Risk factors for haemorrhage in patients with haematological malignancies

Dr Lise Jane Estcourt

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University of Oxford

Trinity Term 2014

A thesis submitted for the Degree of Doctor of Philosophy

This thesis is dedicated to my parents, and my wonderful husband Joe

Abstract

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Haematological malignancies and their treatment lead to prolonged periods of severe thrombocytopenia (platelet count ≤ 50 x 10^9/l). Despite the use of prophylactic platelet transfusions, haemorrhage remains an important complication during this thrombocytopenic period. Within a 30 day period up to 70% of patients have clinically significant haemorrhage (World Health Organization (WHO) grade 2 or above bleeding) and up to 10% have severe or life-threatening haemorrhage (WHO grade 3 or 4 bleeding). Hence our current management of these patients to prevent haemorrhage is sub-optimal. The aim of this thesis was to identify clinical and laboratory factors that may predict the risk of haemorrhage in patients with haematological malignancies and severe thrombocytopenia.

This was achieved via several different study designs and assessed the effect of clinical and laboratory factors on any or clinically significant haemorrhage and their effect on intracranial haemorrhage.

This thesis has demonstrated that there is no consensus on how bleeding is assessed and graded in this patient group. Also it showed that the absolute immature platelet number may be a better alternative to the total platelet count to guide administration of platelet transfusions. Female sex, a previous history of a fungal infection, a high C-reactive protein, a high white cell count, a low platelet count, anaemia, impaired renal function, and recent clinically significant haemorrhage were all found to be independent risk factors for haemorrhage. Patients who were in complete remission from their haematological malignancy had a much lower risk of bleeding.

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Statement of originality

All work in this thesis is my own unless otherwise stated.

The first draft of all published manuscripts arising from this thesis were written by me and then critically appraised by senior co-authors. All publications were peer reviewed.

All statistical analyses were performed by me, and then critically appraised by senior statisticians.

The design, set-up, ethical approval and running of the ATHENA study and InCiTe study were performed by me.

Data for the InCiTe study were collected by staff at all eight-four participating hospitals across the UK.

Data for the ATHENA study were collected by me, as well as research nurses and laboratory technicians in Bristol and Oxford.

Peer-reviewed publications arising from this thesis

Several sections of this thesis have been published in peer-reviewed journals as original research or review articles. I am the first author of all of these publications, and I am allowed to reproduce part or all of the published material in other publications of my own work according to the copyright transfer agreements signed prior to publication.

- **Estcourt LJ**. Why has demand for platelet components increased? A review. *Transfusion Medicine* [In Press]. (Introduction)
- **Estcourt LJ**, Heddle N, Kaufman RM, McCullough J, Murphy MF, Slichter S, *et al*. Differences in the methods of assessing and analysing bleeding outcomes in platelet transfusion trials. On behalf of the BEST (Biomedical Excellence for Safer Transfusion) Collaborative. *Transfusion* 2013; 53(7):1531-1543 DOI: 10.1111/trf.12058. (Chapter 2)
- **Estcourt LJ**, Stanworth, SJ, Harrison P, Powter G, McClure M, Murphy MF, *et al*. Prospective observational cohort study of the association between thromboelastometry, coagulation and platelet parameters and bleeding in patients with haematological malignancies- The ATHENA study. *British Journal of Haematology* 2014 DOI:10.1111/bjh.12928 [Early on-line publication 5th May 2014]. (Chapter 3)
- **Estcourt LJ**, Stanworth SJ, Collett D, Murphy MF. Intracranial haemorrhage in thrombocytopenic haematology patients—a nested case–control study: the InCiTe study protocol. *BMJ Open* 2014;4:e004199. (Chapter 6)

Peer-reviewed publications arising from work related to, but not included in, this thesis

- **Estcourt LJ**, Stanworth S, Doree C, Trivella M, Hopewell S, Murphy MF, et al. Comparison of different platelet count thresholds to guide administration of prophylactic platelet transfusion for preventing bleeding in patients with haematological disorders after chemotherapy or stem cell transplantation (protocol). Cochrane database of systematic reviews (Online) 2014;2014:CD010983.
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- Butler C, Doree C, **Estcourt L**, Stanworth S, Brunskill SJ, Hopewell S, et al. Pathogen-reduced platelets for the prevention of bleeding. Cochrane Database of Systematic Reviews 2013 Issue 3. Art. No.: CD009072. DOI: 10.1002/14651858.CD009072.pub2.
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Abbreviations

AA	Aplastic anaemia
ADP	Adenosine diphosphate
aGvHD	Acute Graft vs. Host Disease
AIPN	Absolute immature platelet number
AL	Acute leukaemia
ALL	Acute lymphocytic leukaemia
AlloHSCT	Allogeneic haematopoietic stem cell transplant
AML	Acute myeloid leukaemia
APL	Acute promyelocytic leukaemia
aPTT	Activated partial thromboplastin time
AUC	Area under the curve
AutoHSCT	Autologous haematopoietic stem cell transplant
BCSH	British Committee for Standards in Haematology
BEST	Biomedical Excellence for Safer Transfusion
BM	Bone marrow
BPSU	British Paediatric Surveillance Unit
BSBMT	British Society of Blood and Marrow Transplantation
CI	Confidence Interval
CF	Clauss Fibrinogen
CFT	Clot formation time
CML	Chronic myeloid leukaemia
CRF	Case report form
CRO	Contract research organization
CRP	C-reactive protein
CT	Clot time
CTCAE	Common toxicity criteria for adverse events
DIC	Disseminated intravascular coagulation

EBMT	European Bone Marrow Transplantation
EDTA	Ethylenediaminetetraacetic acid
GvHD	Graft vs. Host Disease
HASS	Haematology active surveillance system
HDU	High dependency unit
HL	Hodgkin's lymphoma
HLA	Human leucocyte antigen
HNA	Human neutrophil antigen
HPA	Human platelet antigen
HR	Hazards ratio
HSCT	Haemopoietic stem cell transplant
ICH	Intracranial haemorrhage
IPF	Immature platelet fraction
IPS	Infection probability score
IQR	Inter-quartile range
ISTH	International Society of Thrombosis and Haemostasis
ITU	Intensive treatment unit
LRT	Likelihood ratio test
MCF	Maximum clot firmness
MPV	Mean platelet volume
MDS	Myelodysplastic syndromes
ML	Maximum lysis
MRC	Medical Research Council
NCCN	National Comprehensive Cancer Network
NHL	Non-Hodgkin's lymphoma
NHSBT	NHS Blood and Transplant
NICE	National Institute for Health and Care Excellence
NPV	Negative predictive value
NR	Not reported

OR	Odds ratio
PCs	Platelet components
PFA-100®	Platelet function analyser-100
PPTxs	Prophylactic platelet transfusions
PPV	Positive predictive value
P-Rx	Pathogen-reduced platelets
PT	Prothrombin time
RBC	Red blood cell
RCT	Randomised-controlled trial
RNA	Ribonucleic acid
ROC	Receiver operating characteristic
ROTEM®	Rotational thromboelastometry
RR	Relative Risk
SaBTO	Advisory Committee on the Safety of Blood, Tissues and Organs
SAPS	Simplified Acute Physiology Score
SCBU	Special care baby unit
SHOT	Serious Hazards of Transfusion Scheme
SIRS	Septic inflammatory response syndrome
SOFA	Sequential organ failure assessment
TPC	Total platelet count
TRALI	Transfusion related acute lung injury
TRIM	Transfusion-related immune modulation
UKOSS	UK Obstetric Surveillance System
vCJD	Variant Creutzfeld Jacob Disease
VOD	Veno-occlusive disease
WCC	White cell count
WHO	World Health Organisation

1 Introduction

Haematological malignancies and their treatment lead to prolonged periods of severe thrombocytopenia (platelet count ≤ 50 x 10^9/l).[1-5] During this thrombocytopenic period patients are at risk of severe and life-threatening haemorrhage.[6,7] Platelet transfusions are used in modern clinical practice to prevent and treat haemorrhage in thrombocytopenic patients with haematological malignancies, and the majority of these platelet transfusions are given to prevent haemorrhage.[8] However, despite the current use of prophylactic platelet transfusions, haemorrhage remains an important complication in patients with haematological malignancies who are severely thrombocytopenic. Within a 30 day period, up to 70% of patients have clinically significant haemorrhage (usually defined as World Health Organization (WHO) grade 2 or above bleeding) and up to 10% have severe or life-threatening haemorrhage (usually defined as WHO grade 3 or 4 bleeding).[6,7]

Platelet components (PCs) are the second most commonly issued blood component.[9] About 312,000 adult doses/year are transfused in the UK,[10] costing about £65 million/year.[11] The use of platelet transfusions in patients with haematological disorders accounts for a large proportion (up to 67%) of all PCs issued (Figure 1.1).[12-14] In the UK, approximately £44 million per year is spent on platelet transfusions given to patients with haematological malignancies.

At the moment, prophylactic platelet transfusions are given when the patient's platelet count falls below a threshold (usually 10 x 10^9/l).[15] However, this practice is unlikely to be the most effective way of using prophylactic platelet transfusions. Differences in baseline

characteristics between patients are not taken in to account, which could increase or decrease a patient's risk of haemorrhage. Patients at low risk of haemorrhage may be receiving platelet transfusions unnecessarily, exposing the patient to the risks of a blood transfusion[16] as well as placing additional strain on the limited supply of platelet components.[17] Patients at high risk of haemorrhage may be receiving insufficient treatment to prevent haemorrhage occurring, which may explain why despite prophylactic platelet transfusions, there remains a high incidence of clinically significant haemorrhage.[6,7]

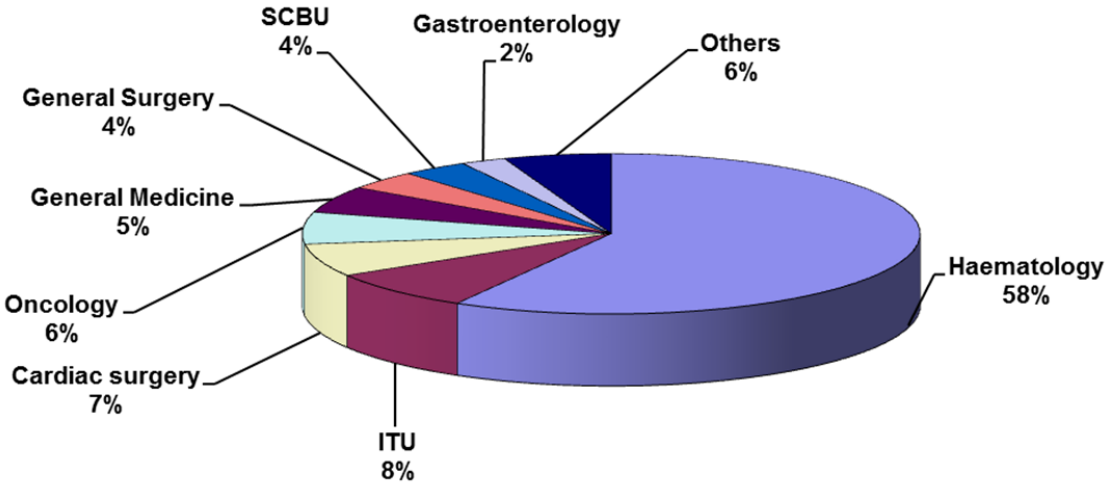


Figure 1.1 Platelet components issued during March 2010 in North West England.

Figure created using data from the North West of England and Wales audit of platelet use and wastage. [13]

ITU = intensive treatment unit; SCBU = special care baby unit.

To create a risk-stratified approach to the use of prophylactic platelet transfusions, there has to be sufficient knowledge about the underlying risk factors.

In the introduction to this thesis, I will give a brief overview of the:

- Incidence and prevalence of haematological malignancies, and their impact on the current trend in platelet component demand
- Problems with meeting any future increase in platelet component demand
- Evidence for the use of prophylactic platelet transfusions from randomised-controlled trials (RCTs)
- Risks associated with giving a platelet transfusion
- Risk factors for haemorrhage in patients with haematological malignancies that have been identified in the literature

I will then describe the aims of this thesis based on this introductory information, as well as the structure of the rest of this thesis.

1.1 Incidence and prevalence of haematological malignancies

Haematological malignancies constituted 8% of all new cancers in the UK (2007-09)[18] and 9% of all new cancers in the US (2008),[19] and their incidence is increasing in both the UK (Figure 1.2) and abroad.

In the US, the incidence of non-Hodgkin's lymphoma (NHL), leukaemia and myeloma has been rising each year over the last 10 years, whereas the incidence of Hodgkin's lymphoma (HL) has remained stable (Table 1.1).

Table 1.1: Estimated incidence and prevalence of haematological malignancies in the US in 2013.*

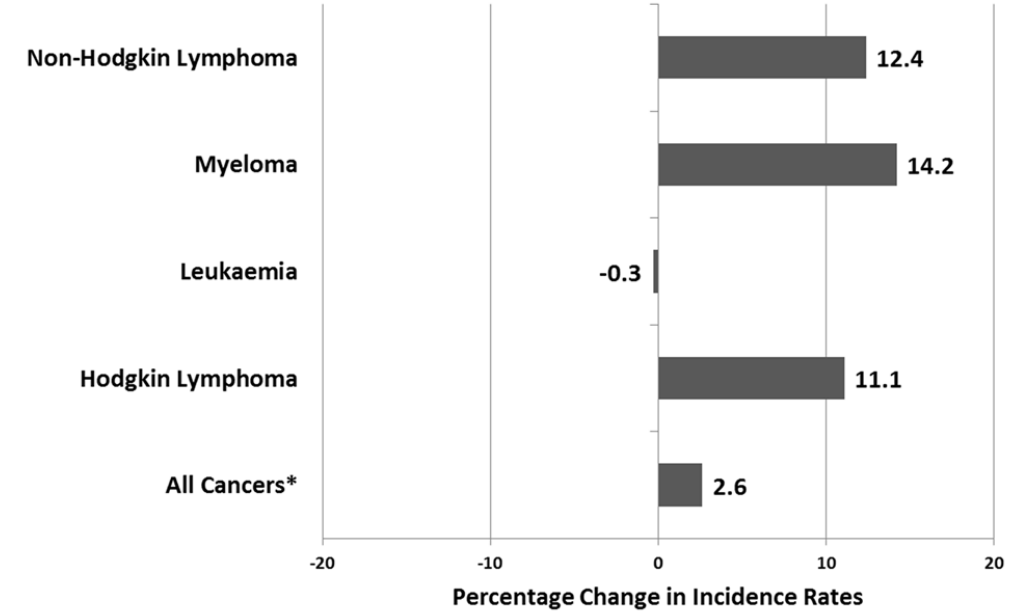
	NHL	Leukaemia	Myeloma	HL	Total
Estimated new cases 2013	69,740	48,610	22,350	9,260	149,960
% of all new cancer cases	4.2	2.9	1.3	0.6	9
Estimated prevalence 2013	509,065	287,963	77,617	181,928	1,056,573
APC incidence/year	+0.5	+0.1	+0.7	+0	-
APC death rate/year	-2.7	-1.0	-1.7	-2.2	-

***Data from the Surveillance, Epidemiology, and End Results Program (SEER) Stat Fact Sheets.[20-23] The annual percentage change (APC) in incidence and mortality rates for both sexes and all races has been age-adjusted to the 2000 US Standard Population using SEER 9 data.

The prevalence of these disorders is also increasing due to improved survival rates (Figure 1.3 & Table 1.1). In the US, it is estimated that in 2013 over one million people are living with a haematological malignancy (Table 1.1). These improved survival rates are due to the introduction of intensive chemotherapy treatments and use of haemopoietic stem cell transplantation (HSCT).[24-27] There has been a dramatic rise in the use of HSCT to treat haematological malignancies (Figure 1.4).

Since 1990, the number of HSCT performed in Europe has risen from 4,200 to over 30,000 annually.[28] The most obvious recent trend has been the increase in allogeneic HSCT with a 50% rise from 2005 to 2011.[28] Treatment with an allogeneic HSCT leads to much more prolonged periods of severe thrombocytopenia ($\leq 50 \times 10^9$/l) than an autologous HSCT, and therefore an increased requirement for platelet components.

A- Male



B - Female

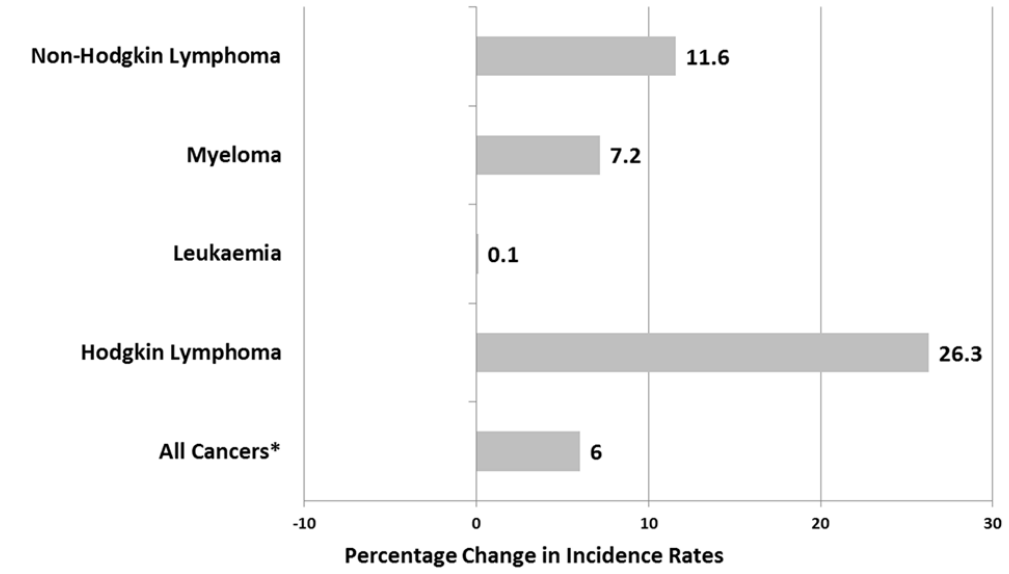
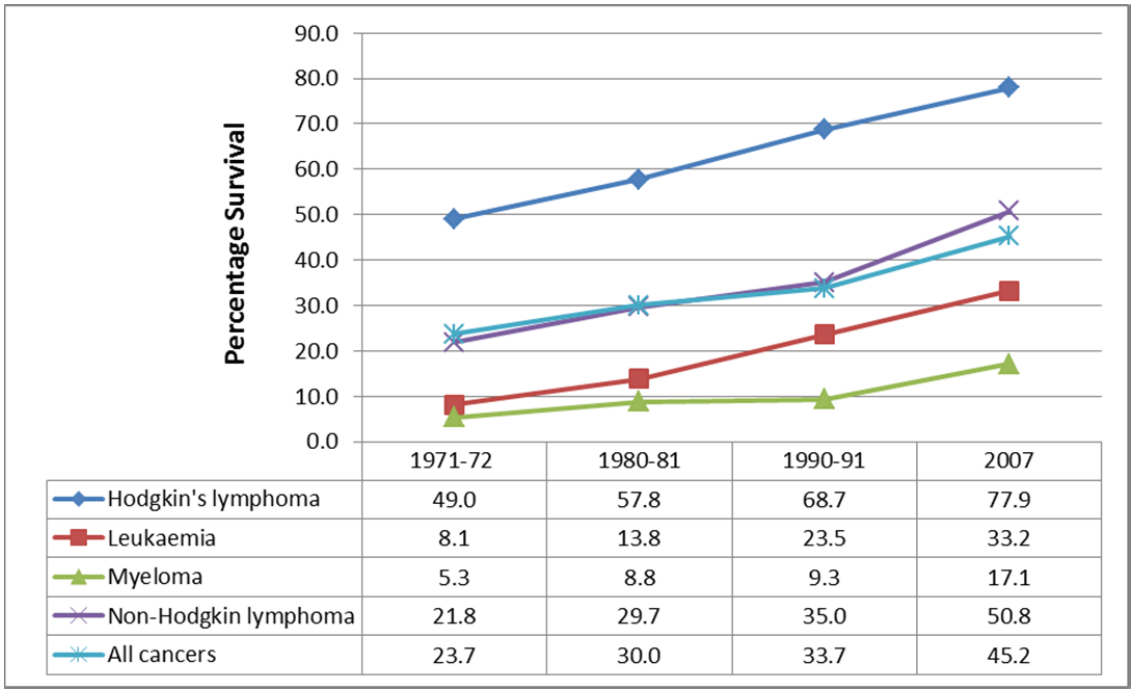


Figure 1.2 Percentage Change in European Age-Standardised Three Year Average Incidence Rates, (A) Males and (B) Females, UK, 1999-2001 and 2008-2010 for the major types of haematological malignancies and all cancers *excluding non-melanoma skin cancer.[29]



	1971-72	1980-81	1990-91	2007
Hodgkin's lymphoma	49.0	57.8	68.7	77.9
Leukaemia	8.1	13.8	23.5	33.2
Myeloma	5.3	8.8	9.3	17.1
Non-Hodgkin lymphoma	21.8	29.7	35.0	50.8
All cancers	23.7	30.0	33.7	45.2

Figure 1.3 Ten year relative survival (%), adults (15-99 years), for patients diagnosed with haematological malignancies and all cancers, England and Wales: survival trends for 1971-2007.
Data for patients diagnosed between 1971-1991 actual survival data, and data for patients diagnosed in 2007 hybrid survival data (predicted).[30,31]

1.1.1 Increase in platelet component demand

This dramatic change in the management of patients with haematological malignancies and the increased prevalence of haematological malignancies are likely to be two of the main drivers for the recent increase in platelet component demand in the UK (Figure 1.5). Once the increasing population has been taken in to account, demand has still increased by 16% in the UK over the last decade, with 4.2 components per 1000 population being issued in 2002, compared to 4.9 components per 1000 population in 2012. This increasing trend has also been seen in Australia where demand has increased by approximately 18% from

2003-2004 to 2012-2013.[32,33] European data is only available until 2008, but again shows a similar trend.[34-37]

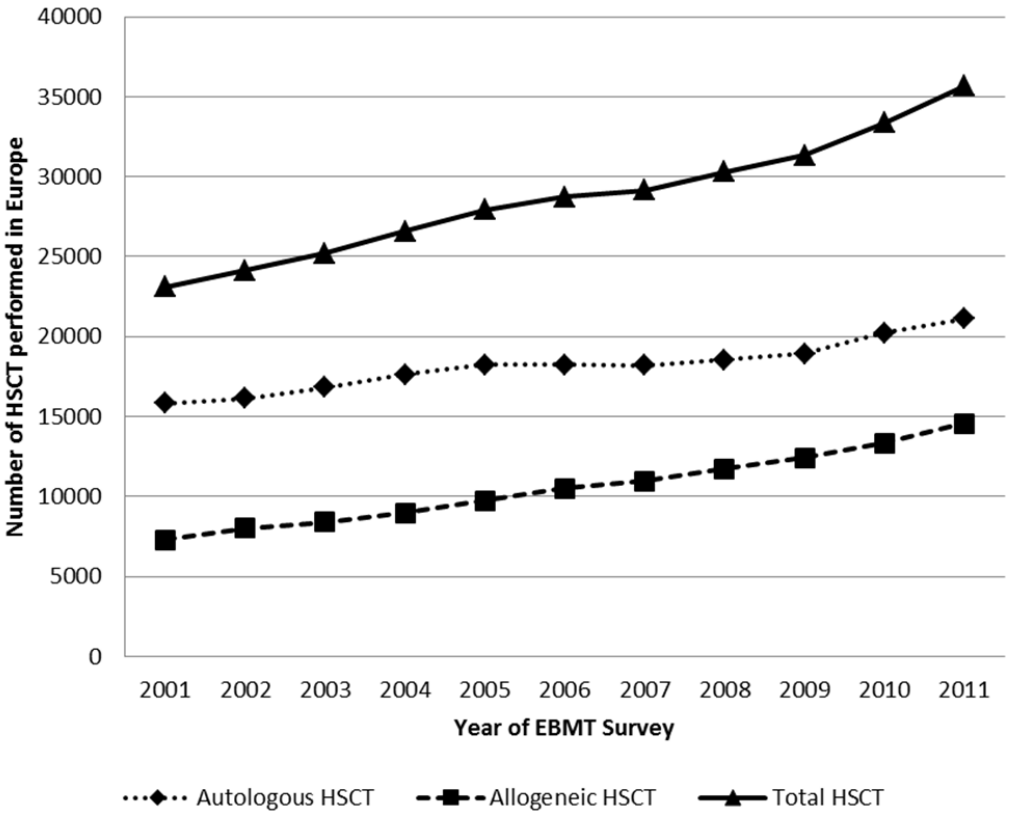


Figure 1.4: Number of Haemopoietic stem cell transplants (HSCT) performed in Europe from 2001 to 2011 according to the annual European Bone Marrow Transplantation (EBMT) Activity Surveys.[28,38-47]

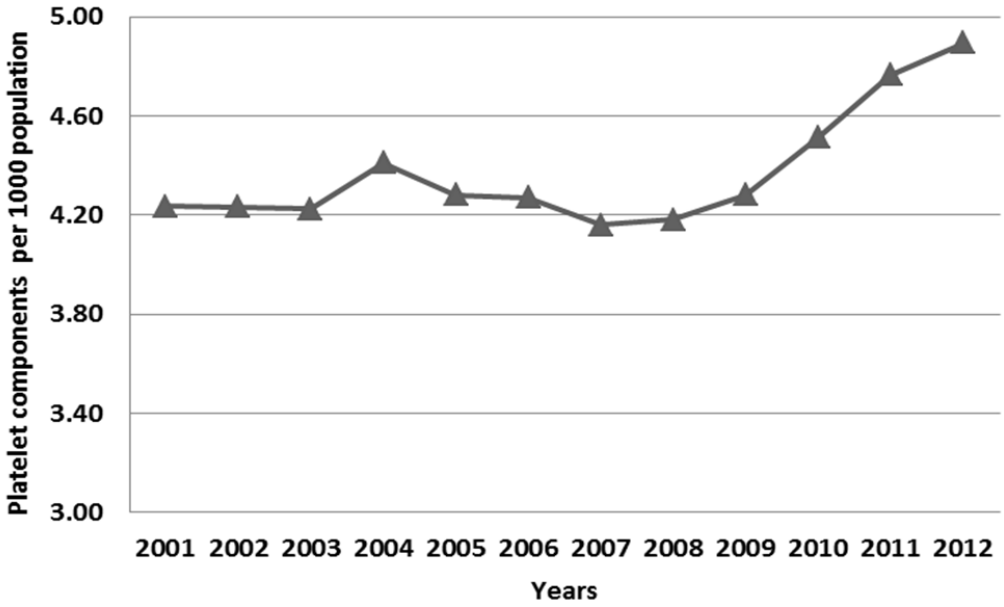


Figure 1.5: Platelet component demand for the UK for the period 2001 to 2012, adjusted to the UK population (per 1000 population). Data from SHOT Annual Reports and Office of National Statistics.[9,48-50]

1.2 Problems with increasing the supply of platelet components

1.2.1 Production of platelet components

There are two main methods for producing platelet components (PCs).

- Apheresis PCs (one donor per transfusion) requires platelet donors to be connected to a cell separator for at least 90 minutes.[51]
- Pooled PCs (four donors per transfusion) derived from the platelets within four whole-blood donations.

In the UK, at least 80% of PCs are produced by apheresis.[51] This policy was introduced to decrease the risk of variant Creutzfeld Jacob Disease (vCJD) transmission by decreasing donor exposure (one donor per platelet transfusion versus four donors per transfusion).[52]

1.2.2 Platelet donors

The number of donors that can provide platelets via apheresis is limited, and there are several reasons for this.

Firstly, the donor population pool is declining. Only 4% of the eligible population donate blood,[53] and of these only 15% who have donated blood within the last two years (active donors) are between 17 and 24 years of age.[54] The proportion of donors in this age group has decreased by 20% over the last decade.[53] To maintain the current number of donors, 200,000 new donors need to be recruited every year.[54]

Secondly, platelet donors have to be regular donors. A large proportion (40%) of regular donors are aged over 55 years of age and tend to live away from large urban areas.[55]

Thirdly, there are additional special requirements to be eligible to be a platelet donor. Donors have to have a high platelet count, they have to have a large enough total blood volume that they can donate two adult platelet components at each donation, they have to be male or female with no evidence of HLA antibodies, and they cannot be taking any medications that could affect platelet function e.g. aspirin.[56]

Fourthly, platelet donations collected by cell separators can only occur at twenty-four specialist centres within England and North Wales.[51] Therefore, not all regular donors will be close to a platelet donation centre.

Lastly, donating platelets takes much longer (90 minutes to 2 hours)[51] and due to the anticoagulant used during the collection process it can lead to unpleasant side-effects such as tingling around the mouth. Therefore, only a proportion of donors who are eligible will be willing to become platelet donors.

1.2.3 **Cell separators**

The machines used to collect platelet donations are very expensive and require specialist nurses to run them. This means that it will be very difficult to increase the number of centres that collect platelet donations via cell separators.

For these reasons, it will be very difficult to match supply to any further increase in demand for platelet components.[17]

1.3 Prophylactic platelet transfusions

The standard practice in most haematology units across the developed world has been to use prophylactic platelet transfusions (PPTxs), often in line with national guidelines.[15,57-62]

1.3.1 Platelet count threshold

The morning platelet count is currently used to indicate when a patient requires PPTxs. When PPTxs were first used routinely, it was standard practice to transfuse them at platelet counts below 20 x 10^9/l in an attempt to prevent haemorrhage.[63] This practice was partly based on the findings of non-randomised studies[64,65] which showed that gross haemorrhage (haematuria, haematemesis and melaena) was present more frequently at platelet counts below 5 x 10^9/l than when the platelet count was between 5 x 10^9/l and 100 x 10^9/l. However, these studies did not clearly support the use of a trigger for PPTxs of 20 x 10^9/l, nor was any threshold effect seen.[64,65] A similar pattern of increased haemorrhage at platelet counts ≤ 5 x 10^9/l has also been seen in three recent RCTs (Figure 1.6).[2,6,7] Despite the lack of evidence, the use of a threshold platelet count of 20 x 10^9/l for PPTxs became widespread in the 1970s and 1980s, and led to a marked growth in demand for platelet concentrates.[66] This increased demand stimulated research to address whether the platelet count threshold could be safely lowered to 10 x 10^9/l.[67-71] The consensus formulated from these trials was that patients should receive PPTxs when the platelet count is < 10 x 10^9/l, unless there are other risk factors for haemorrhage, such as sepsis, concurrent use of antibiotics or other abnormalities of haemostasis[15,57-59] when the threshold should be raised.

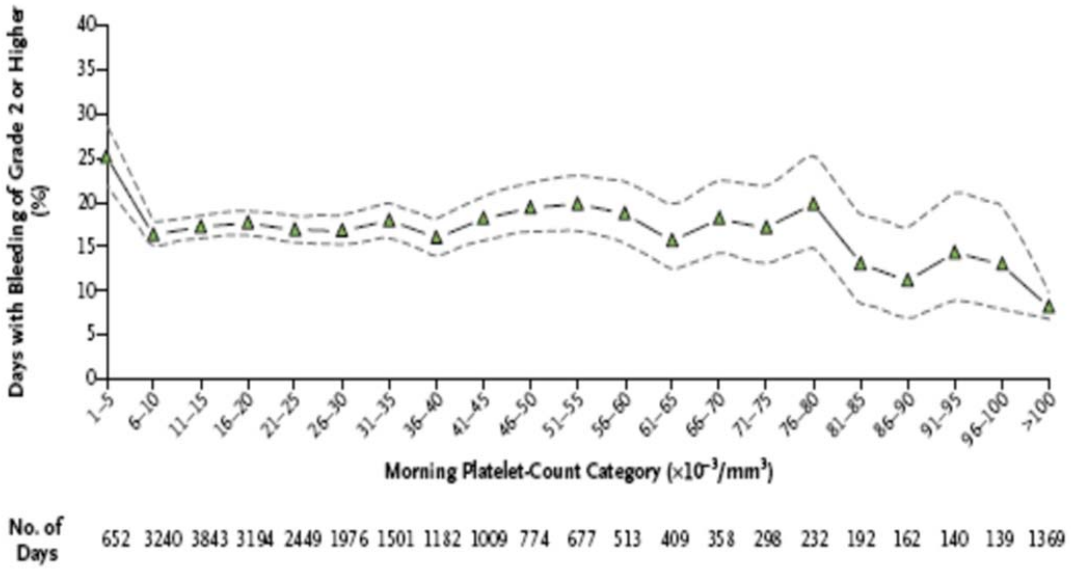


Figure 1.6 Percentage of days on which patients had bleeding of WHO grade 2 or higher, according to the morning platelet count category, with associated 95% confidence intervals (dashed lines). **"Reproduced with permission from (Slichter et al, 2010), Copyright Massachusetts Medical Society.**[6]
Data based on 24,309 days on which both platelet count and bleeding grade were known. No difference in the risk of bleeding (17%) at morning platelet counts between 6 and 80x10^9/l.

It has been suggested that the threshold for PPTxs could be reduced further[15,67] because of the previously mentioned evidence for an increased rate of haemorrhage at a platelet count of ≤ 5 x 10^9/L. However, a major concern in doing this is the reported inaccuracy of current automated counters when the platelet count is very low.[72] This was well demonstrated in a large multicentre study of platelet analyser accuracy when measuring platelet counts < 20 x 10^9/l. [73] Analysers that utilised optical, impedance and immunological methods were compared with the international reference method for platelet counting.[74] Although the optical and impedance methods were roughly equivalent in their accuracy in this study, both methods tended to over-estimate the platelet count.

In a retrospective review of almost 3000 thrombocytopenic adult patients, over a 10-year period, Friedmann et al (2002) showed no relationship between the morning platelet count, or the lowest platelet count of the day, and the risk of severe or life-threatening haemorrhage.[75] This raises the question as to whether a platelet count threshold-defined PPTx approach is appropriate.

1.3.2 Prophylactic vs. therapeutic-only platelet transfusions

Until recently, the overall benefit of PPTxs over a policy to use platelets only therapeutically had not been well established. Only two published RCTs had addressed this question,[76,77] and two further studies had been published as abstracts.[78,79] All of these studies were small trials completed over 30 years ago. The care of haematological patients has changed dramatically since that time, therefore the ability to extrapolate any meaningful results from these studies is limited.

Two recent studies have addressed this question.[2,7] Both studies compared the use of prophylactic platelet transfusions (platelet count trigger ≤ 10 x 10^9/l) with a therapeutic platelet transfusion strategy (platelets only given when patient had clinically relevant haemorrhage) in patients with haematological malignancies.

Both of these studies showed that prophylactic platelet transfusions were effective at reducing clinically significant haemorrhage (defined as WHO grade 2 or above).[2,7] However, the effectiveness of prophylactic platelet transfusions differed between patients receiving autologous HSCT and patients receiving chemotherapy or allogeneic HSCT (Figure 1.7).

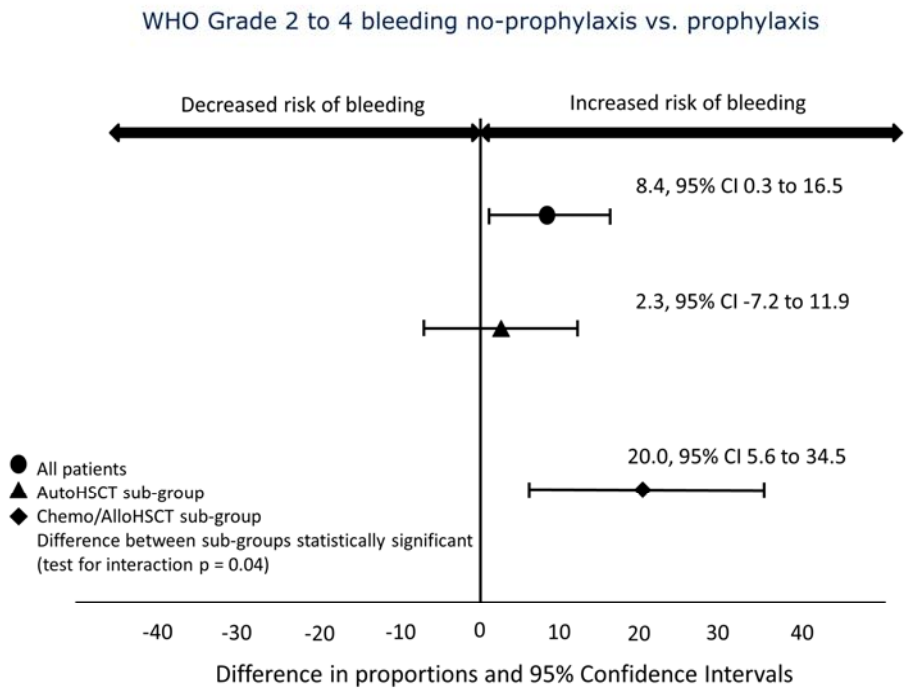


Figure 1.7 Difference in proportion of patients who had bleeding of WHO grade 2 or higher with associated 95% confidence intervals. Data from the TOPPS study.[7]

1.4 Risks of transfusion

Platelet transfusions are also associated with adverse events. Mild to moderate reactions to platelet transfusions include rigors, fever (1 in 5 platelet transfusions), and urticaria (1 in 100 to 1 in 300 transfusions).[80] These reactions are not life-threatening but can be extremely distressing for the patient. Rarer, but more serious sequelae include: transfusion transmitted infections (bacterial sepsis (1 in 10,000 platelet transfusions)[80] and viral infections); transfusion-related acute lung injury (TRALI);[81] and immunomodulatory effects.[82]

1.4.1 Infection

As well as the rare infection risks that all blood products pose such as the transmission of HIV (1 in 140,000 transfusions),[83] Hepatitis B (1 in 790,000 transfusions),[83] and vCJD (4 cases reported),[9] platelet transfusions are much more likely to cause bacterial sepsis. In the UK, since 1996, there have been 77 confirmed cases of transfusion-transmitted-infection (TTI) reported to the Serious Hazards of Transfusion Scheme (SHOT),[10] and 43 of these were bacterial in nature.[10] The majority of these cases (n = 33) involved platelet transfusions and there were 11 associated deaths. Although rare, the high mortality rate associated with bacterial contamination of platelets has led the Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) to investigate risk reduction strategies. These include screening platelets for bacterial contamination (this has already been introduced in the UK) and pathogen reduction of platelets.[84]

1.4.2 Transfusion Related Acute Lung Injury (TRALI)

TRALI was once thought to be a rare event, but it is now known to be a major cause of transfusion-related death with a mortality rate of 9%.[85] It is defined, by SHOT, as acute dyspnoea with hypoxia and bilateral pulmonary infiltrates during or within 6 hours of transfusion, not due to circulatory overload or other likely causes. Plasma components from female donors that contain leucocyte antibodies (HLA or HNA antibodies) had been the main component implicated in the aetiology of TRALI.[86] For this reason, in 2003, the UK Blood Services moved to the use of plasma from male donors for transfusion and also for the re-suspension of pooled platelet products. Since then, platelets have been the main component implicated in cases of TRALI reported to SHOT (Table 1.2). TRALI occurs in 0.04 to 0.16% of all patients transfused,[80] however platelets are much more likely to be implicated in TRALI than red blood cell (RBC) components [OR 7.9; 95% CI, 2.5-24.8,[86] OR 8.16; 95% CI 4.91 to 13.37].[87] Furthermore, patients with haematological disorders have been found to be particularly at risk of developing TRALI. 31% (n = 41) of all suspected TRALI cases reported to SHOT since 2005 have been in haematology/oncology patients (Table 1.2) and a retrospective study of 90 cases of TRALI found that patients with haematological malignancies were particularly at risk of developing TRALI (P = 0.0004).[88] Haematology patients are therefore at a higher risk of developing TRALI due to their underlying condition and the number of platelet transfusions they receive.

