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Intracranial haemorrhage in thrombocytopenic haematology patients—a nested case–control study: the InCiTe study protocol

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ABSTRACT

Introduction: Intracranial haemorrhage (ICH) is one of the most serious side-effects of severe thrombocytopenia in haematology patients. ICH is rare, but can have devastating consequences (death or major morbidity). It is unknown why some patients with severe thrombocytopenia bleed and others do not.

Study aims: Primary aim was to identify risk factors for ICH in patients with haematological malignancies. Secondary aims were to identify short-term outcomes for these patients at 30 days (major morbidity and mortality) and produce a more accurate estimate of ICH incidence in this population. This information is key to identifying means to improve treatment and quality of care.

Methods/analysis: This is a UK-wide case–control study of ICH nested within a 4-year prospective surveillance study set up specifically for the case–control study. Each case will be matched to one control. Cases will be adult haematology patients (≥16 years) who have had any type or severity of ICH who are receiving, about to receive or have just received myeloablative chemotherapy (defined as chemotherapy expected to cause a significant thrombocytopenia <50×10^9/L for >5 days) or a haemopoietic stem cell transplant. Only patients being treated with curative intent will be included. Controls will be patients who fulfil the same inclusion criteria as cases (apart from ICH) and were treated at the same hospital immediately before the index case. Cases and controls will be matched to type of treatment (myeloablative chemotherapy or haemopoietic stem cell transplant). Hospitals across the UK will participate in a monthly email reporting strategy (started June 2011), as to whether a case of ICH occurred during the preceding calendar month. Case and control forms will be sent to any hospital reporting an eligible case. Conditional logistic regression will be used to calculate ORs. Denominator data for incidence estimates will use national registry data.

Study Registration: ISRCTN05026912 (prospective registration). NIHR Portfolio (UKCRN ID 10712).

Strengths and limitations of this study

- Case–control studies are an efficient way of studying rare outcomes.
- Nested case–control design minimises selection bias.
- UK-wide study.
- Data on exposures based on data from medical records. This minimises recall bias but risk that data may not have been recorded.

intensive chemotherapy), it is important to gain a greater understanding of the risk factors for life-threatening haemorrhage. This case–control study concentrates on intracranial haemorrhage (ICH) because it is the most serious type of bleed caused by significant thrombocytopenia. If an ICH does not cause death, it may lead to significant long-term morbidity. However, this complication of severe thrombocytopenia (defined as a platelet count ≤50×10^9/L) is rare, its exact incidence is uncertain and predisposing risk factors are unknown.

There have been many case reports in the literature of ICH in patients with haematological disorders, but there has been no previous attempt to prospectively study ICH within this patient group and assess whether there are any common factors that predispose patients to this serious side-effect.

Prophylactic platelet transfusions are used to prevent patients with severe thrombocytopenia developing a life-threatening haemorrhage. The use of platelet transfusions in patients with haematological disorders accounts for a large proportion (59%) of all platelets issued in the UK.[1] In patients with haematological malignancies, platelets issued to prevent thrombocytopenic bleeding account for up to 69% of all platelets issued to that patient population.[2 3] Platelet transfusions are not without risk, and therefore a

INTRODUCTION

To advance the quality of care for haematology patients (when they are receiving

reliable marker of a patient's bleeding risk needs to be sought. A number of recently completed studies have highlighted our lack of knowledge regarding risk factors and incidence of severe and life-threatening haemorrhage.[4–7]

The morning platelet count has been used, up to now, to indicate when a patient requires prophylactic platelet transfusions. The consensus outlined in BCSH guidelines on platelet transfusions was that patients should receive a platelet transfusion when the platelet count is $<10\times10^9$/L, unless there are other risk factors for haemorrhage such as sepsis, concurrent use of antibiotics or other abnormalities of haemostasis.[8] A patient's morning platelet count has been shown to be a poor predictor of haemorrhage.[9] In a recent study, the rate of bleeding was similar over a broad range of platelet counts ($6–80\times10^9$/L).[5] This is unsurprising as a patient's platelet count indicates only the presence of a specific number of platelets within the circulation; it does not give any information on the functional activity of these platelets, nor does it provide any information on the other factors that affect the formation of a clot. Why some patients with severe thrombocytopenia bleed and others do not is therefore unknown.

