In the European Union, the EU Directive 2006/17/Ec and 2006/86/EC regulates on the quality and safety issues covering the donation, procurement, testing, processing, preservation, storage, and distribution of human tissues and cells.[97] These directives require all member states to have inspection and accreditation systems in place ensuring that all banks providing these services comply with an agreed set of standards. In the United Kingdom this is implemented by the Human Tissue Act, set up in 2004 and implemented in April 2006.[98] Also, locally in the United Kingdom, the Code of Practice for Tissue Banks published in 2001 covers all establishments providing tissues and cells of human origin for therapeutic use. This forms the basis for the Department of Health accreditation scheme to which all CBBs within the United Kingdom are required to be licensed, with inspections carried out by the Medicines and Healthcare products Regulatory Agency.[99]

In the United States, the Food and Drug Administration (FDA) in 2005 introduced the regulation of the manufacture of unrelated UCB to support the compliance of the Current Good Tissue Practices 21 CFR 1271.210. Later on in 2007, the FDA issued a draft guidance recommending the licensure of CBB for the manufacture of UCB units. This was finally implemented in 2011 and requires that all manufacturers of UCB units need to have an approved Biologics License Application or Investigational New Drug Application in order to be able to import a UCB into the United States. This regulation treats a UCB unit as a biological drug.[100,101]

1.3.3.2 Optimal Size of a Cord Blood Bank

Discussions around cost efficiency and optimal size of UCB units required to provide donors for the majority (80%) of patients in need of an unrelated donor have been ongoing since adult HSC donor registries were first established. The probability of finding an HLA-matched unrelated donor depends not only on the number of loci (i.e., 6/6 or 10/10 loci) and resolution (medium vs. high) of the HLA matching

required, but also on the ethnic background of the patient and the pool of donors to be searched. Since the vast majority of donors currently available in the international registries are of European Caucasoid ethnic background, the probability of finding a 6/6 (or a 10/10) HLA-matched donor for patients from EM backgrounds is significantly reduced.[102]

In UCB transplantation, a higher degree of HLA NetCord mismatches could be tolerated and these transplants could be performed with as little as 3/6 HLA loci matching between the recipient and the CBU. Also, several studies have confirmed that the outcomes of CB transplantation between 4/6 and 5/6 matched donors and recipients seem to be comparable to those seen between fully NetCord matched adult donors.

These results led to the proposition that the required size of UCB inventory could be smaller than that of adult unrelated HSC donors. Most patients could find at least one 4/6 matched donor from the current global CBU inventory.

A study published in 2009 demonstrated that, at least for the United Kingdom, a minimum number of 50,000 banked UCB should be sufficient to provide a UCB NetCord matched unit to approximately 80% of patients.[103–107] The role of high resolution (HR) HLA matching was found not to be significant.[108]

However, it has now been shown that high-resolution HLA-A, B, C, and DRB1 matching may also influence the clinical outcome of UCB transplantation and if these results are confirmed, a larger pool of donors to select the best compatible UCB will be required.[109,110] Therefore, the question as to whether the exact number of banked UCB units would suffice requires further evaluation.

Recent data have shown improved transplant outcomes after HLA-mismatched UCB transplantation where the mismatched antigen in the patient is matched to the donor NIMA, suggesting that when a fully HLA-matched CBU donor is unavailable, a NIMA-matched donor could be chosen.[86,87] If NIMAs are taken into account, additional "virtual" HLA phenotypes of the CBU are available for matching consideration. One locus NIMA substitution for the current matching guidelines for HLA-A, B, or DRB1 loci would add six "virtual" phenotypes, two substitutions would provide 12 "virtual" phenotypes, and three substitutions would provide eight "virtual" phenotypes. One CBU could then provide a maximum of 26 "virtual" phenotypes if all HLA-A, B, and DRB1 loci are included. The NHS-CBB currently has 3000 UCB units listed with maternal HLA phenotypes in the British Bone Marrow Registry (BBMR) and BMDW. These UCB units have the potential of adding 18,000 new phenotypes with one NIMA substitution, 36,000 new phenotypes with two, and 24,000 with three substitutions, giving a total of 78,000 new phenotypes for the CBU registered. If maternal HLA typing was performed on the 15,000 NHS-CBB banked and registered CBUs, a potential 390,000 additional "virtual" CBU phenotypes would be added to the BBMR. By using information on the HLA types of NIMAs it is possible to increase the number of CBU phenotypes available for searching and consequently increase the probability of finding an appropriately matched donor for a patient.

1.3.3.3 Future Challenges

One of the important remaining challenges in UCB transplantation is how to improve engraftment, associated with a slow and often incomplete immune recovery, particularly in adult patients. This limitation, which seems to be primarily due to the insufficient number of HSC and to immunological naïvety of the immunological effectors, such as T cells in the UCB collections, has led to the development of a number of approaches to improve these outcomes. Of these the most successful so far has been the use of two UCB units for an individual patient in order to increase the number of transplanted HSCs.[111–113] This approach has yielded comparable results to those using a single CBU, and has opened the way for using UCB transplantation in older and heavier patients. The initial protocol developed by the Minneapolis group has now been adopted by many centers with or without modifications and has allowed the performance of UCB transplantation in patients not previously considered for the procedure due to their age or weight.

Other developments include the in vitro expansion of UCB HSC but early attempts were not very successful, since it appears that the majority of the protocols used led to the expansion of mainly mature progenitors. More recently, in vitro and in vivo expansion using SDF-1/CXCL12 associated to diprotin A and/or other cytokines, or using Notch-ligand Delta 1, or mesenchymal stem cells (MSCs) has been described.[114,115]

Enhancing the homing capacity of UCB cells by inhibiting the enzymatic activity of CD26/dipeptidylpeptidase IV or by in vivo direct injection of CB cells into the iliac crest has been published and has also gone into phase II clinical trials.[116]

Some investigators have now attempted the infusion of UCB intrabone or in conjunction with CD34+ or third party bone marrow-derived MSCs, with or without CD34+ cells, with limited improvement in engraftment rates.[117,118]

Regardless of these potential new developments, the majority of CBBs are now banking UCB units with greater TNC and a high number of CD34+ cells.

The identification of modifiable prognostic factors for engraftment such as choosing the "best" CBU based on cell dose such as HLA, diagnosis, and presence of HLA antibodies may also contribute to the improvement of this procedure.

Another challenge is to try to improve the immune reconstitution of cord blood transplant patients in order to reduce infections and/or viral reactivation.[119]

2. CONCLUSIONS

Unrelated UCB banking is a complex and highly regulated procedure and involves the collection, processing, testing, banking, listing, selection, and release of frozen UCB units to patients in need of an HSC transplant. Since its inception, it has undergone a significant evolution driven primarily by the clinical results obtained with the use of the banked units.

On the other hand, despite the initial skepticism of many transplant physicians, the success of UCB transplantation that we see today has been aided by development and implementation of stringent standards and international accreditation programs to ensure the safety, quality, and efficacy of these UCB units. In future and if the new experimental protocols for the expansion of HSCs and/or immune effectors prove to be successful, further development of the procedures and standards currently used in the banking of UCB will be required.

As UCB transplantation continues to increase and with the introduction of new clinical protocols, other genetic and epigenetic factors related to the quality and the efficacy of the UCB units and/or related to the recipient of these units may begin to emerge. Also, some of the immunotherapy protocols derived from adult unrelated HSC donors such as expansion of viral-specific T cell, regulatory T cells, NK cells, or MSCs could be applied to UCB transplantation. If this is the case, CBBs will have to consider the operational changes that will be required to collect, process, store, and release these new associated products.

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