Table 1.2: Suspected cases of Transfusion-related acute lung injury reported to SHOT since 2005

Year reported to SHOT	Number of cases		Component implicated (cases with concordant donor antibodies)			
	Total	Haematology/ oncology patients N (%)	FFP N (%)	Platelets N (%)	RBC N (%)	Other N (%)
2005[89]	23	7 (30)	0 (0)	3 (50)	2 (33)	1 (17)
2006[90]	10	2 (20)	0 (0)	1 (33)	1 (33)	1 (33)
2007[48]	24	11 (46)	0 (0)	5 (45)	5 (45)	1 (9)
2008[49]	17	3 (18)	3 (60)	2 (40)	0 (0)	0 (0)
2009[85]	21	6 (29)	2 (25)	3 (37)	2 (25)	1 (13)
2010[91]	15	3 (20)	0 (0)	2 (50)	2 (50)	0 (0)
2011[9]	12	5 (42)	0 (0)	1 (100)	0 (0)	0 (0)
2012[10]	11	4 (36)	0 (0)	1 (25)	2 (50)	1 (25)
Total	133	41 (31)	5 (12)	18 (43)	14 (33)	5 (12)

1.4.3 Immunomodulatory effects

Transfusion-related immune modulation (TRIM) is the phenomenon of transient immunosuppression after allogeneic blood transfusion. It has a confirmed beneficial clinical effect, which is the enhanced survival of renal allografts after allogeneic blood transfusion.[92] The existence of deleterious clinical TRIM effects has been postulated to explain the increased mortality seen in patients receiving blood transfusions according to liberal red cell transfusion thresholds,[93] increased rate of infections post allogeneic transfusion,[93] and decreased overall survival in patients with acute leukaemia receiving more platelet transfusions.[94,95]

1.5 Platelet transfusion refractoriness

Platelet transfusion refractoriness is defined as the repeated failure to obtain satisfactory increases in the platelet count after platelet transfusions. There are multiple causes of platelet refractoriness,[96] which include both immune and non-immune causes.

Platelet transfusion refractoriness due to immune causes is much more common in the multiply transfused patient.[97] Even if HLA antibodies cannot be detected the higher the number of platelet transfusions a patient has received the poorer the platelet count increment (Figure 1.8).

Therefore if we want to maintain a patient's response to platelet transfusions we need to avoid giving platelet transfusions when they are unnecessary.

Figure 1.8 Re-analysis of TRAP study data.[98] Figure A represents all study patients. Figure B represents only those patients who had no detectable HLA antibody.[99]

The figure 1.8 originally presented here cannot be made freely available via ORA because of copyright. The figure was sourced at 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.

1.6 Risk factors for haemorrhage in patients with haematological malignancies: evidence from the literature

Haemostasis *in vivo* is dependent on a complex interaction of platelets, coagulation factors within the plasma, and endothelial cells (Figure 1.9).

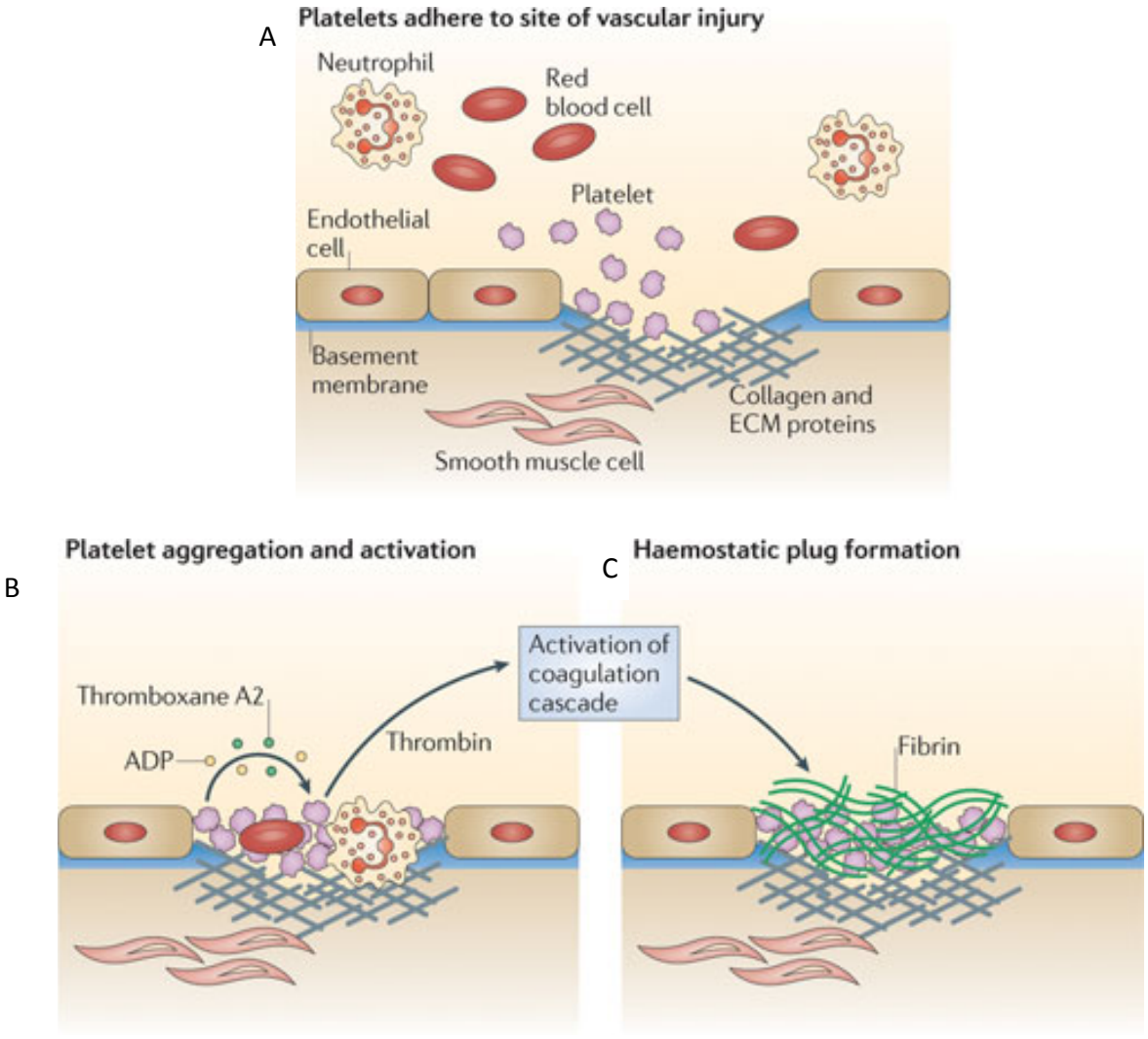


Figure 1.9 Haemostasis, and the role of platelets.[100]

A) Vascular injury exposes collagen and basement membrane proteins. This allows platelets to adhere to the site of injury. B) The adherent platelets aggregate and release platelet activation mediators, such as ADP and thromboxane A2. Following activation the platelets produce thrombin, which catalyses initiation of the coagulation cascade. C) The end result of activation of the coagulation cascade is creation of a cross-linked fibrin mesh which produces a stable clot.

A patient's platelet count indicates only the presence of a specific number of platelets within the circulation, but 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 risk of haemorrhage. We know that some patients with severe thrombocytopenia have many episodes of haemorrhage whereas other patients with a similar level of thrombocytopenia have no episodes of haemorrhage (Figure 1.10).

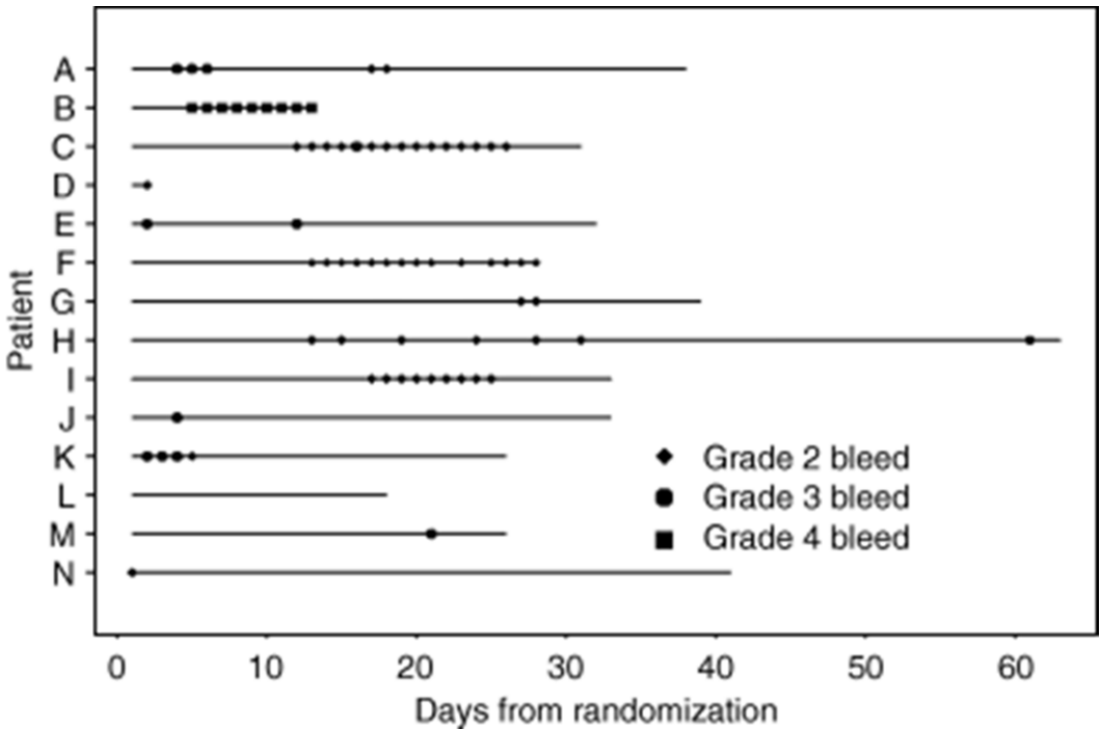


Figure 1.10 Bleeding event chart for a sample of patients from Rebulla et al 1997.[68,101]

All patients had acute myeloid leukaemia and were receiving induction chemotherapy. All patients developed severe thrombocytopenia. Each diamond, circle or square represents a day with bleeding of WHO grade 2 to 4 severity).

In the large national audit of over 3000 platelet transfusion episodes,[8] a large proportion (49%) of platelet components that were given appropriately (according to the audit algorithm) were given above the threshold of $10x10^9$/L (Table 1.3).

Table 1.3 Reasons why prophylactic platelet transfusions were considered appropriate (N=1375) according to the audit algorithm.[8]

Reason	Number of cases	Percentage
Platelet count < 10 x 10^9/L	698	51
Individualised threshold in notes	184	13
Infection	174	13
Platelet count expected to fall before next evaluation	136	10
Bleeding diathesis	56	4
Other*	55	4
Platelet components expire at midnight and platelet count ≤ 20 x 10^9/l	53	4
Severe mucositis	10	<1
Severe leucocytosis	5	<1
Treatment with Anti thymocyte globulin or Bortezomib	4	<1
*Includes factors such as minor bleeding, minor infection, liver dysfunction, hazardous job		

One of the commonest reasons was that the patient had an infection (Table 1.3); this accounted for 13% of all “appropriate” platelet transfusions.

Although many guidelines recommend raising the platelet count threshold for prophylactic platelet transfusions in the presence of risk factors for haemorrhage, these recommendations are based on expert opinion rather than evidence from the literature.[15,57]

The next section will therefore discuss the published literature reporting clinical and laboratory risk factors associated with haemorrhage in patients with haematological malignancies.

1.6.1 Baseline characteristics

1.6.1.1 *Type of haematological malignancy*

Two retrospective observational studies have shown that the risk of haemorrhage is higher in patients with acute promyelocytic leukaemia (APL) than patients with other forms of leukaemia. [102,103] One of these studies,[102] containing 792 patients with acute leukaemia, reported this as an independent risk factor for haemorrhage after performing multivariable analysis [Relative Risk (RR) 4.06 (95% CI 1.63 to 10.13)]. This is not unexpected because this type of leukaemia is commonly associated with coagulation abnormalities.[104]

1.6.1.2 *Characteristics of the haematological malignancy*

One retrospective observational study[105] containing 480 patients undergoing HSCT identified poor risk disease as an independent risk factor for haemorrhage [Odds ratio (OR) 1.84 (95% CI 1.05 to 3.22)]. Good risk disease was classified by the study as: acute leukaemia in first complete remission; chronic myeloid leukaemia (CML) in chronic phase; and chemotherapy sensitive lymphomas. All other disease was classified as poor risk disease.

1.6.1.3 *Type of treatment*

Two studies have shown that a recent HSCT is associated with an increased risk of haemorrhage.[75,106] However, neither study differentiated between autologous and allogeneic HSCT (Table 1.4).

Three studies have reported a higher rate of haemorrhage in patients receiving an allogeneic HSCT compared to an autologous HSCT (Table 1.4). The odds ratios (OR) were similar in all three studies, ranging from 2.2 to 2.8.

Table 1.4 Risks of haemorrhage due to type of treatment (for each risk factor, studies are organised according to the size of the study)

Risk Factor	Study	Study Design	Number patients in study	Multi-variable analysis	Results of analysis*
BM HSCT within 100d vs. Other treatment	Friedmann 2002[75]	Retrospective Observational	2942	Y	OR 1.32 (95% CI 1.22 to 1.43)
Blood & BM HSCT vs. Other treatment	Lawrence 2001[106]	Prospective Interventional	141	Y	P < 0.001
Allogeneic HSCT vs. Autologous HSCT	Gerber 2008[107]	Retrospective Observational	1514	Y	OR 2.17 (95% CI 1.56 to 3.03)
	Nevo 2007[105]	Retrospective Observational	480	Y	OR 2.29 (95% CI 1.11 to 4.77)
	Zumberg 2002[108]	RCT	159	NR	OR 2.8 (95% CI 1.1 to 7.7)

BM = bone marrow; HSCT = haematopoietic stem cell transplant; NR = not reported; RCT = randomised controlled trial; Y = Yes.
*P-value only reported if an odds ratio (OR), hazards ratio (HR) or relative risk (RR) was not reported

Patients receiving an autologous HSCT have a shorter duration of severe thrombocytopenia than patients receiving an allogeneic HSCT.[7] This may be one of the underlying reasons for this increased risk.

1.6.2 Medications

Table 1.5 Risks of haemorrhage due to type of medication (for each risk factor, studies are organised according to the size of the study)

Risk Factor	Study	Study Design	Number patients in study	Multi-variable analysis	Results of analysis*
Anti-fungal medication (Amphotericin)	Friedmann 2002[75]	Retrospective Observational	2942	Y	OR 0.97 (95% CI 0.84 to 1.13)
	Webert 2006[109]	Retrospective analysis of RCT†	255	Y	RR 0.59 (95% CI 0.39 to 0.90)
	Zumberg 2002[108]	RCT	159	Y	P < 0.001
	Lawrence 2001[106]	Prospective Interventional	141	Y	P < 0.001
Antibiotics (Semi-synthetic penicillin)	Friedmann 2002[75]	Retrospective Observational	2942	Y	OR 0.94 (95% CI 0.80 to 1.09)
	Lawrence 2001[106]	Prospective Interventional	141	Y	P = 0.014ǂ
Anticoagulants	Friedmann 2002[75]	Retrospective Observational	2942	Y	OR 0.94 (95% CI 0.09 to 10.12)
	Gerber 2008[107]	Retrospective Observational	1514	Y	OR 3.1 (95% CI 1.8 to 5.5)

RCT = randomised controlled trial; Y = Yes.
*P-value only reported if an odds ratio (OR), hazards ratio (HR) or relative risk (RR) was not reported
† Data from Rebulla *et al*, 1997[68]
ǂ Minor haemorrhage only. No association with major haemorrhage

Five studies reported the effect of different medications on the risk of haemorrhage. Four studies reported the effects of amphotericin (an anti-fungal medication), two studies reported the effects of anticoagulants, and two studies reported the effect of penicillin (Table 1.5).

1.6.2.1 *Antifungal medication*

Four studies reported this relationship, but there was no clear trend (Table 1.5). The largest study found no effect.[75] The second largest study found a protective effect of antifungal medication,[109] whereas the smallest two studies[108] found an increased risk of haemorrhage associated with the use of anti-fungal medication. Only one study has assessed the effect of amphotericin-B administration on platelet membrane glycoproteins *in vivo,* and no effect was seen.[110]

1.6.2.2 *Antibiotics*

Two studies reported this relationship (Table 1.5). Neither study found an effect of antibiotic use on the risk of severe bleeding.[75] One prospective study, which contained 141 patients, found that antibiotic use was associated with an increased risk of minor bleeding.[106]

1.6.2.3 *Anticoagulants*

Two studies reported this relationship (Table 1.5). Only one of the two studies found an effect of anticoagulant use on the risk of haemorrhage. There were significant differences between the two studies in the number of clinical factors that were taken in to account in

the multivariable analysis, and this may account for the difference between the two studies. The study that included many more covariables found no effect.[75]

1.6.3 Laboratory test results

1.6.3.1 *Platelet count*

Three studies reported this relationship (Table 1.6). Two of the studies found a significant relationship, with a lower platelet count leading to higher rates of haemorrhage.[106,109] The other study did not find a significant relationship after the results of multiple other clinical factors were taken in to consideration.[75]

1.6.3.2 *White cell count*

One study[3] reported that a high blast cell count in patients with acute promyelocytic leukaemia led to a higher rate of haemorrhage (Table 1.6). Another study[75] found no statistically significant association between a high total white cell count and the risk of haemorrhage but did find that a low white cell count ($<0.5 \times 10^9$/L) led to a lower rate of haemorrhage.

1.6.3.3 *Bilirubin*

One study found no association between a higher bilirubin level and risk of bleeding (Table 1.6).

1.6.3.4 *Raised creatinine or urea*

Two studies found that a raised creatinine[3] or raised urea[75] (both markers of renal impairment) led to a higher rate of haemorrhage (Table 1.6).

Table 1.6 Risks of haemorrhage associated with different laboratory test results (for each risk factor, studies are organised according to the size of the study)

Risk Factor	Study	Study Design	Number patients in study	Multi-variable analysis	Results of analysis*
Platelet count 0 to 9 x 10^9/l vs. 40 to 49 x 10^9/l	Friedmann *et al*, 2002[75]	Retrospective Observational	2942	Y	OR 1.14 (95% C.I. 0.89 to 1.46) (not transfused) OR 0.98 (95% C.I. 0.69 to 1.39) (transfused)
Platelet count (per 1x10^9/l increase)	Webert *et al*, 2006[109]	Retrospective analysis of RCT†	255	Y	RR 0.96 (95% C.I. 0.93 to 0.98)
Platelet count	Lawrence *et al*, 2001[106]	Prospective Interventional	141	Y	P < 0.001
Low white cell count (<0.5 x 10^9/l)	Friedmann 2002[75]	Retrospective Observational	2942	Y	OR 0.70 (95% CI 0.60 to 0.82)
High white cell count (>75 x 10^9/l)	Friedmann 2002[75]	Retrospective Observational	2942	Y	OR 2.76 (95% CI 0.87 to 8.83)
Peripheral blood blast cell count (≥30 x 10^9/l)	De La Serna 2008[3]	Retrospective analysis of LPA96 and LPA99 trials	732	Y	OR 4.4 (95% CI 1.9 to 10.2)
Bilirubin (per 1mg/dl)	Friedmann 2002[75]	Retrospective Observational	2942	Y	OR 1.00 (95% CI 0.99 to 1.01)
Hypoalbuminaemia (Albumin per 1gm/dl)	Friedmann 2002[75]	Retrospective Observational	2942	Y	OR 1.54 (95% CI 1.33 to 1.79)
Raised creatinine (>124µmol/l)	De La Serna 2008[3]	Retrospective analysis of LPA96 and LPA99 trials	732	Y	OR 24.3 (95% CI 7.5 to 78.1)
Uraemia (urea > 17.9 mmol/l)	Friedmann 2002[75]	Retrospective Observational	2942	Y	OR 1.64 (95% CI 1.40 to 1.92)

RCT = randomised controlled trial; Y = Yes.
*P-value only reported if an odds ratio (OR), hazards ratio (HR) or relative risk (RR) was not reported
† Data from Rebulla *et al*, 1997[68]

1.6.4 **Clinical co-morbidities**

1.6.4.1 ***Infection and fever***

Four studies reported the effect of an infection or fever on the risk of haemorrhage.[75,106,109,111] All four studies reported the effect of an infection, and three of the four studies reported the effect of fever.[75,106,109]

1.6.4.1.1 Infection

Two of the four studies[106,109] found that an infection increased the risk of haemorrhage, whereas the two larger studies[75,107] did not (Table 1.7). Both of the two studies that did find an effect, the association of infection with an increased risk of haemorrhage was only seen with minor haemorrhage, and not major haemorrhage.[106,109]

1.6.4.1.2 Fever

Two of the three studies found that a fever increased the risk of haemorrhage (Table 1.7). Neither of these two studies[106,109] found an association between fever and major haemorrhage. There was an association between clinically significant haemorrhage and fever in one of the studies.[109]

Although platelet transfusion guidelines[15,57] recommend increasing the platelet count threshold for transfusion when a patient is septic, it is unclear from the available literature whether their risk of haemorrhage increases during a septic episode.

Table 1.7: Risks of haemorrhage due to fever or infection (for each risk factor, studies are organised according to the size of the study)

Risk Factor	Study	Study Design	Number patients in study	Multi-variable analysis	Results of analysis*
Infection					
Bacteraemia	Friedmann 2002[75]	Retrospective Observational	2942	Y	OR 1.01 (95% CI 0.81 to 1.26)
Systemic infection	Najima, 2009[111]	Retrospective Observational	622	Y	HR 1.52 (95% CI 0.57 to 4.03)
Clinical infection	Webert 2006[109]	Retrospective analysis of RCT†	255	Y	RR 1.98 (95% CI 1.0 to 3.92)ǂ
Sepsis	Lawrence 2001[106]	Prospective Interventional	141	Y	P = 0.036ǂ
Fever					
(per 1^{0}C rise in temp)	Friedmann 2002[75]	Retrospective, Observational	2942	Y	OR 1.02 (95% CI 0.94 to 1.1)
Fever > 38.5^{o}C WHO grade 2 to 4 haemorrhage	Webert 2006[109]	Retrospective analysis of RCT†	255	Y	RR 3.95 (95% CI 1.90 to 8.20)
Fever > 38.5^{o}C WHO grade 3 to 4 haemorrhage					RR 1.62 (95% CI 0.44 to 5.91)
Fever (not specified)	Lawrence 2001[106]	Prospective, Interventional	141	Y	P < 0.001ǂ

*P-value only reported if an odds ratio (OR), hazards ratio (HR) or relative risk (RR) was not reported
† Data from Rebulla *et al*, 1997[68]
ǂ Minor haemorrhage only. No association with major haemorrhage

1.6.4.2 *Recent haemorrhage*

1.6.4.2.1 Severe haemorrhage (WHO grade 3 or 4)

One large retrospective observational study[75] containing 2942 patients, found that a recent history of severe haemorrhage (WHO grade 3 or 4 within the last five days), significantly increased the risk of a further severe haemorrhage (OR 6.72; 95% CI 5.53 to 8.18).

1.6.4.2.2 Clinically significant haemorrhage (WHO grade 2)

One study[109] that analysed data from a randomised controlled study (RCT) containing 255 patients found that clinically significant haemorrhage did not predict severe bleeding the next day (RR 2.86; 95% CI 0.37 to 21.93). However, the confidence intervals were very wide.

1.6.4.2.3 Minor haemorrhage (WHO grade 1)

The same study[109] that analysed data from the randomised controlled study (RCT) referred to above did find that minor haemorrhage (WHO grade 1) predicted a clinically significant haemorrhage (WHO grade 2 to 4) the following day (RR 2.55; 95% CI 1.18 to 5.49). There was also a trend seen for minor haemorrhage predicting a severe haemorrhage (WHO grade 3 to 4) the following day, but this did not reach statistical significance (RR 2.83; 95% CI 0.95 to 8.40).

1.6.4.3 *Co-morbidities associated with haemopoietic stem cell transplantation (HSCT)*

Four studies have reported on the effect of complications of HSCT on the risk of haemorrhage (Table 1.8). All four studies reported the effects of Graft vs. Host Disease

(GvHD) on risk of haemorrhage. Three of the studies also reported the effect of veno-occlusive disease (VOD) on risk of haemorrhage (Table 1.8).

Table 1.8: Risk factors for haemorrhage associated with haemopoietic stem cell transplantation. (for each risk factor, studies are organised according to the size of the study)

Risk Factor	Study	Study Design	Number patients in study	Multi-variable analysis	Results of analysis*
Graft vs. Host Disease (GvHD)					
Acute GvHD	Gerber 2008[107]	Retrospective Observational	1514	Y	OR 2.4 (95% C.I. 1.8 to 3.3)
	Najima 2009[111]	Retrospective Observational	622	Y	HR 1.41 (95% C.I. 1.01 to 1.97)
	Bleggi-Torres 2002[112]	Post-mortem study	180	NR	OR 0.86 (95% CI 0.45 to 1.62)
	Zumberg 2002[108]	RCT	159	Y	P < 0.049
Veno-occlusive Disease (VOD)					
	Gerber 2008[107]	Retrospective Observational	1514	Y	OR 2.2 (95% CI 1.4 to 3.6)
	Najima 2009[111]	Retrospective Observational	622	Y	HR 2.63 (95% C.I. 0.77 to 9.00)
	Zumberg 2002[108]	RCT	159	Y	P < 0.033

NR = not reported; RCT = randomised controlled trial; Y = Yes.
*P-value only reported if an odds ratio (OR), hazards ratio (HR) or relative risk (RR) was not reported

1.6.4.3.1 Graft versus Host Disease (GvHD)

Acute GvHD (aGvHD) occurs within 100 days of an allogeneic HSCT, and is a major cause of early mortality (within 100 days of HSCT).[113] It causes problems with the skin (erythematous

skin reaction), liver (cholestatic liver disease) and gastrointestinal tract (nausea, vomiting and diarrhoea).[113] It is thought to be caused by a three phase process:[113]

1) Tissue damage due to treatment required prior to receiving donor stem cells
2) Activation of host antigen-presenting cells and activation and proliferation of donor T-cells
3) Release of inflammatory cytokines e.g. interleukin-1

Three of the four studies that reported the association found an increased risk of bleeding with acute GvHD (Table 1.8).

Possible reasons for the increased risk of haemorrhage may be the underlying inflammation present,[114] or increased friability of tissues due to the presence of aGvHD, or both.

1.6.4.3.2 Veno-occlusive Disease (VOD)

VOD is the term used to classify the symptoms and signs of hepatic toxicity caused by the conditioning regimen (treatment given prior to the infusion of stem cells).[115] It is characterised by jaundice, fluid retention and tender hepatomegaly.[115]

Two of the three studies found an increased risk of haemorrhage associated with the presence of VOD (Table 1.8). The results for the only study that did not find an association[111] showed a very wide 95% confidence interval for the hazard ratio. Therefore, a true association between VOD and haemorrhage may have been present, but was not detected.[116]

There are two main possibilities for this increased risk. Firstly, VOD is associated with damage to the vascular endothelium, and release of pro-inflammatory cytokines.[115] Secondly, the main specific treatment for VOD is an anticoagulant (defibrotide).[115]

1.7 Summary

- Patients with haematological disorders are the main recipients of all platelet components issued. The majority of these platelet transfusions are given to prevent haemorrhage (prophylaxis).
- Haematological malignancies are increasing in incidence and prevalence and therefore demand for platelet transfusions will increase if we continue with the current prophylactic platelet transfusion policy
- Platelet components are a scarce resource and should be used judiciously.
- The current approach to decide whether or not a patient should receive a prophylactic platelet transfusion is sub optimal.
- Platelet components are associated with risks to the patient. The safest transfusion is the one not given because it is not needed.
- Any strategy that can safely decrease the need for prophylactic platelet transfusions in haematology patients will have significant logistical and financial implications as well as decreasing patients' exposure to the risks of transfusion.
- Infection is one of the commonest reasons why clinicians give a prophylactic platelet transfusion above the threshold of 10×10^9/L but there is no clear evidence from the literature that fever, sepsis or infection increase the risk of haemorrhage.

1.8 Aims of the research

To identify clinical and laboratory factors that may predict the risk of haemorrhage in patients with haematological malignancies and severe thrombocytopenia.

Information from this research may be used in the future to develop a patient-centred approach to the use of prophylactic platelet transfusions and other haemostatic agents, based upon an individual's baseline characteristics and risk factors for haemorrhage.

1.9 Thesis plan

This thesis is divided in to two main sections:

Section I is concerned with factors that may be associated with any or "clinically significant" haemorrhage. Clinically significant haemorrhage is usually defined as WHO grade 2 or above bleeding.

Section II deals with factors that may be associated with severe or life-threatening haemorrhage.

1.9.1 Introductory section (Chapter 2)

A study was performed to assess how bleeding outcomes were measured in randomised-controlled trials (RCTs) of platelet transfusions. Bleeding is a subjective measure and this study was used to identify whether a "gold standard" for assessing bleeding outcomes in this patient population was available. If present, this measure would then be used to measure the severity of haemorrhage for the remainder of the thesis.

1.9.2 Section I: Factors that may be associated with any or "clinically significant" haemorrhage (usually defined as WHO grade 2 or above bleeding)

1.9.2.1 *Chapter 3 ATHENA Study*

The aim of this study was to evaluate whether rotational thromboelastometry (ROTEM) parameters of extrinsically activated coagulation (EXTEM), intrinsically activated coagulation (INTEM) and the fibrinogen contribution to coagulation [FIBTEM (test based on EXTEM but contains cytochalasin D to inhibit platelets)] or other routinely available platelet parameters [mean platelet volume (MPV), platelet mass, immature platelet fraction (IPF), absolute immature platelet number (AIPN), platelet function analyser-100 (PFA-100)] were better at predicting bleeding than the total platelet count.

1.9.2.2 *Chapter 4. TOPPS sepsis sub-study*

An observational study of a sub-set of patients who participated in the TOPPS trial, an RCT that compared a no-prophylaxis versus prophylactic platelet transfusion strategy.[7] Additional data were collected on over one third of study participants to assess whether sepsis as well as other clinical and baseline characteristics increase a patient's risk of haemorrhage.

1.9.3 Section II: Factors that may be associated with severe or life-threatening haemorrhage

Intracranial haemorrhage (ICH) has been chosen as representative of severe or life-threatening haemorrhage for several reasons. Firstly, although rare, it frequently causes death or significant long-term morbidity. Secondly, its presence or absence can be easily

confirmed using non-invasive radiological techniques. Thirdly, all grading systems that mention site-specific haemorrhage, within this patient population, agree that ICH is a severe or life-threatening haemorrhagic event.

1.9.3.1 *Chapter 5*

A systematic review of cases of ICH in patients with severe thrombocytopenia secondary to a haematological malignancy or its treatment.

1.9.3.2 *Chapter 6*

A case-control study of ICH in patients with a haematological malignancy (InCiTe study).

1.9.4 **Chapter 7**

Summary of the thesis, and future work arising from this thesis

2 What is the best method for assessing haemorrhage in this patient group

2.1 Introduction

There are two important considerations when haemorrhage is used as an outcome measure: the documentation of signs and symptoms of haemorrhage; and, the translation of this information into a clinically meaningful score or grade. This is fundamental to the robustness of a study's results, and valid comparisons between studies can only be drawn if similar outcomes are being compared. Therefore, if haemorrhage is to be used as the main outcome measure, as in this thesis, it is important that it is defined and documented in a consistent and standardised way.

The assessment of haemorrhage involves an element of subjectivity, and the literature indicates wide variability in the methods by which haemorrhage has been assessed and documented in the past. Taken together, these factors may be responsible, in part, for differences in the reported baseline bleeding rates between studies, which have varied in randomised-controlled trials (RCTs) of platelet transfusions from 5% [117] to 70%.[6] Different trials have taken different approaches in an attempt to minimise this bias, such as the use of a standardised tool for assessing bleeding [118]; training of staff [119]; or grading of haemorrhage. [6,68,118,120]

Although variability in the documentation and grading of haemorrhage is known to exist, there has not been a systematic summary of the approaches used to document and grade haemorrhage in platelet transfusion trials. Such a summary would enable me to understand

current practices and select the most appropriate method to assess and grade haemorrhage within this thesis.

Information was sought from the lead investigators of clinical trials of platelet transfusions in association with the Biomedical Excellence for Safer Transfusion (BEST) Collaborative, with the specific aims to:

1) Describe the methods used in the trial protocol to document haemorrhage;

2) Describe the methods for grading bleeding, including the assignment of haemorrhage grades and validation of the process.

2.2 Materials and methods

2.2.1 Identification of studies

Clinical platelet studies were identified via the search strategies used within a recently updated Cochrane review of prophylactic platelet transfusions[121] and that reported by the BEST Collaborative for their systematic assessment of quality of reporting for platelet transfusion studies.[122] The search was limited to RCTs in humans that reported bleeding as a primary or secondary outcome. The primary authors of all identified trials were contacted and were invited to participate in the study by sharing copies of their bleeding assessment tool case report forms (CRFs) together with any protocol, standard operating procedure, or guidance notes describing the procedures for bleeding assessment and documentation. The primary author was contacted up to three times. If the primary author did not respond a second author was also contacted up to three times.

2.2.2 **Data collection**

Data for analysis were taken from three sources. Publications associated with each study were reviewed. The CRFs and any guidance notes were reviewed to assess how the study teams documented signs, symptoms and site of bleeding, severity (grade) of bleeding, and treatment of any bleeding episodes. This was supplemented by information from a questionnaire (Appendix 2.1) developed in conjunction with the Biomedical Excellence for Safer Transfusion (BEST) Collaborative Project Group and piloted by Dr N Heddle, Dr S Stanworth, and Prof. S Slichter (three primary authors who had agreed to participate in the study). The questionnaire was circulated to all authors who had agreed to participate and had provided copies of their CRFs. The questionnaire asked about how study data were collected, the methods used for standardisation and training, and how bleeding was graded.

Summary data elements were identified by the investigators, I reviewed all submitted material including the primary study manuscripts and captured the relevant data summarising it in table format. The information from each study was then sent to the study author for verification of accuracy and completion of any missing data.

2.2.3 **Analysis**

The overall analysis was descriptive with results presented as percentages for categorical data. Missing data were reflected by variation in denominators.

2.3 Results

2.3.1 Overview

The results of the search strategy (Figure 2.1) identified 28 different primary authors.

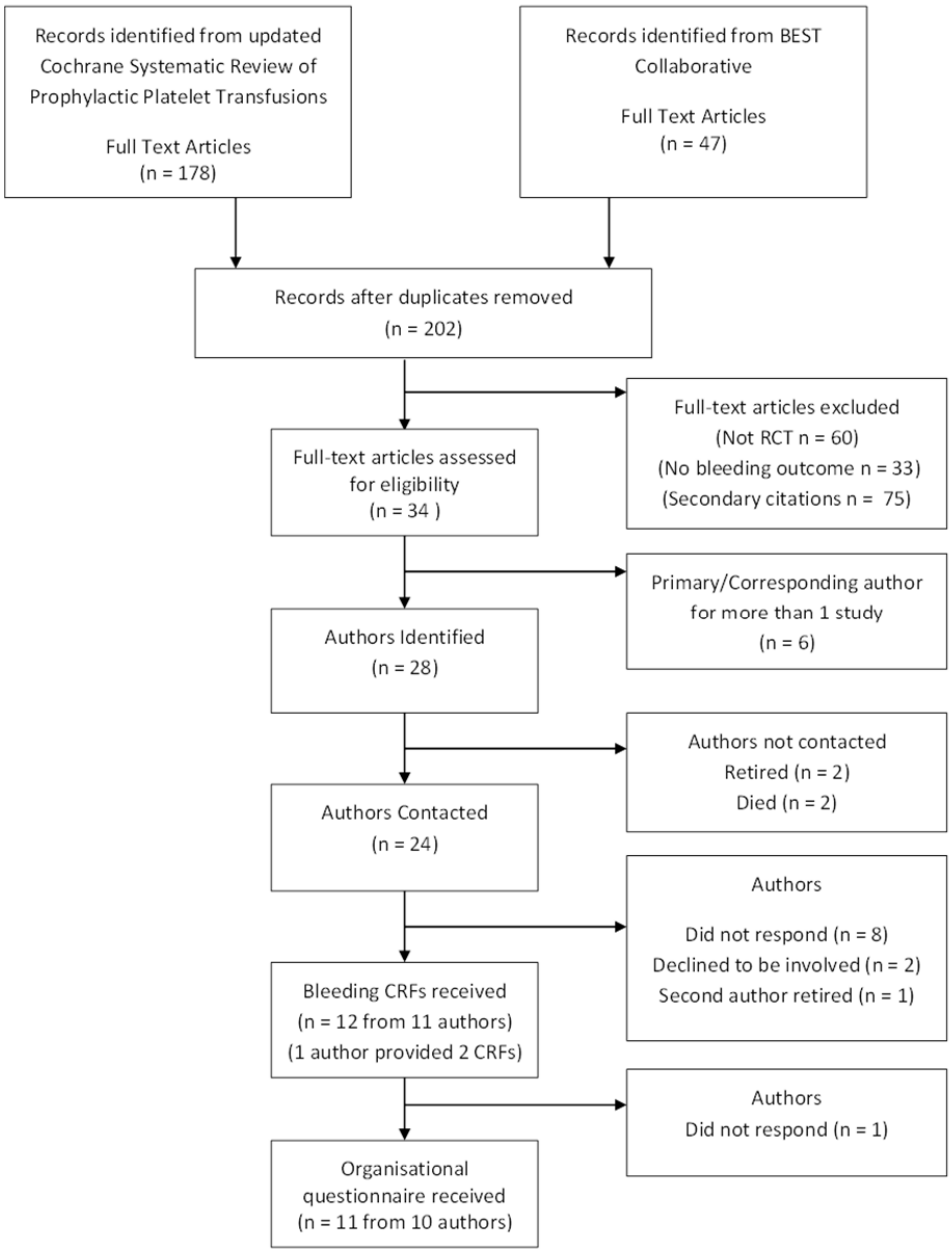


Figure 2.1 Flow diagram for the identification of authors within this study

Four of the authors, who had published studies in the 1970s and 80s, had retired or died.[76,77,123,124] Fifteen primary authors responded, of these, two authors declined to participate,[78,125] the remaining 13 authors[2,5-7,68,69,108,119,126-130] agreed to participate. Nine secondary authors[117,131-138] were contacted, one responded, but had just retired.[131] Two sets of two authors[7,119,127,129] worked in close collaboration and used the same CRFs and one author[139,140] had two different CRFs for separate studies.