Rare complications in haematological disorders are difficult to study and in consequence are often under-researched; our understanding of them is poor; and any interventions used in current clinical practice are rarely based on robust evidence. Routine sources of information are limited or unreliable,[10] and comprehensive studies of uncommon haematological conditions require a large collaboration to identify relatively small numbers of patients. A case–control study nested within a prospective surveillance study will overcome many of these problems. This type of study design has been used successfully to study rare disorders in other patient groups, but this is the first time this study design has been used in haematology patients.[11 12]

A systematic review of the literature (2000–2010) found that cases of ICH in haematology patients have been very poorly reported.[13] Data about postulated risk factors from this systematic review and from known risk factors for ICH in the general population will be collected in this study.

STUDY OBJECTIVES
Primary objective
What factors (eg, age, type of haematological disease, treatment and infection) are associated with an increased risk of developing an ICH?

Secondary objectives
Objective 1: What is the incidence of ICH in thrombocytopenic haematology patients?

Objective 2: What are the short-term outcomes for these patients (eg, death within 30 days of haemorrhage, persistent neurological deficit)?

STUDY DESIGN
This is a case–control study nested within a prospective surveillance system.

The study aims to collect anonymous data about haematological patients who have had an ICH while undergoing intensive chemotherapy or a stem cell transplant. This information is key to identifying means to improve treatment and quality of care.

The British Paediatric Surveillance Unit (BPSU) and the UK Obstetric Surveillance System (UKOSS) have developed a reliable and straightforward methodology to study uncommon disorders of childhood and pregnancy. BPSU surveys have been used to inform national screening committee decisions on antenatal screening.[14] Information will be collected through doctors and specialist nurses in hospitals throughout the UK. An information collection system will be developed for this study and will be based on similar systems in obstetrics (UKOSS). Assistance in the development of this system will be via the British Society of Blood and Marrow Transplantation (BSBMT) and advice from UKOSS. Once this system is in place, it could also be used for future studies of rare disorders and complications within haematology that are difficult to study via any other method.

Recruitment
Patients will be identified through a new haematological surveillance system (Haematology Active Surveillance System) 'HASS', which is based on similar obstetric (UKOSS) and paediatric surveillance systems (BPSU), to study rare haematological disorders.

Case identification
This anonymous descriptive case–control study will be conducted through a monthly case-collection scheme. Each hospital with a haematology department caring for patients with acute leukaemia or transplant patients will identify at least four individuals (representing haematologists, specialist nurses and transplant coordinators) to report to HASS.

The individuals will be identified via BSBMT (principal transplant consultant and transplant coordinator, specialist nurse) and individuals associated with acute myeloid leukaemia (AML)-16 trial or other current Medical Research Council (MRC) leukaemia trials for non-transplant cases (principal investigator for the current leukaemia trials at each centre and research nurses associated with the trials).

Every month, the four nominated individuals will be sent a report card (in an email format). They will be asked to complete a simple Yes/No tick box indicating if any cases have occurred in the previous month (figure 1). We expect that the majority of cards each month will be 'No cases' because ICH is a rare event. 'No cases' responses are extremely important because they allow us to confirm the number of haematology patients in the denominator cohort.
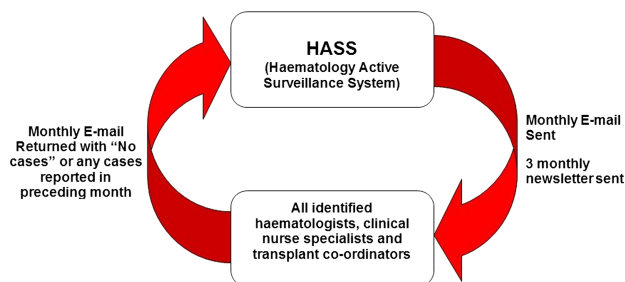
Figure 1 Study design for reporting cases of ICH to the InCiTe study.

Control identification

In order to perform the case–control study, HASS will also collect anonymised information on control patients. Clinicians who report a case will also be asked to identify an appropriate control patient and complete a similar data collection form from their case notes.

Study participants

Cases: All adult patients (≥16 years of age) who have an ICH while receiving or about to receive myeloablative chemotherapy (expected to cause a significant thrombocytopenia $<50\times10^9/L$ for >5 days) or a stem cell transplant within the study period (1 May 2011 to 30 April 2015). Only patients being treated or about to be treated with curative intent will be included (this includes patients who present with an ICH at initial diagnosis or relapse and who would have been treated with curative intent if they had not had an ICH). All types and severities of ICH will be included.

