Desmopressin for treatment of thrombocytopenia or platelet dysfunction

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Abstract

The objective of the work presented in this thesis was to explore the role of potential alternatives to platelet transfusions and specifically to investigate whether desmopressin could be used for treatment of thrombocytopenia or platelet dysfunction. Patients with thrombocytopenia or platelet dysfunction are often treated with platelet transfusions to treat or prevent bleeding. However the evidence for the efficacy of platelet transfusion is limited and there is some evidence of harm. I have focused on thrombocytopenic patients with haematological malignancies or critically ill patients, who are amongst the groups most commonly treated with platelet transfusions.

The aims of this research were to determine:

1. If levels of Von Willebrand factor (VWF) or other measures of haemostasis are predictive of bleeding in severe thrombocytopenia;
2. Whether VWF compensates for thrombocytopenia *in vitro;*
3. The evidence for the efficacy of desmopressin in all patients undergoing surgery or invasive procedures;
4. The evidence for desmopressin for platelet dysfunction or thrombocytopenia;
5. If it is feasible to use desmopressin to treat critically ill thrombocytopenic patients in a clinical trial.

To identify derangements of haemostasis that may signify candidates for alternatives to platelet transfusions, I analysed blood samples from an observational trial of fifty patients with haematological malignancies and profound thrombocytopenia due to intensive chemotherapy. I used a panel of tests to investigate measures of primary haemostasis, thrombin generation, cross-linked fibrin formation and fibrinolysis. Using

multivariable logistic regression, I found no consistent correlation between any measures of haemostasis and the risk of clinically significant bleeding. VWF antigen levels were the best predictor of clinically significant bleeding on the same day (odds ratio 0.31, 95% confidence interval 0.10 to 0.98, p=0.047) but were not predictive of severe bleeding over the 24 hours after the test (odds ratio 0.48, 95% confidence interval 0.10 to 2.34, p=0.36).

In a separate set of experiments, I evaluated thrombus formation under flow in thrombocytopenia. This technique was sensitive to the platelet count . Addition of exogenous VWF to thrombocytopenic blood resulted in improvement in thrombus formation, suggesting that agents that affect or influence VWF pathways might have a role.

Desmopressin can be used to increase VWF levels, so leading on from my laboratory experiments; I used systematic reviews and meta-analyses to assess whether desmopressin could be used in unselected patients to reduce bleeding peri-operatively. I identified 62 randomised controlled trials. Overall there was no evidence of benefit for administering desmopressin to unselected patients. However further analysis of eleven randomised controlled trials that focused on patients with platelet dysfunction found that desmopressin resulted in transfusion of fewer units of red cells (equivalent to a 25% reduction compared to control), less blood loss (equivalent to a 23% reduction compared to control) and a lower risk of requiring a re-operation due to bleeding (Peto odds ratio 0.39, 95% confidence interval 0.18 to 0.84). There was no evidence for an increase in thrombotic events. There was no randomised controlled trial evidence for perioperative desmopressin for patients with thrombocytopenia. These specific research gaps were addressed by designing new clinical trials.

I have commenced a randomised controlled feasibility trial of desmopressin versus placebo for critically ill patients with thrombocytopenia undergoing invasive procedures. This trial is ongoing and is the first randomised trial evaluating peri-procedural desmopressin in thrombocytopenia.

The programme of work arising from this research has the potential to benefit a large number of patients by preventing bleeding and reducing exposure to allogeneic blood components such as platelets. The results presented in this thesis are exploratory but are an important step on a path towards larger trials using desmopressin as an alternative, or adjunct to platelet transfusion.

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Statement of originality

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

I would like to acknowledge Dr L. Estcourt who led the ATHENA study, including collecting blood samples and patient details (Chapter 2). I expanded the ideas for ATHENA II including a more detailed examination of fibrinolysis, von Willebrand factor function, and thrombin generation. I performed the laboratory tests and analysed the results.

I acknowledge the help of Prof J. Gibbins and Dr A. Bye with the design of the platelet depletion technique and the laboratory tests in Chapter 3.

All statistical analyses with the exception of the multivariable logistic regression in Chapter 2 were performed by me. I made the analysis plan for multivariable logistic regression in Chapter 2 and A. Griffiths performed the analysis.

I designed and led the systematic reviews and meta-analyses. Dr C. Dorée designed the search strategies. Data were extracted in duplicate for all meta-analyses and I am grateful to Dr L. Estcourt, Dr C. Brierley, Ms K. Oakland and Dr S. Bennett for their help extracting data. I performed the statistical analyses and they were checked by Dr M. Trivella.

I designed setup, secured funding, and carried out the DRIVE trial with the assistance of the NHS Blood and Transplant clinical trials unit and member of the trial team. The trial was managed by the NHS Blood and Transplant Clinical Trials unit.

Peer reviewed publications arising from this thesis

- **Desborough MJ**, Oakland KA, Brierley C, Bennett S, Doree C, Trivella M, Hopewell S, Stanworth SJ, Estcourt LJ. Desmopressin for minimising red cell transfusion. *Cochrane Database of Systematic Reviews* 2017; **7**: CD001884.
- **Desborough MJ**, Oakland KA, Landoni G, Crivellari M, Doree C, Estcourt LJ, Stanworth SJ. Desmopressin for treatment of platelet dysfunction and reversal of antiplatelet agents: a systematic review and meta-analysis of randomized controlled trials. *Journal of Thrombosis and Haemostasis* 2017; **15**(2): 263-72.
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Abbreviations

A2AP: alpha-2-antiplasmin

ACBG: aortocoronary artery bypass graft

ADAMTS13: a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13

ADP: adenosine diphosphate

ALL: acute lymphoblastic leukaemia

AML: acute myeloid leukaemia

ANOVA: analysis of variance

APACHE: acute physiology and chronic health evaluation

APML: acute promyelocytic leukaemia

aPTT: activated partial thromboplastin time

ASA: American Society of Anesthesiologists

ASD: atrial septal defect

ATHENA: prospective observational cohort study of the association between thromboelastometry, coagulation and platelet parameters in haematological malignancy

ATREAT: American Trial Using Tranexamic Acid in Thrombocytopenia

BSA: body surface area

BSH: British Society for Haematology

CABG: coronary artery bypass graft

cAMP: cyclic adenosine monophosphate

CAT: calibrated automated thrombogram

CENTRAL: Central Register of Controlled Trials

CPB: cardiopulmonary bypass

CI: confidence interval

CINAHL: Cumulative Index to Nursing and Allied Health Literature

CIRSE: Cardiovascular and Interventional Radiology Society of Europe

CLL: chronic lymphocytic leukaemia

cm: centimetre

CML: chronic myeloid leukaemia

COX: cyclooxygenase

CPCI-S: Conference Proceedings Citation Index-Science

CRP: C-reactive protein

CRP-XL: collagen-related peptide

CT: computerised tomography

C-ADP: collagen and adenosine diphosphate

C-EPI: collagen and epinephrine

°C: degrees Celsius

DASH: desmopressin for reversal of antiplatelet drugs in stroke due to haemorrhage

DDAVP: 1-deamino-8-D-arginine vasopressin (desmopressin)

dF: degrees of freedom

$DiOC_6$: 3-3'-dihexyloxacarbocyanine iodide

DRIVE: Desmopressin for procedures or Radiological InterVEntions

EACA: epsilon aminocaproic acid

ECG: electrocardiogram

EDTA: ethylenediaminetetraacetic acid

ELISA: enzyme-linked immunosorbent assay

eNOS: endothelial nitric oxide synthase

ETP: endogenous thrombin potential

EudraCT: European Clinical Trials Database

F: factor

F(a): activated factor

FACS: fluorescence activated cell sorting

FEU: fibrinogen equivalent units

FITC: fluorescein isothiocyanate

g: gram (weight); relative centrifugal force (centrifuge speed)

GP: glycoprotein

GRADE: Grading of Recommendations Assessment, Development and Evaluation

Gs: trimeric G protein

Hb: haemoglobin

Hct: haematocrit

HEME: HEmorrhage MEasurement

HIV: human immunodeficiency virus

hr: hour

HRA: Health Research Authority

ICH-GCP: International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use – Good Clinical Practice

ICTRP: International Clinical Trials Registry

ICU: intensive care unit

IMP: investigational medicinal product

IQR: interquartile range

ISOC: Intensive Care Study of Coagulopathy

ISRCTN: International Standard Randomised Controlled Trial Number

ISTH: International Society on Thrombosis and Haemostasis

ITP: immune thrombocytopenic purpura

iu: international units

IV: inverse variance

kg: kilogram

l: litre

LILACS: Latin American and Caribbean Health Sciences Literature

LTA: light transmission aggregometry

M: molar

mcg: microgram

MD: mean difference

MDS: myelodysplastic syndrome

MEDLINE: Medical Literature Analysis and Retrieval System Online

MFI: mean fluorescence intensity

MHRA: Medicines and Healthcare products Regulatory Agency

min: minute

ml: millilitre

mM: millimolar

mmol: millimoles

M-H: Mantel-Haenszel

m^2: square metres

ng: nanograms

NHS: National Health Service

NICE: National Institute for Health and Care Excellence

nM: nanomolar

nM.min: nanomoles x minutes (area under the thrombin generation curve)

P: phosphorylation

PAI: plasminogen activator inhibitor

PAP: plasmin-alpha-2-antiplasmin

PC: protein C

PE-Cy: phycoerythrin-cyanine

PEG-rHuMGDF: pegylated recombinant human megakaryocyte growth and differentiation factor

PFA: platelet function analyser

PG: prostaglandin

PICC: peripherally inserted central cannula

PKA: protein kinase A

pM: picomolar

pOR: Peto odds ratio

PPP: platelet poor plasma

PRISMA: Preferred Reporting Items for Systematic reviews and Meta-Analyses

PROBLEMA: Prevention of bleeding in hematological malignancies with antifibrinolytic (epsilon aminocaproic acid)

PRP: platelet rich plasma

PT: prothrombin time

RCT: randomised controlled trial

RIC: reduced intensity conditioning

RR: risk ratio

SD: standard deviation

SE: standard error

SRI: Systematic Reviews Initiative

SToP: A randomized controlled trial comparing standard- and low-dose strategies for transfusion of platelets to patients with thrombocytopenia

TAFI: thrombin activatable fibrinolysis inhibitor

TAP: trans-atrial pacing

TBI: traumatic brain injury

TEG MA: thromboelastography maximum amplitude

TF: tissue factor

TIA: transient ischaemic attack

TOPIC: Transfusion of Fresh Frozen Plasma in Non-bleeding Intensive Care Unit Patients

TOPPS: Trial of Prophylactic Platelets

TPO: thrombopoietin

TPA: tissue plasminogen activator

TRAP: thrombin receptor-activating peptide

TREATT: Trial evaluating the safety and efficacy of tranexamic acid in patients with haematological malignancies with severe thrombocytopenia

ttPeak: time to peak thrombin generation

TXA: tranexamic acid

UK: United Kingdom

UKBTS: United Kingdom Blood Transfusion Services

UPA: urokinase plasminogen activator

V2R: vasopressin 2 receptor

VWF: Von Willebrand factor

VWF:Ac: Von Willebrand factor Activity

VWF:Ag: Von Willebrand factor antigen

VWF:CBA: Von Willebrand factor collagen binding assay

VWF:RiCof: Von Willebrand factor ristocetin cofactor

WB: whole blood

WHO: World Health Organization

WPB: Weibel-Palade bodies

wRBC: washed red blood cells

µg: microgram

µl: microlitre

µM: micromolar

µm^3: cubic micrometres

µmol: micromoles

Chapter 1

1 Introduction

1.1 Thrombocytopenia and platelet dysfunction

Platelets play an essential role in maintaining the haemostatic system. They are responsible for the initial formation of a platelet plug following injury (1) and maintaining vascular integrity (2). Platelets also play a key role in regulation of the inflammatory response; atherogenesis; and wound healing (3). The critical role of platelets in haemostasis can be demonstrated by the bleeding phenotype of patients who are thrombocytopenic or who have dysfunctional platelets.

Thrombocytopenia is common among patients with haematological malignancy where it is a feature of intensive chemotherapy; and in critical care where one third patients have a platelet count <100 $x10^9$/l during their admission (4). Thrombocytopenia has many potential causes but for the purposes of this thesis I will focus on patients with haematological malignancies and critically ill patients on intensive care units, who are among the groups who most commonly require platelet transfusion (5-7).

In haematological malignancy, thrombocytopenia is most commonly related to bone marrow failure/infiltration or chemotherapy. The thrombocytopenia associated with chemotherapy is often profound and results in clinically significant bleeding in approximately 50% of patients undergoing intensive chemotherapy or stem cell transplantation (8). For patients with myelodysplastic syndrome, thrombocytopenia (platelet count <100 $x10^9$/l) is estimated to be present in 23 to 93% patients depending on the cohort, with haemorrhage contributing to death in 14 to 24% cases (9).

In critical care, thrombocytopenia may be due to a wide range of causes (Table 1.1); the most common of which are sepsis and medications (4). In this setting, thrombocytopenia is often less profound (4). However there is a greater exposure to invasive procedures where physicians often aim for a higher platelet count to prevent bleeding. Several studies have demonstrated that although the absolute risk of bleeding is low for interventional procedures, the risk of bleeding is increased with thrombocytopenia (Table 1.2).

Platelet dysfunction may be due to a number of causes including uraemia, liver failure, inherited platelet function disorders and cardiopulmonary bypass. However the most common cause is use of antiplatelet drugs. 6 to 8% of the UK population are taking an antiplatelet drug (10) and this rises to approximately 25% in cohorts of patients who have gastrointestinal (11, 12) or intracranial bleeding (13). Antiplatelet therapy is associated with a 20 to 50% increased risk of perioperative bleeding (14-16) and increased rates of rebleeding and mortality in traumatic brain injury (17) and intracerebral haemorrhage (18); and increased rates of rebleeding following lower gastrointestinal haemorrhage (12).

Consequently treatment of thrombocytopenia or platelet dysfunction is of critical importance.

Causes of thrombocytopenia in critical care	
Infection	**Sepsis**
	Disseminated intravascular coagulation
	HIV
Drugs	**Heparin**
	Antibiotics
	Chemotherapy
	ADP receptor inhibitors (e.g. clopidogrel)
	GPIIb/IIIa inhibitors (e.g. abciximab)
	Alcohol
Bone marrow failure	
Major blood loss and haemodilution	
Mechanical fragmentation	Cardiopulmonary bypass
	Intra-aortic balloon pump
	Renal dialysis
	Extracorporeal membrane oxygenation
Immune mediated disorder	Immune thrombocytopenic purpura
	Antiphospholipid syndrome
	Post-transfusion purpura
Microangiopathic haemolytic anaemia	Disseminated intravascular coagulation
	Thrombotic thrombocytopenic purpura
	Haemolytic uraemic syndrome
Hypersplenism	
Liver failure	
Haematinic deficiency	
Other	Cancer
	Inherited thrombocytopenias

Table 1.1 Causes of thrombocytopenia in critical care (19). ADP: adenosine diphosphate. GP: glycoprotein. Most common causes marked in bold.

Name of study	Platelet transfusions for thrombocytopenia n/N (%)	Peri-procedural bleeding		Odds ratio (95% confidence interval)[1]
		Thrombocytopenia n/N (%)	Normal platelet count n/N (%)	
Central venous catheter (platelet count 20 to 50 x10^9/l)				
Mumtaz 2000 (20)	-	3/88 (3%)[2]	1/1680 (<1%)[2]	59.3 (6.1 to 98.8)
Kander 2013 (21)	58/66 (88%)	2/66 (3%)	9/634 (1%)	2.2 (0.5 to 10.3)
Zeidler 2010 (22)	54/142 (38%)	6/142 (4%)	1/268 (<1%)	11.8 (1.4 to 98.8)
			Pooled odds ratio	***10.0 (1.4 to 69.2*)**
Central venous catheter (platelet count <20 x10^9/l)				
Zeidler 2010 (22)	84/92 (91%)	2/92 (2%)	1/268 (<1%)	5.9 (0.5 to 66.2)
			Pooled odds ratio	***5.9 (0.5 to 66.2)***
Arterial puncture (platelet count <100 x10^9/l)				
Darcy 1996 (23)	-	3/18 (17%)	85/877 (10%)	1.9 (0.5 to 6.6)
			Pooled odds ratio	***1.9 (0.5 to 6.6)***
Paracentesis (platelet count <50 x10^9/l)				
McVay 1991 (24)	-	1/20 (5%)	7/278 (3%)	2.0 (0.2 to 17.4)
Lin 2005 (25)	0/55	0/55	0/223	-
			Pooled odds ratio	***2.0 (0.2 to 17.4)***
Liver Biopsy (platelet count 60 to 100 x10^9/l)				
Seeff 2010 (26)	-	3/509 (0.6%)[2]	3/1331 (0.2%)[2]	2.6 (0.5 to 13.1)
Terjung 2003[3] (27)	7/49 (14%)	13/49 (27%)[2]	59/580 (10%)[2]	3.2 (1.6 to 6.4)
Sharma 1982 (28)	13/16 (81%)	0/16	0/24	-
			Pooled odds ratio	***3.1 (1.6 to 5.8)***
Liver Biopsy (platelet count <60 x10^9/l)				
Seeff 2010 (26)	-	4/76 (5%)[2]	3/1331 (0.2%)[2]	24.6 (5.4 to 112.0)
Sharma 1982 (28)	13/13 (100%)	3/13 (23%)[2]	0/24	16.3 (0.8 to 344.9)
			Pooled odds ratio	***22.7 (5.8 to 88.1)***

Table 1.2 Interventional procedures and bleeding risk with thrombocytopenia. Ordered by size of study. All bleeds were non-major bleeds (World Health Organisation grade 2 or less in severity), unless otherwise stated. [1]Odds ratios pooled using Mantel-Haenszel method and random effects model. [2]All bleeds were major bleeds. [3]Platelet count 50 to 100 x10^9/l.