2.3.2 Baseline characteristics of identified studies

Of the 11 authors who returned CRFs (Figure 2.1), eight were the primary authors of multi-centre RCTs and three of these studies were multi-national. Studies were based in Australia, Canada, France, Germany, Italy, Netherlands, Norway, Spain, Sweden, UK and USA. Study size ranged from 82 to 1351 participants. The majority of the CRFs (9/12) and questionnaires (9/11) were from studies performed from 2000 onwards (Table 2.1). Haemorrhage rates varied significantly between the studies varying from 4.2% to 71% for clinically significant haemorrhage (WHO grade 2 or above) and between 0% and 13.8% for severe or life-threatening haemorrhage (WHO grade 3 or 4) (Table 2.2).

Table 2.1 Baseline characteristics of included studies

Study	Type of study	Study period	Country	Number of participants randomised	Intervention	CRF Sent	Questionnaire returned	Study results published
Platelet Threshold Studies								
Heckman 1997[69]	Single centre Parallel RCT	Apr 1991 to Nov 1995	USA	82	Prophylactic platelet transfusions with different transfusion triggers	Y	Y	Y
Trigger[68]	Multicentre Parallel RCT	Mar 1994 to Mar 1997	Italy	276	Prophylactic platelet transfusions with different transfusion triggers	N	Y	Y
Zumberg 2001[108]	Single centre Parallel RCT	Jul 1997 to Dec 1999	USA	159	Prophylactic platelet transfusions with different transfusion triggers	Y	N	Y
Platelet Dose Studies								
SToP[5]	Multicentre Parallel RCT	Oct 2003 to Jun 2007	Canada, Norway & USA	129	Low dose versus standard dose platelet transfusions	Y	Y	Y
PLADO[6]	Multicentre Parallel RCT	Jul 2004 to Dec 2007	USA	1351	Low dose versus standard dose versus high dose platelet transfusions	Y	Y	Y
Tinmouth 2004[128]	Single centre Bayesian approach study	Feb 2001 to Mar 2002	Canada	111	Low dose versus standard dose platelet transfusions	Y	Y	Y

RCT = randomized controlled trial; N = No; NK = not known; NA = not applicable; Y = Yes.
Parallel RCT = patients are randomized to intervention or control.
Crossover RCT = patients receive both the intervention and the control. Randomised to which one they receive first.
Bayesian approach study = differs from the standard frequentist approach to analysis. It starts with the researchers' *a priori* belief about the risk ratio and uses the study data to modify that opinion.

Study	Type of study	Study period	Country	Number of participants randomised	Intervention	CRF Sent	Questionnaire returned	Study results published
Pathogen reduced platelet component studies								
SPRINT[119*†]	Multicentre Parallel RCT	Jul 1999 to Feb 2001	USA	671	Pathogen reduced platelets versus standard apheresis components	Y	Y	Y
Lozano et al 2011[*†]	Multicentre Parallel RCT	Oct 2005 to Jul 2009	France, Spain, Sweden & UK	242	Pathogen reduced platelets versus standard platelet components	NA	NA	Y
MIRACLE[130‡]	Multicentre Parallel RCT	Dec 2005 to Sep 2007	France	118	Pathogen reduced platelets versus standard platelet components	Y	Y	Y
TriPlate[139†]	Multicentre Parallel RCT	Mar 2007 to Jan 2009	Netherlands	295	Pathogen reduced platelets versus standard platelet components	Y	Y	Y
PrePAReS[140†]	Multicentre Parallel RCT	Started Nov 2010	Netherlands	In progress	Pathogen reduced platelets versus standard platelet components	Y	Y	N
IPTAS[141‡]	Multicentre Parallel RCT	Started Dec 2008	Italy	In progress	Pathogen reduced platelets versus standard platelet components	Y	N	N
Platelet age studies								
PPIP[129§]	Multicentre Crossover RCT	Started Sep 2007	UK	Completed	2 to 5 day versus 6 to 7 day old platelet transfusions	NA	NA	N
Therapeutic only versus prophylactic platelet transfusions								
TOPPs [7]	Multicentre Parallel RCT	Aug 2006 to Aug 2011	Australia & UK	600	Prophylactic versus therapeutic only platelet transfusions	Y	Y	Y
Nuerenberg trial[2]	Parallel RCT	Feb 2005 to May 2010	Germany	396	Prophylactic versus therapeutic only platelet transfusions	Y	Y	Y

* Study has the same methodology as the SPRINT study on which CRFs and questionnaire were returned
†Cerus pathogen-reduced platelet components (Ultraviolet-A in presence of amotosalen, S-59, in Intercept Blood System)
‡Caridian pathogen-reduced platelet components (Ultraviolet-A in presence of riboflavin, B2 in Mirasol Pathogen Reduction Technology)
§ Study has the same methodology as the TOPPs study [12] on which CRFs and questionnaire were returned

Table 2.2 Variability in bleeding rates in all randomised controlled trials of platelet transfusions that performed daily bleeding assessments for the duration of the study.

Study	Intervention	Number of patients in each arm	Percentage of patients with any bleeding	Percentage of patients with significant bleeding/ WHO grade 2 or above	Percentage of patients with WHO grade 3 or 4 bleeding
Platelet threshold studies					
Diedrich *2005*[125]	**< 10 x 10^9/l**	**79**	**-**	**18**	**4**
	< 30 x 10^9/l	**87**	**-**	**15**	**7**
Trigger[68]	< 10 x 10^9/l	135	-	21	11*
	< 20 x 10^9/l	120	-	20	9*
Heckman 1997[69]	< 10 x 10^9/l	37	95	46	-*
	< 20 x 10^9/l	41	90	17	-*
Zumberg, *et al* 2002[108]	< 10 x 10^9/l	78	95	27	-*
	< 20 x 10^9/l	81	98	26	-*
Therapeutic only versus prophylactic platelet transfusions					
Nuerenberg trial [2]	Therapeutic	197	-	42.0	6.0
	Prophylactic	194	-	19.0	2.0
TOPPS[7]	Therapeutic	300	-	50.0	2.0
	Prophylactic	298	-	43.0	0.3
Platelet dose studies					
Sensebe *2004*[117]	**Standard dose**	**48**	**19**	**4**	**-**
	High dose	**48**	**10**	**6**	**-**
Tinmouth 2004[128]	Low dose	56	-	11	-*
	Standard dose	55	-	7	-*
SToP[5]	Low dose	58	91	52	14
	Standard dose	61	79	49	10
PLADO[6]	Low dose	417	-	71.0	12.0
	Standard dose	423	-	69.0	9.0
	High dose	432	-	69.9	10.0
Pathogen reduced platelet component studies					
Janetzko *2005*[135]	**p-Rx; apheresis**	**22**	**64**	**-**	**-**
	Standard apheresis	**21**	**71**	**-**	**-**
TriPlate[139]	p-Rx; PAS III; BC	85	32	13	6*
	PAS III; BC	94	15	4	0*
	Plasma; BC	99	19	7	1*
SPRINT[119]	p-Rx; apheresis	318	89.6	-	4.1
	Standard apheresis	327	84.7	-	6.1

* Study did not use the WHO grading system. p-Rx = pathogen reduced platelet components; PAS III = platelet additive solution III; BC = buffy coat. Authors from the studies highlighted in bold did not participate in this study.

2.3.3 **Methods of recording haemorrhage – the bleeding assessment**

Based on the 11 questionnaires returned, the bleeding assessment consisted of at least a formalised bleeding assessment and a clinical examination of the patient (Table 2.3). In all but two cases (9/11) this was done on a daily basis. One study performed the assessment twice a day,[2] and the other study [130] performed the assessment twice daily on the day of any platelet transfusion and once daily on the day following the platelet transfusion.

In 6 of the 11 studies,[2,5-7,119,139] the bleeding assessment was performed by trained research nurses or other research investigators at least part of the time. In 4 out of 11 studies the bleeding assessor was reported to be blinded to treatment allocation.[6,119,130,142] In only one study was the effectiveness of this blinding assessed,[119] where research nurses were asked to detect study units from a panel of 10 platelet components (a mixture of study and conventional units) and then complete a questionnaire.

Table 2.3 Assessment of haemorrhage/bleeding. Results of the 11 questionnaires returned

Study	Assessment of bleeding					Frequency of assessment	Same time each day	Type of bleeding assessors				Blinding of bleeding assessors	Effectiveness of blinding assessed
	Patient notes/chart	Formalized bleeding assessment	Examination of patient	Patient questionnaire	Nurse questionnaire			Medical staff	Nurses	Trained research nurses	Other research investigators*		
MIRACLE[130]	Y	Y	Y	N	N	Twice daily on day of Tx, once daily on day after Tx	N	Y	Y	N	N	Y	N
Heckman 1997[69]	Y	Y	Y	N	N	Daily	Y	Y	Y	N	N	N	-
STOP[5]	Y	Y	Y	N	N	Daily	Y	N	N	Y	Y	Y	N
SPRINT[119]	Y	Y	Y	Y	Y	Daily	Y	N	N	Y	N	Y	Y†
HOVON-Triplate[139]	Y	Y	Y	N	N	Daily	N	Y	N	N	N	N	-
PrePAReS[140]	Y	Y	Y	Y	Y	Daily	Y	N	N	N	Y	N	-
Trigger[68]	Y	Y	Y	NR	NR	Daily	N	Y	N	N	N	N	-
PLADO[6]	Y	Y	Y	Y	Y	Daily	Y	N	N	Y	N	Y	N
TOPPs[7]	Y	Y	Y	Y	Y	Daily	Y	Y	N	Y	Y	N	-
Tinmouth 2004[128]	Y	Y	Y	Y	N	Daily	N	Y	N	N	N	N	-
Nuerenberg trial[2]	Y	Y	Y	Y	Y	Twice daily	Y	Y	Y	N	Y	N	-

Y = Yes; N = No; NR = Not reported; Tx = transfusion

* I did not ask for further details on who these investigators were † Research nurses asked to detect study units from a panel of 10 platelet components (mixture of study and conventional units).

2.3.3.1 *Site and severity of haemorrhage*

(Table 2.4, Table 2.5 & Table 2.6)

Information on the CRFs in all studies was collected using a mixture of check boxes (yes/no) and open-ended questions (which may be more difficult to analyze). In all of the studies conducted during the last decade (9/12),[2,5-7,108,119,128,130,139,141] the CRFs consisted of tick box or Yes/No responses although (2/9)[128,130] had a significant number (> 25%) of open-ended questions.

There was significant variability among CRFs in the amount of detail related to the site and severity of haemorrhage (Table 2.4 & Table 2.5). Definitions appeared to vary most between studies in relation to bleeding into the skin and subcutaneous tissue. Pathology[143] and dermatology[144] textbooks differentiate between petechiae, purpura and ecchymoses according to the size of the haemorrhage into the skin (petechiae < 2mm, purpura 2mm to 10mm and ecchymoses > 10mm) and all are forms of hematoma. However, the definitions of these variables in many of the studies differed from each other and did not conform with textbook definitions. The term purpura was often used to define any type of skin bleeding larger than petechiae. Three studies distinguished between purpura that were less than or greater than one inch.[6,119,140] One study distinguished between hematomas that were less than or greater than 1cm.[130] Two studies[5,118] distinguished between purpura and ecchymoses with the distinction between the two being 2cm[118] or 1 inch.[5]

All studies provided suggested definitions for different severity scores of bleeding at some anatomical sites, but the details varied between studies (Table 2.5). For example,

all studies asked about bleeding from the mouth, but three did not ask about the duration of bleeding.[68,108,139] Two studies asked about red blood cells (RBCs) in CSF (cerebro-spinal fluid) only seen on microscopy,[6,140] and one study[6] asked about RBCs in other body cavity fluid on microscopic examination. Ten studies[2,5-7,108,119,128,130,139,140] assessed bleeding severity by the need for RBC transfusion to treat bleeding above routine requirements (83.3%; 10/12). Fifty per cent (6/12) of the studies[6,118,119,130,140,141] required information on whether bleeding was associated with haemodynamic instability. Twenty-five per cent (3/12) of the studies documented whether bleeding was associated with a significant fall in haemoglobin (Hb).[6,128,140] Two studies[5,140] also defined severity of bleeding by need for interventions other than RBC transfusion e.g. other medications or procedures (Table 2.6).

None of the platelet threshold studies[68,69,108] (Table 2.2) classified skin bleeding as clinically significant bleeding, and all these studies had a lower rate of haemorrhage than the platelet dose studies[5,6,128] and studies assessing pathogen-reduction technologies.[119,139]

Table 2.4 Site of haemorrhage documented in the 12 case report forms (CRFs)

Site of bleeding Documented on CRF	No. of CRFs	Specific types of haemorrhage at each anatomical site	No. of CRFs*
Mouth	12/12	-	12/12
GI	12/12	Melaena	12/12
		Haematemesis	12/12
		Haematochezia	9/12
CNS	11/12	-	11/11
Urogenital	11/12	Haematuria	11/11
		Vaginal	10/11
Nose	11/12	-	11/11
Eye	10/12	Retinal	10/10
		Conjunctival	3/10
		Vitreous	1/10
Pulmonary (Hemoptysis)	10/12	-	10/10
Skin	10/12	Petechiae	9/10
		Purpura	8/10
		Ecchymoses	4/10
Insertion site	9/12	-	9/9
Musculo-skeletal	8/12	Haematoma	8/8
		Joint bleed	3/8
Body cavities	5/12	-	5/5
Associated with surgery	4/12	-	4/4
Other (please specify)	7/12	-	7/7
*The denominator is the number of CRFs that reported haemorrhage at that anatomical site			

Table 2.5 Severity of haemorrhage documented in the 12 case report forms (CRFs)

Site of bleeding Documented on CRF	No. of CRFs that reported haemorrhage at that site	Specific types of haemorrhage at each anatomical site	No. of CRFs*
Mouth	12	Duration	9/12
		Intervention	3/12
GI	12	Number of separate bleeding occasions	2/12
		Intervention	3/12
CNS	11	Neurological symptoms/signs	10/11
		Intervention	3/11
Urogenital	11	Severity of haematuria	9/11
		Severity of vaginal bleeding	9/11
		Intervention	3/11
Nose	11	Duration	9/11
		Intervention	3/11
Eye	10	Visual impairment	9/10
		Ophthalmology review	3/10
Pulmonary (Hemoptysis)	10	Intervention	4/10
Skin	10	Spread	5/10
		Size	8/10
		Number	4/10
Insertion site	9	-	-
Musculo-skeletal	8	-	-
Body cavities	5	Severity of bleeding	5/5
		Intervention	2/5
Associated with surgery	4	-	-
Other (please specify)	7	-	-
*The denominator is the number of CRFs that reported haemorrhage at that anatomical site			

Table 2.6 Procedures/interventions or transfusions given to treat haemorrhage documented in the 12 case report forms (CRFs)

Procedure/Intervention/Transfusion	No. of CRFs	Sub-categorisation of procedure/Intervention/Transfusion	No. of CRFs*
Any	10/12	-	-
Endoscopy	3/12	Colonoscopy	2/3
		Bronchoscopy	2/3
Bladder irrigation	2/12	-	-
Nasal	1/12	Packing	1/1
		Cauterization	1/1
Pericardiocentesis	1/12	-	-
Transfusion	10/12	RBCs	10/10
		FFP	3/10
Factor concentrates	3/12	Factor VIIa	2/3
Medications	2/12	Tranexamic acid	2/2
		DDAVP	2/2
		Aminocaproic acid	2/2
Topical fibrin glue	1/12	-	-

*The denominator is the number of CRFs that reported a particular intervention/procedure or transfusion

2.3.4 Methods of achieving consistency in the assessment of haemorrhage – the bleeding assessment

Eight of the 11 studies reported the training of bleeding outcome assessors, prior to commencement of the study.[5,6,68,118,119,130,140,145] Four of the studies provided further training once the study had started,[118,119,130,145] but only two of these studies indicated that training occurred on a regular basis.[118,119] Six of the studies reported more than one trained bleeding assessor at each site[5,6,68,118,119,145] and four of these mentioned that trained assessors were present at weekends or that there was a back-up assessor to cover sick leave.[5,6,118,119]

Seven of the studies reported providing guidance notes to the bleeding assessors in addition to the actual CRFs.[5,6,68,118,119,130,140] But there was great variability in the level of detail provided in these notes for the five studies that shared this information. Two provided practical information on how to complete the CRFs[118,130] and two included 'easy to read' definitions of the different types of bleeding.[118,140] For two studies the notes were expanded versions of the World Health Organization (WHO) grading criteria.[5,119]

Duplicate bleeding assessments were performed in three of the studies.[69,118,145] This was performed in 50% of cases for one study,[69] approximately 10% of cases for the second study[118] and only in cases of severe or life-threatening bleeding for the third study.[145] Whether the second bleeding assessor was blinded from the results of the first bleeding assessor was not asked in the questionnaire. In all three cases the results of these assessments were fed back to the assessors, as a form of on-going education.

Two studies reported other specific methods of decreasing inter-observer variability. These included dummy bleeding assessment scenarios during training days[118] and clinical investigator oversight and final review of all daily bleeding assessment forms.[119] (In this study, all CRFs were monitored against primary source documents by Contract Research Organization (CRO) research monitors; discrepant data were queried and reviewed by the clinical investigator at each site; and the primary clinical investigator at each site rendered the final assessment of daily bleeding grade.)

All of the studies[5,6,119] that have reported haemorrhage rates over 50% employed specially trained research nurses to perform the bleeding assessment.

2.3.5 **Grading of haemorrhage**

Six of the 11 authors used the WHO grading system[146] in their most recent platelet transfusion studies.[5,6,118,127,130,140] Three of the authors reported using the Rebulla system [68] or a variant of this scale.[108,128] One study used a modified scoring system that included elements of the WHO and Rebulla classifications.[145] One study [69] used a system devised by Ajani et al,[147] but this has not been used in more recent platelet transfusion studies.

Different grading systems can lead to different baseline levels of bleeding. This can be seen in data from the SPRINT study (Table 2.7). Patients were graded daily using the WHO grading system but the trained study personnel also reported any bleeding as a side-effect using the Common Toxicity Criteria for Adverse Events (CTCAE) system. Although the overall incidence of bleeding was similar between the two grading systems,[148] which reflects no difference in the number of bleeds being reported using the

two systems, there were significant differences in how they were graded. There were fewer grade 2, 3 or 4 bleeds using the CTCAE system, which may be explained by the observation that occult blood in the urine could be graded as WHO grade 2 and that, apart from bleeding into the skin, to classify bleeding as CTCAE grade 2 it required an intervention of some sort.

Table 2.7 Comparison of CTCAE and WHO grades (Data from SPRINT Trial[119] – including previously unpublished data)

Severity of Bleeding	P-Rx (N = 318)		Control (N = 327)	
	CTCAE* N (%)	WHO* N (%)	CTCAE* N (%)	WHO* N (%)
Any Bleeding	285 (90)	296 (93)	277 (85)	295 (90)
Grade 1	147 (46)	110 (35)	164 (50)	105 (32)
Grade 2	65 (20)	173 (54)	48 (15)	170 (52)
Grade 3	63 (20)	12 (4)	57 (17)	14 (4)
Grade 4	10 (3)	1 (<1)	8 (2)	6 (2)

CTCAE = Common Toxicity Criteria for Adverse Events
WHO = World Health Organization
P-Rx = Pathogen reduced platelets
* Maximum bleeding grade the patient experienced during the study

2.3.5.1 *WHO grading system[146]*

The WHO system is now used in the majority of studies. The original grading system[146] classified petechiae as grade 1; mild blood loss as grade 2; gross blood loss as grade 3 and debilitating blood loss as grade 4. None of the terms were defined further. All of the authors who defined significant bleeding classified it as grade 2 or above. All of the authors who defined life-threatening bleeding classified it as grade 4. But, all of the

authors who were using the WHO grading system had refined it from the original formulation[146] in different ways. This led to variability in the way the same bleed would be graded between different studies (Table 2.8).

Some of these differences could lead to the same bleed being categorized as grade 2 in one study and grade 1 or no bleeding in another study. For example, 3/6 studies[5,119,130] defined occult blood >1+ in the urine as WHO grade 2 bleeding. 3/6 studies[118,119,130] defined grade 2 vaginal bleeding as bleeding saturating more than 2 pads per day, whereas the other 3 studies[5,6,140] defined abnormal vaginal bleeding more than spotting as grade 2 bleeding. 3/6 studies[6,118,140] defined epistaxis that lasted more than 30 minutes as grade 2 bleeding whereas the other 50%[5,119,130] only regarded bleeding that lasted an hour as grade 2 bleeding. There was also variability in the way that petechiae, purpura, haematomas and ecchymoses were defined between studies. Two of the studies defined diffuse petechiae as WHO grade 2 bleeding.[118,119] Retinal bleeding that did not cause visual compromise was classified as grade 1 in one study [119] and grade 2 in the other five studies.[5,6,118,130,140] Finally, the definition of haemodynamic compromise varied between studies: two studies[6,140] categorised a drop in systolic or diastolic blood pressure of 30mmHg as a grade 3 bleed; whereas, two further studies [118,119] regarded this as a grade 4 bleed.

Table 2.8 Major differences in WHO grading between studies

Study	Occult blood in stool	Microscopic blood in urine		Grade 2 skin bleeding			Retinal bleeding without visual compromise	Grade 2 vaginal bleeding	Grade 2 epistaxis/ bleeding from mouth	Haemodynamic instability	
		Grade 1	Grade 2	Petechiae	Purpura	Ecchymoses				Definition	Grade
MIRACLE[130]	Grade 1	1+	> 1+	N	> 1 cm	N	Grade 2	> 2 saturated pads/day	> 1 hr	N	-
PrePAReS[140]	Grade 1	Positive	N	N	> 1 inch	N	Grade 2	Abnormal vaginal bleeding*	> 30 mins	30-50mmHg fall >50mmHg fall/ 50% fall in BP	3 4
SToP[5]	Grade 1	1+	> 1+	N	N	> 10 cm	Grade 2	abnormal vaginal bleeding*	> 1 hr or packing	N	-
SPRINT[119]	Grade 1	1+	> 1+	Generalized	> 1 inch	N	Grade 1	> 2 saturated pads/day	> 1 hr	> 30mmHg fall	4
PLADO[6]	N	N	N	N	> 1 inch	N	Grade 2	Abnormal vaginal bleeding > spotting	> 30 mins	30- 50mmHg fall >50mmHg fall/> 50% fall	3 4
TOPPs[118]	N	N	N	Diffuse	> 5	> 10cm or multiple > 2cm	Grade 2	unexpected vaginal bleeding saturating 2 pads/24 hrs	> 30 mins	> 30mmHg fall	4

Information from questionnaire, guidance notes sent with CRF and published article

N = Not defined/present in the study's grading system

* Unexpected bleeding out of normal cycle OR bleeding heavier than normal OR breakthrough bleeding (patient on hormonal therapy) more than spotting

2.3.5.2 *RBC transfusion requirements*

10 of the 11 authors used RBC requirements to grade bleeding,[5,6,68,69,108,119,129,130,139,140,143,149,150] and five of these authors reported a standardised transfusion policy operating in the study.[2,7,68,69,140] Without a standardised policy the same type of bleed could be graded as grade 2 or 3 depending on the transfusion decision by the treating physician/research centre.

2.3.5.3 *Conversion of bleeding data into a bleeding grade*

The majority of studies assigned a bleeding grade manually (9/11) (Table 2.9). Three studies (3/11) assigned the grade using a validated computer algorithm[6,118,140] and two[6,118] of these used this single system of grading. In one of these studies[118] validation of the algorithm was performed by comparing 100 patients via both manual and computer methods, the other study[6] did not report the method of validation. The third study[140] performed both manual and computer algorithm methods of grading bleeding. Of those that graded bleeding manually only two studies[68,130] did not check the grading using a second person or method. All of the studies that checked their results via a second method or person resolved any disagreement by adjudication.

This adjudication could have involved: debate between study personnel (four studies); [69,139,140,145] referral to the Chief Investigator/Principal Investigator (two studies);[119,145] or referral to an independent third party/parties (two studies).[5,128]

Table 2.9 Table showing how platelet transfusion studies converted data about bleeding in to a bleeding grade

Study	Initial assignment of bleeding grade	Bleeding assessor & grader of bleeding the same person*	Person who graded bleeding blinded to the intervention*	Bleeding grade checked by second person/method	Method of checking	Person who graded bleeding blinded to the intervention	Person who graded bleeding was blinded to the initial grade	Was adjudication† performed if differences in bleeding grade
MIRACLE[130]	Manual	Y	Y	N	-	-	-	-
Heckman 1997[69]	Manual	Y	N	Y	Manual	N	NR	Y
SToP[5]	Manual	Y	Y	Y	Manual	Y	Y	Y
Triplate[139]	Manual	Y	N	Y	Manual	N	N	Y
PrePAReS[140]	Manual	N	Y	Y	Computer algorithm†	-	-	Y
SPRINT[119]	Manual	Y	Y	Y	Manual	Y	N	Y
Trigger[68]	Manual	Y	N	N	-	-	-	-
PLADO[6]	Computer algorithm‡	-	-	N	-	-	-	-
TOPPs[118]	Computer algorithm‡	-	-	N	-	-	-	-
Tinmouth 2004[128]	Manual	Y	N	Y	Manual	Y	Y	Y
Nuerenberg trial[2]	Manual	N	N	Y	Manual	N	NR	Y

Y = Yes; N = No; NR = Not reported; - = Not applicable

* Question was only answered if method of assessing bleeding was manual

† This was resolved mainly by discussion between the two graders or between the graders and Principal Investigator/Chief Investigator

‡ Validated prior to commencement of the study

2.4 Discussion

This study has shown that the methods used to assess, document and grade haemorrhage in platelet transfusion trials display considerable heterogeneity, yet haemorrhage is increasingly defined as a primary outcome. The variability extended from the nature of the staff acting as bleeding assessors (e.g. trained and blinded research nurses), type of bleeding data recorded, through to the different methods for assigning bleeding grade (e.g. computer algorithm vs. manual). A key question is to what extent these different methodological approaches contribute to the variability in the frequency of haemorrhage reported between studies. Although no formal analysis of the effect of the methodological differences on the reported bleeding outcomes were possible in this study, some general trends in the variations of bleeding outcomes can be identified, which have implications for researchers and their readers.

Variability in bleeding frequencies between studies was most apparent for minor and moderate bleeds (WHO grade 1 & 2 bleeding) (Table 2.2). This raises questions about how different research groups have defined these types of bleeds, and what information, including education and training, is provided for bleeding assessors. As shown in Table 2.4 and Table 2.5, different studies have taken different approaches to define sites and severity of bleeding. These variations could affect the number of grade 2 bleeds and may explain some of the variability in the number of WHO grade 2 bleeds between different studies (Table 2.2). For example, bleeding classified as grade 2 in some studies

(microscopic blood in urine or spreading petechiae), was not classified at a similar grade of bleeding (grade 0 or 1, respectively) in another study.

Another reason for variation in rates of bleeding relates to how different research groups have provided education and training for bleeding assessors. Only a few trials indicated the preparation of specific information sheets with examples of different bleeding events, as a practical means of supporting researchers and ensuring consistent application of the different definitions. Dedicated training with guidance notes for researchers and follow-up training would be further expected to reduce inter-observer variability, but processes for providing specific training and education to assessors were only described in three studies by McCullough,[119] Stanworth,[118] and Wandt.[2] The use of dedicated research staff might facilitate any training and education in a trial, as for the PLADO,[6] SPRINT,[119] TOPPs,[118] and StoP[5] trials, but the use of research staff will presumably inflate the cost of the overall trial by comparison to using ward or clinical staff.

All trials converted bleeding data into bleeding grades. Although most of the recent studies used the WHO classification to grade bleeding, each study appeared to describe variations in the criteria. Although often minor, these variations could affect the number of grade 1 and 2 bleeds reported between different studies (Table 2.2). For example, all studies that did not classify skin bleeding as grade 2 bleeding had a lower overall rate of bleeding. Also, the baseline rate of bleeding differed between the StoP[5] and PLADO[6] studies. Part of this difference may be explained by differences in the grading of epistaxis (1 hr vs. 30mins) and skin bleeding (purpura/ecchymoses of 10cm vs. 2.54cm) that was

classified as grade 2 bleeding. Some studies reported that occult bleeding was important to document and grade, for example, the SPRINT study[119] graded faecal occult blood and haematuria that was only dipstick positive as bleeding, however the Kerkhoff study did not.[139] In the SPRINT study[119] the most common type of bleeding was genitourinary (32.1%), whereas other studies that did not document microscopic blood loss had a much lower rate of genitourinary bleeding. For example, in studies that included skin bleeding as grade 2 bleeding muco-cutaneous bleeding was the most common,[6,7,151] whereas gastrointestinal blood loss was the most common type in those that excluded skin bleeding[68,128]

Most studies reported using a manual method of bleeding adjudication for assigning grade, but few data are available about how this works in practice and experience from the recent trial SToP[5] suggests real difficulties achieving consensus. Computer algorithms may provide a more consistent method of assigning bleeding grade, but without details on validation of these methods, it is unclear exactly whether computer algorithms deliver more accurate grading than manual methods, although reproducibility is likely to be higher than manual methods.

Only a few studies included the need for specific interventions as a guide to severity. The use of interventions to manage bleeding is one of the first decisions to be made by clinicians when faced by a patient with bleeding. However, a difficulty with using this approach might be the desire to minimise interventions in these patients who are often profoundly neutropenic (e.g. endoscopy/colonoscopy), and the inevitable variation in access to interventions and use of interventions which may also change with time e.g.

use of recombinant factor VIIa where the evidence for overall lack of effectiveness has become clearer.[149]

2.4.1 Implications of this study for the research community

The analysis of methodologies in this study has raised a number of points which should help trialists, in the future, at the study design stage, when training staff and when reporting results. Whilst it seems obvious that study protocols should provide clear definitions of all relevant bleeds, wider agreement and consensus on the definitions of bleeding events and which bleeding events are clinically meaningful would help improve the consistency of reporting, and support direct comparison of results between studies. Some of these key areas for agreement could be fairly easily considered by the international community. For example, some protocols suggest that all petechiae are WHO grade 1, and clinically insignificant; but it seems likely that many clinical haematologists when faced with a patient with spreading or generalized petechiae and severe thrombocytopenia would treat the patient with a platelet transfusion. It also seems difficult to suggest that nose bleeding up to 1 hour is of no importance or concern, particularly to the patient. However, some studies always grade this type of bleeding as WHO 1 and therefore clinically insignificant.

Some studies have collected information on occult bleeding. But if these types of bleeds are considered important to collect, which is perhaps open to discussion, then specific questions on the CRFs need to capture this information, to minimise any risk of under-

reporting (this has been seen in other settings, for example the systematic under-reporting of acute lung injury).[150]

These issues also raise the question as to whose perception should be considered if standardisation is attempted: the physicians, the patients, or both? There has been little reported work evaluating how patients feel about the different types of bleeding, as a recent systematic review has demonstrated.[152] Patient perception of bleeding is especially important in those studies that rely on patients recording bleeding outcomes in the outpatient setting, away from medical oversight.

The methods of data collection need to be considered in a trial protocol, including the role of bleeding assessors. The method of training of staff or researchers undertaking bleeding assessments has been poorly documented in many trials. Assessment of bleeding will always be subjective, to a degree, and therefore ways to improve consistency within and between studies is crucial, and trial protocols should describe the training programs and strategies taken to support consistency in the recording of bleeding. Achieving blinding in platelet transfusion studies can be challenging, unless the platelet components are identical, due to problems with blinding the medical staff caring for the patient. The only blinding that is likely to be readily achievable is that of the bleeding assessor, if they are independent from the care of the patient. However, the bleeding assessor is also usually the person collecting the clinical and laboratory transfusion data, and therefore studies comparing prophylactic versus therapeutic policies or platelet transfusion thresholds would require the data for bleeding and

transfusion to be collected by separate individuals to maintain blinding. This would have major resource implications.

The methodology for defining and assigning bleeding grades is important, and any differences from previous trials should be indicated. The WHO score was developed as a tool for adverse event reporting in cancer patients, and although widely used, it has never been validated for specific use in clinical trials of transfusion. However, its widespread use and acceptance may provide a degree of post-hoc validation. Indeed, one study has shown good agreement (90%; 136/151 days) between self-assessment and medical grading of bleeding.[153] If it is to continue to serve as the international consensus scoring tool for this purpose agreement on the exact format of the WHO grading score should be considered by the international research community, as this would remove one level of uncertainty when comparing results between studies. For example, consensus on the need to collect data on occult types of bleeding or these could be identified separately e.g. grade 1^{Occult} or grade 2^{Occult}. An alternative would be to develop and use a new type of bleeding scale which has recently been reported, although with any new scale it would take time to assess its clinical acceptability.[154] At a minimum, if bleeding is a main outcome measure,[155] all trials of platelet transfusion should include specific information on bleeding grade definitions and educational/training support.

2.4.2 **Limitations of this study**

Although this study aimed to review all randomised controlled platelet transfusion trials, it was limited by only a proportion of authors responding to a request for the CRFs or

completion of the questionnaire, despite repeated requests. Despite this caveat, information was obtained from the majority of the current major researchers in this field. This study is therefore likely to provide a comprehensive picture of the current methodologies.

The analysis in this study was descriptive; and this study was not designed to quantify risk factors responsible for variability in bleeding. Estimates of how much the differences in grading could affect bleeding rates, could only be undertaken if individual patient data were available, and recorded in sufficient detail for different grading systems to be compared.

2.5 Summary

This study has identified differences in how the recording of signs and symptoms of bleeding is documented and how bleeding grades are assigned between studies.

There is currently no gold standard for the assessment and grading of bleeding. However, consistency can be improved by minimising:

- Inter-observer variability of bleeding assessors
    - Training of staff
    - Provide guide-notes and definitions of types of bleed
- Variability in grading the same severity of bleed by using a validated computerized algorithm

International consensus is required to produce agreement on definitions of type of bleed and criteria to allocate bleeding to a specific grade. Until then, a well-recognized grading system (e.g. modified WHO grading system)[7] should be used.

This thesis will therefore use the same methodology for assessing and grading haemorrhage that was used in the TOPPS study.[7]

Section I

Factors that may be associated with any or “clinically significant” haemorrhage (usually defined as WHO grade 2 or above bleeding)

3 Prospective observational cohort study of the association between thromboelastometry, coagulation and platelet parameters and bleeding in patients with haematological malignancies- The ATHENA Study.

3.1 Introduction

As discussed in chapter 1, in current practice, the decision to transfuse platelet components prophylactically is primarily based on the platelet count determined from daily blood tests. However, previous studies have shown that the risk of bleeding the following day is the same over a wide range of platelet counts.[6,7] Bleeding remains prevalent in patients with haematological malignancies despite the use of the platelet count to direct platelet transfusion prophylaxis.[2,6,7] In some patient groups, for example patients receiving an autologous stem cell transplant, bleeding occurred at a similar frequency and severity in patients receiving platelet prophylaxis directed by platelet count to those not on prophylaxis regimes.[7,156] The most plausible explanations for these observations are either that the platelet count is an inadequate marker of platelet haemostatic function in this clinical setting, or that other defects in haemostasis contribute significantly to bleeding.

3.2 Aim of the study

This study's aim was to evaluate whether other laboratory markers of haemostasis showed a stronger association with bleeding than the platelet count in severely thrombocytopenic patients receiving treatment for haematological malignancies.

In order for laboratory markers to be clinically useful in directing treatments to prevent bleeding, each marker must be detectible using rapid, robust and inexpensive laboratory tests. Therefore, this study evaluated a panel of markers that can be detected using point-of care format ROTEM® thromboelastometry and Platelet Function Analyser (PFA)-100® devices or by using automated coagulation and haematology analyser tests that are in widespread use in other clinical settings.

3.3 Methods and Materials

The ATHENA study (ISRCTN81226121) was an observational cohort study of 50 consecutive adults with haematological malignancies admitted for intensive chemotherapy or haematopoietic stem cell transplant at the Oxford or Bristol haematology centres between September 2010 and September 2012.