Controls: Are patients who fulfil the same inclusion criteria as cases (apart from the presence of ICH) and were treated immediately before the index case. Cases will be matched to type of treatment (chemotherapy or haemopoietic stem cell transplantation).

This methodology has been used by UKOSS successfully in previous studies and has been shown to produce a comparison group similar in characteristics to the population as a whole.

Data collection

On receiving a case report, the central team will dispatch case and control data collection forms to the clinician. The data collection forms will seek additional information on risk factors in both cases and controls, and the management and outcome of the ICH. Cases and controls will be allocated a central HASS identification number. No names, addresses, dates of birth, hospital numbers or National Health Service (NHS) numbers will be sought. Respondents will only be asked to record the unique HASS identification number in order to facilitate elimination of duplicates.

If the completed forms are not received back by the central team after 4 weeks, a written reminder will be sent out. If there is still no response after a further 4 weeks, a further set of forms will be sent to the centre (to ensure that non-return is not due to the forms going missing) and the clinician will be contacted by telephone. After a further 4 weeks, the clinician will receive a further written reminder and telephone call.

All information will be anonymous and will be completed from the patient's case notes. The studies thus only involve the provision of information after the acute event has occurred. The patients' management will not be changed in any way by inclusion of their data in the study, and patients will not be contacted at any point by the central research team or by local collaborating clinicians. All the data requested in the case and control forms are data that are routinely recorded within patients' medical records.

The response rate from reporting clinicians will be monitored throughout the course of the study, as part of the routine operation of HASS. A three-monthly newsletter will be produced to inform all the reporting clinicians of the response rate to the study, and the number of cases reported.

Rationale for questions to be asked within the data collection form

Clinical factors associated with increased risk of ICH in the general population

Age: The risk of ICH increases with age. In a systematic review of the literature, a relative risk (RR) of ICH 1.97 for every 10 years increase in age (95% CI 1.79 to 2.17).[15] In a pooled prospective study of over 21 000 individuals, there was an RR 2.06 for every 10 years increase in age (95% CI 1.76 to 2.51).[16]

Sex: Some studies have shown a significantly higher rate of ICH in men. RR in general population male versus female for ICH 3.73 (95% CI 3.28 to 4.25).[15] No difference was seen in Sturgeon et al.[16]

Ethnic origin: Young and middle-aged blacks have a substantially higher risk of subarachnoid haemorrhage (SAH) or intracerebral haemorrhage than whites of a similar age (2.1×risk of SAH (95% CI 1.3 to 3.6); 1.4×risk of intracerebral haemorrhage (95% CI 0.9 to 2.1)[17]: in Sturgeon et al's[16] study RR 2.56 (95% CI 1.8 to 3.65)).

Smoker: Current smoker RR 1.31 (95% CI 1.09 to 1.58).[15] No difference seen in Sturgeon et al.[16]

Hypertension: Patients with systolic blood pressure (BP) ≥160 mm Hg or diastolic BP ≥110 mm Hg RR 5.55 (95% CI 3.07 to 10.0).[16] In Ariesen et al's[15] study 2003 RR 3.68 (95% CI 2.52 to 5.38).

Diabetes: In Ariesen et al's[15] study 2003 RR 1.3 (95% CI 1.02 to 1.67). No difference seen in Sturgeon et al.[16]

Site of haemorrhage: Site of haemorrhage is a predictor of functional outcome in the general population.[18] In a recent study, poor outcomes after ICH in patients with AML were associated with four independent risk factors, three of which were associated with the site of the haemorrhage. Brainstem haemorrhage (p=0.001), SAH (p=0.017) and extradural haemorrhage (p=0.014).[19]

Volume of haemorrhage: In the general population, the volume of the haematoma is one of the most important predictors of mortality and functional outcome after ICH.[18 20 21]

Glasgow Coma Scale (GCS) at time of haemorrhage: In the general population, this was another important predictor of functional outcome. FUNC score GCS <9, a much worse outcome.[18]

Clinical factors associated with haemorrhage in haematology patients

A recent history of severe bleeding: In a review of almost 3000 thrombocytopenic adult patients, over a 10-year period, Friedmann et al[9] showed a significant relationship between a history of recent bleeding (within the previous 5 days) and occurrence of significant haemorrhage (OR 6.72; 95% CI 5.53 to 8.18).