1.2 Platelet transfusion

1.2.1 Platelet transfusion strategies

Treatment strategies may involve prophylactic treatment to prevent spontaneous bleeding (predominantly for severe thrombocytopenia); prophylactic treatment before surgery, or a procedure where bleeding is expected; or treatment of the bleeding patient (Figure 1.1). Allogeneic platelet transfusions are often administered to provide a pool of functioning platelets. However these transfusions have significant limitations and it is possible other agents, such as desmopressin may prove to be effective alternatives, or adjuncts, to platelet transfusion.

Platelet transfusions are often administered to treat thrombocytopenia and less commonly platelet dysfunction (29). The majority of platelet transfusions are administered for prophylaxis against spontaneous bleeding (5-7) where the platelet transfusion threshold is approximately 10 $x10^9$/l (29, 30). UK regional audits demonstrated that approximately 14% platelet transfusions were administered before a procedure or surgery (5) where the platelet transfusion threshold is approximately 50 $x10^9$/l (20 to 100 $x10^9$) depending on the type of procedure) (29, 30); and 22 to 27% were administered for the treatment of bleeding (5-7) where the transfusion threshold is also approximately 50 $x10^9$/l (20 to 100 $x10^9$/l depending on the type of surgery or procedure) (29, 30).

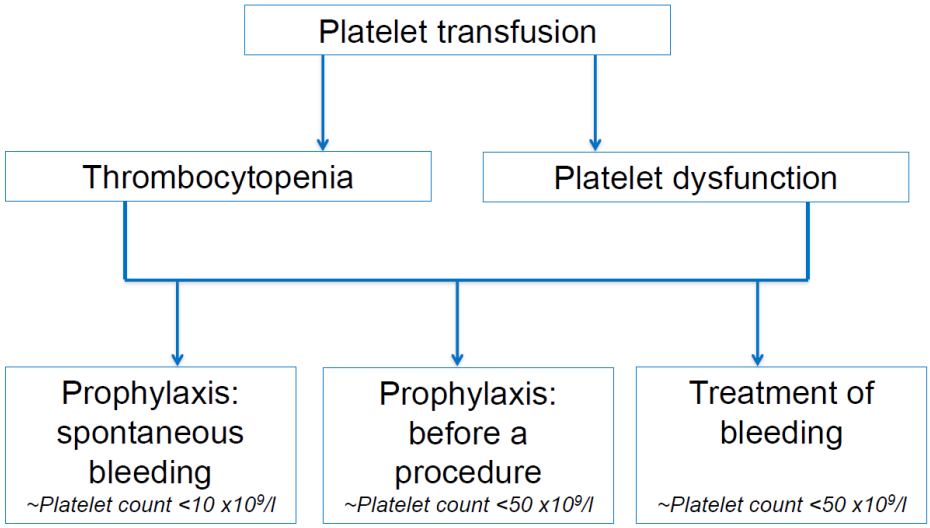


Figure 1.1 Rationale for platelet transfusion

1.2.2. Efficacy of platelet transfusion

A systematic review synthesising the data from six trials of prophylactic platelet transfusion versus on demand transfusion for patients undergoing myelosuppressive chemotherapy or stem cell transplantation found that clinically significant bleeding was less common amongst patients treated with prophylactic platelet transfusions than those receiving on demand therapy (31). The occurrence of life-threatening bleeding is rare in this patient group and there is insufficient evidence to determine whether there is an increase in the risk of severe or life-threatening bleeding if platelet transfusion is only given on demand, although it seems likely that other patient factors may be more relevant to explain risk of major bleeding. From the largest of these trials to date (Trial of Prophylactic Platelets; TOPPS) (8), the authors reported in subgroup analyses that in recipients of autologous haematopoietic stem cell transplantation, there was no difference in bleeding between those on the two different platelet transfusion strategies (32). It is also important to consider that non-bleeding patients with chronic bone marrow failure do not benefit from prophylactic platelet transfusion (29, 30) and

platelet transfusion may be harmful in the setting of thrombotic thrombocytopenic purpura and heparin-induced thrombocytopenia (33). In the developing world, platelet transfusions may be used for treatment of thrombocytopenia in dengue fever. Clinical trial data suggest that platelet transfusion does not reduce bleeding and is associated with a higher incidence of adverse events than no transfusion (34).

The evidence base for platelet transfusion before interventional procedures or surgery is very limited. There are no completed randomised controlled trials of platelet transfusion thresholds in this setting to date. There is broader recognition in guidelines that some interventional procedures such as bone marrow aspirate and trephine may not require 'cover' with platelet transfusions (29, 30). However, there is still widespread use of platelet transfusions pre-procedure (5).

For patients taking antiplatelet agents, transfusion of two to three units of platelets to reverse antiplatelet agents in the event of major bleeding or emergency surgery is recommended in several guidelines (35, 36). These recommendations are largely based on studies demonstrating that impaired platelet aggregation due to antiplatelet agents can be corrected *in vitro* with the addition of donor platelets (37, 38); and a single arm clinical trial that administered two units of platelets pre-operatively to patients on dual antiplatelet therapy (39). This strategy has been called into doubt by a recent trial examining the use of platelet transfusions for reversal of antiplatelet agents in the setting of spontaneous intracerebral haemorrhage (40). Patients were randomised to receive standard care or platelet transfusion (one unit for those taking aspirin or two units for those taking other antiplatelet agents). There was no difference in bleeding between the groups but those transfused with platelets had a significant increase in the risk of mortality or a poor functional outcome at three months.

Platelets are usually stored for 5 to 7 days before transfusion. One explanation for the relatively poor efficacy of platelet transfusions is that platelet haemostatic and secretory function diminishes within hours of blood storage and by 5 to 7 days, there is a marked reduction in agonist-stimulated aggregation of liquid-stored platelets regardless of their storage medium (41, 42).

1.2.3 Risks of platelet transfusion

Platelet transfusions are associated with diverse risks such as transfusion reactions, bacterial contamination and viral transmission, which may include emerging infections such as Zika virus, which are hard to detect (43). Platelet transfusions require compatibility testing, and may take time to request and obtain for administration (not all hospital blood banks have a ready supply of platelets for all blood groups). There is a relative lack of standardisation between platelet units with variability in platelet reactivity between donors (44) and differences in platelet function following storage (45). Even in the emergency setting of major traumatic bleeding when policies for timely use of blood components should be optimised, the average time to administration of platelets has been reported to be 2 to 3 hours (46). Alternative agents that could be given more quickly may allow procedures to go ahead more rapidly, or arrest bleeding sooner. Patients who refuse blood, such as Jehovah's Witnesses, would also benefit from alternatives to platelet transfusion that do not contain blood components. Another group who may benefit from alternatives to platelet transfusion are those who are refractory to platelet transfusions (47). Lastly, in the developing world, access to safe blood components may be limited. The prevalence of transfusion-transmitted infections in each blood transfusion is much higher in low income countries than in high income countries (HIV 0.85% versus 0.002%, hepatitis B 3.59% versus

0.02% and hepatitis C 1.07% versus 0.02%) (48), and safe, cheap alternatives to platelet transfusion would be appealing.

1.2.4 Guidelines

The British Society for Haematology (BSH) Guidelines (29) make the following recommendations for platelet transfusion thresholds:

- Platelets should not be given as a matter of routine for: bone marrow aspirate or trephine biopsy; traction removal of central lines; or peripherally inserted central cannula (PICC) placement.
- Central venous lines (tunnelled or un-tunnelled) can be inserted when the platelet count is >20 $x10^9$/l
- Lumbar puncture can be performed when the platelet count is ≥40 $x10^9$/l
- Liver biopsy and surgery can be performed when the platelet count is ≥50 $x10^9$/l.
- Epidural catheter insertion, or removal, when the platelet count is ≥80 $x10^9$/l.

By contrast, Cardiovascular and Interventional Radiology Society of Europe (CIRSE) guidelines recommend a platelet transfusion threshold of 50 $x10^9$/l for all procedures including central line insertion/removal and PICC placement (49).

National Institute for Health and Care Excellence (NICE) guidelines do not recommend platelet transfusion at all before low risk procedures such as bone marrow aspirate and trephine, or central venous catheter insertion. NICE recommend consideration of a platelet transfusion threshold of 50 $x10^9$/l for patients undergoing invasive procedures or surgery, rising to 75 $x10^9$/l in high risk cases (or 100 $x10^9$/l if surgery at a critical site) (30).

This lack of consensus is reflected in clinical practice where audits frequently show large variations in triggers for platelet transfusion. An audit in the United Kingdom in 2010 showed 23 to 36% were administered outside guidelines (7).

1.3 Haemostasis and the risk of bleeding

Platelets are one important element of haemostasis but there are many other factors which also contribute towards normal clot formation. It is possible that other factors associated with haemostasis may compensate, or contribute to the bleeding risk in thrombocytopenia. Haemostasis can be split into broad categories, which are discussed in sections 1.3.1 to 1.3.6: endothelial activation; primary haemostasis; thrombin generation; formation of cross-linked fibrin; and fibrinolysis. Tests which can be used to assess haemostasis and risk of bleeding in the laboratory are outlined in table 1.3.

Test	Sample	Endothelium	Platelet adhesion [shear]	Platelet aggregation	Coagulation	Fibrinogen	Fibrinolysis	Sensitive to platelet count
Multiplate®	WB/PRP	-	-	Yes	-	-	-	Yes
LTA	PRP	-	-	Yes	-	-	-	Yes
Flow cytometry	WB/PRP	-	-	Yes	-	-	-	-
PFA	WB	-	Yes	Yes	-	-	-	Yes
Viscoelastic tests	WB/PRP	-	-	-	Yes [limited]	Yes	Yes [weak]	[depending on conditions]
CAT	PRP/PPP	-	-	-	Thrombin generation potential	-	-	[depending on conditions]
Thrombus formation under flow	WB/PRP	[possible]	Yes	Yes	[possible]	[possible]	-	Yes
PT/aPTT	PPP	-	-	-	Yes [limited]	-	-	-
Clauss fibrinogen	PPP	-	-	-	-	Yes	-	-
D-dimer or PAP complexes*	PPP	-	-	-	-	-	Reflect *in vivo* fibrinolysis[1]	-
Clot lysis assays	WB/PPP	-	-	-	-	-	Yes	-

Table 1.3 Summary of haemostasis tests. [1]Measure of *in vivo* fibrinolysis but also rises in proportion with fibrin deposition so may also indicate thrombin and fibrin generation. aPTT:

activated partial thromboplastin time, CAT: calibrated automated thrombogram, LTA: light transmission aggregometry, PAP: plasmin-alpha-2-antiplasmin, PFA: platelet function analyser, PPP; platelet poor plasma, PRP: platelet rich plasma, PT: prothrombin time, WB: whole blood (50-58).

1.3.1 Endothelium

The role of endothelium in haemostasis is the most difficult to measure clinically. Expression of thrombomodulin on endothelial cell surfaces results in activation of protein C, maintaining blood in an anticoagulated state (59). Likewise, endothelial nitric oxide and prostaglandin (PG)I_2 release, prevent platelet activation (60).

In the event of inflammation or endothelial injury, collagen is exposed and Von Willebrand Factor (VWF) is released from endothelial Weibel-Palade bodies (61). Under high shear forces, this results in platelet adhesion and activation (62). Release of tissue factor from endothelium and mononuclear cells triggers the clotting cascade and the endothelium acts as a surface for the initial reactions in the clotting cascade (63).

The role of endothelium in thrombocytopenia is unclear. Platelets appear necessary for maintenance of endothelial integrity and vascular leak often results in petechiae (2). In the presence of sepsis, there is an additional compromise to endothelial integrity (64) resulting in an increased bleeding risk (65).

1.3.1.1 Laboratory assessment of endothelial function

The haemostatic role of the endothelium remains the most challenging part of haemostasis to measure. While there are no tests in clinical practice at present, it is possible to assess the role of the endothelium in haemostasis by lining a flow chamber with cultured endothelial cells and assessing thrombus formation under flow (66). With the development of techniques for culturing circulating endothelial outgrowth cells from patients with bleeding disorders (67), it is possible these could be used to line a

flow chamber in the future to permit assessment of endothelium. However this is a labour intensive technique mainly suited to research.

1.3.2 Primary haemostasis

Primary haemostasis is the process leading to formation of a platelet plug (Figure 1.2). Platelets are usually maintained in the quiescent state by endothelial PGI_2 and nitric oxide (60). Following endothelial injury, subendothelial collagen and fibronectin are exposed and endothelial VWF is released. Many triggers for VWF release have been identified including adrenaline, adenosine diphosphate (ADP), thrombin, collagen, histamine, histones and vasopressin (61, 68). VWF is immobilised on endothelial collagen and binds to platelet glycoprotein (GP)Ib receptors initiating the process of adhesion (69). Following this, platelets become firmly tethered to collagen through their collagen receptors (70). Initial activation of platelets is partially through activation of collagen receptors (most notably GPVI) and partially through activation of protease activated receptors by thrombin (generated by the coagulation cascade) (71).

A series of intracellular signalling events results in release of alpha granules (containing serotonin, ADP and P-Selectin) and release of thromboxane A_2 (60). The release of these agonists results in autocrine and paracrine activation of platelets. Oral antiplatelet agents inhibit parts of this process, with aspirin inhibiting cyclooxygenase, preventing synthesis of thromboxane A_2; and agents such as clopidogrel, ticagrelor and prasugrel inhibiting the ADP receptor ($P2Y_{12}$) (72). The final part of platelet aggregation is activation of the GPIIb/IIIa (fibrinogen) receptor, which mediates aggregation (73). Deficiency, or dysfunction, of VWF (74), platelets (75) or collagen (76) can result in a bleeding phenotype.

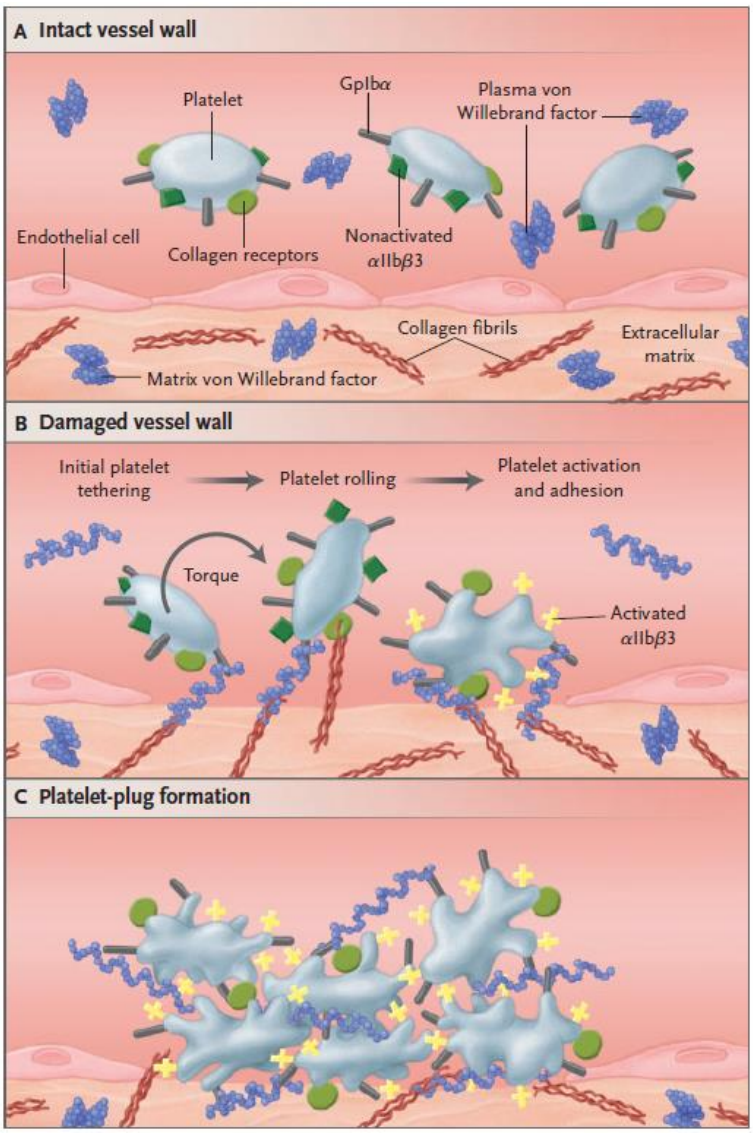


Figure 1.2 Von Willebrand factor and primary haemostasis. Figure reproduced from *Mannucci PM. Treatment of Von Willebrand's Disease. N Engl J Med 2004; 351: 683-94* (77). Copyright© Massachusetts Medical Society. **A**: In normal blood, coiled Von Willebrand factor (VWF) and platelets co-exist in the circulation. Sub-endothelial matrix collagen and VWF are separated from platelets by the endothelium. **B**: When there is damage to endothelium, collagen and VWF are exposed to shear forces from the circulation. VWF unravels, exposing its A1 domain to platelet glycoprotein 1b (Gp1bα) resulting in tethering of platelets to VWF. Platelets roll in the direction of flow before interacting with collagen (most notably through their glycoprotein VI receptors) and adhering to the endothelium. Activation of platelets (represented by irregular margins) leads to a conformational change in glycoprotein IIb/IIIa (αIIb/β3) (represented by yellow crosses). **C**: At high shear forces this enhances the affinity of glycoprotein IIb/IIIa for VWF (at low shear forces, fibrinogen binds to the activated glycoprotein IIb/IIIa receptors).