3.3.1 Participants

3.3.1.1 *Inclusion criteria*

Patients were eligible if they were:

- Adults (≥ 16 years)
- With a confirmed diagnosis of a haematological malignancy

- Being treated as an inpatient with intensive chemotherapy or an haematopoietic stem cell transplant
- Thrombocytopenic (total platelet count (TPC) ≤ $50x10^9$/L) at admission, or were expected to become thrombocytopenic for ≥ 5 days during inpatient treatment
- Able to comply with study monitoring

3.3.1.2 *Exclusion criteria*

Patients were excluded from the study if they:

- Had an inherited disorder of haemostasis
- Required anti-thrombotic medication during the period of thrombocytopenia
- Had a prior diagnosis of immune thrombocytopenia.

Potential participants were approached at admission, checked for eligibility and invited to give written informed consent prior to study enrolment in accordance with UK Regional Ethics Committee approval 10/H0505/47 (Appendix 3.1).

3.3.1.3 *Care of study participants*

All study participants were managed using standard institutional protocols for the duration of the study. This included prophylactic transfusion of 1 adult platelet unit on each day that a morning blood sample showed a total platelet count ≤ 10 x 10^9/L. Additional platelet units were administered in response to bleeding of WHO Grade ≥ 2 and to prevent bleeding before invasive procedures.

3.3.1.4 *Duration of study for participants*

For each participant, the study observation period was from the first day of thrombocytopenia, until platelet count recovery (unsupported total platelet count > 50 x 10^9/L for >3 days), hospital discharge, death, or for 30 days if there was prolonged thrombocytopenia.

3.3.2 Study Assessments

During the observation period, participants underwent daily bleeding assessments and measurement of the total platelet count. Further blood samples were collected thrice weekly for additional coagulation tests into Vacutainer® blood collection tubes (Becton Dickinson, Plymouth, UK) containing either 0.109 M buffered sodium citrate (for ROTEM, PFA-100®, prothrombin time (PT), activated partial thromboplastin time (aPTT) and Clauss fibrinogen (CF) tests) or 7.2 mg EDTA (for platelet parameters). The whole blood laboratory tests were performed within 2 hours of specimen collection. For the plasma coagulation tests, blood was centrifuged twice within 2 hours of specimen collection and plasma was stored at -80^{o}C for later batched analysis at the Oxford Haemophilia and Thrombosis Centre.

3.3.2.1 *Measurement of bleeding*

Bleeding symptoms were recorded during a daily interview by a research nurse using a standardised questionnaire identical to that used in a previous randomized controlled trial of platelet transfusion policy in haematological malignancies.[7] A computerised algorithm was used to classify bleeding symptoms according to modified World Health

Organization (WHO) criteria (Appendix 2.1) in which WHO grades 1-4 indicate bleeding with increasing severity.[7,118]

3.3.2.2 ***Analysis of blood samples***

3.3.2.2.1 Platelet parameters

Platelets were tested using a Sysmex XE2100 automated haematology analyser (Sysmex, Kobe, Japan) to determine the following platelet parameters:

- Total platelet count (TPC)
- Mean platelet volume (MPV)[157]
- Immature platelet fraction (IPF- the percentage of circulating platelets with above-threshold RNA)[158-160]
- Absolute immature platelet number (AIPN- the concentration of immature circulating platelets, calculated as IPF x TPC)[159,160]

3.3.2.2.1.1 *Mean platelet volume (MPV)*

This platelet parameter was selected because larger platelets have been shown to be more metabolically and enzymatically active *in vitro*.[161-163] *In vivo*, a higher MPV has been associated with prothrombotic tendencies in cardiovascular disease, and has also been associated with a lower risk of bleeding in thrombocytopenic patients.[161,164]

3.3.2.2.1.2 *Immature platelet fraction and absolute immature platelet number*

These platelet parameters were chosen because younger platelets have been shown to be more functionally active than older platelets, and this factor has been shown to be

independent of the size of the platelets.[163] Using the Sysmex XE2100 automated analyser detection of immature platelets uses an analogous mechanism to the detection of immature red blood cells (reticulocytes).[165] RNA within the platelets is stained with a florescent dye and the proportion of highly fluorescent platelets is then measured.[165]

3.3.2.2.2 Platelet Function

Platelet function was assessed by determining the PFA-100® closure times (Siemens Healthcare Diagnostics GmBH, Marburg, Germany) with the 150µm aperture Collagen/ADP (C-ADP) cartridge, and with the INNOVANCE® PFA P2Y (aperture 100µm, membrane coated with 20 µg Adenosine diphosphate (ADP), 5 ng prostaglandin E1 and 459 µg calcium chloride). The PFA-100® simulates platelet function at high shear stress.[166] Two types of ADP cartridge were used to assess whether the smaller membrane aperture of the INNOVANCE® cartridge made the results less dependent on platelet count in thrombocytopenia. PFA-100® tests using CADP cartridges with standard aperture size (150µm) are markedly affected by thrombocytopenia.[167] However, Caraco, et al had shown that at platelet counts less than 30 x 10^9/l, an experimental 100-µm aperture had given the best assessment of platelet function.[167] The recently available INNOVANCE® PFA P2Y cartridge was therefore potentially more robust to the effects of thrombocytopenia because of its smaller membrane aperture (100µm), but was untested in this patient group.

3.3.2.2.3 Rotational thromboelastometry

Rotational thromboelastometry is a whole blood clotting test that assesses the viscoelastic properties of clot formation under low sheer stress.[168,169]

Thromboelastometry was performed on a ROTEM® delta instrument (TEM International, Munich, Germany). The properties of the clot, such as speed of clot formation (clot time and clot formation time), the strength of the clot (maximum clot firmness), and the stability of the clot (maximal lysis) were recorded graphically. These measurements are dependent upon an interaction between platelets, clotting factors and fibrinogen.[169-171]

The clot time (CT) is the time from the start of the analysis until the start of fibrin formation, and is equivalent to the activated partial thromboplastin time or prothrombin time. It is mainly affected by levels of coagulation factors.

The clot formation time (CFT) is the time from start of fibrin formation until the clot firmness is 20mm, and measures the ability of fibrin to polymerise.[172]

Maximum clot firmness (MCF) is the firmness of the clot at its peak and is mainly affected by platelets and fibrin.

Maximum lysis (ML) represents the maximum reduction in clot firmness within 60 minutes. A small amount can be normal due to clot retraction. Hyperfibrinolysis is present if the ML is greater than 15%.[173]

Three different ROTEM® tests were performed: the EXTEM (thromboplastin initiated coagulation); INTEM (contact factor initiated coagulation); and FIBTEM (thromboplastin initiated coagulation with the platelet inhibitor cytochalasin D) activating reagents in accordance with the manufacturer's instructions.[168]

For EXTEM and INTEM, the clot time (CT), clot formation time (CFT), maximum clot firmness (MCF) and maximal lysis (ML) were derived from the thromboelastometry traces. For FIBTEM only maximum clot firmness (MCF) and maximal lysis (ML) were derived from the thromboelastometry traces.

3.3.2.2.4 Other coagulation tests

The PT, aPTT and Clauss Fibrinogen were determined using a STA-R Evolution® Expert Series Hemostasis System (Diagnostica Stago S.A.S., Asnières sur Seine, France) using reagents and methodology according to the manufacturer's instructions.

3.3.3 **Statistical analysis**

The sample size of 50 patients was pre-specified to enable inclusion of patients with different types of haematological malignancy and treatment. Since this was a pilot study assessing previously unexplored associations between laboratory parameters and bleeding, a power calculation was not performed. Statistical analysis was performed with STATA version 11.2 (StataCorp, Texas, USA). All data were non-parametric and were expressed as medians and interquartile ranges [IQRs]. Laboratory parameters were analysed as continuous variables. Associations between laboratory parameters and bleeding during the following 1 day and during the following 2 days after each blood sample were tested using a logistic regression model clustered on patient identity to account for repeated measures.[174] This type of analysis increases the power of the study to detect a difference because the effect of a change in a laboratory parameter is measured within the same patient.[174] Laboratory parameters other than the total

platelet count were considered potentially predictive of bleeding if there was a statistically significant odds ratio (Wald test $P < 0.05$) for bleeding when adjusted for the total platelet count using a multivariable logistic regression model clustered on patient identity. Receiver operating characteristic (ROC) curves were derived for the total platelet count and other potentially predictive laboratory parameters and were used to calculate, area under the curve (AUC) and 95% confidence intervals (CIs). Sensitivity, specificity, positive predictive values and negative predictive values were also calculated for various pre-specified thresholds of the total platelet count and any potential alternative parameter. No adjustment for confounders such as underlying patient characteristics (e.g. type of treatment or haematological malignancy) was made because in current clinical practice, these variables are not considered when using the total platelet count to guide administration of platelet transfusions.[15]

3.4 Results

The 50 participants enrolled into the study had mean age 51.0 years (range 20 to 70 years) and 33 were male. The underlying diagnosis was leukaemia in 16/50 participants, lymphoma in 14/50, myeloma in 9/50 and other haematological disorders in 11/50. Treatment was with intensive chemotherapy for 4/50 participants, myeloablative allograft for 10/50, reduced intensity conditioning allograft for 23/50 and autograft for 13/50. One participant, who received induction chemotherapy for acute myeloid leukaemia, was reallocated to outpatient management after study enrolment and was

withdrawn from the study. The remaining 49 participants were followed throughout their study observation periods, for a total of 760 days (Figure 3.1).

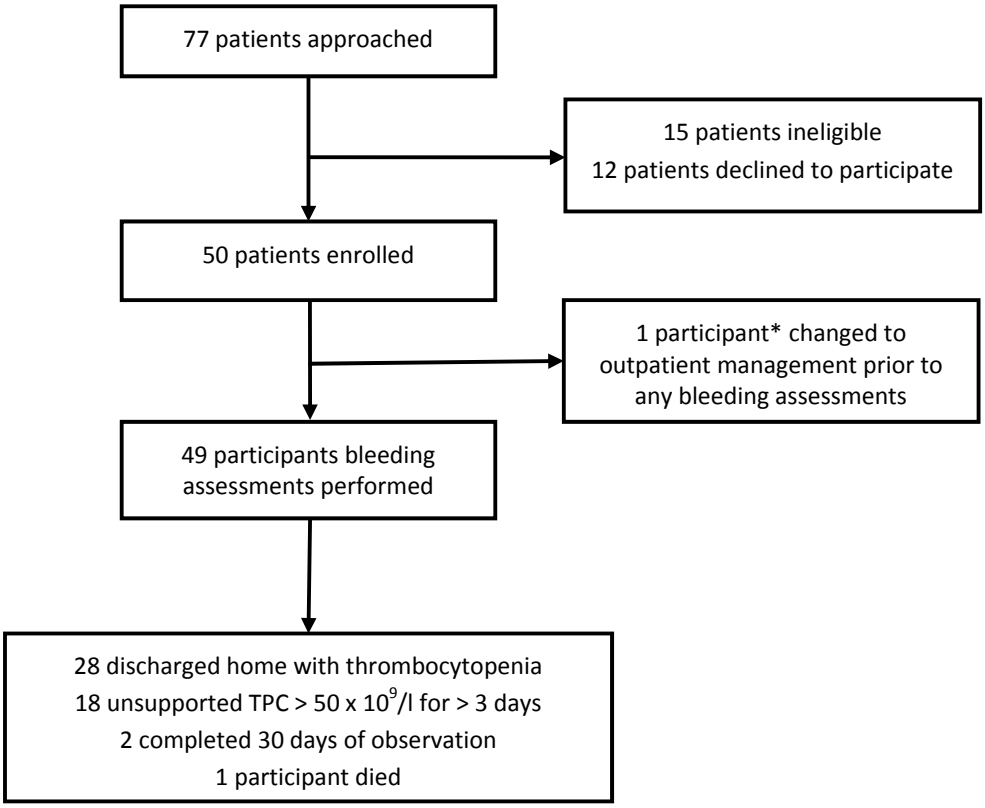


Figure 3.1 ATHENA Study flow diagram

TPC = total platelet count

Bleeding symptom data and the total platelet count on the preceding day were available for a total of 757 (99.6%) and 754 (99.2%) study days respectively. The majority of minor bleeding episodes were associated with the skin (petechiae or bruising) or mucosal surfaces (epistaxis and oropharyngeal bleeding) (Table 3.1). The majority of clinically significant bleeding (WHO Grade 2 or above) was also associated with the skin and

mucosal surfaces. Visible haematuria was another common cause of clinically significant bleeding (Table 3.1). This therefore shows a similar pattern of bleeding to that seen in previous platelet transfusion trials (Chapter 2).

Table 3.1 Types of haemorrhage that patients experienced during the study period.

Type of Haemorrhage	WHO Grade 1	WHO Grade 2 or above
Oropharyngeal	75	5
Epistaxis	57	2
Haemoptysis	-	2
Eye	1	0
Musculoskeletal/deep tissue bleeding	0	0
Abnormal bleeding from invasive or procedure sites	-	5
Visible blood in urine	-	10
Haematemesis	-	2
Visible blood in stool	0	2
Melaena	-	3
Abnormal vaginal bleeding	0	1
Petechiae/purpura	28	6
Bruising	42	2
CNS	-	0
Bleeding in body cavities	0	0
Total	**203**	**40**

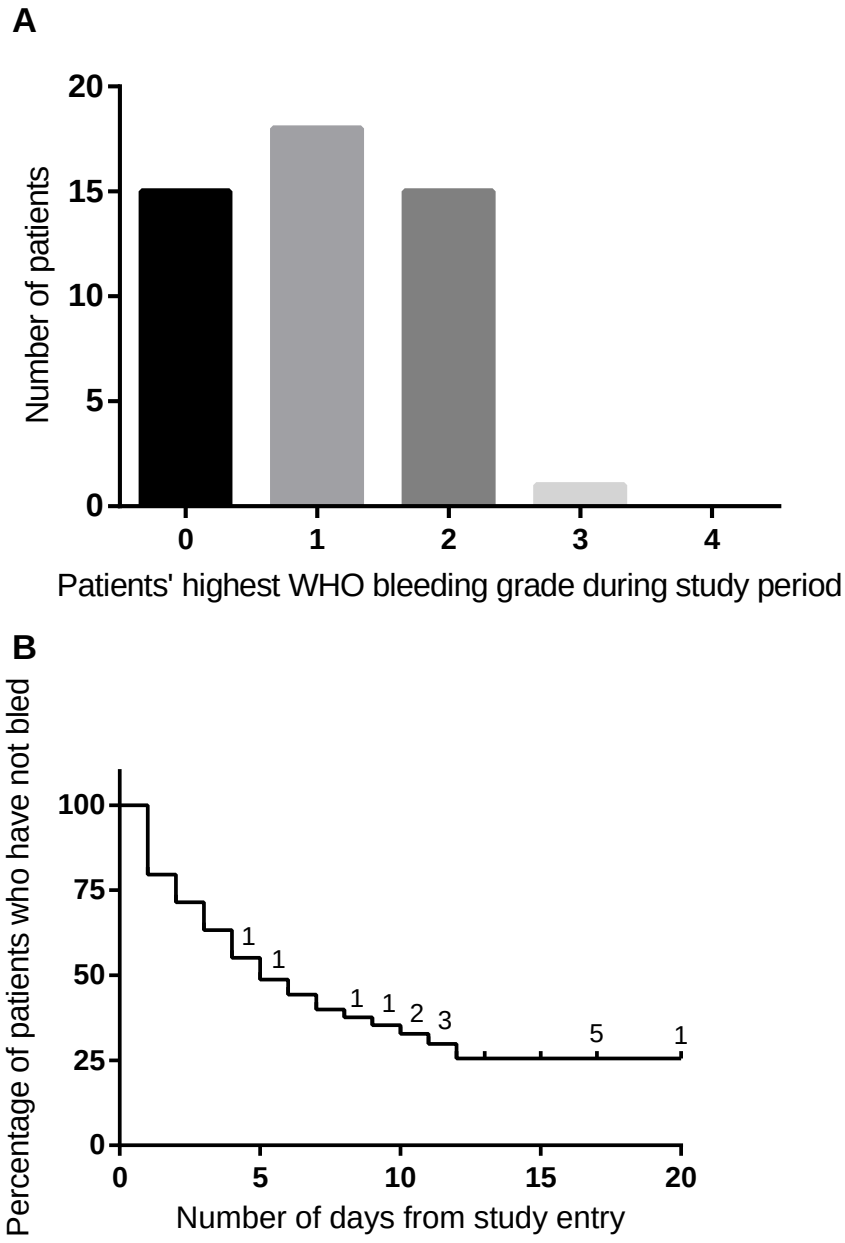


Figure 3.2. The characteristics of bleeding symptoms in the 49 study participants.

A: The highest WHO bleeding grade for each participant during the study observation period.
B: Kaplan-Meier survival curve showing time from the start of thrombocytopenia (TPC ≤ 50 x 10^9/L to first bleeding episode in days. Numbers on graph represent censored patients (number of patients who had completed the study at that time point but had not bled).

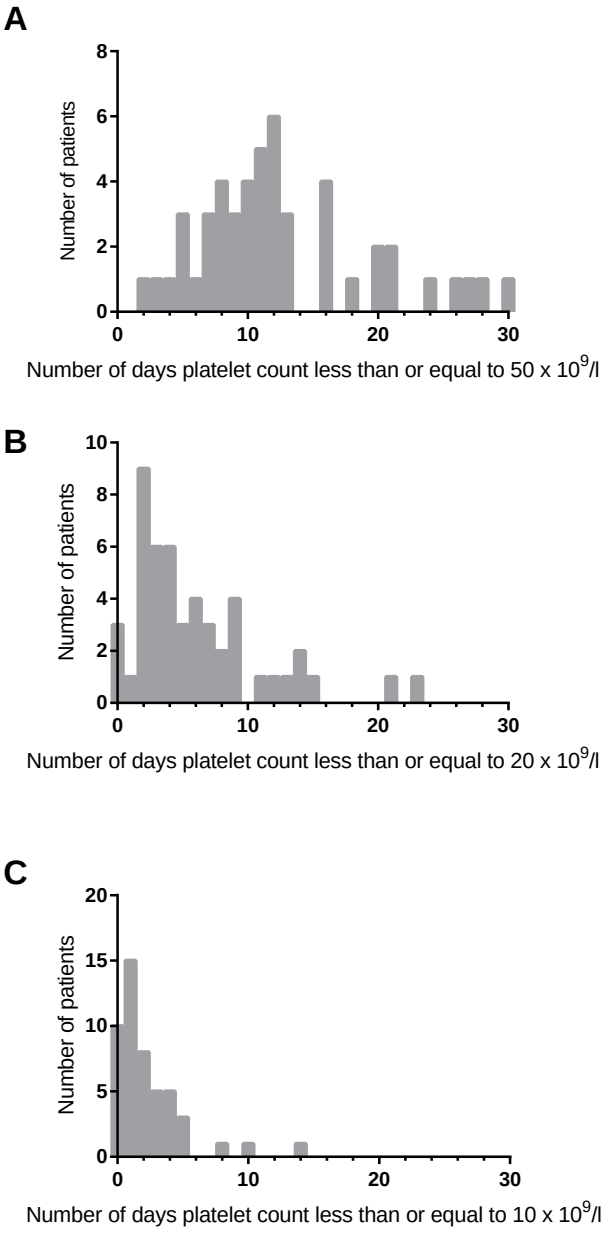


Figure 3.3. The duration of thrombocytopenia in the 49 study participants.

A: Distribution of the number of days the participants had TPC ≤ 50 x 10^9/L during the study period.
B: Distribution of the number of days the participants had TPC ≤ 20 x10^9/L during the study period.
C: Distribution of the number of days the participants had TPC ≤ 10 x10^9/L during the study period.

The participants had a median 3 days of bleeding of any WHO severity (IQR 0 to 6). A total of 65% of participants experienced at least one bleeding episode of WHO Grade I severity but 33% experienced at least one episode of bleeding of WHO Grade ≥2 (Figure 3.2). The median interval between the start of thrombocytopenia and the first bleeding episode was 5 days (IQR 2 to 10 days; Figure 3.2).

The median total duration of thrombocytopenia was 11 days (IQR 8 to 16) for total platelet count ≤ 50 x 10^9/L and 4 days (IQR 2 to 8) for total platelet count ≤ 20x10^9/L (Figure 3.3).

The participants received a total of 175 adult platelet units within 163 separate transfusion episodes during the study observation period, of which 124/175 (71%) were administered for prophylaxis, 34/175 (19%) in response to bleeding, and 17/175 (10%) to prevent bleeding during an invasive procedure.

The type of platelet component was not specified. All platelet components were leucocyte-reduced, up to 80% were collected by apheresis, and common hospital practice was to transfuse ABO and RhD identical platelets. Two patients received HLA matched platelets because of a previous history of platelet refractoriness. The 24 hour platelet increments were calculated for 162 of the 163 separate transfusion episodes in which 175 adult platelet units were administered. The median 24 hour platelet count increment was 11x10^9/L**.** In the 1 day interval after prophylactic platelet transfusions, any haemorrhage occurred on a total of 24 days (21% of all evaluable days after prophylactic transfusion) and WHO Grade ≥2 bleeding on 3 days (2.7%) (Table 3.2).

A total of 157 red cell components were given within 100 separate transfusion episodes during the study observation period. Only 1 transfusion was given to treat haemorrhage. Median pre-transfusion haemoglobin was 81g/L (IQR 78 to 88g/L).

Table 3.2: Presence of haemorrhage* after administration of platelet transfusions, categorised in to reasons for administration of platelet transfusion.

Reason for platelet Transfusion	Any haemorrhage present on day following platelet transfusion N (%)	WHO grade 2 bleeding present on day following platelet transfusion N (%)	Any haemorrhage present on either of the two days following platelet transfusion N (%)	WHO grade 2 bleeding present on either of the two days following platelet transfusion N (%)
Prophylaxis	24/112 (21)	3/112 (3)	33/110 (30)	4/110 (4)
Pre-procedure	4/12 (33)	1/12 (8)	5/12 (42)	1/12 (8)
Therapeutic	22/33 (67)	9/33 (27)	25/33 (76)	11/33 (33)

* Haemorrhage data were available for 158 of 163 separate transfusion episodes given during the study period. Participants were discharged home on the same day as the platelet transfusion for 5 transfusion episodes. Haemorrhage data were available for both of the following two days for 156 separate transfusion episodes.

None of the participants received plasma transfusions during the study period.

Only 4 of the 49 participants did not receive antibiotics during the study period. Median number of days on antibiotics was 10 (IQR 6 to 13). Participants received antibiotics on 478/760 (63%) of study days (Figure 3.4). None of the participants were on anticoagulants or anti-platelet agents during the study period, as per the study protocol, and none of the participants received asparginase as part of their treatment.

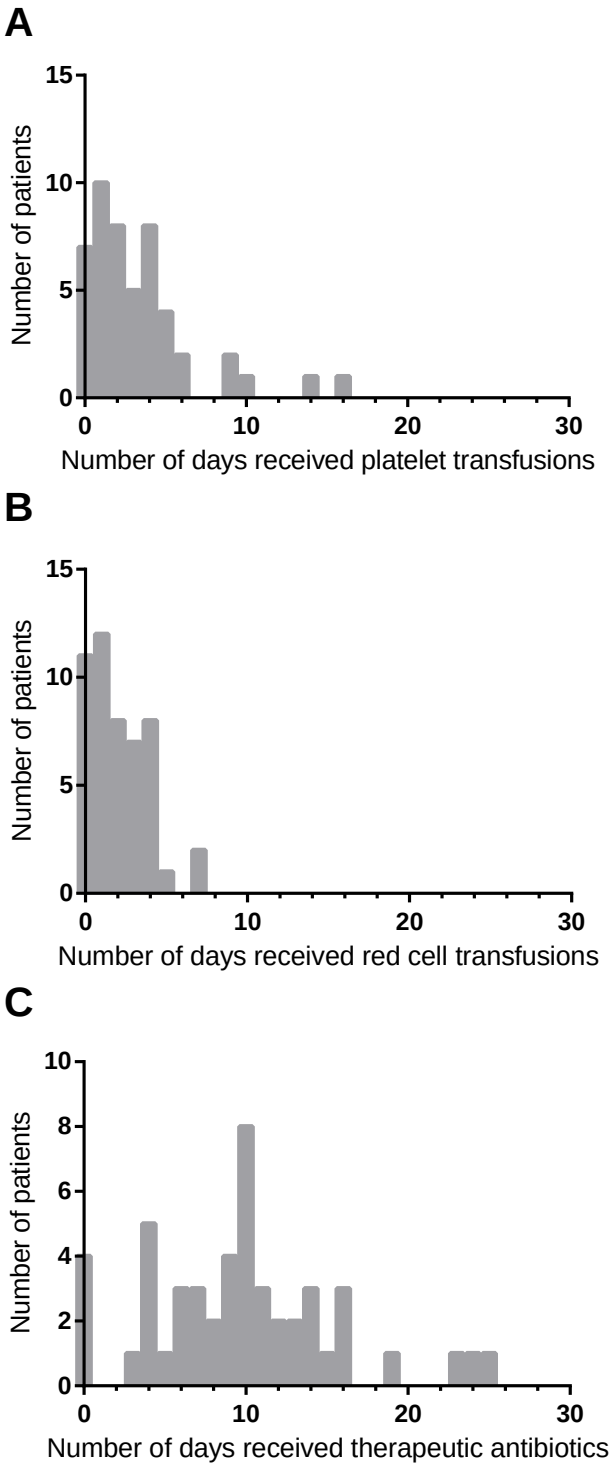


Figure 3.4. Distributions of the number of days the 49 study participants received A) platelet transfusions, B) red cell transfusions and C) antibiotics during the study period.

3.4.1 Completion of laboratory tests

A total of 278 additional blood samples were obtained from the participants during the study. A summary of the laboratory results from the study cohort is shown in Table 3.3.

Table 3.3 Summary of laboratory parameters from 49 study participants from a total of 276 blood samples obtained during the study observation period.

Rotational Thromboelastography					
	Median	**95% CI of median**	**IQR**	**Range**	**Reference interval**
EXTEM CT (s)	59	57 to 61	51 to 70	14 to 329	42 to 74
EXTEM CFT (s)	166	144 to 187	86 to 283	27 to 1474	46 to 148
EXTEM MCF (mm)	45	43 to 46	37 to 52	19 to 73	49 to 71
EXTEM ML (%)	3	2 to 3	1 to 6	0 to 15	0 to 18
INTEM CT (s)	156	154 to 159	144 to 170	19 to 289	137 to 246
INTEM CFT (s)	162	148 to 191	87 to 295	31 to 2513	40 to 100
INTEM MCF (mm)	43	41 to 45	35 to 51	20 to 71	52 to 72
INTEM ML (%)	2	1 to 2	0 to 4	0 to 14	0 to 12
FIBTEM MCF (mm)	23	22 to 24	18 to 29	4 to 51	9 to 25
FIBTEM ML (%)	0	0 to 0	0 to 1	0 to 22	-
Platelet Parameters					
	Median	**95% CI of median**	**IQR**	**Range**	**Reference interval**
TPC (x10^9/l)	26	25 to 28	15 to 48	2 to 380	140 to 400
IPF (%)	4.9	4.4 to 5.2	2.8 to 7.3	0 to 32.7	0.9 to 5.2
AIPN (x10^9/l)	1.12	0.96 to 1.3	0.65 to 2.49	0 to 27.0	2.7 to 12.5
Plasma coagulation tests					
	Median	**95% CI of median**	**IQR**	**Range**	**Reference interval**
PT (s)	13.7	13.2 to 14.1	11.3 to 15.3	10.1 to 67.1	13 to 16
aPTT (s)	31.4	30.6 to 32.4	27.4 to 34.5	19.5 to 67	26 to 36
CF (g/l)	4.3	4.0 to 4.5	3.3 to 5.5	1.29 to >15.0	1.4 to 4.0

CT = clot time; CFT = clot formation time; MCF = maximum clot firmness; ML = % maximal lysis; TPC = total platelet count; IPF = immature platelet fraction; AIPN = absolute immature platelet number; PT- prothrombin time; aPTT- activated partial thromboplastin time; CF = Clauss fibrinogen.

The total platelet count (TPC), immature platelet fraction (IPF) and absolute immature platelet number (AIPN) platelet parameters; the prothrombin time (PT), activated partial thromboplastin time (aPTT), Clauss fibrinogen (CF) and the rotational thromboelastometry (ROTEM)® EXTEM, INTEM and FIBTEM test results were determined for more than 98% of these blood samples. This included the ROTEM® maximum clot firmness (MCF) which may be markedly reduced in samples with a low total platelet count. This indicates that ROTEM® is an appropriate test for evaluating haemostasis in this patient group.

The platelet function analyser (PFA)-100® test reached a closure time endpoint in only 9.3% of samples using the CADP cartridge and in 22.4% of samples using the INNOVANCE® PFA P2Y cartridge when the platelet count was ≤ 50 x 10^9/L. The mean platelet volume (MPV) was not reported by the Sysmex XE2100 analyser in 63.8% of samples with platelet count ≤ 50 x 10^9/L. No further analyses of the PFA-100® closure times or MPV were performed because the poor completion rates of these tests prevented robust statistical analysis.

3.4.2 **Platelet parameters**

There was a significant association between bleeding (any WHO grade) within 1 day of blood sampling and the total platelet count (TPC). For every rise in the TPC of 10 x 10^9/L the odds of bleeding decreased by 24% (OR 0.76, 95% CI 0.62 to 0.93; P=0.008). For every rise in the absolute immature platelet number (AIPN) of 0.5 x 10^9/L the odds of bleeding decreased by 26% (OR 0.74 95% CI 0.61 to 0.90; P=0.002), but this effect was not seen

with the immature platelet fraction (IPF) (OR 0.99, 95% CI 0.93 to 1.06; P = 0.824). There was also a significant association between bleeding (WHO grade ≥2) within 1 day of blood sampling and the AIPN and the TPC but not the IPF (Table 3.4).

Table 3.4 Associations between platelet parameters and WHO grade 2 or above bleeding within 1 day and 2 days of blood sampling.

	Unadjusted Odds Ratio			Odds Ratio adjusted for TPC			Odds Ratio adjusted for AIPN		
	Odds ratio†	95% CI	P value	Odds ratio†	95% CI	P value	Odds ratio†	95% CI	P value
WHO Grade ≥2 bleeding within 1 day of blood sampling									
TPC	0.69	0.52 to 0.92	0.012*	-	-	-	0.83	0.57 to 1.22	0.340
IPF	0.95	0.82 to 1.10	0.458	0.92	0.79 to 1.08	0.318	1.10	0.99 to 1.22	0.073
AIPN	0.48	0.28 to 0.81	0.007**	0.52	0.28 to 0.97	0.038*	-	-	-
WHO Grade ≥2 bleeding within 2 days of blood sampling									
TPC	0.69	0.51 to 0.93	0.014*	-	-	-	0.66	0.41 to 1.05	0.079
IPF	0.99	0.89 to 1.09	0.808	0.97	0.87 to 1.08	0.557	1.09	0.97 to 1.22	0.133
AIPN	0.67	0.49 to 0.93	0.016*	0.77	0.56 to 1.06	0.106	-	-	-

Unadjusted odds ratios (OR) and 95% confidence intervals (CI) for bleeding of WHO grade ≥2 in the day following blood sampling and total platelet count (TPC)(x10^9/l), immature platelet fraction (IPF)(%) and absolute immature platelet number (AIPN) (x10^9/l), as well as OR adjusted for TPC and AIPN. All odds ratios account for repeated measures.

* = P < 0.05; ** = P < 0.01

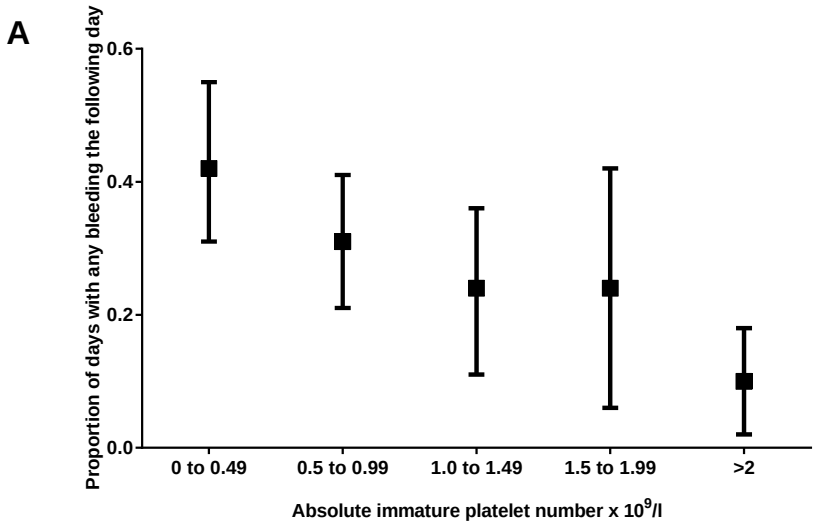
† The odds ratio for TPC is for every 10 x 10^9/L rise in platelet count. The odds ratio for IPF is for every percent rise in IPF. The odds ratio for AIPN is for every 0.5 x 10^9/L rise in AIPN.

Data for the unadjusted TPC was calculated using all 754 study days that reported both the TPC and bleeding assessment data, whereas data for AIPN was only available for 277 days.

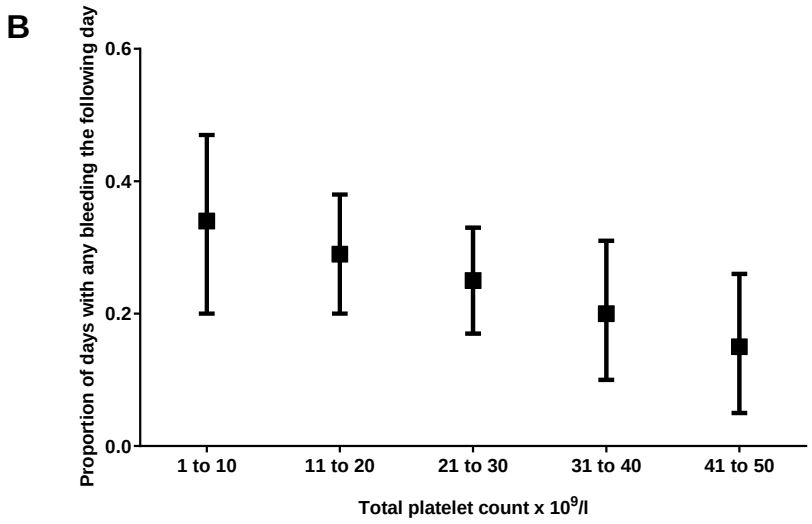
The association between bleeding (WHO grade ≥2) within 1 day of blood sampling and AIPN remained statistically significant after adjustment of AIPN for TPC using logistic

regression analysis clustered on patient identity (OR 0.23 95% CI 0.08 to 0.93; P=0.038; Table 3.4). However, the association between bleeding (WHO grade ≥2) within 1 day of blood sampling and TPC was no longer significant after adjustment for AIPN (Table 3.4). Therefore the best 'model' in this analysis for WHO grade 2 or above bleeding is AIPN alone. The AIPN was not significantly associated with bleeding (WHO grade ≥2) within 2 days of blood sampling after adjustment for TPC (Table 3.4).

The presence of bleeding within 1 day of blood sampling within pre-specified TPC and AIPN categories were estimated with 95% CI (Figure 3.5).



Proportion	0.42	0.31	0.24	0.24	0.1
95% CI	0.31 to 0.55	0.21 to 0.41	0.11 to 0.36	0.06 to 0.42	0.02 to 0.18



Proportion	0.34	0.29	0.25	0.2	0.15
95% CI	0.20 to 0.47	0.20 to 0.38	0.17 to 0.33	0.10 to 0.31	0.05 to 0.26

Figure 3.5: Proportions of the study days after blood sampling in which there was bleeding* of any WHO grade grouped by platelet parameter result.

A: Absolute immature platelet number (AIPN) showing a statistically significant trend between increasing proportions of days with bleeding and lower AIPN (P<0.001). **B:** Total platelet count (TPC) showing a statistically significant trend between increasing proportions of days with bleeding and lower TPC (P=0.015) (Created using data from all 754 study days when both TPC and bleeding assessment data known). Data were adjusted for repeated measures and are expressed as proportions with 95% confidence intervals. *Any bleeding that occurred during the 24 hours from time of blood sampling was recorded.

The presence of bleeding within 1 day of blood sampling within pre-specified TPC and AIPN categories were estimated with 95% CI (Figure 3.5). For AIPN in the range 0 to 0.49 $x10^9$/L, the proportion of study days within 1 day of blood sampling in which there was bleeding of any WHO grade was 42% (95% CI 31 to 55%). The AIPN showed a statistically significant relationship to the proportion of days with bleeding, with lower AIPNs leading to more frequent bleeding (P<0.001; Figure 3.5). For total platelet count in the range 1 to 10 $x10^9$/L the proportion of study days within 1 day of blood sampling in which there was bleeding of any WHO grade was 34% (95% CI 20 to 47%). There was a statistically significant trend between the proportion of days with bleeding and total platelet count (P=0.015; Figure 3.5). A similar trend was seen for WHO grade 2 or above bleeding (AIPN OR 2.29, 95% CI 1.29 to 4.07; p = 0.005: TPC OR 1.35, 95% CI 1.01 to 1.79; p = 0.040).

3.4.3 Thromboelastometry and plasma coagulation test results

There were significant associations between bleeding (any and ≥2 WHO grade) within 1 day of blood sampling and the EXTEM MCF (WHO grade 2 OR 0.93 95% CI 0.88 to 0.97; P = 0.003) and INTEM MCF (WHO grade 2 OR 0.92 95% CI 0.87 to 0.98; P=0.007), but not with the other ROTEM test results (Table 3.5**).** The EXTEM MCF and INTEM MCF were also significantly associated with bleeding (WHO grade ≥2) within 2 days of blood sampling (Table 3.5).