Uraemia: In Friedmann et al's[9] study, uraemia (defined as blood urea nitrogen >50 mg/dL which equals urea >17.9 mmol/L) was associated with an increased risk of bleeding (OR 1.64; 95% CI 1.40 to 1.92).

Recent bone marrow transplantation: In Friedmann et al's[9] study, a recent bone marrow transplant (BMT; under 100 days) was associated with an increased risk of bleeding (OR 1.32; 95% CI 1.22 to 1.43).

Hypoalbuminaemia: In Friedmann et al's[9] study, hypoalbuminaemia (defined as a serum albumin of 2.0 g/dL or lower) was associated with an increased risk of bleeding (OR 1.54; 95% CI 1.33 to 1.79).

Acute graft versus host disease (GvHD): In a single centre retrospective study of 622 allogeneic transplant patients over a 20-year period, 21 cases of ICH were identified. A multivariate analysis with logistic regression identified acute GvHD as the only factor that significantly influenced ICH occurrence.[22] In a single centre randomised controlled trial of platelet transfusions, (n=159, of which 41 had allogeneic transplants), GvHD of any grade was associated with an increased risk of haemorrhage on univariate analysis (OR 2.8; 95% CI 1.2 to 8.2).[23]

Veno-occlusive disease (VOD): Najima et al[22] and Zumberg et al[23] also showed a possible association of VOD of any grade with an increased risk of haemorrhage, although the CIs were very wide (HR 2.63 (95% CI 0.77 to 9.00); OR 4.4; 95% CI 0.6 to 27.8).

Fever: In Webert et al's[24] retrospective analysis of Rebulla et al's[25] data, the presence of an elevated body temperature increased the risk of mild bleeding (grades I and II) by 52% (RR 1.52; 95% CI 1.25 to 1.85; p<0.005). The presence of an elevated body temperature increased the risk of clinically significant bleeding (grades II–IV) by 87% (RR 1.87; 95% CI 1.40 to 2.49; p<0.005). For clinically significant bleeding, the risk of bleeding increased as the temperature increased. Risk of bleeding increased significantly when the patient's body temperature was between 38 and 38.4 (RR 2.43; 95% CI 1.0 to 5.90; p<0.05); and >38.5 (RR 3.95; 95% CI 1.90 to 8.20; p=0.0001). In a previous small study, 9 of 13 patients who bled were febrile at the time of haemorrhage. However, this study was performed when aspirin was still used as an antipyretic.[26 27]

Use of amphotericin: Therapeutic use of amphotericin B is associated with decreased expression of glycoprotein Ib on the surface of stored platelets.[28] This may induce a platelet function defect. In Zumberg et al's[23] study, usage of amphotericin B was associated with an increased risk of bleeding (OR 3.8; 95% CI 1.3 to 10.5).

Use of antibiotics: β lactam antibiotics have been associated with platelet dysfunction.[29 30]

Laboratory factors associated with haemorrhage

C reactive protein: Inflammation has been shown to induce severe haemorrhage in thrombocytopenic mice.[31]

Prothrombin time (PT): Prolonged PT was associated with a poorer outcome after ICH in patients with AML (p<0.001).[19]

Haemoglobin: A low haematocrit has been shown to be associated with an increased risk of bleeding.[32–34]

Persistent thrombocytopenia: Platelets have been shown to provide an endothelial supportive function by plugging gaps in the endothelium of otherwise intact blood vessels. Animal studies have shown that thrombocytopenia is associated with the gradual thinning of the vessel wall endothelium over time, and that, if thrombocytopenia persists, gaps gradually occur between adjacent endothelial cells.[35–37] This thinning and fenestration of the endothelium is accompanied with ongoing and increased use of circulating platelets to prevent the loss of red blood cells through these gaps. In a study of 1402 BMT patients very low platelet counts were significantly associated with bleeding post BMT. The risk of bleeding in a patient with 3–7 (out of 7) days of platelet counts $<10\times10^9$/L in the week preceding the haemorrhage was 40–60% higher than in a patient with 0–2 days with low platelet counts. However, only 8.6% of patients who bled had such profound thrombocytopenia prior to the bleeding episode. In most cases, bleeding episodes started with platelet counts $>20\times10^9$/L.[38 39]