1.3.2.1 Laboratory assessment of primary haemostasis

The most commonly performed tests to assess primary haemostasis are the platelet count (which provides information on platelet numbers, but not function); VWF assays (which provide information on VWF but not platelets; or platelet function assays (which predominantly provide information on platelets). In order to assess the interaction between VWF and platelets, it is necessary to simulate the shear forces present in the circulation. VWF remains in a coiled form until it is exposed to shear forces, exposing its A1 domains (responsible for adhesion to platelet GP1b and collagen); A2 domains (the site of action for the proteolytic enzyme; a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; ADAMTS13); and A3 domains (collagen binding sites) (78, 79). A simple test that can be used to assess the combined effects of VWF and platelet function is a platelet function analyser (PFA) (52). Whole blood is passed at high shear forces (5000 to 6000 s^{-1}) through an aperture which is coated with agonists. As the platelets aggregate in response to the agonists, they occlude the aperture, allowing the calculation of a closure time (52). The PFA is very easy to use but has a low sensitivity and specificity for mild platelet defects (80, 81) and consequently, ISTH guidelines do not recommend its routine use for this purpose in clinical practice (82).

Thrombus formation under flow is a technique that has been used extensively in basic science research (56) and is being used increasingly for clinical research, including assessing the effects of desmopressin (83). In brief, in this technique, platelets labelled with a fluorescent membrane stain are passed through a chamber coated with an agonist (most commonly collagen). The rate of flow can be adjusted to simulate a range of shear forces. Fluorescent microscopy is then used to quantify the amount of platelet adhesion and aggregation. Modifications to this technique can also explore the

combined role of platelet, VWF and coagulation factor function (56). This technique is relatively difficult to use; and there is considerable variation in practice between laboratories that perform this test (84). Consequently, while it has the potential to offer the most complete assessment of coagulation of the available *in vitro* tests, there is still considerable further development will be necessary before it could be used as a routine clinical test.

The techniques for PFA and thrombus formation under flow are described in more detail in Chapter 3.

1.3.3 Thrombin generation

In a series of steps, clotting factor zymogens are cleaved, forming proteolytic enzymes, eventually resulting in generation of thrombin. This model was first described in 1964 and is known as the clotting cascade (85). Initiation of thrombin generation is predominantly through tissue factor (TF) and activated (a) factor (F)VII (86), which form an extrinsic tenase

The extrinsic tenase complex cleaves FX into FXa, which in turn cleaves its cofactor, FV into FVa (87, 88). FVIIa/TF activity is rapidly halted by tissue factor pathway inhibitor when it moves away from a cell surface (89). FXa cleaves prothrombin (FII) in a two-stage process, forming small amounts of thrombin (FIIa) (90). Thrombin generated at this stage, activates FV, FVIII and FXI (91). FXIa cleaves FIX into the proteolytic enzyme FIX, which in complex with its cofactor, FVIIIa, forms an intrinsic tenase, which efficiently cleaves FX into FXa (92). In combination with FVa, this cleaves prothrombin, resulting in generation of high concentrations of thrombin (87).

This process is usually held in check by the natural anticoagulants. When thrombin falls on normal endothelium, it combines with membrane-bound thrombomodulin. The

thrombin/thrombomodulin complex activates protein C (PC) in the presence of its cofactor (activated protein S). Activated PC inhibits FVIII and FV (93); whereas antithrombin predominantly inhibits FIIa and FXa, reducing thrombin generation (94).

The balance of these pro-/anti-coagulant factors determines the amount of thrombin generated (95). Severe deficiencies in most of the procoagulant factors results in a severe bleeding phenotype (96, 97). The role of thrombin generation in thrombocytopenia is unclear. Excess thrombin generation will not directly compensate for a defect in primary haemostasis, although it is possible that it will result in increased fibrin clot strength and platelet activation.

1.3.4 Fibrinogen

Soluble fibrinogen is cleaved into insoluble fibrin by thrombin. These single strands of fibrin are relatively weak, but gain considerable increase in strength following cross-linkage between d-domains by FXIIIa (which is activated by thrombin) (98). Patients with severe hypo-/dys-fibrinogenaemia (99), or those with FXIII deficiency (100), often have a severe bleeding phenotype.

Models of haemostasis using viscoelastic tests often predict that increased fibrinogen will compensate for thrombocytopenia, because they both contribute towards maximum clot firmness (101). However, it remains to be seen whether this is the case.

1.3.5 Fibrinolysis

Fibrinolysis is the process of clot breakdown. Plasminogen is cleaved into the proteolytic enzyme, plasmin, by tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA). These fibrinolysis promoters are inhibited by plasminogen activator (PAI)-1, and to a lesser extent, PAI-2 (102).

Plasmin is also inhibited by activated thrombin activatable fibrinolysis inhibitor (TAFIa), which is formed from TAFI by the action of thrombin in combination with thrombomodulin. It cleaves C-terminal lysine residues from the fibrin surface, decreasing its cofactor activity (103). Lastly, any free plasmin that is generated is rapidly bound to alpha-2-antiplasmin (A2AP), forming plasmin-alpha-2-antiplasmin (PAP) complexes (102). Degradation of fibrin by fibrinolysis results in formation fibrin degradation products, including d-dimers (cross-linked fibrin d-domains) (104). The presence of fibrinolysis in bleeding may represent a primary process, or may be a compensatory response to excess thrombin/fibrin generation as is the case for elevated d-dimers in venous thromboembolism (104).

Clinically significant disorders of fibrinolysis are rare. Deficiencies in PAI-1 (105) and A2AP (106) have been described and associated with a mild bleeding phenotype. The importance of fibrinolysis is emphasised by the use of fibrinolytic agents for treatment of myocardial infarction or ischaemic stroke, where clots are rapidly dissolved, but severe bleeding may occur (107); and in the setting of traumatic haemorrhage where administration of tranexamic acid (an anti-fibrinolytic drug) significantly reduces mortality (108). Platelet poor clots have been shown to be particularly vulnerable to fibrinolysis (109) and it is possible that increase levels of fibrinolysis may contribute towards bleeding risk in thrombocytopenia.

1.3.6 Cell-based model of haemostasis

Haemostasis is complex, and many of the models used to explain it examine each element in isolation. This is partially reflected by the difficulty of creating a single test that can examine all elements of haemostasis.

The interplay between elements of the haemostatic system is well described in the cell-based model of haemostasis (87). This model considers the elements of coagulation in combination, with endothelium, platelets and macrophages acting as surfaces for activation of the clotting cascade and formation of fibrinogen.

The complexities of this system mean that while the risk of bleeding due to thrombocytopenia can be estimated to some extend in the laboratory, there is a need for correlation with clinical data.

1.4 Clinical factors associated with spontaneous bleeding in thrombocytopenia

Platelet count appears to be a relatively poor predictor of bleeding risk in thrombocytopenia (110). The majority of patients with profound thrombocytopenia have haematological malignancies and this section focuses on clinical factors which may contribute towards bleeding.

1.4.1 Sepsis

Inflammation contributes to bleeding in thrombocytopenic animal models (111), suggesting that recognition and treatment of sepsis may reduce the risk of bleeding. The evidence for fever or infection causing bleeding is mixed with some studies reporting an increase in bleeding (65) and others reporting no difference in the risk of bleeding (112-114). Likewise some studies assessing proven infection have demonstrated an increase in the risk of bleeding (113, 114) and others have not (112, 115, 116).

1.4.2 Gender

Female gender is independently associated with an increased bleeding risk for thrombocytopenia patients with haematological malignancies (65, 117, 118). This

increase in risk is still present when cases of menstrual bleeding are excluded. The biological rationale for this increase in risk is unclear.

1.4.3 Underlying haematological disease and treatment

Acute promyelocytic leukaemia is associated with a higher risk of bleeding than other types of leukaemia (118, 119) and is an independent risk factor for haemorrhage (118). Patients with acute promyelocytic leukaemia often have a significant coagulopathy (120) and were not included in the work contained within this thesis and so are not discussed further. One retrospective observational study reported that patients with acute leukaemia in first remission, chronic phase chronic myeloid leukaemia and chemotherapy sensitive lymphomas have a lower risk of bleeding than other underlying diseases (121). Patients undergoing allogeneic stem cell transplantation or induction chemotherapy for acute leukaemia may have a greater risk of bleeding than patients undergoing autologous stem cell transplantation (65, 115, 121-123).

1.4.4 Medications

Medications such as antiplatelet agents and anticoagulants are known to increase bleeding risk (115) but are relatively contraindicated in profoundly thrombocytopenic patients and are rarely used in this setting (124).

1.4.5 Co-morbidities

Both pro- and anti-coagulant factors are synthesised by the liver, and as there is a deficiency in both, haemostasis is often considered to be re-balanced, with a normal capacity to generate thrombin (125). This leads to no increased bleeding risk compared to patients without liver disease, despite abnormalities of laboratory clotting parameters. However thrombocytopenia does reduce thrombin generation in liver disease, so these coagulation abnormalities may contribute towards bleeding in this

setting (126). In the setting of thrombocytopenia, one study has reported that hepatic impairment contributes to bleeding risk (112).

Renal impairment has been reported to be associated with bleeding risk in two studies; one defining renal failure as creatinine >124 μmol/l (117) and the other as uraemia >17.9 mmol/l (112). The increased bleeding tendency in renal failure is multifactorial with evidence of decreased VWF function; defects in platelet secretion and fibrinogen-binding; altered blood rheology; and increased release of platelet inhibitors such as prostacyclin and nitric oxide (127).

1.5 Desmopressin and other alternatives to platelet transfusion

Thrombocytopenia is associated with an increased bleeding risk. However as described in section 1.2, platelet transfusions have limited efficacy and may be associated with serious adverse events. Given the other haemostatic changes that may occur in thrombocytopenia, it is possible that other agents could be more effective than platelet transfusion and have a better adverse event profile. These alternatives are outlined in the sections below with a particular focus on desmopressin (Figure 1.3, and Table 1.4).

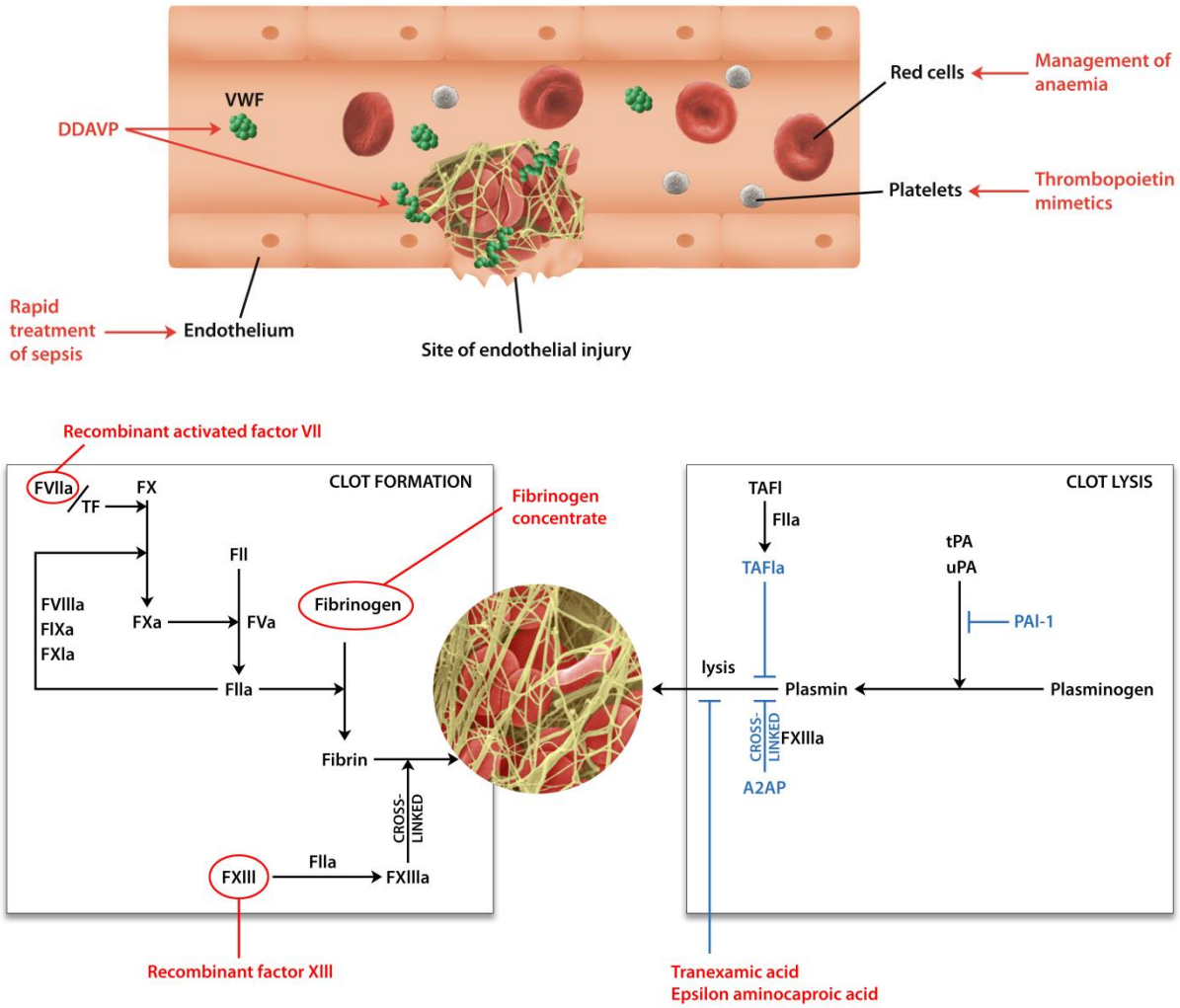


Figure 1.3 Alternatives to platelet transfusion (reproduced with permission from Desborough et al. British Journal of Haematology 2016; **175**(3): 381-92) (128). ***Upper figure***: Following endothelial injury, subendothelial collagen and Von Willebrand Factor (VWF) are exposed. Under shear forces, VWF uncoils and binds to exposed collagen supporting the adhesion of circulating platelets. DDAVP (desmopressin; 1-deamino-8-D-arginine vasopressin) promotes VWF release from endothelial Weibel-Palade bodies, increasing plasma VWF concentrations (129). Rapid treatment of sepsis helps to maintain endothelial integrity (65). Thrombopoietin mimetics directly increase platelet numbers by stimulating megakaryopoiesis (130). Red cell flow through blood vessels pushes platelets into proximity with the endothelium (and also collagen and VWF) promoting platelet adhesion (131). Red cells also provide approximately one third of the phospholipid surface for thrombin generation (132) and red cell incorporation into clots increases their resistance to lysis (133). ***Lower figure****: Clot formation (left box)*. Following endothelial injury, release of tissue factor (TF) and activated (a) factor (F) VII triggers thrombin formation (87). Thrombin (FIIa) is the key enzyme in clot formation resulting in conversion of fibrinogen to fibrin as well as providing a feedback loop, further

amplifying thrombin generation through FVIIIa, FIXa and FXIa. Thrombin also promotes conversion of FXIII to FXIIIa and thrombin activatable fibrinolysis inhibitor (TAFI) to TAFIa, and can directly activate platelets. Formation of fibrin from fibrinogen is the final key step in stabilising the clot and fibrin is further strengthened by cross-linkage by FXIIIa . *Clot lysis (right box).* The key enzyme in clot lysis is plasmin. Conversion of plasminogen to plasmin is driven by tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA). There are three main inhibitors of fibrinolysis, alpha-2-antiplasmin (A2AP) (which is cross-linked to plasmin by FXIIIa) (Fraser*, et al* 2011), TAFIa (which is converted from TAFI by thrombin) and plasminogen activator inhibitor (PAI)-1 (which inhibits the activity of tPA and uPA) (102). Black arrows represent activation and blue arrows inhibition. Alternatives to platelet transfusion are in red text and where appropriate, their site of action is circled.

Platelet transfusion alternative	Stage of development
Thrombopoietin mimetics (134-137)	Licensed for use in ITP. RCTs in haematological malignancy undergoing chemotherapy, MDS and liver disease
Anti-fibrinolytics (138)	In clinical use largely outside the setting of thrombocytopenia. Very limited RCT evidence in setting of haematological malignancy. Several large ongoing RCTs.
Red cell transfusion (139, 140)	No evidence of efficacy from RCTs and some evidence of harm.
Desmopressin (141, 142)	In clinical use largely outside the setting of thrombocytopenia. Clinical case series in the setting of thrombocytopenia. RCTs in setting of platelet dysfunction
Fibrinogen concentrate (143)	In clinical use largely outside the setting of thrombocytopenia or platelet dysfunction. *In vitro* data in setting of thrombocytopenia. Not licensed in most countries outside the setting of congenital hypofibrinogenaemia.
Recombinant factor VIIa (144)	In clinical use largely outside the setting of thrombocytopenia. Limited RCT evidence in setting of stem cell transplantation
Recombinant factor XIII (145)	In clinical use largely outside the setting of thrombocytopenia. Limited RCT evidence in setting of acute leukaemia undergoing induction chemotherapy
Future strategies	Lyophilised platelets: dose escalation study in healthy volunteers underway
	Liposomes: animal trials only
	Engineered nanoparticles: animal trials only
	Infusible platelet membranes: animal trials only
	Platelets generated from stem cells: animal trials only
	Monoclonal antibody fragment against ticagrelor (146).