Table 3.5: Association between ROTEM parameters and plasma coagulation test results and WHO grade 2 or above bleeding with 1 day and 2 days of blood sampling

	Unadjusted Odds Ratio			Odds Ratio adjusted for TPC		
	Odds ratio†	**95% CI**	**P value**	**Odds ratio†**	**95% CI**	**P value**
WHO Grade ≥2 bleeding within 1 day of blood sampling						
EXTEM MCF	0.93	0.88 to 0.97	0.003**	0.95	0.89 to 1.00	0.066
EXTEM ML	0.79	0.62 to 1.02	0.069	0.85	0.65 to 1.11	0.234
INTEM MCF	0.93	0.87 to 0.98	0.007**	0.94	0.87 to 1.01	0.090
INTEM ML	0.69	0.47 to 1.01	0.058	0.75	0.51 to 1.10	0.143
FIBTEM MCF	0.98	0.91 to 1.07	0.705	0.99	0.91 to 1.08	0.861
FIBTEM ML	0.87	0.71 to 1.07	0.187	0.87	0.69 to 1.08	0.209
PT	0.96	0.77 to 1.20	0.706			
aPTT	1.00	0.91 to 1.10	0.995			
Fibrinogen	1.05	0.82 to 1.36	0.689			
WHO Grade ≥2 bleeding within 2 days of blood sampling						
EXTEM MCF	0.93	0.89 to 0.97	0.001**	0.95	0.90 to 1.00	0.041*
EXTEM ML	0.85	0.72 to 1.00	0.060	0.91	0.77 to 1.08	0.286
INTEM MCF	0.93	0.88 to 0.97	0.001**	0.94	0.88 to 1.00	0.057
INTEM ML	0.83	0.65 to 1.06	0.134	0.89	0.70 to 1.14	0.365
FIBTEM MCF	0.99	0.93 to 1.06	0.743	1.00	0.92 to 1.07	0.913
FIBTEM ML	1.03	0.89 to 1.19	0.709	1.03	0.87 to 1.22	0.722
PT	0.94	0.77 to 1.14	0.500			
aPTT	1.05	0.99 to 1.10	0.104			
Fibrinogen	1.05	0.82 to 1.34	0.726			

The associations between ROTEM parameters and bleeding are presented as unadjusted odds ratios and as odds ratios adjusted for TPC. The plasma coagulation test (PT, aPTT and Clauss fibrinogen (g/l)) results are presented as odds ratios unadjusted for TPC. All odds ratios account for repeated measures.
CT = clot time (s); CFT = clot formation time (s); MCF = maximum clot firmness (mm); ML = maximum lysis (%); PT = prothrombin time (s); aPTT = activated partial thromboplastin time (s); s= seconds.
* = P < 0.05; ** = P < 0.01.
† All ROTEM, PT, aPTT and fibrinogen results are presented as the odds ratio for each integer rise in the specific parameter.

Since the ROTEM thromboelastometry MCF is partly dependent on TPC, odds ratios were also calculated for the association between bleeding and MCF after adjustment for TPC. The adjusted odds ratios indicated that there was no longer a statistically significant association between either the EXTEM MCF or the INTEM MCF and bleeding (any and ≥2 WHO grade) within 1 day or within 2 days of blood sampling (Table 3.5**).** There were also no significant associations between PT, aPTT, Clauss fibrinogen and bleeding (any and ≥2 WHO grade) within 1 day or within 2 days of blood sampling (Table 3.5).

3.4.4 Prediction of bleeding using total platelet count (TPC) and absolute immature platelet number (AIPN)

Since AIPN was the only platelet parameter or test result that was associated with bleeding in the study cohort independently of TPC, ROC curves were plotted to compare the sensitivity and specificity of TPC and AIPN as continuous variables for the prediction of bleeding within 1 day of blood sampling. The AIPN had higher values for AUC for bleeding of WHO grade ≥2 (AIPN AUC 0.75; 95% CI 0.66 to 0.84 versus TPC AUC 0.70; 95% CI 0.59 to 0.82, Figure 3.6) but the AUC were very similar for bleeding of any WHO grade (AIPN AUC 0.66; 95% CI 0.59 to 0.73 versus TPC AUC 0.66; 95% CI 0.59 to 0.74).

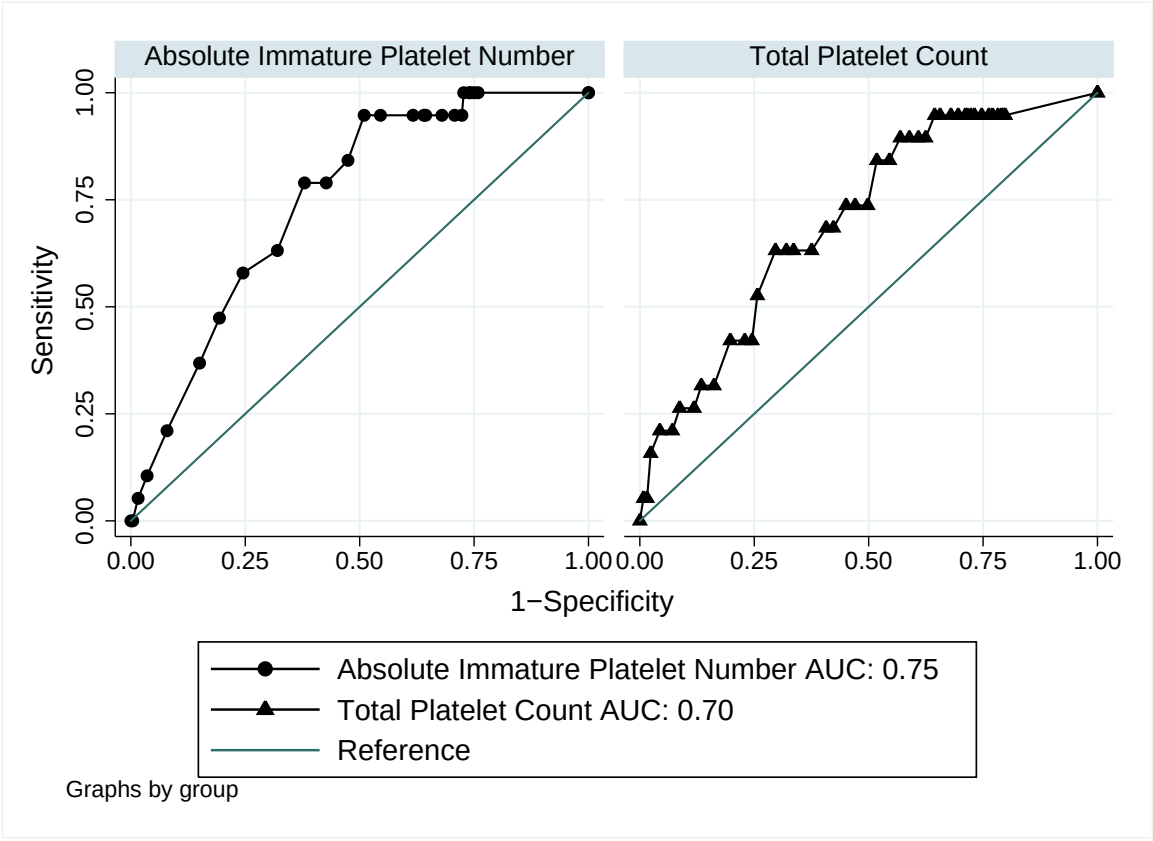


Figure 3.6 Receiver operator characteristic curves for thresholds of absolute immature platelet number and total platelet count (TPC) for prediction of bleeding of WHO grade 2 or above in the day following blood sampling.
ROC curve for total TPC created using data from all 754 study days when both TPC and bleeding assessment data are known. AUC = area under curve

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of different TPC and AIPN thresholds for predicting bleeding are shown in Table 3.6. The TPC had poor PPV for bleeding (33.7% and 8.7% for bleeding of any and ≥2 WHO grade respectively) with a TPC threshold of $10x10^9$/L, which is the threshold usually adopted to direct prophylactic platelet transfusion. The PPV of AIPN at a threshold of $0.5x10^9$/L was higher (42.3% and 17.3% for any and ≥2 WHO grade respectively) than the

Table 3.6 Predictive value of total platelet count (TPC) (x10^9/l) and absolute immature platelet number (AIPN) (x10^9/l) for any bleeding within 1 day of blood sampling

Any Bleeding				
	Sensitivity	**Specificity**	**PPV**	**NPV**
TPC ≤ 10	20.3%	88.1%	33.7%	78.9%
TPC ≤ 20	50.6%	66.5%	30.9%	82.0%
TPC ≤ 30	75.6%	44.2%	28.6%	86.0%
TPC ≤ 40	84.3%	34.0%	27.4%	88.0%
TPC ≤ 50	90.1%	24.6%	26.1%	89.4%
AIPN <0.5	31.0%	85.1%	42.3%	77.7%
AIPN <1	66.2%	57.7%	35.6%	82.9%
AIPN <1.5	83.1%	38.3%	32.2%	86.5%
AIPN <2	90.1%	30.3%	31.4%	89.7%
AIPN <2.5	90.1%	25.9%	30.0%	88.1%
WHO Grade 2 or above Bleeding				
TPC ≤ 10	23.1%	86.7%	8.7%	95.4%
TPC ≤ 20	53.8%	63.5%	7.4%	96.2%
TPC ≤ 30	76.9%	40.6%	6.6%	97.0%
TPC ≤ 40	87.2%	30.8%	6.4%	97.8%
TPC ≤ 50	89.7%	21.8%	5.9%	97.5%
AIPN <0.5	47.4%	83.0%	17.3%	95.5%
AIPN <1	84.2%	54.2%	12.1%	97.9%
AIPN <1.5	94.7%	34.8%	9.8%	98.9%
AIPN <2	100%	26.9%	9.3%	100%
AIPN <2.5	100%	23.3%	8.9%	100%
Data are presented for sensitivity, specificity, positive predictive Value (PPV) and negative predictive value (NPV) of different threshold values for TPC and AIPN.				

TPC threshold of 10x10^9/L. The NPV of the TPC threshold of 10x10^9/L (78.9% and 95.4%

for bleeding of any and ≥2 WHO grade respectively) was similar to that of the AIPN

threshold of $0.5x10^9$/L (77.7% and 95.5% for bleeding of any and ≥2 WHO grade respectively). AIPN thresholds of $≥2x10^9$/L had 100% NPV for bleeding of WHO grade ≥2.

3.5 Discussion

To my knowledge this is the first study that has prospectively evaluated the associations between platelet parameters, other than the total platelet count, and other laboratory markers of haemostasis and bleeding the following day in thrombocytopenic patients undergoing treatment for haematological malignancy.

This study focussed on platelet parameters and other markers of haemostasis that can be detected using rapid, robust and inexpensive laboratory tests. These included the platelet parameters MPV, IPF and AIPN which are determined by automated haematology analysers at the same time as the TPC in the full blood count test. Platelet function was determined using the point-of-care format PFA-100® device[175] and coagulation factor and fibrinogen activity using the widely available PT, aPTT and Clauss fibrinogen tests. Finally, the point-of-care format ROTEM® thromboelastometry device was used to evaluate coagulation factor, fibrinogen and platelet function as well as severe fibrinolysis.[171] ROTEM and similar technologies are used successfully in clinical settings such as cardiac surgery and liver surgery to detect coagulopathy, to direct appropriate treatments to prevent bleeding and to improve clinical outcomes.[176,177]

This study has several important findings that are relevant to the prediction of bleeding in patients with thrombocytopenia during treatment for haematological malignancies. Firstly, measurement of the platelet parameter MPV and platelet function by PFA-100®

closure time is not feasible in this setting because these tests were not completed in a high proportion of study blood samples. PFA-100® closure times are highly dependent on TPC and may not reach endpoint in severely thrombocytopenic samples.[167,175] This study also showed that this effect was not substantially improved by the use of the small aperture INNOVANCE cartridge. Similarly, MPV determined by automated haematology analysers with impedance endpoints requires analysis of large numbers of platelets in each blood sample, which may not be achieved in thrombocytopenic samples using standard instrument protocols.[178] Although this study's data do not exclude a role for the MPV and PFA-100 closure times in predicting bleeding, the inability to reliably detect these markers in this study precludes their use in this clinical setting.

Secondly, for the remaining laboratory tests, in which a very high level of completion was achieved, this study confirmed a significant association between low TPC and increased bleeding within 1 day, and within 2 days of blood sampling. The study's power to detect a difference for the unadjusted TPC was strengthened by the use of data from all 754 study days, compared to only 277 days when adjusted for AIPN. One potential explanation for the relatively poor association between bleeding and TPC in our study is that current clinical practice requires patients with TPC ≤ 10 $x10^9$/L to receive a platelet transfusion on the day of the blood sample. Therefore, the risks of bleeding because of thrombocytopenia after a blood sample with TPC ≤ 10 $x10^9$/L is likely to be lower than expected just by virtue of this intervention. Two recent randomised controlled trials have compared a therapeutic-only versus prophylactic platelet transfusion policy.[2,7] Both

studies showed that prophylactic platelet transfusions reduced bleeding rates overall, but the size of the effect differed between the two studies.

Thirdly, this study also demonstrated a significant association between low ROTEM® MCF and clinically significant bleeding of WHO grade ≥2. The ROTEM® MCF is a composite marker of the haemostatic function of both platelets and fibrinogen,[179] and low ROTEM MCF values caused by hypofibrinogenaemia are associated with increased bleeding in settings such as cardiac surgery.[180] This is unlikely to hold true for low ROTEM MCF in haematological malignancy because there was no significant association between bleeding and the Clauss fibrinogen test result or the ROTEM FIBTEM MCF which also reflects functional fibrinogen.[180] The significant association between ROTEM MCF and bleeding was also no longer observed after adjustment for TPC. This suggests that low MCF in this setting is a direct effect of thrombocytopenia and that this marker has no additional value when compared to the TPC.

Fourthly, this study's finding of no association between bleeding and the ROTEM CT, ROTEM CFT, PT and aPTT test results suggests that coagulation factor defects do not contribute significantly to bleeding in haematological malignancy.

Fifthly, this study's finding of no association between the ROTEM ML test results and bleeding also suggests that severe defects in fibrinolysis are unlikely to contribute to bleeding. However, the ROTEM ML is insensitive to less severe fibrinolysis defects[173] so this coagulopathy cannot be ruled out from our data.

Finally, in contrast to the other laboratory markers of haemostasis evaluated in this study, low AIPN was associated strongly with increased bleeding both within 1 day and 2 days of blood sampling. There was also an approximately linear relationship between AIPN and the proportion of study days on which bleeding occurred in the study participants (Figure 3.5). The association between AIPN and bleeding persisted after adjustment for TPC within 1 day of blood sampling. However, the association between TPC and bleeding lost statistical significance when adjusted for variation in AIPN, suggesting that the AIPN has a more direct association with bleeding.

Both the AIPN and IPF platelet parameters are determined by the Sysmex XE-2100 haematology analyser as part of the full blood count by detecting reticulated platelets which show increased staining with a fluorescent RNA dye.[181] Reticulated platelets are an immature platelet population that are newly synthesised in the bone marrow. Increasing circulating reticulated platelets is an early marker of thrombopoietic regeneration after chemotherapy or haemopoietic stem cell transplantation that precedes increases in the TPC.[158,182] The strong association between low AIPN and increased bleeding in this study suggests that endogenous immature platelets are an important determinant of haemostasis during treatment for haematological malignancies. This is consistent with previous reports that immature platelets have greater haemostatic function than mature or transfused platelets.[183,184] The IPF also provides a measure of circulating immature platelets, although expressed as a proportion of the total circulating platelet count rather than an absolute concentration. Consistent with this, transfusion of donor platelets significantly reduced the IPF in patients with haematological malignancy, but not the

AIPN, at least in part because of a dilution effect from mature platelets in the donor units.[159] Since platelet transfusion was common in this thrombocytopenic study population, it is unsurprising that the circulating immature platelet concentration expressed at the IPF failed to show a consistent association with bleeding.

3.5.1 Strengths of this study

There were two main strengths of this pilot study. Firstly, participants with a wide range of underlying haematological diagnoses and treatments were enrolled and data were collected irrespective of whether patients experienced complications of therapy such as sepsis, mucositis or Graft versus host disease which may potentially alter bleeding risk. Thus, these preliminary findings are likely to be applicable to patients with a broad range of haematological malignancies.

Secondly, assessment of the bleeding outcome measure was performed by trained research nurses and grading was performed by a previously validated computerized algorithm.[7] This ensured a more consistent assessment of the presence and severity of bleeding.[185]

3.5.2 Limitations of this study

This was a small pilot study designed to identify multiple possible laboratory parameters that may be useful in predicting bleeding, and therefore there is a risk that this is a false positive result. This is mitigated by the fact that it is a biologically plausible result and

shows a dose-response relationship (Figure 3.5). Findings from this study do need to be replicated in larger definitive studies to confirm this study's initial results.

3.5.1 Implications of this study

The potential clinical utility of measuring the AIPN in thrombocytopenic patients with haematological malignancy was highlighted by the predictive model. This showed that for prediction of bleeding, the area under the ROC curve for AIPN was greater than for TPC. Moreover, the PPV of an AIPN threshold of 0.5 $x10^9$/L for bleeding was substantially higher than that of the TPC threshold of 10 $x10^9$/L which is currently used to direct prophylactic platelet transfusion, with similar NPV. If confirmed in larger scale studies, these findings suggest that decisions to administer prophylactic platelets based on AIPN and not TPC may improve the appropriateness of platelet transfusion in this group. This has the potential to reduce adverse bleeding events and to avoid unnecessary exposure to platelet components in patients with haematological malignancy.

3.6 Summary

In this study the AIPN was a better predictor of bleeding than the total platelet count. The AIPN should be considered as a potential candidate for guiding the administration of prophylactic platelet transfusions if this study's findings are confirmed in future studies. This study was not sufficiently powered to enable a secondary analysis of whether a patient's baseline characteristics increased their risk of bleeding. This was part of the initial analysis of the next study discussed in Chapter 4.

4 Does sepsis increase the risk of clinically significant bleeding the following day

4.1 Introduction

As described in Chapter 1, sepsis is one of the commonest reasons to increase the platelet count threshold that triggers administration of a prophylactic platelet transfusion (PPTx). Also, according to the current evidence within the literature, there is no consensus on whether sepsis, fever and/or infection increase the risk of bleeding.

4.1.1 Definition of sepsis

There is no international consensus on the definition of neutropenic sepsis,[186,187] current guidelines from the UK and United States differ in their definitions (Table 4.1).

Table 4.1: Definitions of neutropenic sepsis.

	UK (NICE)	US (NCCN)
Single temperature measurement	> 38^{o}C	≥ 38.3^{o}C
Sustained temperature rise (at least one hour)	Not defined	≥ 38.0^{o}C
Neutrophils (x 10^9/l)	≤ 0.5	< 0.5 OR < 1.0 and expected to fall to < 0.5 within next 48 hr
Other	Other signs or symptoms consistent with clinically significant sepsis.	Not defined

NCCN = National Comprehensive Cancer Network
NICE = National Institute for Health and Care Excellence

The only international consensus on a definition for sepsis or a septic inflammatory response syndrome (SIRS) has been developed after studying infection and sepsis in patients who are critically ill.[188,189] SIRS is considered to be present when patients have more than one of the following clinical findings:

1) body temperature > 38°C or < 36°C
2) heart rate > 90 beats per minute
3) respiratory rate > 20 per minute or a $PaCO_2$ < 32 mm Hg
4) White blood cell count of >12 x 10^9/l or < 4 x 10^9/l

Sepsis has been defined as SIRS plus the presence of infection. Severe sepsis as sepsis associated with organ dysfunction, hypoperfusion, or hypotension, and septic shock as sepsis with arterial hypotension, despite adequate fluid resuscitation.[189] These general definitions are now widely used in practice and serve as the basis for numerous clinical trial inclusion criteria.[189]

Other scoring systems that have been used in critical care patients to assess the probability of sepsis cannot be used routinely in patients with haematological malignancies because they use results from invasive monitoring techniques (e.g. arterial oxygen levels – Acute Physiology and Chronic Health Evaluation (APACHE) II Score),[190] blood results that are not routinely measured (e.g. bicarbonate - Simplified Acute Physiology (SAPS) II Score),[191] or require accurate measurements of inspired oxygen administered (Sequential Organ Failure Assessment (SOFA) Score and Infection Probability Score (IPS)).[192,193]

4.2 Aim of the study

The aim of this study was to assess the effect of markers of sepsis on the risk of bleeding the following day. Due to the lack of consensus on the definition sepsis in this patient group multiple factors were collected that could be included within a multivariable model, as well as the SIRS score.

4.3 Methods

4.3.1 Participants

Additional data were collected on all 222 patients included within the TOPPS study (600 participants) who were treated at either Oxford or Glasgow study centres.[7] This was because this sub-study was added to the TOPPS protocol as a substantial amendment in April 2011 after the main study had been initiated (National Research Ethics Service (NRES) Committee South Central – Oxford C (06/Q1606/8)) (Appendix 4.1). Additional data were therefore sought only for observations or results that would have been routinely recorded during their inpatient stay.

4.3.2 Data collection

Prospective data collection was performed by TOPPS research nurses at the Glasgow and Oxford study centres as part of their study assessments. Retrospective data collection was performed by me for all Oxford patients and Mrs Julie Watson (TOPPS research nurse) for all Glasgow patients.

Additional data were collected on:

- Highest and lowest body temperature, systolic blood pressure, and heart rate recorded in the preceding 24 hours
- Any positive microbiology results (blood cultures, urine cultures, sputum cultures, line tip cultures, etc.)
- Any abnormal radiology results (chest x-ray, ultrasound, computerised tomography or magnetic resonance imaging result)
- Any therapeutic antibiotics, antifungal medications or antiviral medications administered in the preceding 24 hours
- Whether the patient was on the intensive care unit, and whether they required inotropes, assisted ventilation or dialysis
- Whether the patient required oxygen
- SIRS Score
- Full blood count (haemoglobin, haematocrit, white cell count and neutrophil count)
- Serum creatinine
- Serum bilirubin
- Any clotting tests performed (prothrombin time (PT), activated partial thromboplastin time (aPTT))

4.3.3 Statistical Analysis

4.3.3.1 *Cox regression multivariable analysis model of time to first bleeding event*

Data for all TOPPS study patients in which all baseline characteristics were known (596 patients) were used to perform the analysis. This analysis was performed to assess whether any baseline characteristics recorded within the TOPPS trial decreased the time to the first WHO grade 2 or above bleeding event. Any variables identified as risk factors for bleeding may not have been statistically significant in the smaller data set, that included only patients at the Oxford and Glasgow study centres, that will be used for this study's main analysis.

All baseline characteristics were treated as categorical variables within this initial analysis because the initial review of the data showed that the trend between age and risk of bleeding was not linear (data not shown). A Cox regression model was chosen so that data from all 596 patients could be used irrespective of whether they had been followed-up for the entire 30 day study period.[194] Univariable analysis was performed using the Cox regression model, and assessments of proportionality were performed.[195] There was no evidence that the proportional hazards assumption had been contravened in the univariable analyses and a multivariable model was therefore created.[195] The initial model included all the baseline characteristics recorded. Backwards elimination of potential risk factors was then performed. The model with and without the potential risk factor was compared using a Likelihood ratio test (LRT). If there was no evidence that the

model with and without the potential risk factor differed (LRT > 0.1) this factor was then removed from the model.[196] Once all risk factors had been identified within the final model interaction tests were performed to assess whether any interactions needed to be taken in to account.[197] No significant interactions were identified.

4.3.3.2 *Multivariable Logistic regression model of risk of bleeding the following day*

An initial univariable logistic regression analysis clustered on patient identity to account for repeated measures was created that included all 222 patients at Oxford and Glasgow study centres, and included all recorded baseline characteristics and the presence or absence of bleeding the preceding day. A multivariable logistic regression model was then created using this data set, this model was used to adjust for the baseline characteristics and presence of bleeding. This data set excluded day 1 data because the bleeding grade would not be known the preceding day (6204 days of data).

The data set for the sepsis analysis only included participant data when at least the full blood count was known. There were 219 patients and 3095 study days included in the analysis. No additional data were available on 3 patients. Univariable logistic regression analyses clustered on patient identity to account for repeated measures were performed that assessed all the additional sepsis risk factor variables. Data were recorded as missing if not reported for a day. The missing variable was not included in the univariable analyses. Due to the risk of collinearity, four potential risk factors were categorised in to

two pre-specified groups of outcomes so that only one of the potential risk factors from that grouping was used in the model.[194] Two groups were created:

- Haemoglobin and haematocrit (haemoglobin was selected to be included in the multivariable analysis because clinicians in the UK use the haemoglobin rather than the haematocrit to guide their management)[194]
- White cell count and neutrophil count (white cell count was selected to be included in the multivariable analysis because less data were missing for this outcome)[194]

The additional sepsis variables were added to the initial multivariable logistic regression model in a step-wise fashion. Variables that were significantly associated with bleeding in the univariable analysis and had the fewest days of missing data were added to the model first.[198] The model with and without the potential risk factor was compared. If there was evidence that the model with and without the potential risk factor differed this factor was then added to the model.[198]

A comparison was made between a multivariable model that included missing variables and a multivariable model that excluded missing variables as part of the sensitivity analysis to ensure there were no obvious biases within the final model.[198] None were found.

4.4 Results

Additional data were available for 219 of the 222 patients recruited from the Oxford and Glasgow study centres (Table 4.2). Data were available for all 147 patients recruited at the Oxford study centre and 72/75 patients recruited at the Glasgow study centre.

4.4.1 Baseline Characteristics

The baseline characteristics were similar to all patients within the TOPPS study (Table 4.2). The only differences noted between the Oxford and Glasgow groups and the overall study were:

- A higher proportion of patients receiving an allogeneic haematopoietic stem cell transplant (HSCT) (23% vs. 14%) and corresponding lower proportion receiving chemotherapy (6% vs 16%)
- A higher proportion of patients with a co-existing disorder or organ failure (13% vs. 7%)

Table 4.2 Baseline Characteristics for Oxford and Glasgow (O&G) study centres and all study data

Characteristic		Data set	No-prophylaxis Median (IQR)	Prophylaxis Median (IQR)	Total Median (IQR)
Age		O&G	55.5 (50.0 to 61.9)	57.0 (IQR 49.3 to 62.2)	55.9 (IQR 49.2 to 62.0)
		All	57.8 (IQR 50.3 to 63.3)	57.8 (IQR 48.8 to 63.5)	57.8 (IQR 49.3 to 63.4)
Platelet count at randomisation (10^9/l)		O&G	42 (IQR 34 to 51)	42 (IQR 31 to 51)	42 (IQR 33 to 51)
		All	41 (IQR 31 to 50)	40 (IQR 28 to 50)	41 (IQR 30 to 50)
Characteristic	**Sub-categories**	**Data set**	**No-prophylaxis N (%)**	**Prophylaxis N (%)**	**Total N (%)**
Female Sex		O&G	42 (37)	42 (39)	84 (38)
		All	103 (34)	108 (36)	211 (35)
Diagnosis	AML	O&G	15 (13)	12 (11)	27 (12)
		All	55 (18)	55 (18)	110 (18)
	Lymphoma	O&G	42 (37)	45 (41)	87 (39)
		All	102 (34)	104 (35)	206 (34)
	Myeloma	O&G	45 (40)	44 (40)	89 (40)
		All	125 (42)	124 (42)	249 (42)
	Other	O&G	11 (10)	8 (7)	19 (9)
		All	19 (6)	16 (5)	35 (6)
Treatment	Chemotherapy	O&G	9 (8)	4 (4)	13 (6)
		All	50 (17)	48 (16)	98 (16)
	Autologous HSCT	O&G	79 (70)	80 (73)	159 (72)
		All	211 (70)	210 (70)	421 (70)
	Allogeneic HSCT	O&G	25 (22)	25 (23)	50 (23)
		All	40 (13)	41 (14)	81 (14)
Relapsed disease		O&G	26 (23)	35 (32)	61 (28)
		All	92 (31)	110/298 (37)	202/599 (34)
Previous HSCT		O&G	7 (6)	9 (8)	16 (7)
		All	20/300 (7)	26/298 (9)	46/598 (8)
Previous fungal infection		O&G	2 (2)	4 (4)	6 (3)
		All	5 (2)	8 (3)	13 (2)
Any co-existing disorder or organ failure		O&G	18 (17)	11 (10)	29 (13)
		All	25 (8)	19/298 (6)	44/599 (7)
Total		O&G	**113 (100)**	**109 (100)**	**222 (100)**
		All	**301 (100)**	**299 (100)**	**600 (100)**

HSCT = haematopoietic stem cell transplant; IQR = interquartile range; N = number; O&G = Oxford and Glasgow

4.4.2 Occurrence of haemorrhage during the study period

The proportion of patients who experienced bleeding was very similar in the Oxford and Glasgow data set and the data set for all patients included in the TOPPS trial (Table 4.3).

Table 4.3 Number of patients who bled during the study period and grade of bleeding for Oxford and Glasgow (O&G) study centres and all study data

Highest bleeding Grade	Data set	No-prophylaxis N (%)	Prophylaxis N (%)	Total N (%)
WHO Grade 0 or 1	**O&G**	56 (50)	63 (58)	119 (54)
	All	149 (50)	170 (57)	319 (53)
WHO Grade 2	**O&G**	55 (49)	45 (41)	100 (45)
	All	145 (48)	127 (43)	272 (46)
WHO Grade 3	**O&G**	2 (2)	1 (1)	3 (1)
	All	4 (1)	1 (<1)	5 (1)
WHO Grade 4	**O&G**	0 (0)	0 (0)	0 (0)
	All	2 (1)	0 (0)	2 (<1)
Total	**O&G**	113 (100)	109 (100)	222 (100)
	All	300 (100)	298 (100)	598 (100)
N = number; O&G = Oxford and Glasgow				

4.4.3 Multivariable Cox regression analysis of baseline characteristics

Within the initial multivariable Cox regression analysis of baseline characteristics for all TOPPS patients that had all baseline characteristics recorded (596 patients), female sex (hazard ratio (HR) 1.45; 95% CI 1.14 to 1.85), the no-prophylaxis treatment arm of the study (HR 1.27; 95% CI 1.00 to 1.62), and a previous history of a fungal infection (HR 2.04; 95% CI 1.03 to 4.05) decreased the time until a first WHO grade 2 or above bleeding event occurred (Table 4.4).

Table 4.4 Multivariable Cox regression analysis for time to first WHO grade 2 or above bleeding episode for all 596 TOPPS patients that had all data on baseline characteristics available.

Risk Factor		Hazard ratio*	95% CI
Treatment arm **No-prophylaxis vs. prophylaxis**		1.27	1.00 to 1.62
Gender **Male vs. Female**		0.69	0.54 to 0.88
Previous HSCT		0.94	0.58 to 1.53
Previous fungal infection		2.04	1.03 to 4.05
Age (categorised) compared to baseline age < 40 years	**<40**	-	-
	40 to < 50	0.63	0.38 to 1.05
	50 to < 60	0.89	0.57 to 1.39
	≥ 60 years	1.07	0.69 to 1.64
Diagnosis Compared to lymphoma	**Lymphoma**	-	-
	Myeloma	0.75	0.56 to 1.01
	Other	1.10	0.69 to 1.76
Type of treatment	**AutoHSCT**	-	-
	Chemo/ AlloHSCT	0.77	0.49 to 1.22

*Adjusted for age, treatment arm, gender, diagnosis, type of treatment, previous fungal infection and previous stem cell transplant
AlloHSCT = allogeneic haematopoietic stem cell transplant; AutoHSCT = autologous HSCT; Chemo = chemotherapy

4.4.4 Multivariable logistic regression analysis of baseline characteristics and presence of WHO grade 2 to 4 bleeding

Within the univariable logistic regression analysis for the presence of bleeding the following day, female sex, previous fungal infection and presence of WHO grade 2 or above bleeding increased the risk of bleeding the following day whereas a diagnosis of myeloma decreased the risk of bleeding (Table 4.5).

Table 4.5 Crude odds ratios (univariable) for WHO grade 2 to 4 bleeding the following day, clustered on patient ID to account for repeated measures.

Risk Factor		Number of patients	Number of days of observation	Odds ratio	95% CI	P-value (Wald test)
Treatment arm **No-prophylaxis vs. prophylaxis**		113/222	3216/6451	1.44	0.90 to 2.32	0.130
Centre **Glasgow vs. Oxford**		75/222	2245/6451	0.58	0.33 to 1.01	0.056
Gender **Male vs. Female**		84/222	4069/6451	0.45	0.28 to 0.71	0.001**
Relapsed disease **Yes vs. No**		61/221	1756/6421	1.09	0.64 to 1.86	0.740
Previous HSCT **Yes vs. No**		16/222	448/6451	2.03	0.82 to 5.06	0.127
Previous fungal infection **Yes vs. No**		6/222	166/6451	3.24	1.16 to 9.01	0.024*
Age (categorised) compared to baseline age < 40	**<40**	23/222	677/6451	-	-	-
	40 to < 50	40/222	1160/6451	0.68	0.28 to 1.63	0.381
	50 to < 60	79/222	2271/6451	0.97	0.50 to 1.89	0.933
	≥ 60 years	80/222	2343/6451	0.76	0.40 to 1.41	0.381
Diagnosis Compared to lymphoma	**Lymphoma**	87/222	2485/6451	-	-	-
	Myeloma	89/222	2628/6451	0.57	0.34 to 0.95	0.033*
	Other	46/222	1338/6451	1.13	0.61 to 2.09	0.695
Type of treatment **Chemo/Allo HSCT vs. AutoHSCT**		159/222	4624/6451	1.45	0.85 to 2.46	0.170
WHO Grade 2 to 4 bleed		222	269/6204	20.5	14.96 to 28.07	<0.001**

* p-value <0.05 **p-value <0.01
AlloHSCT = allogeneic haematopoietic stem cell transplant; AutoHSCT = autologous HSCT;
Chemo = chemotherapy

Treatment arm was not statistically significant but was included in the initial multivariable model because it has been statistically significant in the total TOPPS data set.

All the factors that were identified in the univariable model remained statistically significant in the multivariable model: male gender (odds ratio (OR) 0.48; 95% CI 0.34 to 0.68); previous fungal infection (OR 3.21; 95% CI 1.75 to 5.89); myeloma (OR 0.57; 95% CI 0.38 to 0.86); and grade 2 to 4 bleeding present (OR 16.56; 95% CI 11.86 to 23.13) (Table 4.6). Treatment arm was also statistically significant in the multivariable model (OR 1.43; 95% CI 1.00 to 2.04) (Table 4.6).

Table 4.6 Multivariable logistic regression model for bleeding present the following day clustered on trial number to account for repeated measures for 222 patients within the TOPPS trial treated at either the Oxford or Glasgow centres.

Risk Factor		Odds ratio‡	95% CI	P-value (Wald test)	Odds ratio†	95% CI	P-value (Wald test)
Treatment arm No-prophylaxis vs. prophylaxis		1.64	1.05 to 2.578	0.031*	1.43	1.00 to 2.04	0.046*
Gender Male vs. Female		0.40	0.26 to 0.63	<0.001**	0.48	0.34 to 0.68	<0.001**
Previous fungal infection Yes vs. No		4.78	2.09 to 10.91	<0.001**	3.21	1.75 to 5.89	<0.001**
Diagnosis Compared to lymphoma	**Lymphoma**	-	-	-	-	-	-
	Myeloma	0.49	0.29 to 0.83	0.007*	0.57	0.38 to 0.86	0.007*
	Other	0.81	0.45 to 1.44	0.468	0.82	0.52 to 1.30	0.401
Grade 2 to 4 bleeding present		-	-	-	16.56	11.86 to 23.13	<0.001**

* p-value <0.05 **p-value <0.01
‡Adjusted for treatment arm, gender, previous fungal infection, diagnosis
†Adjusted for treatment arm, gender, previous fungal infection, diagnosis, grade 2 to 4 bleed

4.4.5 Multivariable logistic regression analysis of baseline characteristics, presence of WHO grade 2 to 4 bleeding and additional risk factors

Table 4.7 Crude odds ratios (univariate) for WHO grade 2 to 4 bleeding the following day for additional data collected within this study, clustered on patient ID to account for repeated measures. Data on number of days of missing data also reported

Risk Factor	Categories of risk factor	Number of days of observation N =3095	Odds ratio	95% CI	P-value (Wald test)
C reactive protein (mg/l)	0 to <50	1653	-	-	-
	50 to <100	541	1.76	1.22 to 2.56	0.003**
	100 to <150	275	3.19	2.01 to 5.08	<0.001**
	≥150	376	4.96	3.20 to 7.70	<0.001**
	Missing	250	-	-	-
Haemoglobin (g/l)	<86	269	-	-	-
	86 to 95	805	0.61	0.41 to 0.92	0.019*
	96 to 105	998	0.30	0.19 to 0.47	<0.001**
	≥106	1023	0.20	0.11 to 0.36	<0.001**
Haematocrit (%)	≤23.0	64	-	-	-
	23.1 to 26.0	391	0.46	0.26 to 0.81	0.007**
	26.1 to 30.0	1251	0.30	0.16 to 0.55	<0.001**
	30.1 to 36.9	1299	0.14	0.07 to 0.28	<0.001**
	≥37.0	90	0.06	0.01 to 0.30	0.001**
White cell count (x 10^9/L)	<0.5	1367	-	-	-
	0.5 to 0.9	373	0.67	0.47 to 0.97	0.035*
	≥1.0	1356	0.33	0.21 to 0.50	<0.001**
Neutrophil count (x 10^9/L)	<0.1	1153	-	-	-
	0.1 to 0.4	515	1.03	0.75 to 1.42	0.863
	0.5 to 0.9	360	0.43	0.26 to 0.72	0.001**
	1.0 to 2.4	555	0.34	0.18 to 0.63	0.001**
	≥2.5	453	0.30	0.17 to 0.56	<0.001**
	Missing	59	-	-	-

Risk Factor	Categories of risk factor	Number of days of observation N =3095	Odds ratio	95% CI	P-value (Wald test)
Albumin (g/l)	**<26**	258	-	-	-
	26 to 30	693	0.71	0.34 to 1.47	0.351
	31 to 35	948	0.61	0.29 to 1.28	0.173
	≥36	1071	0.40	0.19 to 0.83	0.017*
	Missing	125	-	-	-
Bilirubin (µmol/l)	**0 to 20**	2407	-	-	-
	21 to 33	253	1.26	0.76 to 2.10	0.375
	34 to 50	115	1.20	0.55 to 2.60	0.643
	≥51	98	4.09	1.60 to 10.46	0.003**
	Missing	222	-	-	-
Creatinine (µmol/l)	**0 to 90**	2310	-	-	-
	91 to 120	459	1.23	0.70 to 2.15	0.475
	121 to 180	238	3.63	2.13 to 6.19	<0.001**
	181 to 240	57	3.73	1.17 to 11.94	0.026*
	>240	15	9.33	4.91 to 17.75	<0.001**
	Missing	17	-	-	-
Plt count (x 10^9/L)	**≤10**	767	-	-	-
	11 to 20	744	0.55	0.40 to 0.75	<0.001**
	21 to 40	797	0.40	0.27 to 0.57	<0.001**
	41 to 50	180	0.12	0.04 to 0.34	<0.001**
	>50	563	0.08	0.02 to 0.20	<0.001**
	Missing	44	-	-	-
Oxygen treatment	**Yes**	293	2.83	1.58 to 5.06	<0.001**
	Missing	151	-	-	-
Antibiotics	**Yes**	1828	2.72	1.76 to 4.21	<0.001**
	Missing	139	-	-	-
Antifungals	**Yes**	453	1.68	1.04 to 2.70	0.033*
	Missing	141	-	-	-

Risk Factor	Categories of risk factor	Number of days of observation N =3095	Odds ratio	95% CI	P-value (Wald test)
ITU/HDU	**Yes**	44	4.73	2.28 to 9.84	<0.001**
	Missing	139	-	-	-
SIRS Score	**0 to 1**	1036	-	-	-
	2	1171	2.05	1.32 to 3.17	0.001**
	3 to 4	710	2.95	1.79 to 4.86	<0.001**
	Missing	178	-	-	-
Temperature (^{o}C)	**≤37.5**	2148	-	-	-
	37.6 to 38.0	449	2.96	1.96 to 4.45	<0.001**
	38.1 to 38.5	186	5.41	3.17 to 9.22	<0.001**
	≥38.6	143	5.38	3.10 to 9.30	<0.001**
	Missing	169	-	-	-

In the univariable analysis of additional risk factors for bleeding C-reactive protein (CRP), haemoglobin, haematocrit, white cell count, neutrophil count, albumin, bilirubin, creatinine, platelet count, oxygen therapy, ITU admission, therapeutic antibiotics, therapeutic antifungal medication, SIRS score and temperature were all associated with bleeding the following day (Table 4.7).