Definition of end of study/study power

The study will be completed at the end of 4 years if a sufficient number of cases have been reported to allow detection of an OR of 2 with 80% power at a 5% significance level for the risk factors of fever, amphotericin B usage and antibiotic usage. This will require a minimum of 136 cases (for the variables stated above), using the incidence data of these variables from previous studies.[9 40] We had originally planned the study length to provide sufficient cases and controls to detect with 80% power at the 5% level an OR of 2.5 for a range of analyses of associated factors. This would have required a minimum of 78 cases using the incidence data referred to above. An interim review of this study by an expert epidemiologist advised that the study should remain open for longer than the planned 2-year period. This was because the majority of ORs identified from previous studies of ICH in the general population

detected significant OR for individual factors of between 1.5 and 2.[9 15–17 19 22–24] Our original minimum study number would have been insufficient to detect an OR of 2 with sufficient power.

The true incidence of ICH is unknown and there are a wide range of estimates from the literature, from 0.5% to 6.9%.[5 22 25 41–43] The study initially expected that the true estimate was between 1% and 2%, with a prediction of 120–240 cases within a 2-year period. This was an overestimate of the number of cases actually reported once the study had started, and the study duration has therefore been extended to 4 years after a review of initial recruitment by the study management group.

Denominator data

One of the secondary objectives is to determine the incidence of ICH in patients with a haematological malignancy or receiving a stem cell transplant. According to the BSBMT registry, there were 2939 transplants performed in 2008. All stem cell transplants performed in the UK have to be reported via BSBMT to the European Group for Blood and Bone Marrow Transplantation (EBMT). We will be able to obtain accurate denominator data via BSBMT for transplant patients.

Accurate denominator data for patients with acute leukaemia will be obtained via a variety of sources including numbers recruited to MRC acute leukaemia trials over the designated time period. The majority of patients diagnosed with leukaemia in the UK are recruited to an acute leukaemia trial.

ANALYSIS

Descriptive information will be presented as frequencies or proportions with CIs.

ORs with 95% CIs will be calculated and adjusted for confounders and effect modifiers using conditional logistic regression.

Incidence rates will be calculated with 95% CIs. See preceding section regarding denominator data. If the true incidence of ICH is 0.5%, then an accurate estimate of incidence will be obtainable with 60 cases from a study population of approximately 12 000 (transplant patients (approximately 6000 cases over 2 years) and non-transplant patients (approximately 6000 cases over 2 years) combined). This would provide an estimate of the incidence of ICH of 0.5% with a 95% CI of 0.39% to 0.64%. An estimate of incidence will not use data from the whole 4-year-study period. This is because fewer hospitals had been recruited and were actively participating in the study's first year.

Consent

It will not be practicable to obtain consent for data collection from individual patients, as this would prevent the achievement of one of the objectives of the study, namely to document the number of patients who suffer from this complication in the UK. Accurate measurement of incidence requires documentation of all cases occurring in the UK.

The National Information Governance Board (formerly Patient Information Advisory Group (PIAG)) considers that organisations seeking to use patient information for research purposes without consent should seek anonymised or pseudoanonymised data only and not any personally identifiable information.

Accordingly, this study will not collect names, addresses, postcodes, dates of birth, hospital numbers or NHS numbers in order to maintain patient confidentiality. Collection of data in this way in the absence of consent is unlikely to cause significant harm.

Data security

The security of all data will be maintained by storage on NHS Blood and Transplant's (NHSBT) secure network, accessible only by the key researchers and responsible members of NHSBT who may require access to data to ensure compliance with regulations. Access by any other individuals for the purposes of any other study will only be allowed after a successful application to a research ethics committee.

Dissemination of study information

The study has been prospectively registered on the Clinical Trials website http://www.controlled-trials.com (ISRCTN05026912).

The data from this study will be analysed and the results published as soon as possible in a scientific journal after study completion. The information will be published and distributed to all participating clinicians; it will also be available on the HASS website as well as being presented at scientific meetings.