Table 1.4 Stages of development for alternatives to platelet transfusion (adapted with permission from Desborough et al. British Journal of Haematology 2016; **175**(3): 381-92). ITP – immune thrombocytopenic purpura, MDS – myelodysplastic syndrome, RCT – randomised controlled trial

1.5.1 Thrombopoietin mimetics

Agents that stimulate platelet production, rather than replace platelets as transfusions, are one potential alternative. Thrombopoietin (TPO) mimetics licensed for use in the UK at present are romiplostim and eltrombopag. These agents act by stimulating megakaryopoiesis resulting in increased thrombopoiesis. TPO mimetics have been used to treat thrombocytopenia in a large number of settings, most notably immune thrombocytopenic purpura (ITP). In the setting of ITP they have been shown to increase platelet counts but were not found to reduce the incidence of serious bleeding events compared to placebo (137). Patients with liver disease on antiviral therapies have also been examined, although in this group, an unacceptable increased risk of both bleeding and thrombosis occurred following TPO mimetic administration (134, 147). There is early promise for the use of TPO mimetics in the treatment of myelodysplastic syndrome (MDS) where their administration improved bleeding rates (130, 136, 148). Previous trials in the setting of haematological malignancy undergoing intensive chemotherapy found no difference in the duration of thrombocytopenia but did note a reduction in platelet transfusions (135). These trials were halted, as the TPO mimetic in question, pegylated recombinant human megakaryocyte growth and differentiation factor (PEG-rHuMGDF) was found to cause anti-TPO antibodies in both patients and volunteers and was withdrawn from further development (149). TPO mimetics may cause an increase in blast count in this setting but there is no evidence for an increased risk of progression to acute myeloid leukaemia (130). Bleeding was only reported in one of these trials with an overall reduction in clinically significant bleeding from 50% to 25% with TPO mimetics, although this trial was underpowered for both this outcome and the more important outcome of preventing serious or life-threatening bleeding (150). Five further trials of TPO mimetics in haematological malignancy are presently

underway, which are aiming to recruit 424 patients in total. These agents are expensive and, have been found to be associated with an increased risk of thrombosis (134). The administration of TPO mimetics for patients with aplastic anaemia is of particular interest, as in addition to their effects in improving the platelet count; TPO mimetics may also have a disease-modifying effect in this setting (151).

1.5.2 Anti-fibrinolytic drugs

Tranexamic acid and epsilon aminocaproic acid are synthetic lysine analogues which block the lysine binding sites on plasminogen, preventing it from interacting with fibrin and thus from being converted to plasmin and from cleaving its substrate (152). These agents result in resistance to clot lysis and increased clot strength. The formation of plasmin may have other effects outside the clotting system such as immune and endothelial activation (153) and cleavage of VWF (154). It is unclear if these anti-fibrinolytic agents exert part of their effect through mechanisms other than inhibition of clot lysis.

There are limited specific data on use of anti-fibrinolytic agents for preventing bleeding for patients with thrombocytopenia. This approach is appealing, as these agents are cheap and have been shown to be effective in a number of other patient settings (not necessarily characterised by thrombocytopenia such as trauma) with minimal adverse events (108, 155). A recent systematic review focussing on the use of tranexamic acid in patients with haematological malignancies identified three trials examining tranexamic acid and one trial examining epsilon-aminocaproic acid, all of which were of low quality, and small sample size (138). Three large trials are presently underway, one of epsilon aminocaproic acid (PROBLEMA) and two of tranexamic acid (TREATT and ATREAT) for the prevention of bleeding in thrombocytopenic patients undergoing intensive

chemotherapy or stem cell transplantation. These trials in total are expected to recruit 1276 patients and are expected to be completed by December 2020.

1.5.3 Red cell transfusions

There is an increasing body of laboratory evidence indicating the role of red cells in promoting normal haemostasis (131-133, 156). Anaemia has been associated with a prolonged bleeding time (157, 158), with correction of anaemia normalising the results (159). Conversely, erythrocytosis is associated with the risk of pathological thrombus formation (160, 161). Red cell transfusion remains the most readily available means for rapid correction of anaemia with laboratory studies demonstrating that red cell transfusion may promote haemostasis (162, 163). In many conditions, re-bleeding after an initial bleeding event is associated with an increased risk of mortality (11, 164) and some physicians opt to transfuse red cells or correct anaemia with an aim to improve haemostasis (165). However clinical data on whether correction of anaemia prevents bleeding are sparse. Consequently, there is a scientific and clinical need to determine whether transfused red cells improve haemostasis and reduce the risk of bleeding.

The results of a recent large meta-analysis indicate that the use of higher haemoglobin thresholds for triggering transfusion (most commonly 90 to 100 g/l) do not improve bleeding or thrombotic outcomes compared to lower haemoglobin thresholds (most commonly 70 to 80 g/l) (139). Liberal transfusion was particularly problematic for patients with cirrhosis where it resulted in a substantially increased risk of both rebleeding and mortality (140).

1.5.4 Increasing thrombin generation

Activated factor VII *(*FVIIa) triggers thrombin generation in combination with tissue factor. The recombinant version of FVIIa (rFVIIa; eptacog alfa) has been used in a

number of settings to control bleeding. It has a short half-life of 2.7 to 3.5 hours and consequently repeated infusions are necessary. A trial of three doses of intravenous rFVIIa to control bleeding post-stem cell transplant found that 40 μg/kg and 120 μg/kg made no difference to the risk of severe or life-threatening bleeding, whereas, there was a reduction in bleeding using a dose of 80 μg/kg. The event rate for severe bleeding is low and these results are likely to be imprecise. rFVIIa was associated with an increased risk of thromboembolic events in this trial, which are difficult to manage in the setting of thrombocytopenia (166). Intrapulmonary rFVIIa has been used following allogeneic stem cell transplantation to control bleeding from alveolar haemorrhage but no clear benefit has been demonstrated (167). The limited efficacy, side effect profile and expense of this agent limit its use as an alternative to platelet transfusion (144).

1.5.5 Increasing cross-linked fibrinogen

In vitro work combining administration of fibrinogen concentrate and rFVIIa demonstrated normalisation of whole blood clot formation in an *in vitro* model of ITP and suggested the two agents may have a synergistic effect (143). Trials administering fibrinogen concentrate to thrombocytopenic pigs demonstrated an improvement in maximum clot firmness (101). No trials of fibrinogen concentrate have been performed for treatment of platelet dysfunction or thrombocytopenia. I am not aware of any trials using cryoprecipitate for the treatment of thrombocytopenia but it is likely that it would have similar effects to fibrinogen concentrate.

FXIII is partially secreted by platelets (Mitchell, *et al* 2014), so thrombocytopenia is likely to limit delivery of FXIII to sites of bleeding. Recombinant FXIII is used for the treatment of congenital FXIII deficiency (168). It has also been used empirically in cardiothoracic surgery with mixed results for control of blood loss (169, 170) and in

cancer surgery where a pilot trial demonstrated an improvement in clot firmness and a small reduction in bleeding (171). A single randomised controlled trial of FXIII supplementation for patients with acute leukaemia from 1982 found no difference in the risk of bleeding after administration (145).

1.5.6 Future developments

Future developments may include use of lyophilised platelets; haemostatic (platelet-bridging) particles; liposomes; engineered nanoparticles; infusible platelet membranes; or platelets generated from stem cells (128). Animal studies have demonstrated some evidence that MEDI2452, a monoclonal antibody fragment, can be used to reverse the effects of ticagrelor (146).

1.5.7 Desmopressin

Desmopressin (also known as DDAVP or 1-deamino-8-D-arginine vasopressin) is a synthetic version of the posterior pituitary hormone vasopressin. It was first synthesised by Milan Zaoral in Prague in 1967 (172), with the first clinical trials in 14 patients with diabetes insipidus published the following year (173). It went on to be mass produced by Ferring pharmaceuticals (launched as Minirin® in 1972) and the first manuscript using desmopressin to treat haemophilia A and Von Willebrand disease was published by Pier Mannucci in 1977 (174).

1.5.7.1 Desmopressin mechanism of action

Desmopressin acts by binding to endothelial vasopressin 2 receptors (V2R), stimulating release of VWF and factor VIII from Weibel-Palade bodies (175-177) (Figure 1.4). Weibel-Palade bodies also contain tPA, which promotes plasmin generation following desmopressin administration (178). However this does not appear to cause clinically significant hyper-fibrinolysis, as the plasmin is rapidly conjugated to A2AP (179). VWF protects FVIII from degradation, so the increased levels of VWF result in a prolonged

FVIII half-life (180, 181). Desmopressin is a potentially appealing treatment for thrombocytopenia, as VWF is necessary for the initial adhesion of platelets to the endothelial surface (77). It may be possible to increase the number of platelets that adhere to damaged endothelium by increasing the concentration of VWF, optimising the function of the platelets that are present. Desmopressin may also directly enhance platelet function, although it is unclear if this mechanism is related to increased levels of VWF and factor VIII or if there might be another mechanism independent of these factors. Desmopressin may contribute towards formation of procoagulant, coated platelets; enhanced platelet-dependent thrombin generation; increases in intracellular sodium and calcium ion flux; and increased formation of procoagulant microparticles (182).

Desmopressin also causes nitric oxide release from endothelial cells by inducing endothelial nitric oxide synthase activation (183). This can cause vasodilation, manifesting as facial flushing, tachycardia and hypotension (184). V2R are present on renal collecting ducts, and stimulation of collecting duct V2Rs results in translocation of aquaporin-2 from intracellular stores to the apical plasma membrane (185). This may result in fluid retention and hyponatraemia in a similar mechanism to that caused by the syndrome of inappropriate antidiuretic hormone secretion. Post-marketing surveillance data estimates the incidence of hyponatraemic seizures at <1 in 10,000 (184). These events have predominantly occurred in pregnant women or children under the age of two years who were not adequately monitored (186). Tachyphylaxis occurs after repeated administration of desmopressin because stores of VWF in Weibel-Palade bodies become exhausted following repeated administration of desmopressin. A

second dose of desmopressin administered 24 hours after an initial dose produces approximately 30% less increase in VWF antigen than the initial dose (187).

Desmopressin has greatest bioavailability when administered intravenously or subcutaneously (188), with VWF levels peaking at 200 to 300% of normal levels after approximately 30 minutes (189) and remaining elevated for up to 8 hours (190). The plasma half-life is 2.2 to 2.7 hours, regardless of whether it is administered intramuscularly or intravenously (189), but may be prolonged 2 to 3-fold in renal failure (191).

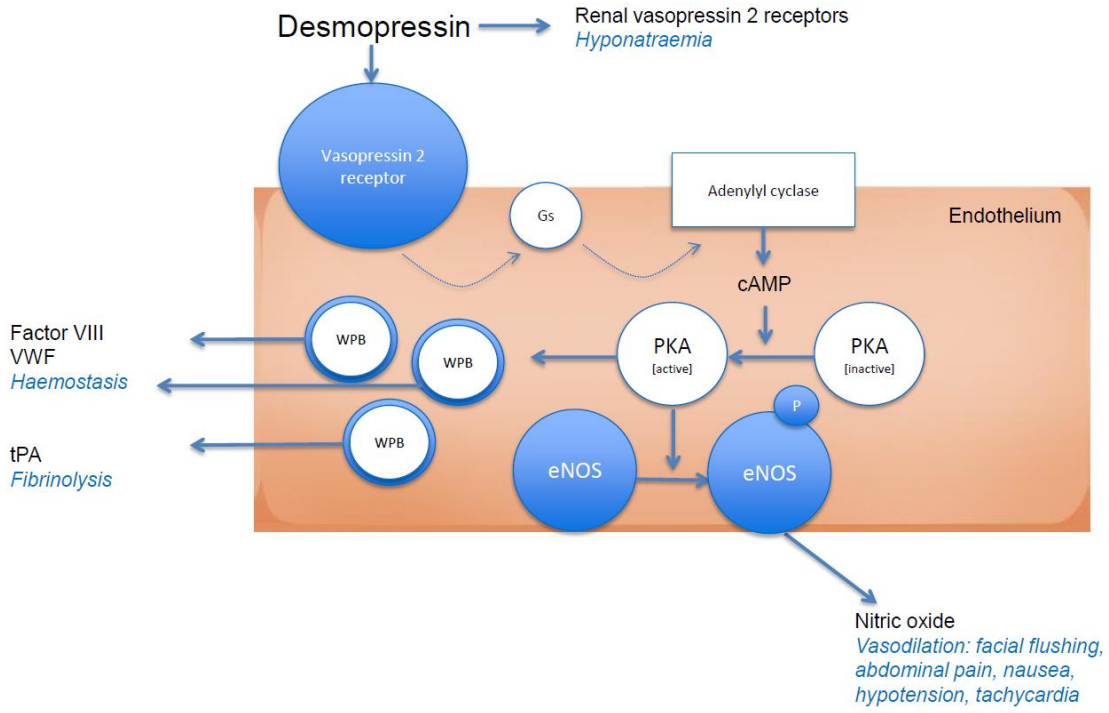


Figure 1.4 Desmopressin mechanism of action. Adapted with permission from *Kaufmann et al. J Thromb Haemost 2003; **1**: 682-9* (192). Desmopressin binds to endothelial vasopressin 2 receptors leading to activation of a trimeric G-protein (Gs), activation of adenylyl cyclase, production of cyclic adenosine monophosphate (cAMP), and activation of protein kinase A (PKA) (177). Activation of PKA stimulates exocytosis of Weibel-Palade bodies (WPBs), leading to release of Von Willebrand Factor (VWF), factor VIII and tissue plasminogen activator (tPA). It also induces endothelial nitric oxide synthase (eNOS) activation, by phosphorylation (P) of

residue Ser1177, leading to nitric oxide production (183). The effects of desmopressin are highlighted in italics.

1.5.7.2 Desmopressin evidence base

For patients taking aspirin (193, 194) or GPIIb/IIIa inhibitors (195), several studies have demonstrated that PFA closure time is reduced following administration of desmopressin. A multicentre, worldwide retrospective study reviewing 423 patients with inherited platelet disorders undergoing surgery, found that desmopressin, alone or in combination with tranexamic acid, was associated with the lowest rates of bleeding (196). There have been several randomised controlled trials of desmopressin for platelet dysfunction, which are reported in detail in Chapter 5.

There is relatively little evidence of the use of desmopressin in thrombocytopenia. One randomised trial compared intranasal desmopressin to platelet transfusion for treatment of cirrhotic patients with platelet counts between 30 and 50 $x10^9$/l undergoing dental extractions. This trial was too small to demonstrate efficacy, as only a single bleeding event occurred (197). Intranasal desmopressin has poor bioavailability compared to intravenous or subcutaneous desmopressin and it is unclear how these patients responded to treatment. One single arm trial assessing the efficacy of desmopressin for 15 bleeding patients (platelet count range 2 to 71 $x10^9$/l) with haematological malignancies found that bleeding was arrested in all cases, although it was most effective for those with a platelet count ≥30 $x10^9$/l (198). Another single arm trial examining the effects of desmopressin for 5 children with haematological malignancies and one with ITP (platelet count range 10 to 34 $x10^9$/l) found that bleeding stopped in all cases following administration of desmopressin (199). Neither trial included a comparator arm limiting assessment of efficacy. By contrast another

study of 15 patients with thrombocytopenia (platelet count range 1 to 53 $x10^9$/l) found that desmopressin made no difference to template bleeding time compared to placebo (200).

1.5.7.3 Desmopressin in clinical guidelines

Desmopressin is commonly used in the treatment of mild to moderate haemophilia A and von Willebrand disease and is also recommended for the treatment of some inherited platelet disorders (29, 201, 202).

Outside the setting of inherited bleeding disorders desmopressin is recommended in the following guidelines:

- European guideline on management of major bleeding and coagulopathy following trauma: "We suggest that desmopressin (0.3 μg/kg) be administered in patients treated with platelet-inhibiting drugs or with von Willebrand disease. We do not suggest that desmopressin be used routinely in the bleeding trauma patient" (203).
- American Society of Anesthesiologists (ASA) practice guidelines for perioperative blood management: "Both the consultants and ASA members agree that, in patients with excessive bleeding and platelet dysfunction, consider the use of desmopressin" (204).
- European Society of Anaethesiology management of severe postoperative bleeding guidelines: "Following discontinuation of CPB [cardiopulmonary bypass], patients with severe aortic stenosis or drug- or CPB-induced platelet dysfunction may benefit from desmopressin" (205).
- Society of Thoracic Surgeons and the Society of Cardiovascular Anesthesiologists Blood Conservation Clinical Practice Guidelines: "Use of 1-deamino-8-D-arginine vasopressin (DDAVP) may be reasonable to attenuate excessive bleeding and

transfusion in certain patients with demonstrable and specific platelet dysfunction known to respond to this agent (e.g. uremic or CPB-induced platelet dysfunction, type I von Willebrand's disease)" (206).

- Neurocritical Care Society and Society of Critical Care Medicine guideline for reversal of antithrombotics in intracranial hemorrhage: "We suggest consideration of a single dose of desmopressin (DDAVP) in intracranial hemorrhage (0.4 mcg/kg intravenously) associated with aspirin/COX [cyclooxygenase]-1 inhibitors or ADP receptor inhibitors" (207).

1.6 Summary

Bleeding associated with thrombocytopenia or platelet dysfunction is a common problem. There are limitations to platelet transfusions which are the most commonly used treatment at present. To determine the best alternate treatment, it will be necessary to assess the other underlying disorders of haemostasis that are present in patients. It is possible that adjusting another haemostatic factor may compensate for the bleeding tendency in thrombocytopenia or platelet dysfunction. VWF appears to be a good target for this because it is a key part of primary haemostasis. Increased VWF levels may result in more platelet adhesion and aggregation and could bypass the effects of antiplatelet agents. One method to increase VWF levels in patients is through administration of desmopressin, a synthetic version of the posterior pituitary hormone vasopressin. This results in release of VWF from endothelial stores. This drug has been used for 40 years for the treatment of inherited bleeding disorders and there is good evidence for its safety. The overarching objective of the work described in this thesis is to investigate whether desmopressin could be used for treatment of thrombocytopenia or platelet dysfunction.

1.7 Aims of the research

The aims of this research were to determine:

1. If levels of VWF or other measures of haemostasis are predictive of bleeding in severe thrombocytopenia;
2. Whether VWF compensates for thrombocytopenia *in vitro;*
3. The evidence for the efficacy of desmopressin in all patients undergoing surgery or invasive procedures;
4. The evidence for desmopressin for platelet dysfunction or thrombocytopenia;
5. If it is feasible to use desmopressin to treat critically ill thrombocytopenic patients in a clinical trial.