In the multivariable analysis (Table 4.8) anaemia, impaired renal function, deranged liver function (raised bilirubin > 50µmol/L), a low platelet count, female sex, a previous history of fungal infection, a low white count, WHO grade 2 or above bleeding the preceding day, and a raised CRP were all independently associated with an increased risk of bleeding the following day. Within the Oxford and Glasgow data set only one episode of vaginal bleeding occurred, therefore this does not explain the reason that women have a

Table 4.8 Multivariable logistic regression model for bleeding present the following day clustered on trial number to account for repeated measures for 219 patients within the TOPPS trial treated at either the Oxford or Glasgow centres who had additional sepsis data.

Risk Factor	Categories of risk factor	Model 1 includes missing values as a variable N = 3095 days			Model 2 excludes all days when data for any variable is missing N = 2637 days		
		Odds ratio	95% CI	P-value (Wald test)	Odds ratio	95% CI	P-value (Wald test)
Treatment arm No-prophylaxis vs. prophylaxis		1.36	0.93 to 1.99	0.108	1.40	0.93 to 2.13	0.110
Gender Male vs. Female		0.46	0.33 to 0.66	<0.001**	0.47	0.32 to 0.68	<0.001**
Previous fungal infection Yes vs. No		2.26	1.32 to 3.88	0.003**	2.36	1.34 to 3.79	0.002**
Diagnosis	**Lymphoma**	-	-	-	-	-	-
	Myeloma	0.85	0.56 to 1.30	0.460	0.76	0.48 to 1.21	0.253
	Other	0.71	0.45 to 1.11	0.135	0.70	0.43 to 1.15	0.159
Grade 2 to 4 bleeding	**Yes**	5.71	3.98 to 8.18	<0.001**	5.29	3.57 to 7.84	<0.001**
	Missing	4.10	0.93 to 18.06	0.063	-	-	-
Platelet count (x 10^9/L)	**≤10**	-	-	-	-	-	-
	11 to 20	0.69	0.49 to 0.99	0.045*	0.70	0.48 to 1.02	0.068
	21 to 40	0.54	0.38 to 0.78	0.001**	0.61	0.41 to 0.92	0.018*
	41 to 50	0.22	0.08 to 0.62	0.004**	0.28	0.09 to 0.81	0.019*
	>50	0.18	0.07 to 0.44	<0.001**	0.16	0.06 to 0.49	0.001**
	Missing	0.32	0.39 to 2.60	0.286	-	-	-
Haemoglobin (g/l)	**<86**	-	-	-	-	-	-
	86 to 95	0.84	0.55 to 1.27	0.407	0.71	0.46 to 1.10	0.124
	96 to 105	0.50	0.32 to 0.78	0.003**	0.44	0.28 to 0.68	<0.001**
	≥106	0.53	0.31 to 0.89	0.017*	0.48	0.28 to 0.83	0.008**
White cell count (x 10^9/L)	**<0.5**	-	-	-	-	-	-
	0.5 to 0.9	0.68	0.48 to 0.97	0.035*	0.69	0.44 to 1.08	0.101
	≥1.0	0.49	0.34 to 0.72	<0.001**	0.53	0.36 to 0.78	0.001**

Risk Factor	Categories of risk factor	Model 1 includes missing values as a variable N = 3095 days			Model 2 excludes all days when data for any variable was missing N = 2637 days		
		Odds ratio	95% CI	P-value (Wald test)	Odds ratio	95% CI	P-value (Wald test)
Bilirubin (µmol/l)	**0 to 50**	-	-	-	-	-	-
	≥51	2.39	1.04 to 5.52	0.041*	1.94	0.85 to 4.42	0.115
	Missing	1.35	0.86 to 2.13	0.197	-	-	-
C reactive protein (mg/l)	**0 to <50**	-	-	-	-	-	-
	50 to <100	1.20	0.86 to 1.67	0.293	1.09	0.76 to 1.57	0.629
	≥100	1.83	1.30 to 2.57	0.001**	1.59	1.13 to 2.26	0.009**
	Missing	1.50	0.87 to 2.56	0.142	-	-	-
Creatinine (µmol/l)	**0 to 120**	-	-	-	-	-	-
	121 to 240	1.70	1.07 to 2.71	0.024*	1.83	1.14 to 2.93	0.012*
	>240	6.96	3.10 to 15.65	<0.001**	7.58	3.24 to 18.73	<0.001**
	Missing	0.31	0.04 to 2.34	0.257	-	-	-
Model 1 includes missing values as a variable (219 patients observed for 3095 days). Model 2 excludes missing values as a variable and only includes (218 patients and 2637 days of observation) in which all variables were known.							

higher risk of bleeding. The risks were unchanged when this bleed was excluded from the analysis.Once all these factors had been taken in to consideration fever the previous day, ITU/HDU admission, SIRS score, treatment with antibiotics, treatment with antifungals, and treatment with oxygen were not independently associated with bleeding within this model.

4.5 Discussion

This analysis has identified that once multiple other variables have been taken in to account the one factor that remained as an independent risk factor for bleeding and was associated with sepsis was the C-reactive protein (CRP). The presence of a CRP greater

than or equal to 100 increased the odds of bleeding the following day (OR 1.83; 95% CI 1.30 to 2.57). No other factor considered in this study that was directly related to sepsis or infection (fever the previous day, therapeutic antibiotics or antifungal medication, SIRS score, or ITU admission) remained as an independent risk factor for bleeding once this had been taken in to account. The CRP is a marker of inflammation and mouse models have shown that severely thrombocytopenic mice only bled when there was co-existing inflammation.[114]

A white cell count ≥ 1 x 10^9/l halved the odds of bleeding the following day (OR 0.49; 95% CI 0.34 to 0.72), compared to a white cell count < 0.5 x 10^9/l. This may be a marker that the patient's bone marrow is recovering after the treatment they have received. Patients who have a higher white cell count may therefore have a higher proportion of functionally active immature platelets in their circulation, leading to a reduced risk of bleeding. In Chapter 3 it was shown that an absolute immature platelet count greater than or equal to 2 x 10^9/l had a 100% negative predictive value for bleeding. This finding is therefore consistent with the ATHENA study.

A haemoglobin greater than 95g/l was associated with a decreased risk of bleeding, compared to a haemoglobin less than 86g/l. This has been postulated to occur, and has been shown in previous animal model studies, and small studies in healthy volunteers.[199-202] The hypothesis of why haemoglobin affects the risk of bleeding is not completely clear, however two main mechanisms have been proposed to explain this phenomenon. Firstly, the number of platelets deposited on the sub-endothelial surfaces is proportional to the haematocrit.[203,204] It is possible that at a higher haematocrit level red blood cells

remain in the central part of the flow of blood pushing platelets into closer contact with the endothelium.[205,206] This might then lead to an increase in the amount of interaction between platelets and the vessel wall.

Secondly, the platelet function analyser (PFA)-100® is known to be affected by the haematocrit.[207] The haematocrit may affect platelet function because red blood cells release ADP during the formation of a haemostatic plug. ADP activates platelets and would therefore lead to increased platelet aggregation.[200,205]

Serum creatinine and serum bilirubin were independent risk factors for bleeding in this study and are markers of renal and liver function impairment. Renal impairment and low albumin have been associated with bleeding in a large retrospective observational study.[75]

Female sex has been associated with an increased risk of bleeding in a previous study of intracranial haemorrhage (ICH) in acute leukaemia patients.[102,208]

The presence of WHO grade 2 to 4 bleeding the preceding day was a strong predictor for bleeding the following day. This has also been demonstrated in the Friedmann study for severe and life-threatening bleeding.[75]

Of interest, once all the other factors had been taken in to consideration whether patients received platelet transfusions only to treat bleeding or whether platelet transfusions were given when the platelet count fell below 10×10^9/l had no effect on the risk of bleeding the following day. However, the total platelet count still had a strong effect on whether bleeding occurred the following day (Table 4.8).

4.5.1 Strengths of this study

One of the strengths of this study was the rigorous nature in which bleeding was assessed and graded compared to other studies that have assessed risk factors for bleeding.[7,118]

4.5.2 Limitations of this study

This study could only use data that was routinely recorded, and was recorded in a sufficient number of patients to enable robust statistical analysis.

Tests that are not currently in routine practice, for example the absolute immature platelet number could not be assessed in this study.

4.5.3 Implications of this study

The CRP is a simple laboratory test that could be used to determine whether a patient requires additional transfusion support to prevent bleeding when they have an infection. This finding needs to be confirmed in other studies prior to advising physicians to use the CRP as a guide as to whether a patient requires additional haemostatic treatments/platelet transfusions.

Whether treatment of anaemia with a liberal red cell transfusion strategy is beneficial is currently being tested in a randomised controlled trial (RCT). This trial is currently recruiting patients and is comparing a restrictive versus liberal red cell transfusion strategy in patients receiving a haematopoietic stem cell transplant (HSCT).[209]

4.6 Summary

- Once other independent risk factors have been taken in to consideration, the C-reactive protein was the only factor directly related to sepsis that increased the risk of bleeding the following day
- Fever the preceding day, septic inflammatory response syndrome (SIRS) score, administration of therapeutic antibiotics or antifungal agents, or admission to a critical care unit were not independent risk factors for bleeding.
- Other factors that were independently associated with an increased risk of bleeding were:
    - Clinically significant bleeding (WHO grade 2 to 4)
    - Female sex
    - Anaemia
    - Low white cell count
    - Abnormal renal function
    - Previous fungal infection
    - Abnormal liver function
- These findings need to be confirmed in other data sets or studies prior to recommending their use in guiding the use of platelet transfusions.

Section II

Factors that may be associated with severe or life-threatening haemorrhage (usually defined as WHO grade 2 or above bleeding)

5 Intracranial haemorrhage (ICH) in patients with a haematological disorder requiring intensive chemotherapy and/or a stem cell transplant: a systematic review.

5.1 Background

5.1.1 Description of condition

As stated in the introduction haematological malignancies account for between 8 and 9% of all new cancers reported in the UK and US,[18,19] and their incidence and prevalence is increasing.[29] However, the underlying disease or its treatment can lead to prolonged periods of severe thrombocytopenia when patients are at increased risk of severe or life-threatening bleeding.[3-5,7]

Platelet transfusions are used in modern clinical practice to prevent and treat bleeding in thrombocytopenic patients with bone marrow failure. In patients with haematological malignancies, platelets issued to prevent thrombocytopenic bleeding account for up to 70% of all platelets issued to that patient population.[210] Platelet transfusions are not without risk and therefore a reliable marker of a patient's bleeding risk needs to be sought.

The morning platelet count has been used, up to now, to indicate when a patient requires prophylactic platelet transfusions (to prevent severe and life-threatening

haemorrhage). However, a patient's morning platelet count has not been shown to be a good predictor of haemorrhage.[6,211]

5.1.2 Why is it important to do this review?

Intracranial haemorrhage (ICH) is the most serious type of bleed caused by significant thrombocytopenia. If an ICH does not cause death it often leads to significant long-term morbidity. This review therefore concentrates on ICH as the primary outcome measure. There have been many case reports in the literature of ICH in patients with haematological disorders but there has been no previous attempt to systematically review this literature and assess whether there are any common factors that predispose patients to this serious side-effect.

5.2 Objectives

Two main objectives:

- To provide an estimate of the incidence of intracranial haemorrhage in this patient population.
- To determine whether there are any emerging factors that predict whether a patient is at a higher risk of developing an intracranial haemorrhage. This will include an estimate of the risks of ICH when possible.

5.3 Methods

5.3.1 Protocol and registration

Details of the inclusion criteria and methods for the analysis were pre-specified and documented in a protocol [PROSPERO (CRD 42011001447)] and published on-line (http://www.crd.york.ac.uk/prospero/).

5.3.2 Criteria for considering studies for this review

5.3.2.1 *Types of studies*

Randomised controlled trials (RCTs), controlled studies, cohort studies, case series and case reports were included in this review. Reviews, animal studies and laboratory studies were excluded from the review. Any type of interventional or observational study was included that mentioned the presence or absence of ICH. ICH could be mentioned as a primary or secondary outcome of the study, or as an adverse event.

5.3.2.2 *Types of participants*

Patients of any age were included if they had haematological disorders that required intensive chemotherapy or a haemopoietic stem-cell transplant (HSCT) and were expected to become significantly thrombocytopenic ($<50 \times 10^9$/L for ≥ 5 days).
If trials consisted of mixed populations of patients only data from the haematological sub-groups were used. If sub-group data for haematology patients was not provided (after contacting the authors of the trial), the trial was excluded if fewer than 80% of participants had a haematological disorder. Studies were also excluded if the participants

were receiving palliative treatment; fewer than 33% of participants were expected to become significantly thrombocytopenic, or patients had immune thrombocytopenia.

5.3.2.3 *Types of intervention*

There was no limitation to the type of intervention included in the studies.

5.3.2.4 *Time Limit*

Only cases published in the literature during a ten year period were reviewed (1st January 2001 to 31st December 2010), this is because of changes in haematological practice prior to 2001, including the introduction of all-trans retinoic acid in to the routine management of patients with acute promyelocytic leukaemia (APL).

5.3.3 Types of outcome measures

5.3.3.1 *Primary outcomes*

- Incidence of intracranial haemorrhage (ICH).
- Relative measures of association (relative risks/odds ratio/hazards ratios) of the potential risk factor (e.g. age, sex, underlying diagnosis, treatment, baseline laboratory results and co-morbidities) and occurrence of ICH.
- Number and underlying characteristics of cases of ICH.

5.3.3.2 *Secondary outcomes*

- Mortality due to ICH within 30 days of occurrence of ICH.
- Morbidity due to ICH within 30 days of occurrence of ICH.

- Types of ICH (extra-dural, sub-dural, sub-arachnoid, intra-parenchymal, intra-ventricular)

5.3.4 Search methods for identification of studies

The search strategy for MEDLINE is available in the published protocol and Appendix 5.1. The search terms were adapted for use with other bibliographic databases in combination with database-specific filters for controlled trials, where these were available. There were no language restrictions. Studies published between January 2001 and December 2010 were sought. The following electronic bibliographic databases were searched.

5.3.4.1 *Search methods*

Five electronic databases (MEDLINE (1990 to Feb 2011); EMBASE (1990 to Feb 2011); Cochrane Central Register of Controlled Trials (CENTRAL); CINAHL (1990 to Feb 2011); Transfusion Evidence Library) were searched (Appendix 5.1). Database searching was augmented by hand-searching reference lists of all included studies and relevant review articles by one reviewer (LE).

5.3.5 Data collection and analysis

5.3.5.1 *Selection of Studies*

All electronically derived citations and abstracts of papers identified by the review search strategy were initially screened for relevancy by one reviewer (LE). Studies clearly irrelevant were excluded at this stage.

The full texts of all potentially relevant trials were then formally assessed for eligibility by one reviewer (LE), and 20% of these were independently checked by a second reviewer (SB). Any disagreement was resolved by discussion. Further information was sought from the authors where articles contained insufficient data to make a decision about eligibility. Authors of 14 excluded studies were contacted for additional information, of these, 4 authors responded and 1 provided additional information. All eligibility criteria failed by excluded studies was recorded (Appendix 5.7).

5.3.5.2 *Data Extraction and Management*

Data extraction was conducted twice by one author (LE), and 20% of the data was independently checked by a second author (SB). Potential disagreements between the review authors were resolved by consensus. The review authors were not blinded to names of authors, institutions, journals, or the outcomes of the trials. Data from the studies were extracted by the review authors using a standardised data extraction form designed specifically for use in this review (Appendix 5.2). This data included general information about the study, characteristics of study participants at baseline, laboratory results at time of haemorrhage, and clinical factors at time of haemorrhage.

Both full-text versions and abstracts of eligible studies were used to retrieve the data. Publications reporting on more than one trial were extracted using one data extraction form for each trial. Trials reported in more than one publication were extracted on one form only.

5.3.6 **Dealing with missing data**

Authors of 52 included studies were contacted for additional information, of these, 13 authors responded and 9 provided additional information.

Some studies included patients with haematological disorders as well as patients with solid tumours. If data could be extracted for a haematological 'subgroup' from the trial general data, this was done; if not, authors were contacted in order to retrieve information on haematology patients.

5.3.7 **Data synthesis**

A narrative synthesis of the findings from the admissible studies, structured around the type of study design and type of outcome was performed.

I anticipated that there would be limited scope for meta-analysis because of the range of different exposures and outcomes measured. The decision about whether or not to combine the results of individual studies depended on an assessment of heterogeneity as well as study design. For dichotomous outcomes the numbers of outcomes in exposure and control groups were recorded. The exposure effect measures across individual studies were estimated as the relative effect measures [hazards ratio (HR) with 95% confidence intervals (CI)]. In studies with rare outcomes (< 5%) the p-value of the odds ratio (OR), RR and HR are similar and it was assumed that the p-value of the OR would approximate the p-value of the HR when the HR was unknown. Reported or estimated p-values were used to calculate the standard error and natural logarithm of the HR. These data were incorporated into meta-analyses using the generic inverse variance method, if

appropriate. The p-value of the odds ratio was only calculated when the ratio between the number of exposed and unexposed participants in a study was between 0.5 and 1.5 to reduce the risk of erroneous results. For narrative reports of potential risk factors, when the results could not be combined in to a meta-analysis only results from studies containing more than 50 participants and at least 20 patients in both exposed and control groups were reported. This reduced the risk of detecting an effect by chance in small studies or sub-groups.

For continuous outcomes, the mean and standard deviations were recorded. No meta-analyses were performed for continuous outcomes in this review.

5.3.8 **Assessment of heterogeneity**

The decision about whether or not to combine the results of individual studies depended on an assessment of heterogeneity.

The I^2 statistic was used to quantify the amount of possible heterogeneity ($I^2 \leq 30\%$ minor heterogeneity, $I^2 > 30\%$ to $\leq 75\%$ moderate heterogeneity, $I^2 > 75\%$ considerable heterogeneity). Potential causes of heterogeneity were explored by sensitivity and subgroup analyses when possible. Meta-analyses were not performed if there was considerable heterogeneity. A fixed-effect model was used if $I^2 < 50\%$, and a random-effects model was used if I^2 was between 50 and 75%. If studies were considered sufficiently homogeneous in their study design, a meta-analysis was carried out and the statistical heterogeneity assessed. Statistical heterogeneity of exposure/outcome effects

between trials was initially assessed by using a Chi² test with a significance level at P < 0.1.

5.3.9 Risk of Bias and assessment of quality

A formal assessment of bias was not performed. This has changed from the original protocol. The vast majority of included studies did not have ICH as a primary or secondary outcome. This meant that whether studies were randomised or not was irrelevant to this review. Formally assessing risk of bias in these studies would not add to the clarity or quality of this review. Studies were therefore categorised into cohort studies which included interventional studies (randomised-controlled trials (RCTs), non-randomised controlled trials, un-controlled trials), and observational studies, (including post-mortem studies); case-control studies; case-series; and case studies (Appendix 5.3). There is also no consensus on how to assess risk of bias in case series or case studies which this review includes.[212-217] I therefore decided not to formally assess risk of bias but instead reported the type of study, whether ICH was a primary or secondary outcome, and whether all ICH were reported or only deaths due to ICH were reported. Studies were not excluded based on the quality of research methods.

5.3.10 Assessment of reporting biases

Publication bias (small study bias) was explored by the generation of a funnel plot when a meta-analysis contained more than 10 trials.[218] A formal test of small study bias was

also performed (Harbord test, or an Egger's test if a Harbord test could not be performed with the available study data).[219]

5.3.11 Subgroup analysis and investigation of heterogeneity

A sub-group analysis was planned to be performed on treatment sub-groups e.g. induction treatment/consolidation treatment/autologous transplants/allogeneic transplants. However, this was not performed due to lack of available data.

5.4 Results

5.4.1 Description of studies

Characteristics of included studies see appendices 5.4, 5.5 & 5.6.

Studies were categorised into cohort studies which included interventional studies (RCTs, non-randomised controlled trials, un-controlled trials) and observational studies; case-control studies; case-series; and case studies (Appendix 5.3).

5.4.1.1 *Results of the Search*

The original search (conducted February 2011) identified 7113 potentially relevant citations.

There were 6859 citations after duplicates were removed. 6005 records were able to be excluded on the basis of the abstract by one author (LE). 854 full text articles were assessed for eligibility by one study author and 20% of these were independently checked by a second study author (SB). See also the PRISMA Flow Diagram (Figure 5.1).

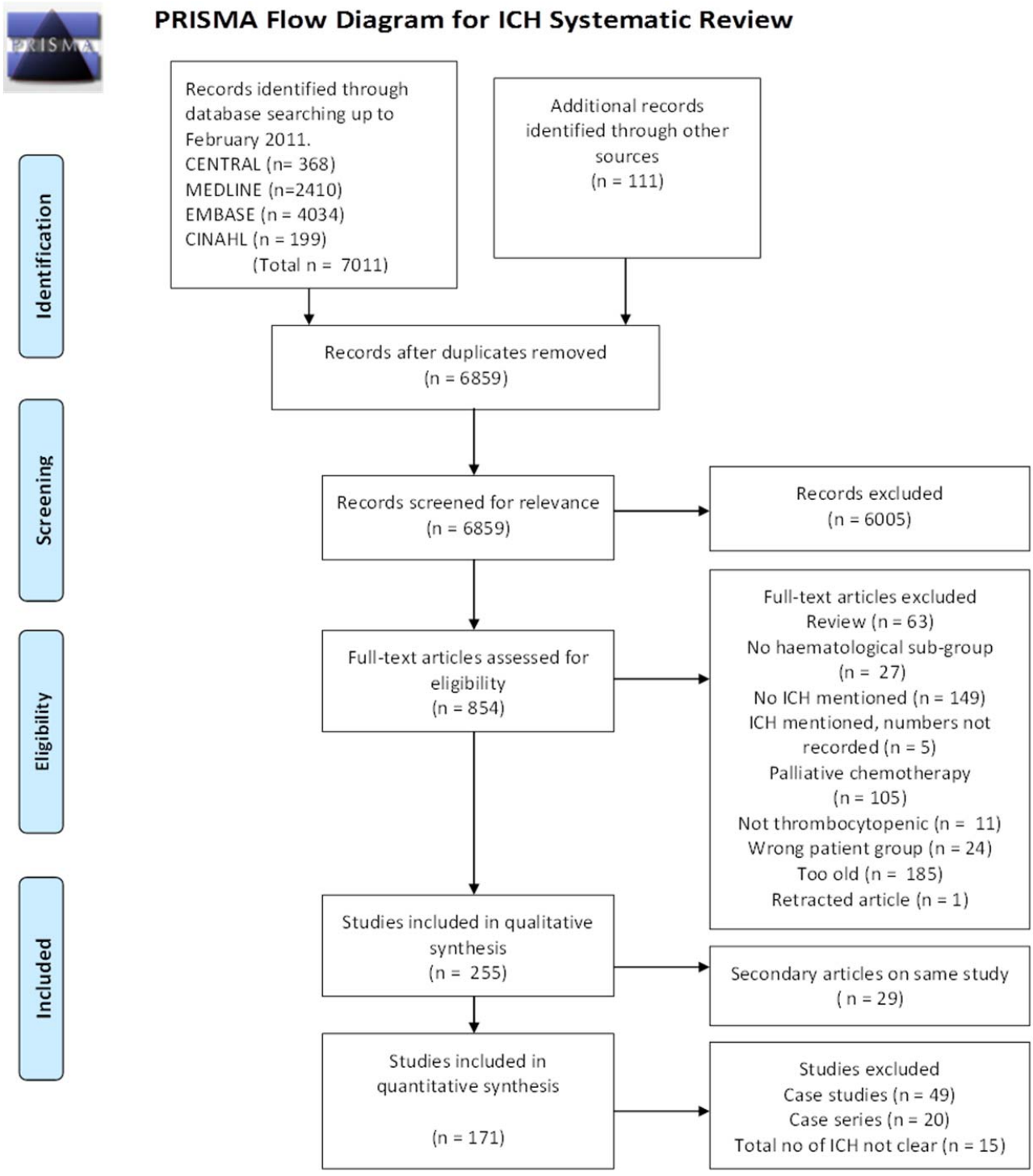


Figure 5.1 PRISMA flow diagram for the systematic review of intracranial haemorrhage

5.4.2 **Included Studies**

For tables of characteristics of the included studies see Appendices 5.4, 5.5 & 5.6.

5.4.2.1 ***Study Type***

There were 255 studies within 284 citations eligible for inclusion within this review (Table 5.1). There were 186 cohort studies within 215 citations, no case-control studies, 20 case-series within 20 citations, and 49 case studies within 49 citations. These 255 studies contained 31,154 participants.

There were 87 interventional studies, these included: 26 RCTs; 11 non-randomized controlled studies; and 50 un-controlled studies (Table 5.1). The type of intervention varied within the studies, and included: different types of chemotherapy (58 studies); different stem cell transplant regimens (16 studies); different supportive regimens (13 studies) (e.g. antibiotics, antifungals, or blood product support).

In 17% (32/186) of the cohort studies ICH was a primary or secondary outcome. In 80% (16/20) of the case series ICH was a primary or secondary inclusion criterion.

In 61% (113/186) of the cohort studies only death due to ICH was reported. In 35% (7/20) of the case series only death due to ICH was reported. In 61% (30/49) of the case studies the ICH led to death.

Table 5.1 Characteristics of studies included within the review

Type of Study	No. of Studies	No. of studies ICH is primary or secondary outcome	No. of studies only deaths due to ICH reported	No. of cases of ICH
Randomised-controlled trial	26	1	8	125
Cohort (Interventional, controlled)	11	0	6	27
Cohort (Interventional, uncontrolled)	50	1	42	90
Observational Cohort	86	26	44	552
Cohort (Unknown study design)	9	0	9	11
Post-mortem study	4	4	4	160
Case Series	20	-	7	145
Case Studies	49	-	30	49
Total	**255**	**32**	**150**	**1159**

5.4.3 Excluded Studies

There were 570 studies excluded on the basis of a full text review. The reasons for exclusion were: study too old (n = 185); no ICH mentioned (n = 149); palliative chemotherapy (n = 105); review (n = 63); no haematological sub-group (n = 27); wrong patient group (n = 24); not thrombocytopenic (n = 11); ICH mentioned, but numbers not recorded (n = 5); retracted article (n = 1) (For a list of excluded studies see Appendix 5.7).

Fifteen cohort studies did not report the presence or absence of ICH adequately. Data from these studies were included within the narrative review but were excluded from any meta-analysis.[220-234]

5.4.4 Primary outcomes

5.4.4.1 *Incidence of intracranial haemorrhage*

Only one of the studies reported an incidence of intracranial haemorrhage,[235] this study has only been reported as an abstract.

The Osselaer study reported incidence rates for 3 different time periods. Time periods 1 and 2 were separated by a one month period and therefore are unlikely to have the same patients in both cohorts, the incidence rates for the first two time periods were reported separately. This study reported an incidence of 1.16 per 1000 patient days at risk in the first study period and 1.21 per 1000 patient days at risk during the second study period. Days at risk were defined in the study as within 48 hours following a platelet transfusion.[235] Performing complex statistical modelling to estimate incidence rates from the reported data in the other studies is beyond the scope of this thesis.

5.4.4.2 *Relative measures of association (relative risks/odds ratio/hazards ratios) of the exposure-outcome of interest*

Only 49% (83/171) of the cohort studies reported any exposure of interest separately for cases and controls (Appendix 5.12.1).[2-4,6,102,103,111,112,119,128,236-308]

Only 4 of these 83 cohort studies reported any relative risks (RR), odds ratios (OR), or hazards ratios of risk factors for ICH.[102,103,111,251] Two further studies[3,306] reported risk

factors for severe or fatal haemorrhage in which the majority of these haemorrhages were ICH (14/18; 77.8%: 24/37; 64.9% respectively). The multivariable analyses performed by these 6 studies adjusted for different variables and did not report all the variables taken in to account when performing their regression analyses.

Data for each univariable analysis of an exposure of interest (e.g. baseline characteristics, treatment, and co-morbidities) were analysed separately. For all of the exposure-outcomes of interest stated below the denominator number of studies is 171.

5.4.4.2.1 Age

6% (10/171) of studies reported the age of cases and controls and included both adults and children or older and younger adults.

Table 5.2 Paediatric versus adult cases of ICH

Study	Definition of adult patients (yrs)	Number of participants	Number (%) of paediatric cases	Number (%) of adult patients	Ratio paed:adult ICH > 1
Slichter 2010[6]	18	1272	14/200 (7.0)	29/1072 (2.7)	✓
De la Serna 2008*	16	732	3/59 (5.1)	34/673 (5.1)	×
McCullough 2004	16	645	1/22 (4.6)	6/623 (1.0)	✓
De Botton 2004	18	455	1/31 (3.2)	9/424 (2.1)	✓
Ordered in descending size of study. *Includes fatal haemorrhage other than ICH. ×No ✓Yes ⦸ No cases in study					

Six studies containing 3129 participants reported whether cases or controls were paediatric or adult patients.[3,6,119,248,257,282] None of the studies could be incorporated into a meta-analysis. Three of the four studies that contained more than 50 participants

demonstrated a higher proportion of ICH in paediatric patients compared to adult patients (Table 5.2).

Four studies containing 825 participants reported whether cases or controls were aged under or over 60 years of age.[3,244,248,268] None of these studies could be incorporated in to a meta-analysis. Only one study contained at least 50 participants, with at least 20 participants in both exposed and control groups (Table 5.3). This study showed a higher number of ICH in patients aged over 60 years of age.[3]

Table 5.3 Number of cases of ICH in patients aged over 60 years of age versus patients aged under 60 years of age

Study	Number of cases	Number of participants	Number (%) of cases aged < 60yrs	Number (%) of cases aged > 60yrs
De la Serna 2008	37*	732	25/543 (4.6)	12/130 (9.2)
*Includes fatal haemorrhage other than ICH				

Two further studies reported OR or RR and there was no statistically significant difference seen (Table 5.4).

Table 5.4 Odds ratio or relative risk of ICH associated with age of the patient

Study	Number of cases	Number of participants	Criteria	Analysis	Risk estimate (95% CI)	P value
Kim 2004	41	792	Age ≥40 vs. <40	RR (Multivariate)	1.58 (0.68 to 3.68)	0.282
Chang 2007	12	78	Age ≥65	OR (Univariate)	0.83 (0.2 to 3.44)	0.801
Ordered in descending size of study						

5.4.4.2.2 Sex

27% (46/171) of studies containing 7356 participants reported the sex of cases and controls.[2-4,102,103,111,112,119,128,242,243,248,259,260,263-265,268,269,273-277,280-285,292,294,295,297,298,300,301,305,307,308] Eleven of these 46 studies, containing 3960 participants could be combined in to a meta-analysis (Figure 5.2).[2,4,5,102,111,119,128,275,283,284,301] This showed that women had a higher risk of ICH than men (Hazards ratio (HR) 1.87 (95% CI 1.18 to 2.77); p = 0.002.

There was no evidence of heterogeneity (I^2 = 0%), and there was no evidence of small study effects (Visual inspection of funnel plot or Egger's test P=0.421).[219]

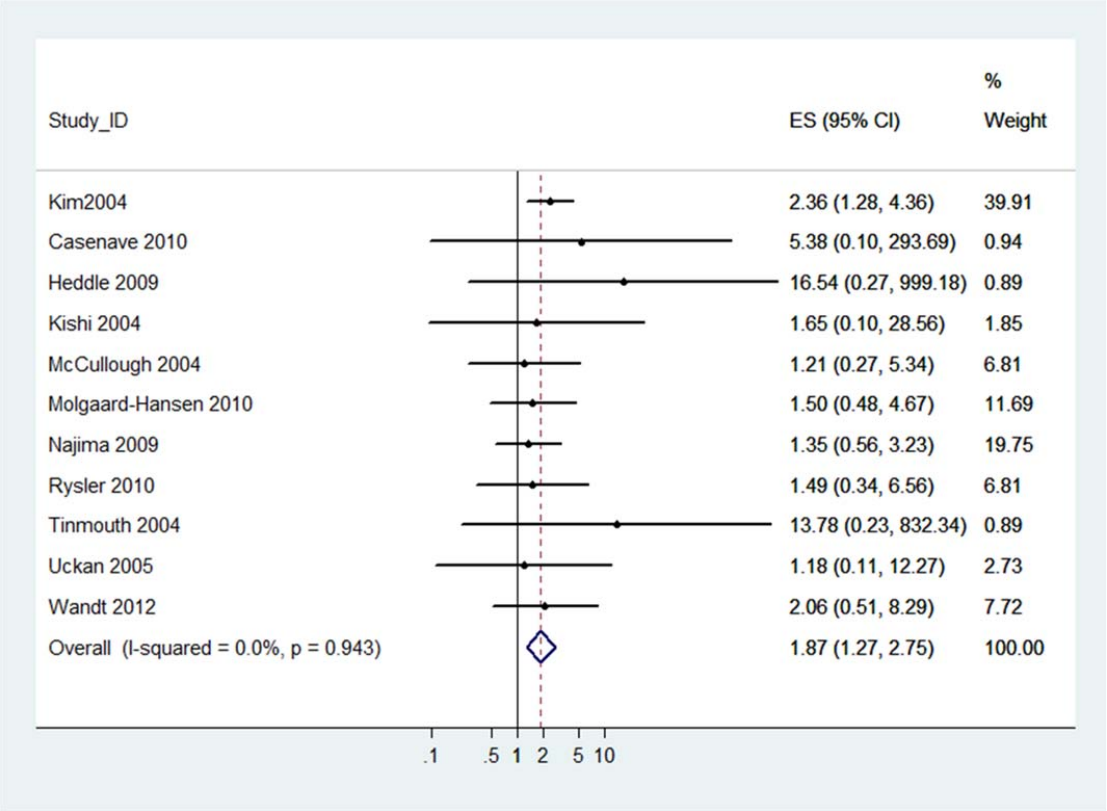


Figure 5.2 Forest plot presenting hazards ratios for ICH for women versus men

However, this pattern of risk was not seen in the studies excluded from the meta-analysis (Table 5.5). Eleven of the remaining 36 studies contained more than 50 participants, four of these studies contained no cases,[255,286,309,310] and only one of the remaining seven studies had a higher proportion of women with ICH than men.[306]

Table 5.5 Female vs. male cases of ICH (11 studies not included in meta-analysis that included more than 50 participants)

Study	Number of cases	Number of participants	Number (%) of cases female	Number (%) of cases male	Ratio female:male ICH > 1
Chen 2009	51	841	14/361 (3.8)	37/480 (7.7)	×
De La Serna 2008	37*	732	14/360 (3.9)	23/372 (6.2)	×
Creutzig 2006	0	473	0/220 (0.0)	0/253 (0.0)	⃠
Yanada 2007	18*	279	9/123 (7.3)	9/156 (5.8)	✓
Weber 2008	2	165	0/54 (0.0)	2/111 (1.8)	×
Breccia 2010	5	105	1/52 (1.9)	4/53 (7.5)	×
Callow 2002	0	98	0/36 (0.0)	0/62 (0.0)	⃠
Kikuchi 2008	1	91	0/27 (0.0)	1/64 (1.6)	×
Mitchell 2003	0	85	0/33 (0.0)	0/52 (0.0)	⃠
Narimatsu 2008	0	77	0/46 (0.0)	0/31 (0.0)	⃠
Imaizumi 2010	2	58	0/27 (0.0)	2/31 (6.5)	×
Ordered in descending size of study. *Includes fatal haemorrhage other than ICH ×No ✓Yes ⃠No cases in study					

5.4.4.2.3 Underlying Diagnosis

5.4.4.2.3.1 *Acute leukaemia*

25% (42/171) of studies containing 6927 participants included patients with both acute leukaemia and other haematological disorders.

2,4,111,112,119,128,236,238,240,242,245,246,249,250,253,258,261,262,264,265,271-273,275,278-281,283,285,286,288,289,292,295,298,301-303,305,307 Five of these 42 studies containing 1432 participants could be combined in to a meta-analysis (Figure 5.3). This showed that patients with acute leukaemia had a higher risk of ICH than patients with other haematological disorders (HR 2.29 (95% CI 1.21 to 4.33) p = 0.011. There was moderate heterogeneity (I^2 = 39.2%), however both fixed and random effects showed a similar effect.