REFERENCES

1. Pendry K, Davies T. An audit of the use and wastage in the North West of England and North Wales—where have all the platelets gone? *Blood Transfus Matters* 2011;34:17–19.
2. Estcourt LJ, Birchall J, Lowe D, *et al*. Platelet transfusions in haematology patients: are we using them appropriately? *Vox Sang* 2012;103:284–93.
3. Qureshi H, Lowe D, Dobson P, *et al*. National comparative audit of the use of platelet transfusions in the UK. *Transfus Clin Biol* 2007;14:509–13.
4. Heddle NM, Cook RJ, Tinmouth A, *et al*. A randomized controlled trial comparing standard and low dose strategies for transfusion of platelets (SToP) to patients with thrombocytopenia. *Blood* 2009;113:1564–73.
5. Slichter SJ, Kaufman RM, Assmann SF, *et al*. Dose of prophylactic platelet transfusions and prevention of hemorrhage. *N Engl J Med* 2010;362:600–13.
6. Wandt H, Schaefer-Eckart K, Wendelin K, *et al*. Therapeutic platelet transfusion versus routine prophylactic transfusion in patients with haematological malignancies: an open-label, multicentre, randomised study. *Lancet* 2012;380:1309–16.
7. Stanworth SJ, Estcourt LJ, Powter G, *et al*. A no-prophylaxis platelet transfusion strategy for hematologic cancers. *N Engl J Med* 2013;368:1771–80.
8. BCSH. British Committee for Standards in Haematology: guidelines for the use of platelet transfusions. *Br J Haematol* 2003;122:10–23.
9. Friedmann AM, Sengul H, Lehmann H, *et al*. Do basic laboratory tests or clinical observations predict bleeding in thrombocytopenic oncology patients? A reevaluation of prophylactic platelet transfusions. *Transfus Med Rev* 2002;16:34–45.
10. Dixon J, Sanderson C, Elliott P, *et al*. Assessment of the reproducibility of clinical coding in routinely collected hospital activity data: a study in two hospitals. *J Public Health Med* 1998;20:63–9.
11. Fitzpatrick KE, Sellers S, Spark P, *et al*. Incidence and risk factors for placenta accreta/increta/percreta in the UK: a national case-control study. *PLoS ONE* 2012;7:e52893.
12. Scott CA, Bewley S, Rudd A, *et al*. Incidence, risk factors, management, and outcomes of stroke in pregnancy. *Obstet Gynecol* 2012;120(2 Pt 1):318–24.
13. Estcourt LJ, Brunskill S, Doree C, *et al*. Intracranial hemorrhage in patients with a hematological disorder requiring intensive chemotherapy and/or a stem cell transplant: a systematic review (Abstract 0563). *Haematologica* 2012;97(Suppl 1):228.
14. Nicoll A, Lynn R, Rahi J, *et al*. Public health outputs from the British Paediatric Surveillance Unit and similar clinician-based systems. *J R Soc Medicine* 2000;93:580–5.
15. Ariesen MJ, Claus SP, Rinkel GJE, *et al*. Risk factors for intracerebral hemorrhage in the general population: a systematic review. *Stroke* 2003;34:2060–5.
16. Sturgeon JD, Folsom AR, Longstreth WT Jr, *et al*. Risk factors for intracerebral hemorrhage in a pooled prospective study. *Stroke* 2007;38:2718–25.
17. Broderick JP, Brott T, Tomsick T, *et al*. The risk of subarachnoid and intracerebral hemorrhages in blacks as compared with whites. *N Engl J Med* 1992;326:733–6.
18. Rost NS, Smith EE, Chang Y, *et al*. Prediction of functional outcome in patients with primary intracerebral hemorrhage: the FUNC score. *Stroke* 2008;39:2304–9.
19. Chen CY, Tai CH, Tsay W, *et al*. Prediction of fatal intracranial hemorrhage in patients with acute myeloid leukemia. *Ann Oncol* 2009;20:1100–4.
20. Broderick JP, Brott TG, Duldner JE, *et al*. Volume of intracerebral hemorrhage. A powerful and easy-to-use predictor of 30-day mortality. *Stroke* 1993;24:987–93.
21. Hemphill JC III, Bonovich DC, Besmertis L, *et al*. The ICH score: a simple, reliable grading scale for intracerebral hemorrhage. *Stroke* 2001;32:891–7.
22. Najima Y, Ohashi K, Miyazawa M, *et al*. Intracranial hemorrhage following allogeneic hematopoietic stem cell transplantation. *Am J Hematol* 2009;84:298–301.