Chapter 2

2. Predictors of bleeding in thrombocytopenia

2.1 Introduction

Profound thrombocytopenia and bleeding are common for patients with haematological malignancies undergoing intensive chemotherapy (8). However the platelet count is a poor predictor of bleeding risk in this setting (53, 110, 123). In some groups, such as those undergoing autologous stem cell transplantation, there is little difference in bleeding risk for those treated with prophylactic platelets or transfused only in the event of bleeding (8). It is possible that other factors involved in haemostasis might augment or replace the platelet count as a measure of bleeding risk (section 1.3). It is also possible that clinical factors such as sepsis might play an important role in determining bleeding risk (65, 111) (section 1.4). As reviewed in section 1.5 this might guide use of alternatives to platelet transfusion, or help to guide platelet transfusion.

2.1.1 Aims

The aim of this chapter was to assess whether measures of haemostasis or infection were predictors of bleeding for patients with haematological malignancy undergoing intensive chemotherapy or stem cell transplantation.

2.2 Methods and materials

Blood samples from the prospective observational cohort study of the association between thromboelastometry, coagulation and platelet parameters in haematological malignancy (ATHENA; ISRCTN81226121) were used for these experiments. ATHENA was led by Dr L. Estcourt, who has published results on the predictive value of total platelet count, immature platelet count and rotational thromboelastometry for the

prediction of bleeding in this patient cohort previously (53). ATHENA recruited 50 consecutive adults with haematological malignancies admitted for intensive chemotherapy or haematopoietic stem cell transplantation at the Oxford or Bristol haematology centres between September 2010 and September 2012.

For ATHENA II, I analysed additional patient plasma samples from ATHENA with a focus on VWF, thrombin generation, fibrinogen, fibrinolysis and bleeding risk. As previously described (53), potential patients were checked for eligibility on admission to hospital and were approached and invited to give written consent prior to study enrolment if they were eligible in accordance with UK Regional Ethics Committee approval 10/H0505/47.

2.2.1 Participants

Participants had to meet all the inclusion criteria and none of the exclusion criteria to be included in the trial.

2.2.1.1 Inclusion criteria

- Adults (age 16 years or more)
- Confirmed diagnosis of a haematological malignancy
- Inpatient being treated with intensive chemotherapy or stem cell transplantation
- Thrombocytopenic at admission (platelet count less than 50 $x10^9$/l) or expected to become thrombocytopenic for at least 5 days during inpatient treatment
- Able to comply with study monitoring

2.2.1.2. Exclusion criteria

- Known inherited disorder of haemostasis
- Antithrombotic therapy during period of thrombocytopenia

- ITP

2.2.2 Care of study participants

The ATHENA study was observational and patients were managed according to standard protocols for both hospitals involved in this trial. This included transfusion of platelets if total platelet count was less than 10 $x10^9$/l or at a higher threshold if a patient bled or an interventional procedure was planned.

2.2.3 Duration of the study for participants

For each patient the duration of the study was from the first day of thrombocytopenia (total platelet count less than 50 $x10^9$/l) until platelet recovery (defined as an unsupported total platelet count of at least 50 $x10^9$/l for three consecutive days); hospital discharge; death; or after 30 days of follow-up – whichever occurred first.

2.2.4 Study assessments

During the observation period, participants had daily bleeding assessments and assessment of total platelet count. Blood samples were collected three times weekly and blood test results were correlated with bleeding assessments.

2.2.5 Measurement of bleeding

Bleeding in malignancy is often assessed using the World Health Organization (WHO) scale. This is relatively subjective with five simple categories:

- Grade 0: no bleeding
- Grade 1: petechiae
- Grade 2: mild blood loss
- Grade 3: gross blood loss
- Grade 4: debilitating blood loss

In order to make bleeding assessments reproducible, this study used a modified version of the WHO bleeding scale (Table 2.1), which was validated in TOPPS (8). Bleeding symptoms were recorded daily by a research nurse using a standardised questionnaire. A computerised algorithm was used to classify bleeding symptoms according to the modified WHO bleeding scale (table 2.1). I considered bleeding to be clinically significant if it was of WHO grade 2 or more. This measure is commonly used in clinical trials (8, 208, 209) and is recommended as a trigger for prophylactic transfusion of platelets for severely thrombocytopenic patients (platelet count <30 $x10^9$/l) in the NICE transfusion guidelines (30).

Modified World Health Organization Bleeding Scale	
Grade 1	Mild bleeding not meeting any of the definitions below
Grade 2[1]	Ecchymosis (lesions > 10 cm in size)
	Haematoma
	Epistaxis > 1 hour in duration or packing required
	Retinal haemorrhage without visual impairment
	Abnormal vaginal bleeding (not normal menses) using > 2 pads/day
	Melaena, hematemesis, haemoptysis, haematuria or haematochezia
	Bleeding from invasive sites or musculoskeletal bleeding
Grade 3	As for grade 2 bleeding but also associated with haemodynamic compromise or requiring red cell transfusion
Grade 4	Debilitating bleeding including retinal bleeding with visual impairment[2]
	Non-fatal central nervous system bleeding with neurological signs and symptoms
	Fatal bleeding

Table 2.1 Modified World Health Organization bleeding scale (209). [1]bleeding not requiring red blood cell transfusion over routine transfusion needs. [2]field deficit or visual loss confirmed by an ophthalmologist.

2.2.6 Analysis of blood samples

Blood was collected into Vacutainer® blood collection tubes (Becton Dickinson, Plymouth, UK) containing 0.109 M buffered sodium citrate (or 1.8 mg/ml ethylenediaminetetraacetic acid [EDTA] for assessment of platelet count). Platelet count was assessed within two hours of blood collection. All other blood samples were centrifuged at 1760g for 20 minutes; then the PPP fraction was separated and

centrifuged at 10,000g for 2 minutes. PPP was divided into aliquots and frozen at -80°C for analysis in batches to avoid thawing and refreezing of samples.

Platelet count, VWF antigen (VWF:Ag), thrombin generation, prothrombin time (PT), Clauss fibrinogen, clot lysis assays, PAP complexes, d-dimer and C-reactive protein (CRP) were assessed for every patient blood sample to assess the correlation with bleeding risk. VWF activity (VWF:Ac), VWF collagen binding (VWF:CBA), tPA, PAI-1, A2AP, uPA, TAFIa and FXIIIa assays were performed on the single blood sample for each patient with the highest level of fibrinolysis (measured by PAP complexes). This was to check for evidence of fibrinolysis-induced VWF dysfunction (154) and to gain greater understanding of the regulation of fibrinolysis in these patients.

In previous work performed by Dr L. Estcourt, PFA closure time with C-ADP was recorded for a subset of patients in this cohort (53). I compared the recorded closure times of patients with VWF:Ag levels <2.0 iu/ml, with those with VWF:Ag levels >3.0 iu/ml. The PFA method is reported in section 3.2.6 but for the purposes of this chapter, pre-generated results were used for the analysis.

2.2.6.1 Automated assays

Total platelet count was assessed using a Sysmex XE2100 automated haematology analyser (Sysmex, Kobe, Japan). Clauss fibrinogen (STA®-liquid Fib kit, Stago, France) (58), A2AP (STA® - STACHROM® ANTIPLASMIN kit, Diagnostica Stago, France), d-dimers (STA® - LIATEST® D-DI kit; Diagnostica Stago, France) and PT (Neoplastine R®, Diagnostica Stago, France) were performed on a STA-R Evolution Analyser® (Diagnostica Stago, France). The international sensitivity index for Neoplastine R® was 0.95 with a mean normal PT of 14 seconds. FXIII activity (Berichrom® FXIII kit, Siemens, Marburg, Germany), VWF:Ag (vWF Ag® Kit, Siemens, Marburg, Germany) and VWF:Ac

(Innovance® VWF Ac kit, Siemens, Marburg, Germany) (210) were performed on a Sysmex CS-5100 (Siemens, Erlangen, Germany). These assays were fully automated. Bilirubin, creatinine and CRP were recorded from routine clinical care performed by the Departments of Clinical Chemistry and Haematology at the John Radcliffe Hospital, Oxford and the Bristol Royal Infirmary.

2.2.6.2 Thrombin generation

Thrombin generation was assessed by calibrated automated thrombinography (CAT) as described by Hemker *et al* (211). Thrombin generation reflects the balance of pro- and anti-coagulant clotting factors; an advantage over the PT and aPTT which only measure pro-coagulant factors. The balance between procoagulant and anticoagulant factors is an important determinant of haemostasis and hence whether or not there is a bleeding risk (212-215).

A combination of 5 pM recombinant human TF and 4 μM phospholipid were used as activators with the coagulation reaction triggered by the addition of calcium chloride which was added in combination with Z-Gly-Gly-Arg-amino-methyl-coumarin, a thrombin-sensitive fluorogenic substrate (all reagents from Thrombinoscope BV, Maastricht, The Netherlands). Analysis was performed using a Fluoroskan Ascent FL microplate fluorometer and luminometer (Thermo Scientific, Helsinki, Finland) and Thrombinoscope version 5 software (Thrombinoscope, BV, Maastricht, The Netherlands) to extrapolate the following parameters:

- Lag-time (time from coagulation activation to initiation of thrombin generation);
- Peak thrombin generation;
- Time to peak (ttPeak; time from coagulation activation to peak thrombin generation);

- Endogenous thrombin potential (ETP; the area under the thrombin generation curve).

At low concentrations of tissue factor (less than 1 pM), contact activation can limit interpretation of results. The intermediate dose of tissue factor (5 pM) used in these experiments is less sensitive to contact activation (216). Thrombin generation triggered by an intermediate dose of tissue factor provides information on both extrinsic and intrinsic clotting cascades (with the exception of FXI, FXII and the contact pathway) (217). However, thrombin generation triggered by intermediate doses of tissue factor is less sensitive to concentrations of FVIII and FIX than thrombin generation triggered by low dose tissue factor (218) and less sensitive to protein C and protein S than thrombin generation supplemented with thrombomodulin (219).

2.2.6.3 Enzyme-linked immunosorbent assays

PAP complexes (PAP TECHNOZYM®, Diagnostica Stago, France), tPA (Asserachrom® tPA, Diagnostica Stago, France), TAFIa (Asserachrom® TAFIa/TAFIai, Diagnostica Stago, France), PAI-1 (Asserachrom® PAI-1, Diagnostica Stago, France), uPA (Human PLAU/Urokinase-type Plasminogen activator, Sigma-Aldrich, St Louis, Maryland, United States) and VWF:CBA (ZYMUTEST vWF:CBA ELISA, Hyphen BioMed, Epsom, Surrey) were analysed using a sandwich enzyme-linked immunosorbent assay (ELISA) technique. As VWF:CBA values were expected to be high, an additional 1:4 dilution of plasma in buffer was performed so that values could be read from the centre of the standard curve. Results were read using a Multiskan® FC microplate photometer (Thermo Scientific, UK) according to the manufacturers' instructions. All samples were analysed in duplicate and were assessed against a reference standard curve along with a

blank sample (buffer only) and two lyophilised control samples with known concentrations of each enzyme.

2.2.6.4 Clot lysis assays

Clot lysis assays were performed on PPP. I analysed all samples at The Institute of Medical Sciences, University of Aberdeen using a procedure which has been validated in previous experiments (102). Samples were analysed in duplicate.

The principle of this test is that the addition of thrombin, calcium and phospholipid to PPP results in conversion of all the soluble fibrinogen in solution into insoluble fibrin (220). Fibrin strands increase the optical density of the sample. Results can be compared to a control of normal plasma with known fibrinogen concentration. Measurements were taken every minute for four hours using a Multiskan® FC microplate photometer (Thermo Scientific, UK). As fibrinolysis proceeded, the fibrin concentration was reduced. I extracted the 50% lysis time, which was the time taken for fibrin to be reduced to 50% of its peak value.

Each sample that was analysed had 30% PPP, 16 μM phospholipids (L-alpha-phosphatidylcholine/L-alpha-phosphatidylserine; Avanti, Alabaster, Alabama, United States), 0.1 units/ml thrombin (Sigma-Aldrich, Gillingham, United Kingdom), 10.6 mM calcium chloride (BDH Laboratory Supplies, Dawsonville, Georgia, United States) and 300 pM tPA (Genentech, San Francisco, California, United States). The volume was equalised between samples using Tris buffered saline (Tris 10 mM, Sodium chloride 140 mM and Tween 0.01%). The optimum dose of tPA was calculated by assessing samples without tPA; 180pM tPA (the usual optimal dose for pooled normal plasma) and 300 pM tPA to find a point where 50% lysis could be measured within four hours.

2.2.7 Normal ranges

I used pre-established normal ranges for platelet count, VWF:Ag, PT, Clauss fibrinogen, CRP and FXIII. For thrombin generation parameters, clot lysis assays, PAP complexes, tPA, uPA, PAI-1, A2AP and TAFIa, I calculated normal ranges by assessing 25 normal controls and generating 95% confidence intervals around the mean.

2.2.8 Statistical analysis

Summary statistics were calculated for all variables. For continuous variables, which were approximately normally distributed (platelet count, VWF:Ag, lag time, peak thrombin generation, ETP, ttPeak and 50% lysis time), means and standard deviations were presented; for non-normal data (PT, Clauss fibrinogen, PAP complexes, d-dimers, CRP, tPA, uPA, PAI-1, A2AP, TAFIa and PFA closure time), medians and interquartile ranges (IQR) were presented instead. Dichotomous data (antibiotics, antifungal drugs and clinical evidence of infection) were expressed as n (%). Sensitivity, specificity, positive predictive values and negative predictive values were extracted where appropriate. These analyses were performed using Prism version 7.0 (www.graphpad.com).

To take account of repeated measures for each patient when comparing bleeding and non-bleeding patients, I used statistical tests which took account of clustering. Normally distributed data were compared with t-tests with robust standard errors (also known as the Huber-White sandwich method). For non-normal data, I used the extended Mantel-Haenszel method. Univariate logistic regression with clustered standard errors was used to compare dichotomous variables between bleeding and non-bleeding patients using the *logistic* function in Stata. For all of these tests, each patient was considered to be a single cluster. These analyses were performed using Stata version 14 (www.stata.com). To account for multiple comparisons for tests comparing bleeding

versus non-bleeding patients, the Bonferroni correction was applied (221). There were 15 different comparators with a desired alpha level of 0.05. Consequently, a p-value of <0.003 (0.05/15) was considered to be statistically significant for these comparisons.

2.2.8.1 Multiple imputation and multivariable logistic regression

Multivariable logistic regression was used to assess the combined contribution of each test to bleeding risk. Bleeding is a binary outcome and multivariable logistic regression models take account of multiple variables (predictors) (222). The following potential predictors were assessed: platelet count, VWF:Ag, peak thrombin generation, fibrinogen, 50% lysis time, bilirubin, creatinine and evidence of infection. These parameters were selected because they have been shown in previous studies to be associated with risk of bleeding or because they represented one of the independent aspects of haemostasis outlined in Section 1.3. Additionally bilirubin and creatinine were added in case the results were confounded by renal failure or liver failure (Section 1.4.5). Clinical evidence of infection was included to assess the role of sepsis (Section 1.4.1). Outcomes were: WHO grade 1+ bleeding on the same day; WHO grade 2+ bleeding on the same day; WHO grade 1+ bleeding in the 24 hours following the blood sample; and WHO grade 2+ bleeding in the 24 hours following the blood sample.

Multiple imputation was used in order to reduce bias and imprecision from missing data (223). Missing data on predictors and outcomes were assumed to be *missing at random*. Five complete datasets were imputed via predictive mean matching with the "mice" (224) package in R (www.R-project.org). This method of multiple imputation is particularly effective when datasets contain non-parametric data (225).

For each of the four outcomes, a multilevel logistic regression model containing the eight predictors was fitted separately to each imputed dataset (using R's "lme4" (226)

package). The results were then combined across the five imputed datasets to create pooled odds ratios with 95% confidence intervals, which took into account uncertainty associated with imputation of missing data. A p-value of <0.05 was considered statistically significant.

2.3. Results

2.3.1 Patient characteristics

The characteristics of the patient cohort included in this study have previously been described (53) but are summarised here.

Fifty patients were enrolled into the study. Forty-nine patients were analysed, because one patient was switched to outpatient management after recruitment into the study, so was withdrawn from the study (Figure 2.1).

Patient characteristics are summarised in Table 2.2. All included patients had haematological malignancies and were undergoing intensive chemotherapy or stem cell transplantation. 34/49 (69%) had at least one bleeding episode; with 16/49 (33%) having at least one episode of clinically significant bleeding (WHO grade 2+). There were no instances of fatal (WHO grade 4) bleeding. Infection was common with 45/49 (92%) patients receiving antibiotics for treatment of infection during the study period. The patient cohort received a total of 175 units of platelets (two patients were treated with HLA-matched platelets) and 157 units of red cells. None of the patients received fresh frozen plasma, anticoagulants, antiplatelet drugs, tranexamic acid or asparaginase.