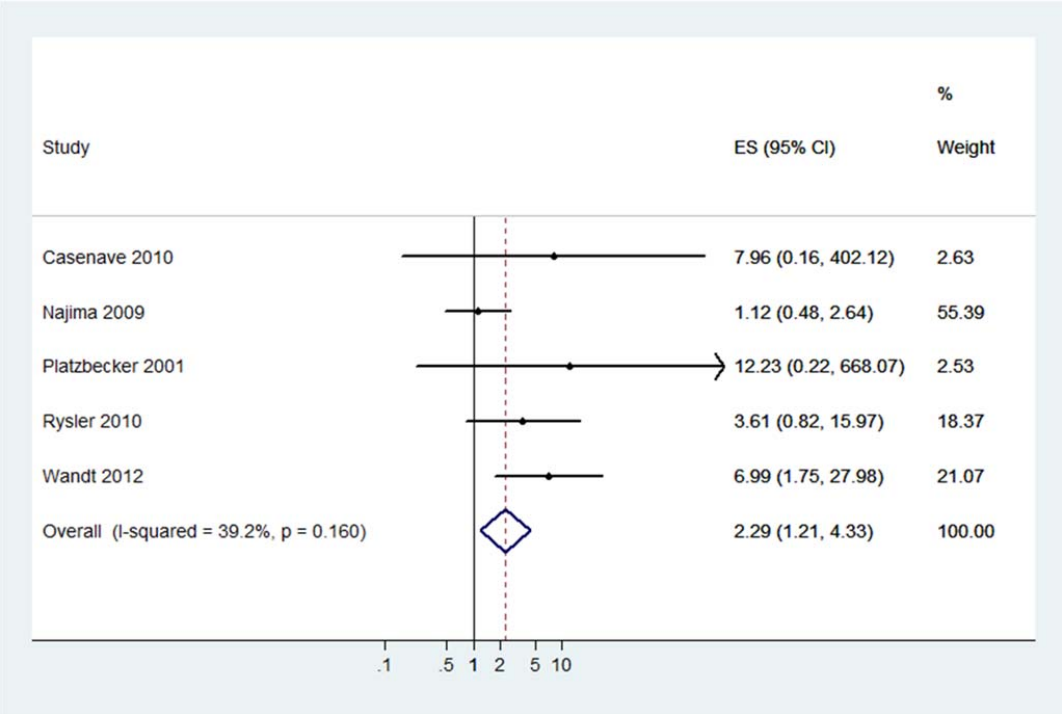


Figure 5.3 Forest plot presenting hazards ratios for ICH for patients with acute leukaemia versus other haematological disorders.

There was a trend to the same pattern of risk in the studies excluded from the meta-analysis (Appendix 5.12.2). Thirteen of the remaining 34 studies contained more than 50 participants with at least 20 participants in both exposed and control groups. Eight of

these 13 studies had a higher proportion of cases of ICH in patients with acute leukaemia versus other haematological disorders.

10 studies[102,103,237,249,254,255,281,284,287,304] containing 3222 participants included patients with both acute promyelocytic leukaemia (APL) and other acute leukaemias. Two of these studies containing 1633 participants could be combined in to a meta-analysis.[102,103] This showed that patients with APL had a higher risk of ICH than patients with other types of acute leukaemia (HR 7.37 (95% CI 2.96 to 18.39) P < 0.001. There was no significant heterogeneity (I^2 = 22.7%). Four of the remaining 8 studies[254,255,284,309] contained more than 50 participants, and three contained more than 20 participants with APL. These studies showed a similar pattern to the meta-analysis (Appendix 5.12.3).

5.4.4.2.3.2 *Myeloma*

10% (17/171) of studies[2,4,111,119,128,240,245,258,265,275,279,283,285,286,289,295,303] containing 3387 participants included patients with both myeloma and other haematological disorders. Two of the studies containing 160 participants could be combined in to a meta-analysis.[128,303] This showed no statistically significant difference in the risk of ICH (HR 0.15 (95% CI 0.01 to 2.44) p = 0.180). This lack of difference may be due to the small number of participants included within the meta-analysis, however the other studies did not appear to show fewer bleeding events in patients with myeloma (Appendix 5.12.4).

5.4.4.2.3.3 *Other Diagnoses*

None of the studies that included patients with aplastic anaemia and other haematological disorders (Appendix 5.12.5); lymphoma and other haematological

disorders (Appendix 5.12.6); myelodysplasia and other haematological disorders (Appendix 5.12.7) chronic myeloid leukaemia and other haematological disorders (Appendix 5.12.8) could be combined in to a meta-analysis.

5.4.4.2.4 Disease status

No studies reported participants with different types of disease status, and the disease statuses were known for both cases and controls.

5.4.4.2.5 Treatment

5.4.4.2.5.1 *Autologous stem cell transplant*

11% (18/171) of studies[2,4,119,128,239,240,245-247,253,259,261,266,289-291,293,305] reported participants treated with both autologous haemopoietic stem cell transplant (HSCT) and other types of treatment (chemotherapy or allogeneic HSCT). 16 studies[4,119,239,240,245-247,253,259,261,266,289-291,293,305] containing 4213 participants included patients treated with both autologous and allogeneic transplants. Three of these studies containing 695 participants could be combined in to a meta-analysis.[4,119,259] This showed that patients treated with an autologous HSCT had a lower risk of ICH than patients treated with an allogeneic HSCT (HR 0.088 (95% CI 0.026 to 0.295) $P < 0.001$. There was no significant heterogeneity ($I^2 = 0\%$).

However, there was no overall trend in those studies excluded from the meta-analysis (Table 5.6).

Table 5.6 Autologous HSCT vs. allogeneic HSCT cases of ICH (6 studies not included in meta-analysis that included more than 50 participants)

Study	Number of Cases	Number of Participants	Number (%) of cases with autologous HSCT	Number (%) of cases with allogeneic HSCT	Ratio of AutoHSCT: AlloHSCT ICH < 1
Coplin 2001	19	1245	2/342 (0.6)	17/903 (1.9)	✓
Pihusch 2002	21	447	5/83 (6.0)	16/364 (4.4)	×
Barlogie 2006	1	297	0/261 (0.0)	1/36 (2.8)	✓
Faraci 2002	3	272	1/87 (1.1)	2/185 (1.1)	×
Batlle 2005	1	185	1/141 (0.7)	0/44 (0.0)	×
Weber 2008	2	165	0/54 (0.0%)	2/111 (1.8%)	✓
×No ✓Yes ⃠No cases in study					

Six studies[2,4,119,128,239,245] containing 1740 participants included patients treated with both autologous HSCTs and chemotherapy. Two of these studies containing 591 participants could be combined in to a meta-analysis.[2,4] This showed that patients treated with an autologous HSCT had a lower risk of ICH than patients treated with chemotherapy (HR 0.125 (95% CI 0.036 to 0.433) P = 0.001. There was no significant heterogeneity (I^2 = 0%). A similar pattern was seen in those studies excluded from the meta-analysis (Appendix 5.12.7).

5.4.4.2.5.2 *Allogeneic stem cell transplant*

3% (5/171) of studies[4,119,239,245,249] containing 888 participants included patients treated with both allogeneic HSCT and chemotherapy. Two of these studies containing 520 participants could be combined in to a meta-analysis.[4,119] This showed no statistically significant difference in the risk of ICH, however the 95% confidence intervals were very

wide (HR 2.27 (95% CI 0.80 to 26.47) p = 0.126). There was no significant heterogeneity (I^2 = 0%). Of those studies excluded from the meta-analysis all showed a higher rate of ICH in the allogeneic HSCT group compared to the chemotherapy group (Table 5.7).

Table 5.7 Allogeneic HSCT vs. chemotherapy cases of ICH (3 studies not included in meta-analysis that included more than 50 participants)

Study	Number of Cases	Number of Participants	Number (%) of cases with allogeneic HSCT	Number (%) of cases with chemotherapy	Ratio of AlloHSCT: Chemotherapy ICH > 1
McCullough 2004	7	330	5/178 (2.8)	2/152 (1.3)	✓
Barlogie 2006	1	247	1/36 (3)	0/211 (0)	✓
Rysler 2010	7	200	5/100 (5)	2/100 (2)	✓
×No ✓Yes ⃠No cases in study					

5.4.4.2.5.3 *Radiation containing regimens*

1% (2/171) of studies[111,251] containing 700 participants included patients treated with or without radiation. These studies were not incorporated into a meta-analysis. Neither study showed a statistically significant effect of radiation on risk of ICH (Table 5.8).

Table 5.8 Odds ratio or hazard ratio of ICH associated with radiation containing regimen

Study	Number of cases	Number of participants	Criteria	Analysis	Risk estimate (95% CI)	P value
Najima 2009	21	622	Radiation containing regimen	HR (Multivariate)	1.53 (0.51 to 4.59)	0.443
Chang 2007	12	78	Cranial irradiation	OR (Univariate)	1.28 (0.3 to 5.42)	0.736

5.4.4.2.6 Baseline laboratory results

5.4.4.2.6.1 *Platelet count at presentation*

5% (8/171) of studies[3,102,269,274,282,284,299,308] containing 2451 participants reported platelet count at presentation. None of the studies could be incorporated in to a meta-analysis. One study reported an obvious relationship between a lower platelet count at presentation and a higher risk of ICH (Table 5.9). However, the other studies did not (Table 5.10).

Table 5.9 Odds ratio, relative risk or hazard ratio of ICH associated with baseline laboratory results

Study	Number of cases	Number of participants	Criteria	Analysis	Risk estimate (95% CI)	P value
Platelet count at presentation						
Kim 2004	41	792	Plts < 35 vs. > 35	RR (Multivariate)	3.28 (1.43 to 7.6)	0.005
White cell count at presentation						
Chang 2007	12	78	WCC ≥ 200 vs. < 200	OR (Multivariate)	6.18 (1.02 to 37.38)	0.047
Kim 2004	41	751	WCC ≥ 50 vs. < 50	RR (Multivariate)	3.30 (1.46 to 7.45)	0.004
Yanada 2007	18*	279	WCC ≥ 20 vs. < 20	OR (Multivariate)	3.61 (1.14 to 11.4)	0.029
Coagulation abnormalities at presentation						
Kim 2004	41	792	INR ≥ 1.5 vs. < 1.5	RR (Multivariate)	3.29 (1.25 to 8.67)	0.016
Kim 2004	41	792	aPTT ≥ 38 vs. < 38s	RR (Multivariate)	2.26 (0.99 to 5.21)	0.054
Yanada 2007	18*	279	Fibrinogen < 1 vs. ≥ 1g/dL	OR (Multivariate)	3.28 (1.17 to 9.19)	0.024
*Includes fatal haemorrhage other than ICH						

Table 5.10 Platelet count below a threshold at presentation vs. platelet count above a threshold at presentation cases of ICH (4 studies that included more than 50 participants)

Study	Platelet count threshold	Number of Cases	Number of Participants	Number (%) of cases with platelet count below threshold	Number (%) of cases with platelet count above threshold	Ratio platelet count below threshold:platelet count above threshold ICH >1
De la Serna 2008	40*	37	732	32/563 (5.7)	5/168 (3.0)	✓
Molgaard-Hansen 2010	20	12	525	2/108 (1.9)	10/417 (2.4)	×
Testi 2005	40	3	110	1/80 (1)	2/27 (7)	×
Nowacki 2002 (PM)	25	58	143	54/97 (56)	4/46 (9)	✓

*Includes fatal haemorrhage other than ICH
PM = Post-mortem study
×No ✓Yes ⃠No cases in study

There were no obvious differences between the participants within the studies to explain these differences.

5.4.4.2.6.2 *White cell count at presentation*

8% (14/171) of studies[3,102,251,252,254,257,269,270,274,284,288,299,306,308] containing 5048 participants reported white cell count (WCC) at presentation. All of these studies were studies in which all of the participants had acute leukaemia. None of the studies could be combined in to a meta-analysis. All three studies that reported an odds ratio or risk ratio showed a statistically significant association with a higher WCC and increased risk of ICH (Table 5.9). Two studies included patients with acute leukaemia[102,251] and one study included patients with APL[306].

The other studies also appeared to show a higher risk of bleeding with higher WCC (Appendix 5.12.10).

5.4.4.2.6.3 *Coagulation abnormalities at presentation*

4% (6/171) of studies[3,102,243,282,306,307] containing 1925 participants reported coagulation abnormalities at presentation. None of the studies could be combined in to a meta-analysis. All of the studies suggested that coagulation abnormalities increased the risk of ICH (Table 5.9 & Appendix 5.12.11). In 4 of these 6 studies, containing 1130 participants only patients with APL were included.[3,243,282,306] In the other study containing more than 50 participants[102] an INR ≥ 1.5 was associated with a higher risk of bleeding (Table 5.9).

5.4.4.2.6.4 *Albumin at presentation*

One study[3] containing 732 participants with APL reported albumin at presentation. No statistically significant relationship was reported between the albumin at presentation and risk of ICH.

5.4.4.2.6.5 *Haemoglobin at presentation*

One study[3] containing 732 participants with APL reported haemoglobin at presentation. No statistically significant relationship was reported between the haemoglobin at presentation and risk of ICH.

5.4.4.2.7 Co-morbidities

5.4.4.2.7.1 *Abnormal renal function*

2% (4/171) of studies[3,244,280,307] containing 793 participants reported this outcome. None of these studies could be incorporated in to a meta-analysis. Two studies contained more than 50 participants and both showed a markedly higher rate of bleeding in the patients with renal impairment (Table 5.11).

Table 5.11 Renal impairment at presentation vs. no renal impairment at presentation cases of ICH (2 studies that included more than 50 participants)

Study	Definition of renal impairment	Number of Cases	Number of Participants	Number (%) of cases with renal impairment	Number (%) of cases without renal impairment	Ratio renal impairment:no renal impairment ICH >1
De la Serna 2008	Creatinine ≥ 1.4mg/dL	37*	732	7/14 (50)	30/718 (4.2)	✓
Bug 2007	Creatinine > 1.2 mg/dL	4	53	4/20 (20)	0/33 (0)	✓
*includes fatal haemorrhage other than ICH ×No ✓Yes ⃠No cases in study						

5.4.4.2.7.2 *Graft versus Host Disease (GvHD)*

5.4.4.2.7.2.1 Acute Graft versus host disease

10% (17/171) of studies[4,111,112,242,264,276,277,280,285,291,292,294,295,298,301,304,305] containing 1146 participants reported this outcome. Two studies[111,305] were incorporated in to a meta-analysis. This showed a statistically significant increase in the risk of ICH in patients with acute GvHD (HR 1.45 (95% CI 1.04 to 2.03) p = 0.029). However, there was moderate heterogeneity (I^2 = 48.6%). However, the three studies that contained more than 50 participants that were not incorporated into the meta-analysis did not show this effect.

The only study that reported the grade of acute GvHD was the Najima study (grade III/IV). Differences between grades of acute GvHD included in studies may explain the difference.

Table 5.12 Acute graft versus Host Disease (GvHD) vs. no GvHD cases of ICH (3 studies excluded from meta-analysis that included more than 50 participants)

Study	Number of Cases	Number of Participants	Number (%) of cases with acute vGvHD	Number (%) of cases with no acute GvHD	Ratio of acute GvHD: no acute GvHD ICH > 1
Pihusch 2002	21	447	8/250 (3.2)	13/197 (6.5)	×
Rysler 2010	5	100	1/47 (2.1)	4/53 (7.5)	×
Bleggi Torres 2002 (PM)	23	180	23/76 (30)	35/104 (34)	×
PM = Post-mortem study ×No ✓Yes ⦸No cases in study					

5.4.4.2.7.2.2 Chronic Graft versus Host Disease

6% (10/171) of studies[4,112,242,264,276,277,280,285,294,298] containing 370 participants reported this outcome. None of these studies could be incorporated in to a meta-analysis. Only one post-mortem study contained more than 50 participants, and there was no evidence that chronic GvHD increased the risk of ICH [chronic GvHD (11/48; 23%) no chronic GvHD (47/132; 36%)].[112]

Table 5.13 Odds ratio, relative risk or hazard ratio of ICH associated with co-morbidities

Study	Number of cases	Number of participants	Criteria	Analysis	Risk estimate (95% CI)	P value
Veno-occlusive disease (VOD)						
Najima 2009	21	622	VOD (Yes/No)	HR (Multivariate)	2.63 (0.77 to 9.00)	0.125
Preceding CNS abnormalities						
Najima 2009	21	622	Prior CNS events (Yes/No)	HR (Multivariate)	1.78 (0.58 to 5.40)	0.312
Infection						
Najima 2009	21	622	Systemic infection (Yes/No)	HR (Multivariate)	1.52 (0.57 to 4.03)	0.399
Fever						
De La Serna 2009	37*	732	Fever at presentation	OR (Multivariate)	NR	0.85
Preceding haemorrhage						
Kim 2004	41	792	Haemorrhage score ≥ 1 vs. 0 at presentation	RR (Multivariate)	1.37 (0.50 to 3.75)	0.538
NR = not reported * includes fatal haemorrhage other than ICH						

5.4.4.2.7.3 *Veno-occlusive disease (VOD)*

One study[111] reported this outcome (Table 5.13). No statistically significant relationship was reported between the presence of VOD and risk of ICH, however the 95% confidence interval was wide.

5.4.4.2.7.4 *Preceding CNS abnormalities*

One study[111] reported this outcome (Table 5.13). No statistically significant relationship was reported between the presence of preceding CNS abnormalities and risk of ICH.

5.4.4.2.7.5 *Fever/sepsis at presentation*

4% (6/171) of studies[3,102,103,111,243,274] reported fever or infection at presentation.

5.4.4.2.7.5.1 Fever

Four studies[3,102,243,274] reported fever. None of the studies could be incorporated in to a meta-analysis. Only one of the studies reported a relative risk and found no effect of fever at presentation (Table 5.13). The other studies contained fewer than 50 participants, or fewer than 20 participants had fever.

5.4.4.2.7.5.2 Sepsis/Infection

Two studies[103,111] reported this outcome. Neither study reported a statistically significant relationship between the presence of infection and risk of ICH, and only one of these studies reported an odds ratio (Table 5.13).

5.4.4.2.7.6 *Preceding haemorrhage*

One study[102] reported this outcome (Table 5.13). No statistically significant relationship was reported between the presence of bleeding at presentation and the risk of ICH.

5.4.4.3 ***Number and underlying characteristics of cases of intracranial haemorrhage***

There were 1159 cases of ICH within all 255 included studies. The characteristics of the cases were very poorly reported (Table 5.14).

Table 5.14: Missing Data on 1159 cases within 255 studies

Baseline Characteristics	**% missing data**
Diagnosis	26.4
Age Paediatric (< 18yrs) vs. Adult	32.4
Treatment Chemotherapy/stem cell transplant/at diagnosis	31.1
Disease status	52.8
Gender	65.0
Outcomes	
Mortality	**18.4**
Morbidity	78.1
Type of ICH	78.8
Possible Risk Factors	
Platelet count at time of ICH	72.6
Graft vs. Host Disease	78.1
White cell count at diagnosis	80.7
Disseminated intravascular coagulation/Coagulopathy	82.1
Preceding CNS abnormalities	84.5
Infection/Fever	84.5
Preceding trauma	92.2
Anticoagulation	94.7

Characteristics with less than 20% missing data are highlighted in **bold**. Only mortality fulfils this criterion.
NB: Data on all other factors pre-specified in the protocol were missing for more than 95% of cases.

Table 5.15: Baseline characteristics and risk factors reported in more than 20% of cases

Characteristic	Overall N = 1159 (%)
Age	
Paediatric (< 18yrs)	192 (16.6)
Adult (All)	592 (51.1)
Unknown	375 (32.4)
Gender	
Male	219 (18.9)
Female	187 (16.1)
Unknown	753 (65.0)
Diagnosis	
Acute leukaemia (All)	613 (52.9)
Acute myeloid leukaemia (All)	447 (38.6)
Acute promyelocytic leukaemia	194 (16.7)
Acute lymphocytic leukaemia	83 (7.2)
Chronic myeloid leukaemia	66 (5.7)
Lymphoma (All)	47 (4.1)
Non-Hodgkin's lymphoma	35 (3.0)
Hodgkin's lymphoma	1 (0.1)
Myeloma	8 (0.7)
Severe aplastic anaemia	67 (5.8)
Myelodysplastic syndromes	25 (2.2)
Other	26 (2.3)
Unknown	306 (26.4)
Disease status	
Active disease	516 (44.5)
Newly diagnosed	291 (25.1)
Relapsed/ refractory	92 (7.9)
Partial remission	4 (0.3)
Complete remission	33 (2.8)
Unknown	612 (52.8)
Treatment	
Chemotherapy	322 (27.8)
Haematopoietic stem cell transplant (HSCT) (All)	378 (32.6)
Autologous HSCT	32 (2.8)
Allogeneic HSCT	209 (18.0)
None (Intracranial haemorrhage at diagnosis)	98 (8.5)
Unknown	361 (31.1)

Characteristic	Overall N = 1159 (%)
Platelet count at time of intracranial haemorrhage (x 10^9/l)	
> 50	19 (1.6)
< 50	205 (17.7)
< 20	111 (9.6)
< 10	40 (3.5)
> 20 (not otherwise specified)	42 (3.6)
Thrombocytopenic (not otherwise specified)	51 (4.4)
Unknown	842 (72.6)

Due to the large amount of missing data, it is difficult to make any firm conclusions about risk factors associated with the cases reported within the literature (Table 5.15). However, it is interesting to note that over 70% (614/853) of cases whose diagnosis was known had acute leukaemia. Over 90% (516/547) of cases whose disease status was known had active disease. Only 4% (32/798) of cases whose treatment was known were receiving an autologous HSCT. The platelet count at the time of ICH is unknown for the majority of cases. No conclusions can be drawn about the small number of cases with a platelet count > 50 x 10^9/L as this may be an artefact of this study's inclusion and exclusion criteria.

(See Appendices 5.6 & 5.8 to 5.11 for characteristics of cases).

5.4.5 Secondary outcomes

5.4.5.1 *Mortality due to ICH*

Overall mortality was reported for 81.6% of cases (946/1159) (Table 5.16). Of these 73.9% (699/946) died, and 88.4% (618/699) of these deaths were due to ICH. However, 59% (150/255) of the studies (444/946 cases) only reported deaths due to ICH. If only

those studies that reported all cases of ICH were reviewed 50.8% (255/502) of cases died, and 92.9% (237/255) of these deaths were due to ICH.

Table 5.16: Morbidity and Mortality associated with intracranial haemorrhage (ICH)

Characteristic	Overall N = 1159 (%)	Cases within studies that reported deaths due to ICH only N = 444 (%)	Cases within studies that reported all ICH N = 502 (%)	Cases within studies that did not report mortality N = 213 (%)
Death (All)	699 (60.3)	444 (100.0)	255 (50.8)	0 (0.0)
Death Due to ICH	618 (53.3)	381 (85.8)	237 (47.2)	0 (0.0)
Death unrelated to ICH	64 (5.5)	46 (10.4)	18 (3.6)	0 (0.0)
Death (cause unknown)	17 (1.5)	17 (3.8)	0 (0.0)	0 (0.0)
Alive (All)	247 (21.3)	0 (0.0)	247 (49.2)	0 (0.0)
Alive (Neurological impairment)	17 (1.5)	0 (0.0)	17 (3.4)	0 (0.0)
Alive (Normal)	37 (3.2)	0 (0.0)	37 (7.4)	0 (0.0)
Alive (Deficit unknown)	193 (16.7)	0 (0.0)	193 (38.4)	0 (0.0)
Unknown	213 (18.4)	0 (0.0)	0 (0.0)	213 (100.0)

5.4.5.2 *Morbidity due to ICH*

Overall, 247/946 cases survived, but morbidity was very poorly reported. The morbidity associated with ICH was unknown in 78.1% (193/247) of cases that survived (Table 5.16).

5.4.5.3 *Types of ICH (extradural, subdural, subarachnoid, intra-parenchymal, intra-ventricular)*

The type of ICH was poorly reported, and was unknown in 79% of cases (913/1159) (Table 5.17). The most common type reported was intra-parenchymal haemorrhage (62.3%, 154/246). The type of ICH was much more likely to be reported in the case

studies or case series than the cohort studies (RCT, non-randomised interventional trial, and observational studies).

Table 5.17: Types of intracranial haemorrhage (ICH) reported number (%). Overall and for different types of study

Type of ICH	Overall N = 1159	Randomised Controlled trial N = 125	Non-randomised interventional trial N = 117	Observational Cohort study N = 563	Post-mortem study N = 160	Case Series N = 145	Case Studies N = 49
Subdural	77 (6.6)	7 (5.6)	6 (5.1)	27 (4.8)	4 (2.5)	27 (18.6)	6 (12)
Extradural	3 (0.3)	1 (0.8)	0 (0.0)	1 (0.2)	0 (0.0)	0 (0.0)	1 (2.0)
Intra-parenchymal	154 (13.3)	5 (4.0)	1 (0.9)	72 (12.8)	28 (17.5)	27 (18.6)	21 (43)
Intra-ventricular	26 (2.2)	0 (0.0)	0 (0.0)	14 (2.5)	1 (0.6)	6 (4.1)	5 (10)
Subarachnoid	50 (4.3)	5 (4.0)	0 (0.0)	24 (4.3)	12 (7.5)	6 (4.1)	3 (6)
Case has >1 type	58 (5.0)	3 (2.4)	0 (0.0)	26 (4.6)	14 (8.8)	8 (5.5)	7 (14)
Unknown	913 (78.8)	111 (88.8)	110 (94.0)	454 (80.6)	130 (81.2)	88 (60.7)	20 (41)

5.5 Discussion

5.5.1 Summary of evidence

This systematic review of a decade of the literature has shown that ICH is usually reported as an adverse event within studies, and it is usually poorly described. Despite this, this review has highlighted some interesting findings that need to be confirmed within further studies. Potential risk factors include: a higher risk of bleeding in paediatric patients; a higher risk of bleeding in women; a higher risk of bleeding in patients with

acute leukaemia; a lower risk of bleeding in patients receiving an autologous HSCT; a higher risk of bleeding in patients with a high white cell count at diagnosis; a higher risk of bleeding in patients with coagulation abnormalities; and a higher risk of bleeding in patients with renal impairment (Table 5.18).

Other factors that may increase a patient's risk of bleeding is whether they have active disease, 94% of cases in this review, in which the disease status was known had active disease.

5.5.2 **Strengths of this review**

There were no obvious biases within the review process. A wide search was conducted, the relevance of each paper was carefully assessed, and no restrictions were made for the language in which the paper was originally published.

5.5.3 **Limitations of this review**

This study was unable to provide an estimate of the incidence of ICH because only one study reported this and this study was only reported as an abstract.

Reporting of ICH in the published literature may be biased. For example, studies which included patients with acute promyelocytic leukaemia (APL), which is well known to have a high incidence of haemorrhage, were more likely to report the presence or absence of ICH. Overall, 27% of acute myeloid leukaemia (AML) patients within the included studies were known to have APL (2690/9937), whereas the actual incidence of APL is approximately 10% of new cases of AML.[311-313]

All the meta-analyses reported were only univariate analyses. They are therefore at risk of confounding. A confounder is associated with the risk factor and causally related to the outcome. A potential risk factor may not be a true association. A classic example is that people who carry matches have a greater chance of developing lung cancer; the confounder being smoking.[314] Carrying matches does not cause cancer but being a smoker means you are more likely to carry matches and more likely to develop lung cancer.

5.6 Summary

This systematic review has highlighted some potential risk factors for ICH in patients with haematological malignancies (Table 5.18). Due to the large amount of missing data within this review, no firm conclusions about risk factors can be made, and these putative risk factors need to be corroborated by other studies. This systematic review led to the development of a UK-wide case-control study of ICH in haematological disorders (InCiTe study) (Chapter 6).[315]

Table 5.18: Summary of potential risk factors for intracranial haemorrhage

Risk Factor	Studies	Number of participants	Meta-analysis	Narrative analysis	Hazard ratio (95% CI)
Baseline Characteristics					
Age (Paediatric vs. adult)	6	3129	No	Paediatric patients may have a higher risk of ICH	-
Sex (Female vs. male)	46	7359	Yes (11 studies) (3960 participants)	Studies excluded from meta-analysis did not confirm this finding	1.87 (1.18 to 2.77)
Underlying diagnosis (AL vs. other diagnoses)	42	6927	Yes (5 studies) (1432 participants)	Studies excluded from meta-analysis showed a similar trend to meta-analysis	2.29 (1.21 to 4.33)
Underlying diagnosis (APL vs. other ALs)	10	3222	Yes (2 studies) (1633 participants)	Studies excluded from meta-analysis showed a similar trend to meta-analysis	7.37 (2.96 to 18.39)
Treatment					
AutoHSCT vs. alloHSCT	16	4213	Yes (3 studies) (695 participants)	Largest study excluded from meta-analysis showed a similar trend (1245 participants), the other studies showed no overall trend	0.09 (0.03 to 0.30)
AutoHSCT vs. chemotherapy	6	1740	Yes (2 studies) (591 participants)	Studies excluded from meta-analysis showed a similar trend to meta-analysis	0.13 (0.04 to 0.43)
Baseline laboratory results					
WCC at presentation > 100 (AL studies)	5	3264	No	Patients with high WCC at presentation have a higher risk of bleeding	-
WCC at presentation > 10 (APL studies)	3	900	No	Patients with high WCC at presentation may have a higher risk of ICH	-
Coagulation abnormalities at presentation (APL studies)	4	1130	No	Patients with APL with coagulation abnormalities at presentation have a higher risk of ICH	-
Co-morbidities					
Abnormal renal function	4	793	No	Patients with abnormal renal function may have a higher risk of ICH	-

6 Intracranial haemorrhage in thrombocytopenic haematology patients. A nested case-control study.

6.1 Introduction

To advance the quality of care for haematology patients (when they are receiving 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 thrombocytopenia 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.[13] In patients with haematological malignancies, platelets issued to prevent thrombocytopenic bleeding account for up to 69% of all platelets issued to that patient population.[8,316] Platelet transfusions are not without risk and therefore a 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.[2,5-7]

The morning platelet count has been used, up to now, to indicate when a patient requires prophylactic platelet transfusions. The consensus from the BCSH guidelines was that patients should receive a platelet transfusion when the platelet count is $<10 \times 10^9$/L unless there are other risk factors for haemorrhage such as sepsis, concurrent use of antibiotics or other abnormalities of haemostasis.[15] A patient's morning platelet count has been shown to be a poor predictor of haemorrhage.[75] In a recent study the rate of bleeding was similar over a broad range of platelet counts (6 to 80 x 10^9/l).[6] This is unsurprising as a patient's platelet count indicates only the presence of a specific number of platelets within the circulation, but 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 at the moment.

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,[317] 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.[318,319]

A systematic review of the literature (2000 to 2010) has been performed (Chapter 5) which showed that cases of ICH in haematology patients have been very poorly reported.[320] Data about postulated risk factors from this systematic review and from known risk factors for ICH in the general population were asked about within the data collection forms.

6.2 Study Objectives

6.2.1 Primary Objective

What factors (e.g. age, type of haematological disease, treatment, infection) are associated with an increased risk of developing an intracranial haemorrhage (ICH)?

6.2.2 Secondary Objectives

What are the short-term outcomes for these patients (e.g. death within 30 days of haemorrhage, persistent neurological deficit)?

6.3 Study Design

This is a case-control study nested within a prospective surveillance system.

The study is collecting anonymous data about haematological patients who have had an intracranial haemorrhage (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 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.[321] Information is being collected through doctors and specialist nurses in hospitals throughout the UK. An information collection system has been developed for this study and will be based on a similar systems in obstetrics (UKOSS). Assistance in the development of this system was via the British Society of Blood and Marrow Transplantation (BSBMT) and advice from UKOSS. Now 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.

6.3.1 **Recruitment**

Patients are being identified through a new haematological surveillance system (Haematology Active Surveillance System) "HASS", which was based on similar obstetric (UKOSS) and paediatric surveillance systems (BPSU), to study rare haematological disorders.

6.3.2 **Case Identification**

This anonymous descriptive, case-control study is being conducted through a monthly case-collection scheme. Each hospital with a haematology department caring for patients with acute leukaemia or transplant patients were asked to participate in the study.

The individuals within each haematology department were identified via BSBMT (principal transplant consultant and transplant co-ordinator, specialist nurse) and individuals associated with AML-16 trial or other current MRC (Medical Research Council) leukaemia trials for non-transplant cases (principal investigator for the current leukaemia trials at each centre and research nurses associated with the trials) using the MRC leukaemia trials e-mail list.

Once a local investigator had agreed to participate and the hospital had given Research and Development (R&D) approval, then every month four nominated individuals are sent a study report card (in an e-mail format). They are asked to complete a simple Yes/No tick box indicating if any cases have occurred in the previous month. I expected that the majority of cards each month would be “No cases” because ICH is a rare event. “No cases” responses are extremely important because they allow the study to confirm the number of haematology patients in the denominator cohort.

6.3.3 **Control Identification**

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

6.3.4 **Study Participants**

Cases: All adult patients (≥ 16 years of age) who had an ICH while receiving or about to receive myeloablative chemotherapy (expected to cause a significant thrombocytopenia < 50 x 10^9/L for > 5 days) or a stem cell transplant within the study period (1st May 2011 to 30th April 2015). Only patients being treated or about to be treated with curative intent are 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 intracranial haemorrhage). All types and severities of intracranial haemorrhage are included.

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

6.3.5 **Data Collection**

On receiving a case report, the central team dispatch case and control data collection forms to the clinician. The data collection forms seek additional information on risk factors in both cases and controls, and the management and outcome of the ICH. Cases and controls are allocated a central HASS identification number. No names, addresses,

dates of birth, hospital numbers or NHS numbers are 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 four weeks, a written reminder is sent out. If there is still no response after a further four weeks, a further set of forms is sent to the centre (to ensure that non-return is not due to the forms going missing) and the clinician is contacted by telephone. After a further four 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. Thus, this study only involves the provision of information after the acute event has occurred. 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 is being monitored throughout the course of the study, as part of the routine operation of HASS. A three-monthly newsletter is being produced to inform all of the reporting clinicians of response rate to the study, and number of cases reported.

6.3.6 **Rationale for questions to be asked within the data collection form**

6.3.6.1 ***Clinical Factors associated with increased risk of ICH in the general population***

Age: Risk of ICH increases with age. In a systematic review of the literature a RR of ICH 1.97 for every 10yr increase in age (95% CI 1.79 to 2.17).[322] In a pooled prospective study of over 21000 individuals there was a RR 2.06 for every 10yr increase in age (95% CI 1.76 to 2.51).[323]

Sex: Some studies have shown a significantly higher rate of ICH in men. RR in general population male vs. female for ICH 3.73 (95% CI 3.28 to 4.25).[322] No difference was seen in Sturgeon *et al (2007).*[323]

Ethnic Origin: Young and middle-aged blacks have a substantially higher risk of sub-arachnoid (SAH) or intra-cerebral haemorrhage than whites of a similar age [2.1 x risk of SAH (95% CI 1.3 to 3.6); 1.4 x risk of intra-cerebral haemorrhage (95% CI 0.9 to 2.1):[324] *In* Sturgeon *et al* study RR 2.56 (95% CI 1.8 to 3.65)].[323]

Smoker: Current smoker RR 1.31 (95% CI 1.09 to 1.58) (Ariesen *et al, 2003).*[322] No difference seen in Sturgeon *et al (2007).*[323]

Hypertension: Patients with systolic blood pressure (BP) ≥ 160mmHg or diastolic BP ≥110mmHg RR 5.55 (95% CI 3.07 to 10.0).[323] In Ariesen *et al* study 2003 RR 3.68 (95% CI 2.52 to 5.38).[322]

Diabetes: In Ariesen *et al* study 2003 RR 1.3 (95% CI 1.02 to 1.67).[322] No difference seen in Sturgeon *et al (2007).*[323]

Site of haemorrhage: Site of haemorrhage is a predictor of functional outcome in the general population.[325] In a recent study, poor outcomes after ICH in AML patients were associated with four independent risk factors, three of which were associated with the site of the haemorrhage. Brainstem haemorrhage (P = 0.001), sub-arachnoid haemorrhage (SAH) (P = 0.017), and extra-dural haemorrhage (EDH) (P = 0.014).[103]

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.[325-327]

Glasgow coma scale (GCS) at time of haemorrhage: In the general population this was another important predictor of functional outcome. The FUNC score is a functional outcome risk stratification score that has been developed and validated in the general population. This scoring system stratifies patients into whether they presented with a GCS less than 9 or not. Those patients with a lower GCS had a much worse outcome.[325]

6.3.6.2 *Clinical Factors associated with haemorrhage in haematology patients*

A recent history of severe bleeding: In a review of almost 3,000 thrombocytopenic adult patients, over a 10-year period, Friedmann showed a significant relationship between a history of recent bleeding (within the previous five days) and occurrence of significant haemorrhage (OR 6.72; 95% Cl, 5.53 to 8.18).[75]

Uraemia: In Friedmann's 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% Cl, 1.40 to 1.92).[75]

Recent bone marrow transplantation: In Friedmann's study a recent bone marrow transplant (under 100 days) was associated with an increased risk of bleeding (OR 1.32; 95% CI, 1.22 to 1.43).[75]

Hypoalbuminaemia: In Friedmann's study, hypoalbuminaemia (defined as a serum albumin of 2.0 gm/dL or lower) was associated with an increased risk of bleeding (OR 1.54; 95% CI, 1.33 to 1.79).[75]

Acute Graft vs. 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.[111] 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% C.I. 1.2 to 8.2).[108]

Veno-occlusive disease (VOD): Najima *et al* and Zumberg *et al* also showed a possible association of VOD of any grade with an increased risk of haemorrhage, although the confidence intervals were very wide (Hazard ratio 2.63 (95% CI 0.77 to 9.00) (Najima *et al*, 2009);[111] (OR 4.4; 95% CI 0.6 to 27.8) (Zumberg *et al*, 2002).[108]

Fever: In Webert *et al*'s retrospective analysis[109] of Rebulla *et al*'s data[68] the presence of an elevated body temperature increased the risk of mild bleeding (WHO grade 1 & 2) 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 (WHO grade 2 to 4) 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 to 38.4^{o}C (RR 2.43; 95% CI (1.0 to 5.90); p< 0.05); and > 38.5^{o}C (RR 3.95; 95% CI (1.90 to 8.20); p=0.0001). In a previous small study 9 out of 13 patients who bled were febrile at the time of haemorrhage. However, this study was performed when aspirin was still used as an anti-pyretic.[123,328]

Use of amphotericin: Therapeutic use of amphotericin B is associated with decreased expression of glycoprotein Ib on the surface of stored platelets.[110] This may induce a platelet function defect. In Zumberg's study usage of amphotericin B was associated with an increased risk of bleeding (OR 3.8; 95% CI 1.3 to 10.5).[108]

Use of antibiotics: Beta lactam antibiotics have been associated with platelet dysfunction.[329,330]

Presence of disseminated intravascular coagulation (DIC): DIC is strongly associated with acute promyelocytic leukaemia. Patients who have DIC associated with their leukaemia are known to be at a high risk of severe bleeding.[331] In this study the International Society of Thrombosis and Haemostasis (ISTH) definition of overt DIC was used to define cases of DIC.[332]

6.3.6.3 *Laboratory factors associated with haemorrhage*

CRP: Inflammation has been shown to induce severe haemorrhage in thrombocytopenic mice.[114]

Prothrombin Time: Prolonged PT was associated with a poorer outcome after ICH in AML patients ($P < 0.001$).[103]

Haemoglobin: A low haematocrit has been shown to be associated with an increased risk of bleeding.[199,200,202]

Persistent Thrombocytopenia: Platelets have been shown to provide an endothelial supportive function by plugging gaps in the endothelium of otherwise intact blood vessels.[333] 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.[333-335] This thinning and fenestration of the endothelium is accompanied with on-going and increased use of circulating platelets to prevent the loss of red blood cells (RBCs) through these gaps. In a study of 1402 bone marrow transplant (BMT) patients very low platelet counts were significantly associated with bleeding post BMT.[336,337] The risk of bleeding in a patient with 3 to 7 (out of 7) days of platelet counts $<10 \times 10^9$/l in the week preceding the haemorrhage was 40 to 60% higher than a patient with 0 to 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 $>20 \times 10^9$/l.[336,337]

6.3.7 Definition of End of Study/Study Power

The study will be completed at the end of four years if a sufficient number of cases have been reported to allow detection of an odds ratio (OR) of 2.0 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 (Table 6.1).[75,99]

Table 6.1 Variables and the number of cases required to detect relative risks (RR) of 1.5 to 3 with both 80% and 90% power

Variable	Estimated Incidence from studies	Number of cases required to detect with 80% power				Number of cases required to detect with 90% power			
		RR 3	RR 2.5	RR 2	RR 1.5	RR 3	RR 2.5	RR 2	RR 1.5
General factors									
Fever	0.36[1]	55	78	135	399	71	101	177	521
Amphotericin B	0.44[2]	55	77	133	387	72	101	174	506
Uraemia	0.15[3]	77	115	212	670	100	150	277	876
Antibiotics	0.34	55	78	136	404	72	102	178	528
Transplant related Factors (estimated 50% cases will be transplant cases)									
Acute GvHD	0.25[4]	59	86	154	470	77	112	201	613
VOD	0.14[5]	81	120	222	705	105	157	290	920
RR = relative risk									

I 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 two year period. This was

[1] Estimates for fever (0.39) Friedmann *et al* 2002 and (0.32) Slichter *et al* 2005.
[2] Estimates for usage of Amphotericin B (0.404) Friedmann *et al* 2002 and (0.48) Slichter *et al* 2005
[3] Estimate of incidence of uraemia and usage of antibiotics Friedmann *et al* 2002
[4] Estimate of incidence of acute GvHD (Grade III to IV) Cahn *et al* 2005
[5] Estimate of incidence of VOD Coppell *et al* 2010

because the majority of odds ratios (OR) identified from previous studies of ICH in the general population detected significant OR for individual factors of between1.5 to 2.[75,103,108,109,111,322-324] The 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%.[6,68,111,112,338,339] The study initially expected that the true estimate was between 1 to 2%, with a prediction of 120 to 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 four years after a review of initial recruitment by the study management group.