23. Zumberg MS, del Rosario ML, Nejame CF, *et al*. A prospective randomized trial of prophylactic platelet transfusion and bleeding incidence in hematopoietic stem cell transplant recipients: 10,000/µL versus 20,000/µL trigger. *Biol Blood Marrow Transplant* 2002;8:569–76.
24. Webert K, Cook RJ, Sigouin CS, *et al*. The risk of bleeding in thrombocytopenic patients with acute myeloid leukemia. *Haematologica* 2006;91:1530–7.
25. Rebulla P, Finazzi G, Marangoni F, *et al*. The threshold for prophylactic platelet transfusions in adults with acute myeloid leukemia. Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto. *N Engl J Med* 1997;337:1870–5.
26. Higby DJ, Cohen E, Holland JF, *et al*. The prophylactic treatment of thrombocytopenic leukemic patients with platelets: a double blind study. *Transfusion* 1974;14:440–6.
27. Estcourt L, Stanworth S, Doree C, *et al*. Prophylactic platelet transfusion for prevention of bleeding in patients with haematological disorders after chemotherapy and stem cell transplantation. *Cochrane Database Syst Rev* 2012;5:CD004269.
28. Sloand EM, Kumar P, Yu M, *et al*. Effect of amphotericin B and fluconazole on platelet membrane glycoproteins. *Transfusion* 1994;34:415–20.
29. Fletcher C, Pearson C, Choi S, *et al*. In vitro comparison of antiplatelet effects of beta-lactam penicillins. *J Lab Clin Med* 1986;108:217–23.
30. Burroughs SF, Johnson GJ. Beta-lactam antibiotic-induced platelet dysfunction: evidence for irreversible inhibition of platelet activation in vitro and in vivo after prolonged exposure to penicillin. *Blood* 1990;75:1473–80.
31. Goerge T, Ho-Tin-Noe B, Carbo C, *et al*. Inflammation induces hemorrhage in thrombocytopenia. *Blood* 2008;111:4958–64.
32. Valeri CR, Cassidy G, Pivacek LE, *et al*. Anemia-induced increase in the bleeding time: implications for treatment of nonsurgical blood loss. *Transfusion* 2001;41:977–83.
33. Valeri CR, Khuri S, Ragno G. Nonsurgical bleeding diathesis in anemic thrombocytopenic patients: role of temperature, red blood cells, platelets, and plasma-clotting proteins. *Transfusion* 2007;47 (Suppl 4):206S–48S.
34. Blajchman MA, Bordin JO, Bardossy L, *et al*. The contribution of the haematocrit to thrombocytopenic bleeding in experimental animals. *Br J Haematol* 1994;86:347–50.
35. Kitchens CS, Weiss L. Ultrastructural changes of endothelium associated with thrombocytopenia. *Blood* 1975;46:567–78.
36. Blajchman MA, Senyi AF, Hirsh J, *et al*. Hemostatic function, survival, and membrane glycoprotein changes in young versus old rabbit platelets. *J Clin Invest* 1981;68:1289–94.
37. Nachman RL, Rafii S. Platelets, petechiae, and preservation of the vascular wall. *N Engl J Med* 2008;359:1261–70.
38. Nevo S, Vogelsang GB. Acute bleeding complications in patients after bone marrow transplantation. *Curr Opin Hematol* 2001;8:319–25.
39. Nevo S, Enger C, Hartley E, *et al*. Acute bleeding and thrombocytopenia after bone marrow transplantation. *Bone Marrow Transplant* 2001;27:65–72.
40. Slichter SJ, Davis K, Enright H, *et al*. Factors affecting posttransfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients. *Blood* 2005;105:4106–14.
41. Bleggi-Torres LF, Werner B, Gasparetto EL, *et al*. Intracranial hemorrhage following bone marrow transplantation: an autopsy study of 58 patients. *Bone Marrow Transplant* 2002;29:29–32.
42. Antonini G, Ceschin V, Morino S, *et al*. Early neurologic complications following allogeneic bone marrow transplant for leukemia: a prospective study. *Neurology* 1998;50:1441–5.
43. Nevo S, Swan V, Enger C, *et al*. Acute bleeding after bone marrow transplantation (BMT)—incidence and effect on survival. A quantitative analysis in 1,402 patients. *Blood* 1998;91:1469–77.