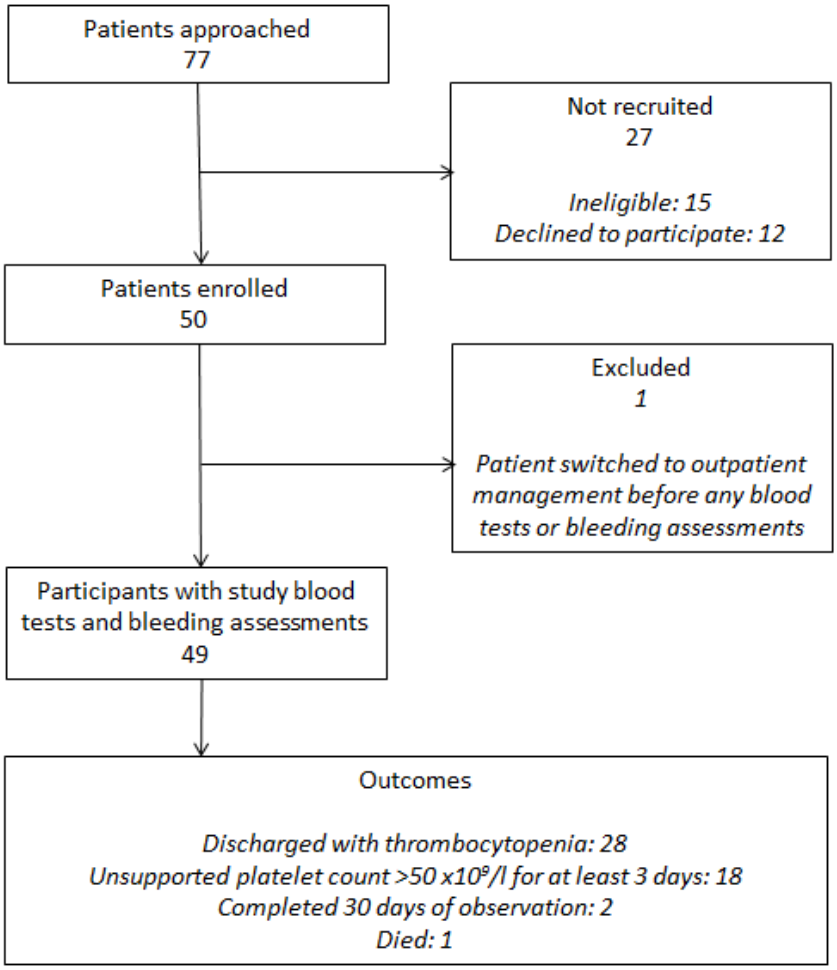


Figure 2.1 Study flow diagram

Demographics		Frequency n (%)
Age		51 (21-70) years[1]
Gender	Female	17 (35%)
	Male	32 (65%)
Disease	ALL	2 (4%)
	AML	12 (24%)
	APML	1 (2%)
	CLL	2 (4%)
	CML	3 (6%)
	Lymphoma	13 (27%)
	Lymphoma and MDS	1 (2%)
	MDS	4 (8%)
	Myelofibrosis	2 (4%)
	Myeloma	9 (18%)
Treatment	Induction chemotherapy	3 (6%)
	Autologous stem cell transplant	13 (27%)
	RIC allogeneic stem cell transplant	23 (47%)
	Myeloablative allogeneic stem cell transplant	10 (20%)
Highest WHO bleeding grade	0	15 (31%)
	1	18 (37%)
	2	15 (31%)
	3	1 (2%)
	4	0
Days of bleeding		3 (0 to 6) days[2]
Time from start of thrombocytopenia to first bleed		11 (8 to 16) days[2]
Antibiotics	Proportion of patients	45 (92%)
	Duration of treatment	10 (6 to 13) days[2]
Platelet transfusions	Prophylaxis against spontaneous bleeding	124 units (71%)
	Prophylaxis before a procedure	17 units (10%)
	Treatment of bleeding	34 units (19%)
	All platelet transfusions	*175 units*
Red cell transfusions	Transfused in absence of bleeding	155 units (99%)
	Treatment of bleeding	2 units (1%)
	All red cell transfusions	*157 units*
Total		**49**

Table 2.2 Patient characteristics. [1]Median (range). [2]Median (interquartile range). ALL: acute lymphoblastic leukaemia, AML: acute myeloid leukaemia, APML: acute promyelocytic leukaemia, CLL: chronic lymphocytic leukaemia, CML: chronic myeloid leukaemia, MDS: myelodysplastic syndrome, RIC: reduced intensity conditioning.

2.3.2 Measures of haemostasis

Blood results were obtained at 226 time-points (median 4, IQR 3 to 6 per patient) from 49 patients. These included 55 time-points with WHO grade 1+ bleeds and 14 with WHO grade 2+ bleeds on the same day as the test. Additionally there were 64 time-points with WHO grade 1+ bleeds and 15 with WHO grade 2+ bleeds in the 24 hours following the tests. Data completeness was 90 to 100% for most outcomes but was 69% for VWF antigen (Table 2.3). Comparison of results in patients with haematological malignancies undergoing intensive chemotherapy with the normal range are summarised in Table 2.4.

Variable	No bleeding [same day]	Bleeding [same day]	No bleeding [next 24hrs]	Bleeding [next 24hrs]
Platelet count	171 (100%)	55 (100%) *14 (100%)*	162 (100%)	64 (100%) *15 (100%)*
VWF antigen	111 (65%)	45 (82%) *12 (86%)*	106 (65%)	50 (78%) *14 (93%)*
Thrombin generation[1]	166 (97%)	52 (95%) *13 (93%)*	160 (98%)	58 (91%) *14 (93%)*
Prothrombin time	164 (96%)	41 (75%) *10 (93%)*	144 (89%)	61 (95%) *12 (80%)*
Fibrinogen	159 (93%)	52 (95%) *13 (93%)*	150 (93%)	61 (95%) *14 (93%)*
50% lysis	171 (100%)	54 (98%) *13 (93%)*	162 (100%)	62 (97%) *15 (100%)*
PAP complexes	164 (96%)	53 (96%) *12 (86%)*	154 (95%)	63 (97%) *13 (87%)*
D-dimers	157 (92%)	53 (96%) *13 (93%)*	148 (91%)	62 (97%) *14 (93%)*
C- reactive protein	171 (100%)	55 (100%) *14 (100%)*	162 (100%)	64 (100%) *15 (100%)*
Total	***171***	***Grade 1: 55*** ***Grade 2+: 14***	***162***	***Grade 1: 64*** ***Grade 2+: 15***

Table 2.3 Data completeness. Results expressed as number of time-points and percentage of data captured for each test (100% indicates that every time point was captured). Bleeding defined as World Health Organization grade 1+ bleeding. World Health Organization grade 2+ bleeds are in italics. PAP: plasmin-alpha-2-antiplasmin complexes. VWF: Von Willebrand Factor. [1]all thrombin generation parameters reported together.

Variable		Normal range
Platelet count (x10^9/l)[1]	22.4 ± 11.4	*150 to 400*
	n=226	
VWF:Ag (iu/ml)	2.31 ± 0.82	*0.5 to 1.5*
	n=156	
Lag time (min)	3.82 ± 1.40	*1.5 to 3.8*
	n=218	
Peak (nM)	294 ± 104	*176 to 406*
	n=218	
ETP (nM.min)	1705 ± 408	*944 to 2048*
	n=218	
ttPeak (min)	6.81 ± 2.02	*3.7 to 6.8*
	n=218	
PT (sec)	14.1 ± 5.2	*12 to 16*
	n=205	
Fibrinogen (g/l)	4.41 ± 1.67	*1.5 to 4.0*
	n=211	
50% lysis (mins)	86.2 ± 22.2	*54 to 136*
	n=225	
PAP (ng/ml)	682.5 (447 to 1086)	*93 to 384*
	n=217	
D-dimers (FEU/l)	862 (492 to 1765)	*0 to 500*
	n=210	
CRP (mg/l)	28.3 (9 to 72.5)	*0 to 5*
	n=226	

Table 2.4 Measures of haemostasis in trial population. Results expressed as mean ± standard deviation or median (interquartile range). CRP: C-reactive protein. ETP: endogenous thrombin potential. PAP: plasmin-alpha-2-antiplasmin complexes. PT: prothrombin time. ttPeak: time to peak thrombin generation. VWF:Ag: Von Willebrand Factor antigen. [1]patients were only eligible for the study when their platelet count was < 50 x10^9/l.

Time-points where there was WHO grade 1+ bleeding (Table 2.5) or WHO grade 2+ bleeding on the same day (Table 2.6) were compared to time-points where there was no bleeding. Likewise, time-points where there was WHO grade 1+ bleeding in the 24

hours following the test (Table 2.7) or WHO grade 2+ bleeding (Table 2.8) are summarised below.

Variable	WHO grade 1+ bleeding	No bleeding	Summary statistic
Platelet count (x10^9/l)	19 ± 9 *n=55*	23 ± 12 *n=171*	p=0.01
VWF antigen (iu/ml)	2.19 ± 0.81 *n=45*	2.36 ± 0.83 *n=111*	p=0.31
Lag time (min)	4.02 ± 1.55 *n=52*	3.75 ± 1.35 *n=166*	p=0.25
Peak thrombin (nM)	292 ± 73 *n=52*	290 ± 88 *n=166*	p=0.71
ETP (nM.min)	1753 ± 325 *n=52*	1695 ± 429 *n=166*	p=0.39
ttPeak (min)	6.92 ± 1.82 *n=52*	6.67 ± 1.87 *n=166*	p=0.32
Prothrombin time (sec)	14.0 ± 3.1 *n=41*	14.2 ± 5.8 *n=164*	p=0.84
Fibrinogen (g/l)	4.58 ± 1.54 *n=52*	4.35 ± 1.71 *n=159*	p=0.48
50% lysis time (min)	90 ± 30 *n=54*	85 ± 19 *n=171*	p=0.30
PAP complexes (ng/ml)	791 (473 to 1208) *n=53*	661 (436 to 1052) *n=164*	p=0.06
D-dimer (FEU/l)	911 (420 to 1873) *n=53*	825 (506 to 1678) *n=157*	p=0.06
C-reactive protein (mg/l)	36.7 (13.4 to 84.2) *n=55*	23.0 (8.0 to 66.3) *n=171*	p=0.49

Table 2.5 World Health Organization (WHO) grade 1+ bleeding on same day versus no bleeding. Normally distributed data expressed as mean ± standard deviation and compared with t-tests with robust standard errors. Non-normally distributed data expressed as median (interquartile range) and compared with the extended Mantel-Haenszel method. P-value <0.003 considered to be statistically significant. ETP: endogenous thrombin potential. PAP: plasmin-alpha-2-antiplasmin. ttPeak: time to peak thrombin generation. VWF: Von Willebrand Factor.

Variable	WHO grade 2+ bleeding	No bleeding	Summary statistic
Platelet count (x10^9/l)	22 ± 8 *n=14*	23 ± 12 *n=171*	p=0.80
VWF antigen (iu/ml)	1.86 ± 0.49 *n=12*	2.36 ± 0.83 *n=111*	p=0.005
Lag time (min)	4.02 ± 1.55 *n=13*	3.75 ± 1.35 *n=166*	p=0.49
Peak thrombin (nM)	292 ± 73 *n=13*	290 ± 88 *n=166*	p=0.63
ETP (nM.min)	1717 ± 313 *n=13*	1695 ± 429 *n=166*	p=0.93
ttPeak (min)	6.53 ± 0.66 *n=13*	6.67 ± 1.87 *n=166*	p=0.30
Prothrombin time (sec)	14.7 ± 3.5 *n=10*	14.2 ± 5.8 *n=164*	p=0.50
Fibrinogen (g/l)	4.68 ± 1.40 *n=13*	4.35 ± 1.71 *n=159*	p=0.45
50% lysis time (min)	94 ± 27 *n=13*	85 ± 19 *n=171*	p=0.24
PAP complexes (ng/ml)	780 (420 to 1178) *n=12*	661 (436 to 1052) *n=164*	p=0.77
D-dimer (FEU/l)	911 (420 to 1873) *n=13*	825 (506 to 1678) *n=157*	p=0.35
C-reactive protein (mg/l)	26.9 (7.6 to 46.7) *n=14*	23.0 (8.0 to 66.3) *n=171*	p=0.75

Table 2.6 World Health Organization (WHO) grade 2+ bleeding on same day versus no bleeding. Normally distributed data expressed as mean ± standard deviation and compared with t-tests with robust standard errors. Non-normally distributed data expressed as median (interquartile range) and compared with the extended Mantel-Haenszel method. P-value <0.003 considered to be statistically significant. ETP: endogenous thrombin potential. PAP: plasmin-alpha-2-antiplasmin. ttPeak: time to peak thrombin generation. VWF: Von Willebrand Factor.

Variable	WHO grade 1+ bleeding	No bleeding	Summary statistic
Platelet count (x10^9/l)	24 ± 11 *n=64*	22 ± 12 *n=162*	p=0.66
VWF antigen (iu/ml)	2.24 ± 0.76 *n=50*	2.42 ± 0.88 *n=106*	p=0.31
Lag time (min)	4.00 ± 1.43 *n=58*	3.79 ± 1.42 *n=160*	p=0.36
Peak thrombin (nM)	284 ± 68 *n=58*	288 ± 95 *n=160*	p=0.70
ETP (nM.min)	1705 ± 355 *n=58*	1697 ± 443 *n=160*	p=0.71
ttPeak (min)	6.89 ± 1.72 *n=58*	6.89 ± 2.31 *n=160*	p=0.30
Prothrombin time (sec)	14.2 ± 2.6 *n=61*	13.8 ± 5.5 *n=144*	p=0.80
Fibrinogen (g/l)	4.79 ± 1.45 *n=61*	4.31 ± 1.77 *n=150*	p=0.09
50% lysis time (min)	89 ± 27 *n=62*	85 ± 19 *n=162*	p=0.28
PAP complexes (ng/ml)	768 (514 to 1134) *n=63*	674 (452 to 1053) *n=154*	p=0.26
D-dimer (FEU/l)	911 (513 to 1934) *n=62*	830 (497.5 to 1678) *n=148*	p=0.39
C-reactive protein (mg/l)	40.0 (12.0 to 75.5) *n=64*	21.2 (6.8 to 59.4) *n=162*	p=0.13

Table 2.7 World Health Organization (WHO) grade 1+ bleeding in 24 hours following test versus no bleeding. Normally distributed data expressed as mean ± standard deviation and compared with t-tests with robust standard errors. Non-normally distributed data expressed as median (interquartile range) and compared with the extended Mantel-Haenszel method. P-value <0.003 considered to be statistically significant. ETP: endogenous thrombin potential. PAP: plasmin-alpha-2-antiplasmin. ttPeak: time to peak thrombin generation. VWF: Von Willebrand Factor.

Variable	WHO grade 2+ bleeding	No bleeding	Summary statistic
Platelet count (x10^9/l)	28 ± 13 n=15	22 ± 12 n=162	p=0.98
VWF antigen (iu/ml)	1.93 ± 0.51 n=14	2.42 ± 0.88 n=106	p=0.04
Lag time (min)	4.62 ± 2.13 n=14	3.79 ± 1.42 n=160	p=0.14
Peak thrombin (nM)	277 ± 77 n=14	288 ± 95 n=160	p=0.53
ETP (nM.min)	1569 ± 349 n=14	1697 ± 443 n=160	p=0.21
ttPeak (min)	7.51 ± 2.51 n=14	6.89 ± 2.31 n=160	p=0.35
Prothrombin time (sec)	14.2 ± 2.2 n=12	14.1 ± 6.5 n=144	p=0.41
Fibrinogen (g/l)	4.75 ± 0.94 n=14	4.31 ± 1.77 n=150	p=0.50
50% lysis time (min)	89 ± 15 n=15	85 ± 19 n=162	p=0.53
PAP complexes (ng/ml)	951 (583 to 1217) n=13	674 (452 to 1053) n=154	p=0.10
D-dimer (FEU/l)	911 (428 to 2500) n=14	830 (498 to 1678) n=148	p=0.30
C-reactive protein (mg/l)	14.2 (4.1 to 57.9) n=15	21.2 (6.8 to 59.4) n=162	p=0.30

Table 2.8 World Health Organization (WHO) grade 2+ bleeding in 24 hours following test versus no bleeding. Normally distributed data expressed as mean ± standard deviation and compared with t-tests with robust standard errors. Non-normally distributed data expressed as median (interquartile range) and compared with the extended Mantel-Haenszel method. P-value <0.003 considered to be statistically significant. ETP: endogenous thrombin potential. PAP: plasmin-alpha-2-antiplasmin. ttPeak: time to peak thrombin generation. VWF: Von Willebrand Factor.

2.3.2.1 Primary haemostasis

All patients included in this study had platelet counts <50 $x10^9$/l. VWF antigen levels were greater than the reference range at 134/156 (86%) time-points. VWF antigen was not below the reference range at any time-point.

There was no significant difference in platelet count (19 ± 9 $x10^9$/l vs 23 ± 12 $x10^9$/l; p=0.01) or VWF:Ag (2.19 ± 0.81 iu/ml vs 2.36 ± 0.83 iu/ml; p=0.31) for time-points with WHO grade 1+ bleeding and time-points with no bleeding (Figure 2.2). There was also no significant difference in platelet count (22 ± 8 $x10^9$/l vs 23 ± 12 $x10^9$/l; p=0.80) or VWF:Ag (1.86 ± 0.49 iu/ml vs 2.36 ± 0.83 iu/ml; p=0.005) between those with WHO grade 2+ bleeding and those with no bleeding.

The results were similar for bleeding over the 24 hours following the blood test. There was no significant difference in platelet count (24 ± 11 $x10^9$/l vs 22 ± 12 $x10^9$/l; p=0.66) or VWF:Ag (2.24 ± 0.76 iu/ml vs 2.42 ± 0.88 iu/ml; p=0.31) for time-points with WHO grade 1+ bleeding and time-points with no bleeding. There was also no significant difference in platelet count (28 ± 13 $x10^9$/l vs 22 ± 12 $x10^9$/l; p=0.98) or VWF:Ag (1.93 ± 0.51 iu/ml vs 2.42 ± 0.88 iu/ml; p=0.04) between those with WHO grade 2+ bleeding and those with no bleeding.