The analysis in this thesis is an interim analysis and the study will be completed in 2015.

6.3.8 **Denominator Data**

In the final analysis 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 2,939 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 (Medical Research Council) 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. This analysis will not be performed until the end of the study in 2015.

6.3.9 **Analysis**

This is an interim analysis of the case-control study after 85 case and control forms have been returned to the study centre. A complete analysis will be performed once the study has been completed in May 2015.

An estimate of the incidence of ICH has not been performed within this interim analysis, and will only be performed once the study has been completed.

A conditional logistic regression (CLR) model was used throughout this analysis for both univariable and multivariable analyses clustered on case identity.

Crude odds ratios with 95% confidence intervals were calculated. A model was then created that included factors that were known in 84/85 cases and controls and had a p-value less than 0.25 in the univariable analysis. This allowed for potential factors that may not be significant in a univariable analysis but are significant in a multivariable analysis.

A model was created in a step-wise fashion adding factors, assessing the effect of the factor using likelihood ratio tests (LRT) ($P < 0.1$ was considered significant) and testing for interactions at each stage.

Variables tested within the model included: gender, previous medical history of hypertension, previous history of fungal infections, complete remission, relapsed or

refractory disease, WCC ≥ 50 x 10^9/L, presence of DIC, platelet count, and previous history of ischaemic heart disease.

Once this model had been created additional risk factors that were reported in fewer cases and controls were added one by one to assess the effect of adding the factor to the model using likelihood ratio tests (LRT) ($P < 0.1$ was considered significant) and testing for interactions at each stage. One potential risk factor (fibrinogen) could not be assessed in this way due to the large amount of missing data.

6.3.10 Ethics and dissemination

This study has been approved by Oxford Research Ethics Committee B (10/H0605/78) (Appendix 6).

This study seeks to collect information about haematological patients with an intracranial haemorrhage. This information is key to identifying means to improve treatment and quality of care.

6.3.10.1 *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 pseudo-anonymised 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.

6.3.10.2 *Data Security*

The security of all data will be maintained by storage on NHS Blood and Transplant's 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.

6.3.10.3 *Dissemination of Study Information*

The study has been prospectively registered on the Clinical Trials website 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.

6.4 Results

Table 6.2 Baseline Characteristics. Number of cases and controls with each characteristic and crude odds ratio for each characteristic with 95% confidence interval (CI) and p-values calculated using univariable conditional logistic regression controlling for case id

Potential risk factor	Cases	Controls	Total	Odds Ratio	95% CI	P value (LRT)
Female vs. Male	40/85	25/85	65/170	2.25	1.14 to 4.44	0.015*
Age ≥ 60 yrs	41/85	39/85	80/170	1.13	0.57 to 2.21	0.732
Other ethnic group vs. white	7/85	10/85	17/170	0.57	0.17 to 1.95	0.363
Obese vs. not obese	17/77	12/81	29/158	1.86	0.74 to 4.65	0.176
Smoker vs. non-smoker	13/69	15/73	28/142	1.00	0.40 to 2.52	1.000
Hypertensive	14/85	7/85	21/170	2.75	0.88 to 8.64	0.065
Diabetic	6/85	10/85	16/170	0.56	0.19 to 1.66	0.282
Ischaemic heart disease	5/85	2/85	7/170	2.50	0.49 to 12.89	0.249
Previous fungal infection	7/85	2/85	9/170	6.00	0.72 to 49.84	0.047*
Previous history of renal impairment	6/85	6/85	12/170	1.00	0.20 to 4.95	1.000
Previous stem cell transplant	2/85	5/85	7/170	0.00	-	-
Previous clinically significant bleed	10/85	9/85	19/170	1.17	0.39 to 3.47	0.781
Previous CNS complications	6/85	5/85	11/170	1.33	0.30 to 5.96	0.705
Refractory to platelet Tx	12/85	4/85	16/170	5.00	1.10 to 22.82	0.016*
Complete remission†	11/85	26/84	37/169	0.29	0.12 to 0.71	0.003**
Relapsed/refractory	24/84	13/84	37/168	3.20	1.17 to 8.73	0.014*

No case or control had a previous medical history of a cerebrovascular accident
LRT = likelihood ratio test
† Includes MRD positive patients * $p < 0.05$ ** $p < 0.01$

6.4.1 **Baseline Characteristics – univariable analysis**

On the univariable analysis, female sex, having a relapsed or refractory haematological disorder, being refractory to platelet transfusions and having a previous fungal infection were all risk factors for ICH (Table 6.2). Whereas, being in complete remission from their haematological disorder meant a patient was less likely to have an ICH (Table 6.2).

6.4.2 **Laboratory results – univariable analysis**

On the univariable analysis, low fibrinogen (< 2.5g/l), a high white cell count (> 50 x 10^9/l), a high C-reactive protein (CRP), impaired renal function (raised creatinine or reduced creatinine clearance), and a low albumin were all associated with an increased risk of an ICH (Table 6.3).

Table 6.3 Laboratory results at time of haemorrhage compared to blood results at D7 after stem cell transplant or D10 after chemotherapy in control patient. Number of cases and controls with each characteristic and crude odds ratio for each characteristic with 95% CI and p-values calculated using univariable conditional logistic regression

Potential risk factor		Cases	Controls	Total	Odds Ratio	95% CI	P value
High PT/INR (INR ≥1.5 or PT > 18 s)		12/84	5/81	17/165	2.75	0.88 to 8.64	0.065
High aPTT/aPTTr (APTTr ≥1.5 or APPT > 40 s)		6/84	5/81	11/165	1.00	0.29 to 3.45	1.000
Fibrinogen < 2.5 g/l		19/53	10/54	29/107	4.33	1.23 to 15.21	0.009**
WCC ≥ 50 x 10^9/l		12/85	1/85	13/170	12.00	1.56 to 92.29	0.001**
CRP ≥100 mg/l		27/70	12/74	39/144	3.14	1.34 to 7.36	0.004**
Haemoglobin ≤85 g/l		29/85	27/84	56/169	0.94	0.48 to 1.86	0.862
Platelet count > 20 x 10^9/l		34/85	47/85	81/170	0.71	0.51 to 0.99	0.041*
Creatinine > 120 µmol/l		16/84	5/83	21/167	6.50	1.47 to 28.80	0.003**
Creatinine clearance ≤ 50 ml/min		11/78	4/82	15/160	4.50	0.97 to 20.83	0.028*
Liver impairment (Bilirubin > 34 µmol/l)		9/85	6/85	15/170	2.00	0.50 to 8.00	0.313
DIC (ISTH definition)		9/85	4/85	13/170	3.50	0.73 to 16.85	0.086
Albumin (g/l)	**≤20**	9/82	1/83	10/165	-	-	<0.001**
	21 to 25	15/82	6/83	21/165	0.34	0.03 to 3.48	
	26 to 30	19/82	21/83	40/165	0.14	0.02 to 1.19	
	31 to 35	21/82	21/83	42/165	0.14	0.02 to 1.16	
	≥36	18/82	34/83	52/165	0.07	0.01 to 0.63	
CRP (mg/l)	**<10**	6/70	22/74	28/144	-	-	<0.001**
	10 to 99	37/70	40/74	77/144	6.11	1.37 to 27.28	
	≥100	27/70	12/74	39/144	14.43	2.84 to 73.27	

aPTT = activated partial thromboplastin time; aPPTr = aPPT ratio; CRP = C-reactive protein; DIC = disseminated intravascular coagulation; INR = international normalised ratio; PT = prothrombin time; s = seconds; WCC = white cell count

6.4.3 Multivariable conditional logistic regression analysis

An initial model was created that included all factors that were known in 84/85 cases and controls and had a p-value less than 0.25 in the univariable analysis. This allowed for potential factors that may not be significant in a univariable analysis but are significant in

a multivariable analysis. Variables tested within the model included: gender, previous medical history of hypertension, previous history of fungal infections, complete remission, relapsed or refractory disease, WCC > 50 x 10^9/l, presence of DIC, platelet count, and previous history of ischaemic heart disease.

Table 6.4 Model 1: Multivariable conditional logistic regression model for pre-specified potential risk factors that were reported in 84 cases and controls

Potential risk factor	Odds Ratio	95% CI	P value
Sex	2.39	1.02 to 5.62	0.045*
Hypertension	3.79	0.86 to 16.70	0.078
Previous fungal infection	6.30	0.62 to 64.50	0.121
Complete remission	0.26	0.08 to 0.82	0.021*
Platelet count ≤ 20 x 10^9/l	2.67	1.14 to 6.28	0.024*
WCC > 50 x 10^9/l	12.93	1.59 to 104.85	0.017*
WCC = white cell count * p < 0.05			

In this model female sex, a platelet count ≤ 20 x 10^9/l, and a white cell count > 50 x 10^9/l were all independent risk factors for developing an ICH (Table 6.4). Patients who were in complete remission from their haematological disorder had a lower risk of ICH (Table 6.4). No potential interactions had been pre-specified in this analysis. The only biologically plausible interaction detected was between gender and hypertension. However, there was only weak evidence that a possible interaction exists (P = 0.08), and this interaction term was therefore not included in the model.

When additional potential risk factors were added to the multivariable model, only one risk factor was found to be statistically significant, and this risk factor was the C-reactive

protein (CRP) (Table 6.5). Other factors that were tested in the model that were not found to be significant were: low albumin, renal impairment (measured by creatinine > 120μmol/l), high PT or INR. Fibrinogen was unable to be tested in the multivariable model due to the large number of case and control pairs in which this potential risk factor was not reported. Creatinine clearance was not tested in this model because it was reported in fewer case-control pairs than the serum creatinine.

Table 6.5 Model 2: Model including only 66 cases and controls, including inflammation as a risk factor (measured by CRP). CRP was only reported for both cases and controls in a sub-set of the data.

Potential risk factor	Odds Ratio	95% CI	P value
Sex	3.34	1.18 to 15.86	0.027*
Hypertension	6.76	0.83 to 55.15	0.075
Previous fungal infection	5.01	0.39 to 63.87	0.215
Complete remission	0.19	0.04 to 0.84	0.028*
Platelet count ≤ 20 x 10^9/l	2.50	0.82 to 7.60	0.106
WCC > 50 x 10^9/l	19.75	1.90 to 204.87	0.012*
CRP > 100 mg/l	4.33	1.18 to 15.86	0.027*

CRP = C-reactive protein; WCC = white cell count
* $p < 0.05$

In this model female sex, and a white cell count > 50 x 10^9/L remained independent risk factors for developing an ICH (Table 6.4). Patients who were in complete remission from their haematological disorder also had a lower risk of ICH (Table 6.4). A platelet count ≤ 20 x 10^9/l was no longer found to be statistically significant, however the 95% confidence

interval was wide. No potential interactions were detected in this analysis. The only additional factor that was found to be statistically significant in this multivariable model was the CRP.

6.4.4 Outcomes of the cases of ICH

In this interim analysis there were 85 cases of ICH.

Forty-seven of the cases (55%) died within 30 days of the ICH. 94% (44/47) of those who died, died due to their ICH (Table 6.6). Thirty-eight cases survived the ICH, however, of these, 13/38 (32%) had a persistent neurological impairment at 30 days or at discharge from hospital. Therefore less than a third of patients who had an ICH survived the event with no long term sequelae (Table 6.6).

Twenty-seven of the cases (32%) had a Glasgow Coma Scale (GCS) less than 9 at presentation. Over eighty percent (22/27, 82%) of patients with a GCS less than 9 died, whereas only 43% (25/58) died if their GCS at presentation was greater than or equal to 9.

Table 6.6. Outcomes of the cases of ICH

Outcome	Number (%)
Death	47/85 (55)
Neurological impairment	13/85 (15)
Neurologically normal	25/85 (29)

The specific volume of haemorrhage was often not reported on the imaging report therefore no comment can be made about whether the volume of haemorrhage affected mortality or functional outcome.

Of those thirteen patients that had a neurological impairment, only two (15%) were independent in all activities of daily living (ADLs) (Table 6.7). Most of the patients had significant neurological impairments requiring long-term care and support (Table 6.7).

Table 6.7. Outcomes of the cases of ICH with neurological impairment (13 cases)

Outcome	Number (%)
Activities of daily living (ADLs)	
Fully independent in all activities of daily living (ADLs)	2/13 (15)
Partially dependent for independent ADLs	6/13 (46)
Partially dependent for basic ADLs	3/13 (23)
Fully dependent	2/13 (15)
Motor impairment	
Facial weakness	3/13 (23)
Limb weakness	8/13 (62)
Sensory impairment	
Altered sensation	2/13 (15)
Visual loss	1/13 (8)
Language	
No impairment	5/13 (39)
Loss of fluency	5/13 (39)
Fragmentary speech	2/13 (15)
Aphasic	1/13 (8)
Cognitive ability	
No impairment	9/13 (69)
Impaired but able to answer simple questions	2/13 (15)
Makes mistakes when asked simple questions	2/13 (15)

6.4.5 Site of the ICH

Table 6.8 Site of the intracranial haemorrhage (ICH)

Type of ICH	Number (%) of cases	Number (%) of cases who died
Subdural	29 (34)	12 (41)
Extradural	1 (1)	0 (0)
Intra-parenchymal	33 (39)	21 (64)
Intra-ventricular	7 (8)	5 (71)
Subarachnoid	9 (11)	5 (56)
Case has > 1 type	6 (7)	4 (67)
Total	85 (100)	47/85 (55)

Patients who had a subdural haemorrhage appeared to have a lower risk of death than other types of ICH (Table 6.8). Patients who had an intra-ventricular or intra-parenchymal haemorrhage appeared to have a higher risk of death than other types of ICH (Table 6.8). Only one patient had an extradural haemorrhage.

Six patients had a brainstem haemorrhage and 3 of these patients died. No formal analysis of the differences between the types of ICH was performed in this interim analysis, because even if true differences were present they were unlikely to be statistically significant.

6.5 Discussion

This is the first study that I know of that has prospectively identified cases of ICH in this patient group. This interim analysis of the case-control study has identified some interesting findings.

Female sex has been found to be a risk factor for ICH (OR 3.34, 95% CI 1.18 to 15.86; P = 0.027). This was also identified as a potential risk factor in the systematic review of ICH (Chapter 5) and the TOPPS sepsis analysis (Chapter 4). A previous retrospective review has also identified female sex as a potential risk factor for bleeding.[208] This differs from the general population. A systematic review of fourteen case-control and eleven cohort studies found that men were much more likely to have an ICH than women (relative risk of ICH 3.73 (95% CI 3.28 to 4.25)).[322]

If patients were in complete remission from their haematological malignancy they were at much lower risk of developing an ICH. Patients with acute promyelocytic leukaemia (APL) commonly have a coagulopathy when they have leukaemic cells present. This is due to direct and indirect activation of the coagulation system and increased fibrinolysis.[104] Patients with other types of haematological malignancy do not have the marked derangements in their coagulation profile seen in patients with APL. However, increased fibrinolysis is not detected by routine coagulation tests.[173] If other types of cancer cells can directly or indirectly cause increased fibrinolysis, this could be a potential mechanism that increases a patient's risk of bleeding, without being detected on routine coagulation. A recent case series has demonstrated increased fibrinolysis and low levels of factor XIII in paediatric patients who have been recently diagnosed.[340]

A high white cell count was independently associated with an increased risk of bleeding. This was also seen in the systematic review (Chapter 5). A high white cell count is associated with the burden of leukaemia present, and can be associated with leucostasis. Both of these factors could predispose to bleeding.

A low platelet count was associated with an increased risk of bleeding. This was not statistically significant in the smaller data set that included CRP as a risk factor. However, there was a wide 95% CI, and with the additional data in the final analysis this factor may become statistically significant.

Although ICH is uncommon the impact of this complication is dramatic. Over 70% of affected patients died or had a significant neurological impairment.

6.5.1 Strengths of this study

Data were collected on a large number of pre-specified potential risk factors identified from the published literature. Cases were identified using a monthly reporting strategy developed for this study. This ensured that all hospitals that had enrolled on to this study (84 hospitals across the United Kingdom) reported all relevant cases. An appropriate conditional logistic regression analysis was performed using data from discordant pairs.

The only other case-control study of ICH that has been performed in haematology patients was in paediatric cases of immune thrombocytopenia.[341] This previous study reported on 40 cases and 80 matched controls reported over a 13 year time period. Seventeen of the cases were identified retrospectively and the prospective identification of cases did not use a stringent monthly reporting system, this could have biased the

selection of cases. Also, in this previous study, a conditional logistic regression analysis was not performed.

6.5.2 Limitations of this study

This is only an interim analysis, and is underpowered to detect certain associations. Only data that had been recorded within the patients' notes were available for this study. This meant that certain types of data, for example, volume of haemorrhage was often not reported.

6.5.3 Implications of this study

This study has shown that this nested case-control study design is feasible to study rare disorders in haematology patients.

6.6 Summary

- Factors that were independently associated with an increased risk of ICH were:
    - Female sex
    - Platelet count (≤ 20 x 10^9/l)
    - High white cell count (> 50 x 10^9/l)
    - C-reactive protein (> 100 mg/l)
- The only additional factor that was associated with a decreased risk of ICH was if the patient was in complete remission from their haematological diagnosis.
- This is only an interim analysis so these results have to be treated with caution until the full data-set can be analysed.

- The majority of patients who had an ICH in this study died. Only 29% of patients survived the ICH without any neurological impairment

7 Thesis summary and future work derived from this thesis

The aim of this thesis was to identify clinical and laboratory factors that may predict the risk of haemorrhage in patients with haematological malignancies and severe thrombocytopenia. This was achieved via several different study designs and assessing the effect of clinical and laboratory factors on any or clinically significant haemorrhage (WHO grade 2 or above) and their effect on intracranial haemorrhage (ICH). This work has provided new data that can be used to design a definitive method for assessing and grading bleeding in this patient group. It has provided a potential alternative candidate to the total platelet count to guide administration of platelet components and has provided evidence for risk factors for bleeding that can be used as the basis of future definitive studies. The main findings and implications are summarised below.

7.1 Assessment of Bleeding

There is currently no gold standard for the assessment and grading of bleeding in platelet transfusion trials (Chapter 2). This has led to major problems comparing the findings from different randomised controlled trials,[121] and consequently impaired the ability to make firm recommendations on how platelet transfusions should be administered. Two recent studies that both assessed a prophylactic versus therapeutic platelet transfusion policy differed in how bleeding was assessed and graded.[2,7] Although both studies supposedly used the same WHO grading system, they differed in the way they had assessed bleeding and also in the way they had modified the grading system.[2,7]

Consistency in assessing bleeding can be improved by minimising: inter-observer variability of bleeding assessors via staff training and provision of guide-notes that include definitions of the types of bleed. Variability in grading the same severity of bleed can also be minimised by using a validated computerised algorithm.

7.2 The absolute immature platelet number (AIPN) as an alternative to the total platelet count to guide administration of platelet transfusions

A low AIPN was associated strongly with increased bleeding both within 1 day and 2 days of blood sampling. There was also an approximately linear relationship between AIPN and the proportion of study days on which bleeding occurred in the study participants (Chapter 3). This association between AIPN and bleeding persisted after adjustment for the total platelet count within 1 day of blood sampling. The association between the total platelet count and bleeding lost statistical significance when adjusted for variation in AIPN, suggesting that the AIPN is a better predictor of bleeding than the total platelet count. Reticulated platelets are an immature platelet population that are newly synthesised in the bone marrow. Increasing circulating reticulated platelets is an early marker of thrombopoietic regeneration after chemotherapy or haemopoietic stem cell transplantation that precedes increases in the TPC by approximately two to three days.[158,182,342,343] If the findings from this study are confirmed it suggests that platelet transfusion support may be stopped two to three days earlier that currently occurs, reducing a patient's exposure to platelet transfusions.

The strong association between low AIPN and increased bleeding in this study suggests that endogenous immature platelets are an important determinant of haemostasis during treatment for haematological malignancies. This is consistent with previous reports that immature platelets have greater haemostatic function than mature or transfused platelets.[183,184] One recent small randomised controlled trial has tested this hypothesis. They compared a prophylactic platelet transfusion policy using components that had a low proportion of immature platelets to components that contained a high proportion of immature platelets.[344] Patients receiving platelet transfusions with high levels of immature platelets required fewer platelet transfusions (statistically significant) and there was a trend towards fewer bleeding episodes. Due to the small size of the study (20 patients in each arm) it is unlikely that the difference in bleeding episodes reached statistical significance – no statistics were reported.[344]

7.3 Risk factors for bleeding

7.3.1 Baseline characteristics

7.3.1.1 *Female Sex*

Female sex has been found to be an independent risk factor for haemorrhage in several of the studies within this thesis. It was associated with a reduced time to first clinically significant haemorrhage (HR 1.45; 95% CI 1.14 to 1.85), an increased risk of clinically significant bleeding the following day (OR 2.13; 95% CI 1.47 to 3.13) (Chapter4). It was also associated with an increased risk of ICH in the case-control study (OR 3.34, 95% CI

1.18 to 15.86; P = 0.027) (Chapter 6), and there was a suggestion that there was an increased risk in the systematic review (HR 1.87; 95% CI 1.18 to 2.77) (Chapter 5).

The reason for this gender difference is currently unexplained, because women have higher levels of factor VIII, von Willebrand factor and fibrinogen[345,346] than men and there is no clear evidence of a difference in the amount of fibrinolytic activity.[347]

7.3.1.2 *Complete remission from haematological malignancy*

If patients were in complete remission from their haematological malignancy they were at much lower risk of developing an ICH. This was found to be an independent risk factor in the case-control study (OR 0.19; 95% CI 0.04 to 0.84) (Chapter 6). This finding is also partially supported by the systematic review (Chapter 5) which found that over 90% of cases where the disease status was known had active disease. One possible explanation for this is that the haematological malignancy induces a coagulopathy. This is well recognized in patients with acute promyelocytic leukaemia (APL) who commonly have a coagulopathy when they have leukaemic cells present. This is due to direct and indirect activation of the coagulation system and increased fibrinolysis.[104] Coagulopathy is also associated with AML M5. Patients with other types of haematological malignancy do not often have the marked derangements in their coagulation profile seen in patients with APL and AML M5. However, increased fibrinolysis and factor XIII deficiency are not detected by routine coagulation tests.[173,340] Factor XIII is a transglutaminase that cross-links fibrin chains and is therefore essential for the formation of a stable clot. A recent case series has demonstrated increased fibrinolysis and low levels of factor XIII in paediatric patients who have been recently diagnosed with a malignancy.[340]

7.3.1.3 *Previous history of fungal infection*

Patients with a previous history of a fungal infection had an increased risk of clinically significant bleeding the following day (OR 2.26; 95% CI 1.32 to 3.88), and a decreased time to the first clinically significant bleeding event (HR 2.04; 95% CI 1.03 to 4.05). It was not found to an independent risk factor for bleeding in the case-control study (Chapter 6). However, the 95% CI was very wide (OR 6.30; 95% CI 0.62 to 64.50) and therefore it may also be a risk factor, but the study is currently underpowered to detect the effect. It is unknown why this could be a possible risk factor at the moment. Some possible reasons could be that:

- Direct tissue damage due to the fungal infection may predispose to bleeding at the that site
- A previous fungal infection is much more likely if a patient has had a larger number of courses of intensive chemotherapy. This treatment could have caused tissue damage or endothelial dysfunction leading to an increased risk of bleeding.

7.3.2 **Sepsis**

Two of the studies have found that a high C-reactive protein (CRP) predisposes to haemorrhage (Chapter 4 and Chapter 6). A CRP greater than or equal to 100 increased the odds of having a clinically significant bleed the following day by 1.83 times (95% CI 1.30 to 2.57) (Chapter 4), and increased the odds of having an ICH by 4.33 times (95% CI 1.18 to 15.86) (Chapter 6). None of the other potential risk factors associated with sepsis or infection (fever the previous day, therapeutic antibiotics or antifungal medication,

SIRS score, or ITU admission) that were considered in the TOPPS sub-study were independent risk factors for bleeding (Chapter 4). This finding fits in with the mouse model reported by Goerge et al that demonstrated that organ haemorrhage only occurred in thrombocytopenic mice at sites of inflammation.[114] The CRP is a simple and cheap marker for inflammation. This means that it could be a good potential candidate for guiding the management of patients with sepsis and severe thrombocytopenia. However, inflammation can be prothrombotic, by the activation of procoagulant proteins, inhibition of fibrinolysis, and down-regulation of natural anticoagulants.[348] For example, a high CRP has been associated with an increased risk of cardiovascular disease,[349] and thrombosis is common in severe sepsis,[350] and other types of malignancy.[351] Thrombosis also occurs in patients with haematological malignancies,[352-360] therefore any future guidance needs to ensure that any management that prevents bleeding does not produce a significant rise in thromboembolic complications.

7.3.3 **Other clinical risk factors**

7.3.3.1 ***Renal impairment***

Renal impairment was associated with a higher risk of bleeding in the TOPPS sub-study (Chapter 4), it was also associated with a higher risk of bleeding in the systematic review (Chapter 5). It is well recognized that uraemic patients develop an acquired platelet dysfunction[361] and impaired platelet-vessel wall interactions.[362] The pathogenesis of this haemostatic dysfunction is complex and includes the effects of uraemic toxins and alterations of the vessel wall.[361,362]

7.3.4 Laboratory risk factors

7.3.4.1 *Low platelet count*

A low platelet has been shown to be a risk factor for bleeding in several of the studies included within this thesis. A platelet count greater than 20 x 10^9/L reduced the odds of clinically significant haemorrhage (WHO grade 2 or above bleeding) the following day by approximately 50% (OR 0.54; 95% CI 0.38 to 0.78) (Chapter 4). A platelet count less than or equal to 20 x 10^9/L increased the odds of an ICH by 2.6 times (95% CI 1.14 to 6.28) in the full case-control data set that included 84 cases and controls, however it did not reach statistical significance in the smaller data set (66 cases and controls). The platelet count is an important contributor to bleeding but, as has been demonstrated in this thesis, it should not be considered in isolation and the AIPN may be a better marker of a patient's risk of bleeding.

7.3.4.2 *Low haemoglobin*

In the TOPPS sepsis sub-study (Chapter 4) a haemoglobin greater than 95g/L was associated with a decreased risk of bleeding, compared to a haemoglobin less than 86g/L. This has been postulated to occur, and has been shown in previous animal model studies, and small studies in healthy volunteers.[199-202] A previous pilot study that compared a restrictive versus liberal red cell transfusion strategy did not demonstrate any difference in rates of bleeding between the two treatment arms, however only sixty patients were enrolled.[205] Although a higher haemoglobin may reduce bleeding this needs to be balanced against the finding that in other patient groups it may increase in-

hospital mortality[363,364] and overall mortality and morbidity.[365] Another pilot study is currently testing a restrictive versus liberal red cell transfusion policy in patients receiving haematopoietic stem cell transplants.[209]

7.3.4.3 *Low white cell count*

In the TOPPS sepsis sub-study (Chapter 4) a white cell count ≥ 1 x 10^9/l halved the odds of bleeding the following day (OR 0.49; 95% CI 0.34 to 0.72), compared to a white cell count < 0.5 x 10^9/l. This may be a marker that the patient's bone marrow is recovering after the treatment they have received. Early neutrophil recovery is seen at a similar time to increases in the number of immature platelets in the circulation.[343] In Chapter 3 a higher absolute immature platelet number was associated with a lower risk of bleeding.

7.3.4.4 *High white cell count*

The case-control study (Chapter 6) found that a high white cell count (> 50 x 10^9/L) was associated with an increased risk of bleeding (OR 19.75; 95% CI 1.90 to 204.87). This trend was also seen in the systematic review (Chapter 5). Symptomatic hyperleucocytosis (leucostasis) is a medical emergency and is associated with abnormalities of haemostasis (disseminated intravascular coagulation), and increased viscosity of the blood. Blast cell aggregates and white blood cell thrombi in the capillaries of the brain have been demonstrated in post-mortem studies.[244] An increased risk of ICH has been demonstrated in patients with leucostasis and is thought to be due to infarction due to thrombus formation with an associated secondary haemorrhage.[366]

7.4 Further work arising from this thesis

7.4.1 BEST Collaborative bleeding assessment form

Due to the work carried out in Chapter 2 highlighting the major differences between platelet transfusion trials on how bleeding was assessed and graded a BEST Collaborative Project Group was set up. I am a member of this project group, and we have designed a bleeding assessment form for future clinical platelet transfusion trials. The form has been developed via international consensus and was developed so that bleeding assessment data is collected in a consistent manner. This form is currently being validated in Canada, the United States and the United Kingdom.

Once this validation process has been completed the next step will be to create a consistent grading system for bleeding.

7.4.2 Fibrinolysis Study (ATHENA II)

This study has been designed to characterise the clinically significant abnormalities in the fibrinolytic system in frozen samples already collected in the ATHENA study (Chapter 3).[367] This pilot study aims to examine a small number of patients in careful detail to document the key abnormalities in fibrinolysis. This is a preliminary study, and if an association is seen between fibrinolysis and bleeding this will guide the development of future studies. These future studies will be developed to assess whether antifibrinolytic drugs may be a useful adjunct to platelet transfusions.

7.4.2.1 *Primary Objective*

To determine the prevalence and severity of fibrinolytic activity in patients with haematological malignancies who are being treated with chemotherapy or a stem cell transplantation as measured by increases in plasmin generation. This will be determined by plasmin-antiplasmin (PAP) complex levels.

7.4.2.2 *Secondary Objectives*

Objective 1: To observe whether there is any relationship between the abnormalities detected and the risk of haemorrhage.

Objective 2: To apply this knowledge to develop future trials of prophylactic strategies including antifibrinolytics.

7.4.2.3 *Study Participants*

Participants were a sample of fifty patients who had been diagnosed with a haematological malignancy, and received myelosuppressive chemotherapy or a stem cell transplant at the Churchill Hospital, Oxford or Bristol Royal Infirmary. These patients were recruited as part of the ATHENA study.[367]

7.4.2.4 *Blood Sampling*

Samples for ROTEM and freezing were processed within 2 hours of a sample being taken. The sample for haemostatic assays was placed in a citrated tube and spun down within two hours of first draw. First at 1,760g for 20 minutes, the supernatant was then extracted and re-spun at 10,000g for a further 2 minutes. The extracted plasma was stored in frozen aliquots at -80^{o}C.

ROTEM samples were processed within two hours of blood draw at 37^{o}C on a ROTEM delta instrument. All pipetting steps and the mixing of reagents with samples were guided by the electronic pipette programme. Three separate ROTEM assays were performed for all patients, the EXTEM, INTEM and FIBTEM.

Assays to be performed on the frozen samples will be:

- Plasma activity levels of α_2-antiplasmin
- D-dimer
- Fibrinogen
- Tissue Plasminogen activator (t-PA)
- Plasminogen activator inhibitor-1 (PAI-1)
- Prothrombin fragments 1 + 2 (PF 1+2)
- Plasmin α2-antiplasmin complex (PAP)
- Thrombin activatable fibrinolysis inhibitor (TAFI)

These investigations have been selected because they have been used in trauma studies to study fibrinolysis in detail[173] and encompass all aspects of the fibrinolytic pathway (Figure 7.1).

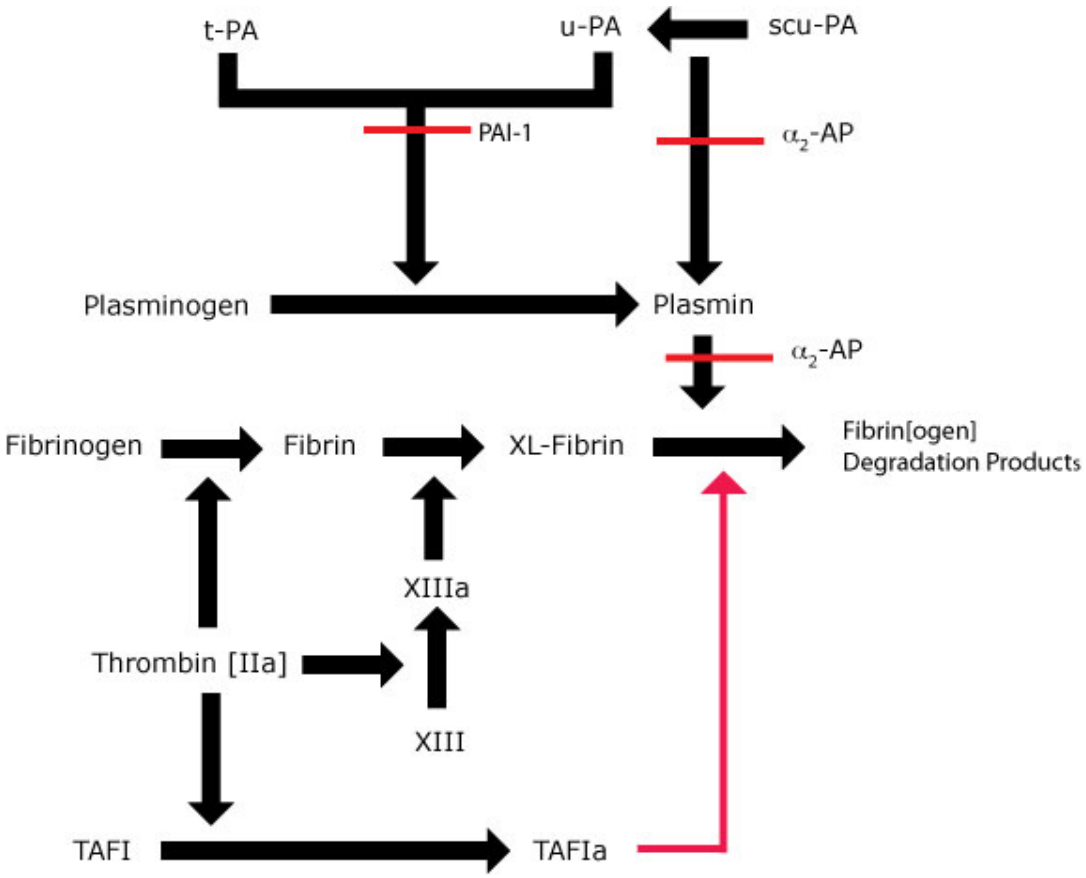


Figure 7.1 Fibrinolytic pathway

This study has been funded by the NHSBT Trust Fund and had already started.

Dr Mike Desborough is performing the investigations and this study will constitute part of his DPhil thesis.

7.4.3 InCiTe Study (Final analysis)

The InCiTe study is not due to be completed until June 2015 (Chapter 6). The study has now had 130 cases of intracranial haemorrhage (ICH) reported to it and is continuing to collect more cases on a monthly basis. This will strengthen and expand the findings from

the interim analysis. An estimate of the overall incidence of ICH will also be performed at this stage.

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