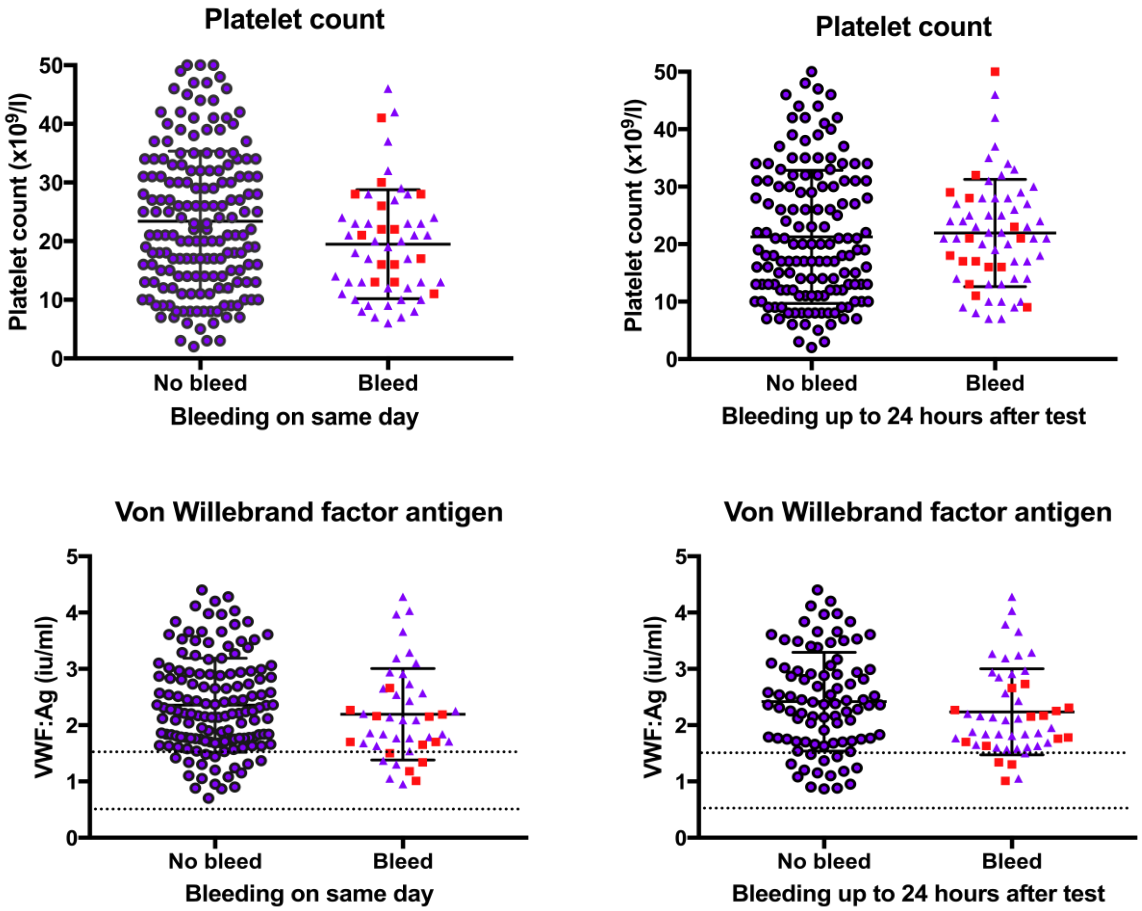


Figure 2.2 Scatter dot plots: primary haemostasis and bleeding. Comparison of platelet count and Von Willerband Factor antigen (VWF:Ag) between patients with, or without bleeding. Figures on the left represent bleeding on the same day the blood test was taken. Figures on the right represent bleeding over the 24 hours following the blood test. For bleeding patients, blue triangles represent bleeds of World Health Organization (WHO) grade 1. Red squares indicate bleeding of WHO grade 2 or more. Error bars represent mean and standard deviation. Dotted lines represent normal range. Lower limit of normal range for platelet count is 150 x10^9/l (not shown). Platelet count n=226; VWF:Ag n=156.

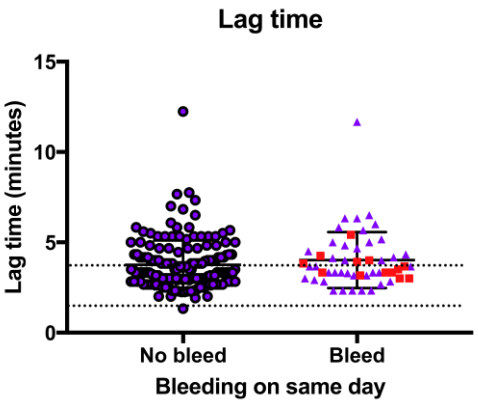
2.3.2.2 Thrombin generation

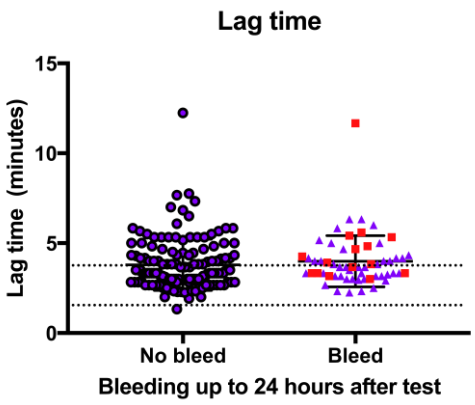
Lag time was less than the reference range at 1/218 (<1%) time-points and greater at 91/218 (42%) time-points. Peak thrombin generation was less than the reference range at 21/218 (10%) time-points and greater at 19/218 (9%) time-points. ETP was less than the reference range at 6/218 (3%) time-points and greater at 33/218 (15%) time-

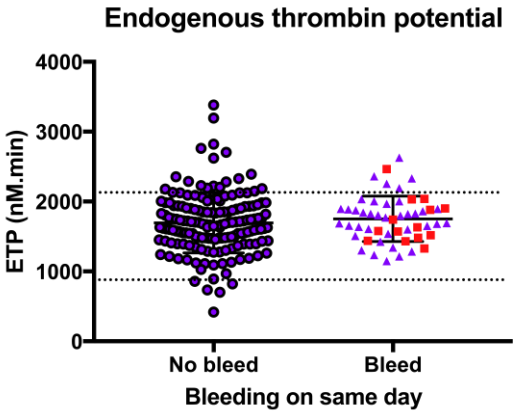
points. Time to peak thrombin generation was less than the reference range at 1/218 (<1%) time-points and greater at 88/218 (40%) time-points.

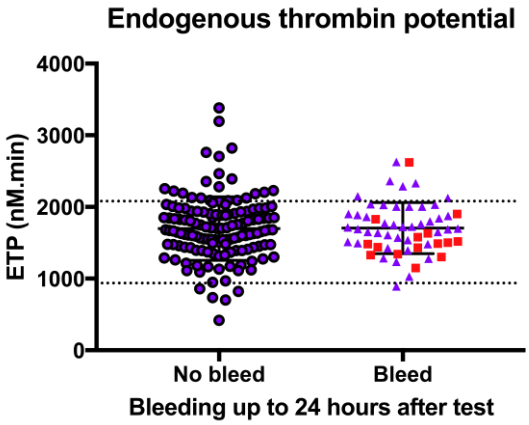
There was no significant difference in lag time (4.02 ± 1.55 minutes vs 3.75 ± 1.35 minutes; p=0.25), peak thrombin generation (292 ± 73 nM vs 290 ± 88 nM; p=0.71), ETP (1753 ± 325 nM.min vs 1695 ± 429 nM.min; p=0.39) or ttPeak (6.92 ± 1.82 minutes vs 6.67 ± 1.87 minutes; p=0.32) for time-points with WHO grade 1+ bleeding and time-points with no bleeding (Figure 2.3). There was also no significant difference in lag time (4.02 ± 1.55 minutes vs 3.75 ± 1.35 minutes; p=0.49), peak thrombin generation (292 ± 73 nM vs 290 ± 88 nM; p=0.63), ETP (1717 ± 313 nM.min vs 1695 ± 429 nM.min; p=0.93) or ttPeak (6.53 ± 0.66 minutes vs 6.67 ± 1.87 minutes; p=0.30) between those with WHO grade 2+ bleeding and those with no bleeding.

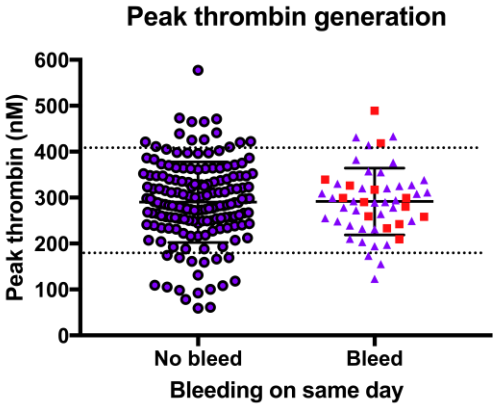
The results were similar for bleeding over the 24 hours following the blood test. There was no significant difference in lag time (4.00 ± 1.43 minutes vs 3.79 ± 1.42 minutes; p=0.36), peak thrombin generation (284 ± 68 nM vs 288 ± 95 nM; p=0.70), ETP (1705 ± 355 nM.min vs 1697 ± 443 nM.min; p=0.71) or ttPeak (6.89 ± 1.72 minutes vs 6.89 ± 2.31 minutes; p=0.30) for time-points with WHO grade 1+ bleeding and time-points with no bleeding. There was also no significant difference in lag time (4.62 ± 2.13 minutes vs 3.79 ± 1.42 minutes; p=0.14), peak thrombin generation (277 ± 77 nM vs 288 ± 95 nM; p=0.53), ETP (1569 ± 349 nM.min vs 1697 ± 443 nM.min; p=0.21) or ttPeak (7.51 ± 2.51 minutes vs 6.89 ± 2.31 minutes; p=0.35) between those with WHO grade 2+ bleeding and those with no bleeding.

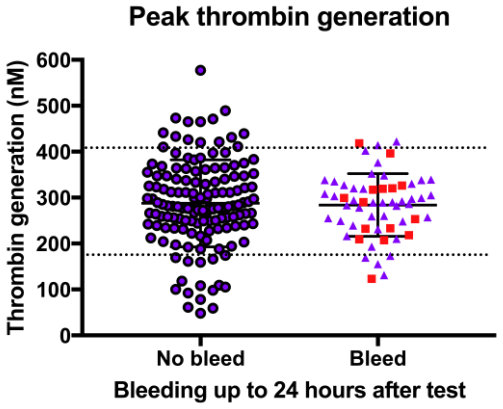

Lag time
Lag time (minutes)
15
10
5
0
No bleed
Bleed
Bleeding on same day

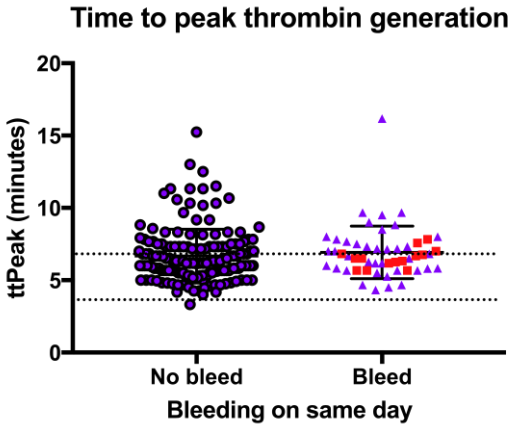

Lag time
Lag time (minutes)
15
10
5
0
No bleed
Bleed
Bleeding up to 24 hours after test


Endogenous thrombin potential
ETP (nM.min)
4000
3000
2000
1000
0
No bleed
Bleed
Bleeding on same day


Endogenous thrombin potential
ETP (nM.min)
4000
3000
2000
1000
0
No bleed
Bleed
Bleeding up to 24 hours after test


Peak thrombin generation
Peak thrombin (nM)
600
500
400
300
200
100
0
No bleed
Bleed
Bleeding on same day


Peak thrombin generation
Thrombin generation (nM)
600
500
400
300
200
100
0
No bleed
Bleed
Bleeding up to 24 hours after test


Time to peak thrombin generation
ttPeak (minutes)
20
15
10
5
0
No bleed
Bleed
Bleeding on same day

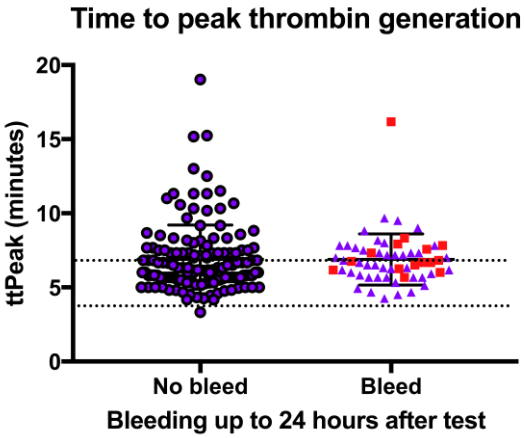

Time to peak thrombin generation
ttPeak (minutes)
20
15
10
5
0
No bleed
Bleed
Bleeding up to 24 hours after test

Figure 2.3 Scatter dot plots: thrombin generation and bleeding. Comparison of thrombin generation parameters between patients with, or without bleeding. Figures on the left represent bleeding on the same day the blood test was taken. Figures on the right represent bleeding over the 24 hours following the blood test. For bleeding patients, blue triangles represent bleeds of World Health Organization (WHO) grade 1. Red squares indicate bleeding of WHO grade 2 or more. Error bars represent mean and standard deviation. Dotted lines represent the normal range. ETP: endogenous thrombin potential. ttPeak: time to peak thrombin generation. n=218 for all parameters.

2.3.2.3 Prothrombin time and fibrinogen

PT was less than the reference range at 33/205 (17%) time-points and greater at 31/205 (16%) time-points. Clauss fibrinogen was less than the reference range at 2/211 (1%) time-points and greater at 120/211 (57%) time-points.

There was no significant difference in PT (14.0 ± 3.1 seconds vs median 14.2 ± 5.8 seconds; p=0.84) or Clauss fibrinogen (4.58 ± 1.54 g/l vs 4.35 ± 1.71 g/l; p=0.48) for time-points with WHO grade 1+ bleeding and time-points with no bleeding (Figure 2.4). There was also no significant difference in PT (14.7 ± 3.5 seconds vs 14.2 ± 5.8 seconds; p=0.50) or Clauss fibrinogen (4.68 ± 1.40 g/l vs 4.35 ± 1.71 g/l; p=0.45) between those with WHO grade 2+ bleeding and those with no bleeding.

The results were similar for bleeding over the 24 hours following the blood test. There was no significant difference in PT (14.2 ± 2.6 seconds vs 13.8 ± 5.5 seconds; p=0.80) or Clauss fibrinogen (4.79 ± 1.45 g/l vs 4.31 ± 1.77 g/l; p=0.09) for time-points with WHO grade 1+ bleeding and time-points with no bleeding. There was also no significant difference in PT (14.2 ± 2.2 seconds vs 14.1 ± 6.5 seconds; p=0.41) or Clauss fibrinogen (4.75 ± 0.94 g/l vs 4.31 ± 1.77 g/l; p=0.50) between those with WHO grade 2+ bleeding and those with no bleeding.

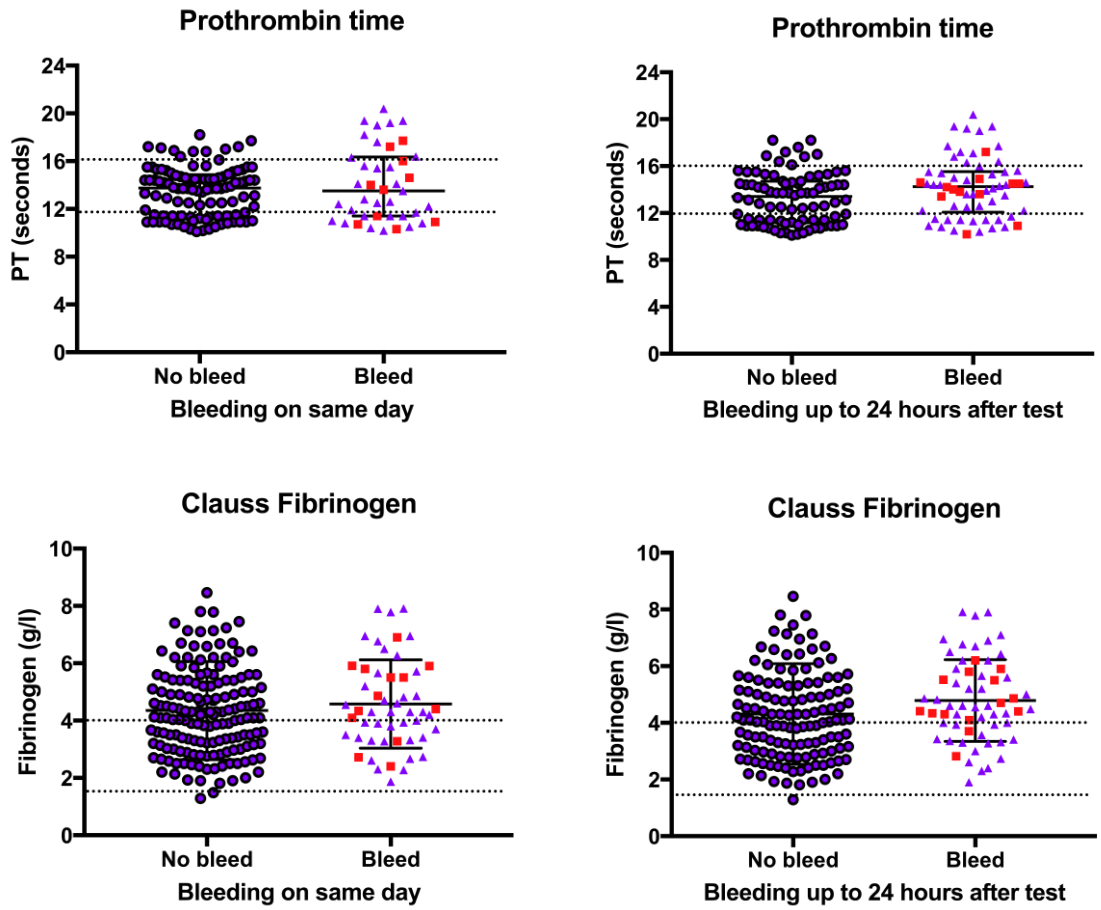


Figure 2.4 Scatter dot plots: prothrombin time, fibrinogen and bleeding. Comparison of prothrombin time (PT) and Clauss fibrinogen between patients with, or without bleeding. Figures on the left represent bleeding on the same day the blood test was taken. Figures on the right represent bleeding over the 24 hours following the blood test. For bleeding patients, blue triangles represent bleeds of World Health Organization (WHO) grade 1. Red squares indicate bleeding of WHO grade 2 or more. Error bars represent mean and standard deviation. Dotted lines represent the normal range. N=205 for prothrombin time and n=211 for Clauss fibrinogen.

2.3.2.4 Fibrinolysis

50% lysis time was less than the reference range at 3/225 (1%) time-points and greater at 8/225 (4%) time-points. PAP complexes were greater than the reference range at 185/217 (85%) time-points. D-dimers were greater than the reference range at 157/217 (75%) time-points. PAP complexes and d-dimer were not below the reference range at any time-point.

There was no significant difference in 50% lysis time (90 ± 30 minutes vs 85 ± 19 minutes; p=0.30), PAP complexes (median 791, IQR 472.5 to 1208 ng/ml vs median 661, IQR 436 to 1052; p-0.06), or d-dimers (median 911, IQR 420 to 1873 FEU/l vs median 825, IQR 506 to 1678 FEU/l; p=0.06) for time-points with WHO grade 1+ bleeding and time-points with no bleeding (Figure 2.5). There was also no significant difference in 50% lysis time (94 ± 27 minutes vs 85 ± 19 minutes; p=0.24), PAP complexes (median 780, IQR 420 to 1178 ng/ml vs median 661, IQR 436 to 1052 ng/ml; p=0.77), or d-dimers (median 911, IQR 420 to 1873 FEU/l vs median 825, IQR 506 to 1678 FEU/l; p=0.35) between those with WHO grade 2+ bleeding and those with no bleeding.

The results were similar for bleeding over the 24 hours following the blood test. There was no significant difference in 50% lysis time (89 ± 27 minutes vs 85 ± 19 minutes; p=0.28), PAP complexes (median 768, IQR 514 to 1134 ng/ml vs median 674, IQR 452 to 1053 ng/ml; p=0.26), or d-dimers (median 911, IQR 513 to 1934 FEU/l vs median 830, IQR 497.5 to 1678 FEU/l; p=0.39) for time-points with WHO grade 1+ bleeding and time-points with no bleeding. There was also no significant difference in 50% lysis time (89 ± 15 minutes vs 85 ± 19 minutes; p=0.53), PAP complexes (median 951, IQR 583 to 1217 ng/ml vs median 674, IQR 452 to 1053; p=0.10), or d-dimers (median 911, IQR 428 to 2500 FEU/l vs median 830, IQR 498 to 1678 FEU/l; p=0.30) between those with WHO grade 2+ bleeding and those with no bleeding.

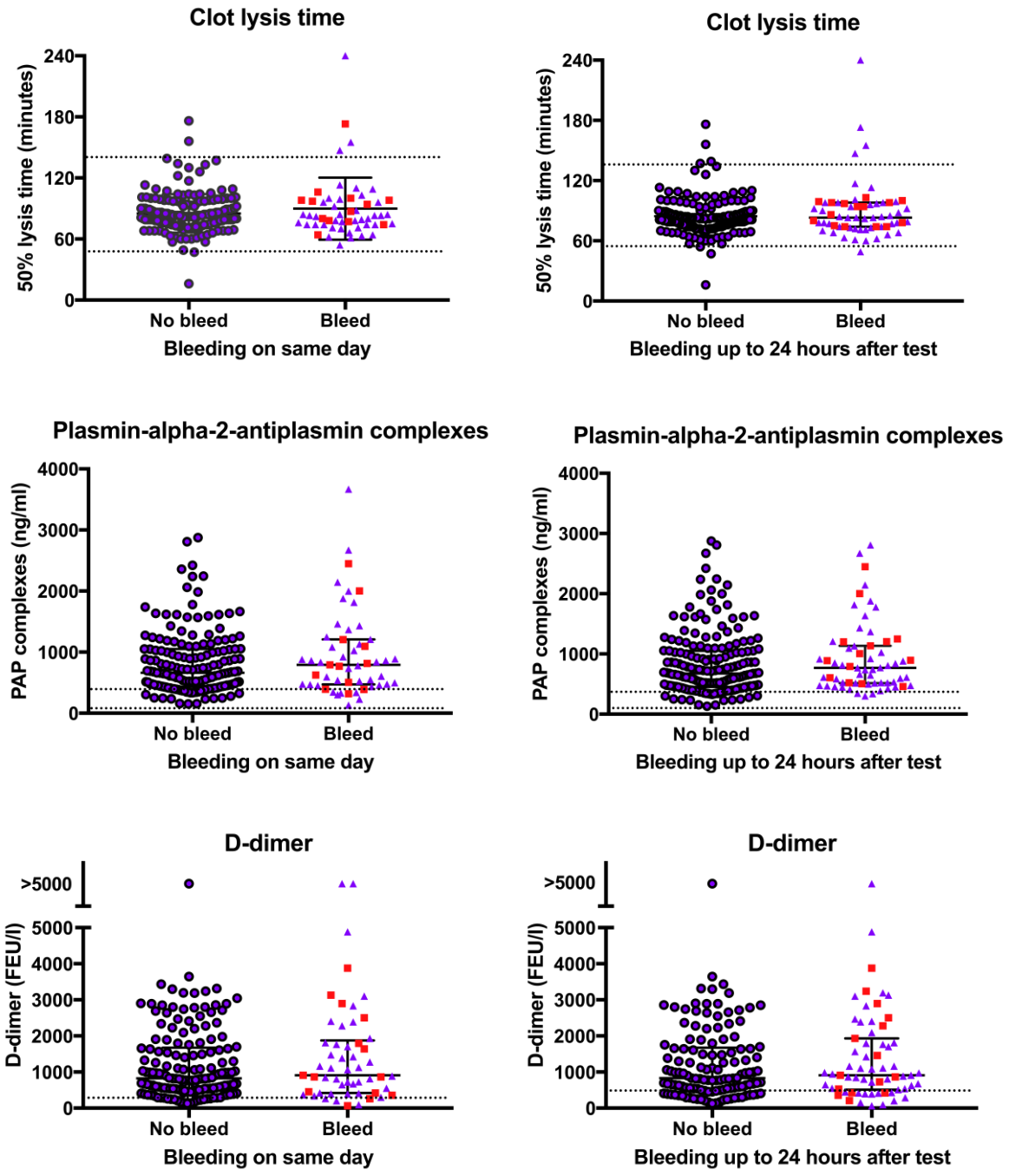


Figure 2.5 Scatter dot plots: fibrinolysis and bleeding. Comparison of measures of fibrinolysis between patients with, or without bleeding. Figures on the left represent bleeding on the same day the blood test was taken. Figures on the right represent bleeding over the 24 hours following the blood test. For bleeding patients, blue triangles represent bleeds of World Health Organization (WHO) grade 1. Red squares indicate bleeding of WHO grade 2 or more. Error bars represent mean and standard deviation for 50% lysis time and. median and interquartile range for PAP (plasmin-alpha-2-antiplasmin) complexes and d-dimer. D-dimer of >5000 FEU/l was above upper limit for detection. Dotted lines represent the normal range. For

d-dimer the lower limit of the normal range is 0 FEU/l (not shown). N=225 for 50% lysis time; n=217 for PAP complexes and n=210 for d-dimers.

2.3.2.5 Infection and inflammation

CRP was greater than the reference range at 186/226 (82%) time-points.

There was no significant difference in CRP (median 36.7, IQR 13.4 to 84.2 mg/l vs median 23.0, IQR 8.0 to 66.3 mg/l; p=0.49) for time-points with WHO grade 1+ bleeding and time-points with no bleeding (Figure 2.6). There was also no significant difference in CRP (median 26.9, IQR 7.6 to 46.7 mg/l vs median 23.0, IQR 8.0 to 66.3 mg/l; p=0.75) between those with WHO grade 2+ bleeding and those with no bleeding.

The results were similar for bleeding over the 24 hours following the blood test. There was no significant difference in CRP (median 40.0, IQR 12.0 to 75.5 mg/l vs median 21.2, IQR 6.8 to 59.4 mg/l; p=0.13) for time-points with WHO grade 1+ bleeding and time-points with no bleeding. There was also no significant difference in CRP (median 14.2, IQR 4.1 to 57.9 mg/l vs median 21.2, IQR 6.8 to 59.4 mg/l; p=0.30) between those with WHO grade 2+ bleeding and those with no bleeding.

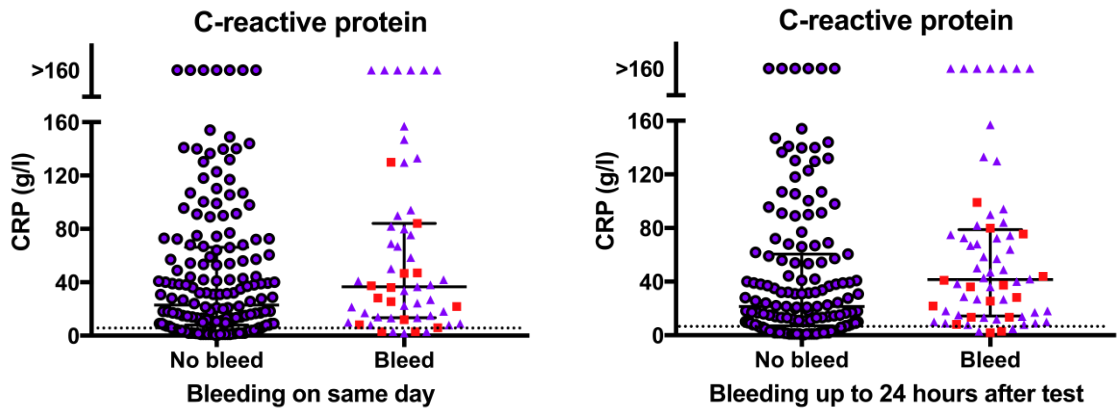


Figure 2.6 Scatter dot plots: C-reactive protein and bleeding. Comparison of C-reactive protein (CRP) between patients with, or without bleeding. Figures on the left represent bleeding on the same day the blood test was taken. Figures on the right represent bleeding over the 24 hours following the blood test. For bleeding patients, blue triangles represent bleeds of World Health Organization (WHO) grade 1. Red squares indicate bleeding of WHO grade 2 or more. Error bars represent median and interquartile range. Dotted linee represents upper limit of normal range (lower limit is 0 mg/l – not shown). CRP >160 mg/l was above upper limit for detection. N=226.

There was no difference in the risk of bleeding for patients taking antibiotics or antifungal drugs (Table 2.9). There was an increase in WHO grade 1+ bleeding over the following 24 hours for patients with clinical evidence of infection but this was not statistically significant (odds ratio 2.79, 95%CI 1.07 to 7.27; p=0.04). There was also no significant difference in WHO grade 2+ bleeding for those with, or without clinical evidence of infection (odds ratio 2.05, 95%CI 0.44 to 9.59; p=0.36).

Clinical factor	WHO grade 1+	WHO grade 2+
Odds of bleed on same day		
Antibiotics	1.63 (0.67 to 3.95)	0.79 (0.27 to 2.29)
	P=0.28	*P=0.66*
Antifungals	2.28 (0.87 to 6.01)	1.20 (0.27 to 5.24)
	P=0.10	*P=0.81*
Evidence of infection	1.74 (0.82 to 3.69)	1.33 (0.44 to 4.06)
	P=0.15	*P=0.62*
Odds of bleed in 24 hours after test taken		
Antibiotics	2.36 (0.91 to 6.14)	1.61 (0.41 to 6.37)
	P=0.08	*P=0.49*
Antifungals	1.83 (0.82 to 4.09)	0.44 (0.05 to 3.83)
	P=0.14	*P=0.45*
Evidence of infection	2.79 (1.07 to 7.27)[1]	2.05 (0.44 to 9.59)
	P=0.04	*P=0.36*

Table 2.9 Markers of infection and bleeding. Statistics: univariate logistic regression odds ratios with 95% confidence intervals with P<0.003 considered statistically significant. [1]95% confidence interval of odds ratio does not cross one, but P-value not statistically significant with Bonferroni correction. WHO: World Health Organization. N=226.

The sensitivity and specificity were low for any of the infection parameters for prediction of bleeding. However absence of infection, (or absence of antibiotic therapy as a surrogate measure) had high negative predictive values for WHO grade 2+ bleeding, although the positive predictive value was low (Table 2.10).

Clinical factor	Sensitivity	Specificity	PPV	NPV
Same day WHO grade 1+ bleeding				
Antibiotics	0.71	0.40	0.27	0.81
Antifungals	0.2	0.90	0.39	0.78
Evidence of infection	0.75	0.37	0.28	0.82
Same day WHO grade 2+ bleeding				
Antibiotics	0.57	0.37	0.06	0.93
Antifungals	0.14	0.88	0.07	0.94
Evidence of infection	0.71	0.35	0.07	0.95
Next day WHO grade 1+ bleeding				
Antibiotics	0.77	0.42	0.37	0.80
Antifungals	0.19	0.89	0.43	0.71
Evidence of infection	0.81	0.39	0.37	0.82
Next day WHO grade 2+ bleeding				
Antibiotics	0.73	0.37	0.08	0.95
Antifungals	0.81	0.39	0.37	0.82
Evidence of infection	0.80	0.34	0.09	0.96

Table 2.10 Sensitivity and specificity of infection for bleeding. PPV: positive predictive value. NPV: negative predictive value. WHO: World Health Organization.

2.3.3 Other assessments of haemostasis

2.3.3.1 Promotors and inhibitors of fibrinolysis

For each patient, the sample with the highest PAP complexes was identified and I analysed the fibrinolytic activators and inhibitors (Figure 2.7). TPA (median 11.8, IQR 6.4 to 15.8 ng/ml) was greater than the reference range in 23/49 (47%) cases; and uPA (median 1.91, IQR 1.38 to 2.33 ng/ml) was greater in 19/49 (39%) cases. There were no instances of low tPA or uPA in the peak fibrinolysis samples.

PAI-1 (median 7.8, IQR 3.7 to 18.2 ng/ml) was less than the reference range in 10/49 (20%) cases. There were no instances of elevated PAI-1. A2AP (median 1.02, IQR 0.93 to 1.11 iu/ml) was less in 8/49 (16%) cases and greater in 4/49 (8%) cases. TAFIa (median 23.0, IQR 14.8 to 34.6 ng/ml) was greater than the reference range in 27/49 (55%) cases and there were no instances of decreased TAFIa. FXIII (median 1.11, IQR 0.88 to 1.25 iu/ml) was within the normal range in all cases.

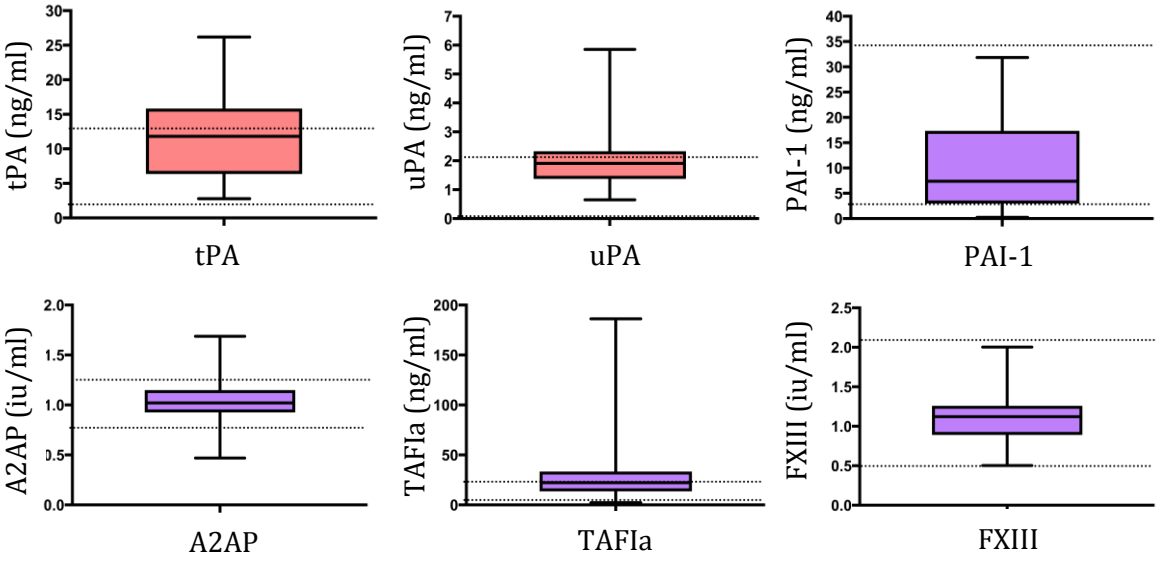


Figure 2.7 Box plots of promotors and inhibitors of fibrinolysis. Promotors in red; inhibitors in blue. Box plots indicate median, interquartile range and range. Dotted lines represent normal range. tPA: tissue plasminogen activator, uPA: urokinase plasminogen activator, PAI-1: plasminogen activator inhibitor-1, A2AP: alpha-2-antiplasmin, TAFIa: activated thrombin activatable fibrinolysis inhibitor.

2.3.3.2 Von Willebrand factor function

VWF function was analysed at a single timepoint for each patient with the highest PAP complexes to check for fibrinolysis-induced platelet dysfunction. VWF:Ag, VWF:Ac and VWF:CBA were greater than 0.5 iu/ml (the lower limit of normal) in all cases. VWF:Ag and VWF:Ac (Pearson's rank 0.84, 95%CI 0.69 to 0.92, R^2=0.70; p<0.001); and VWF;Ag

and VWF:CBA (Pearson's rank 0.67, 95%CI 0.42 to 0.83, R^2=0.45; p<0.001) were significantly positively correlated (Figure 2.8).

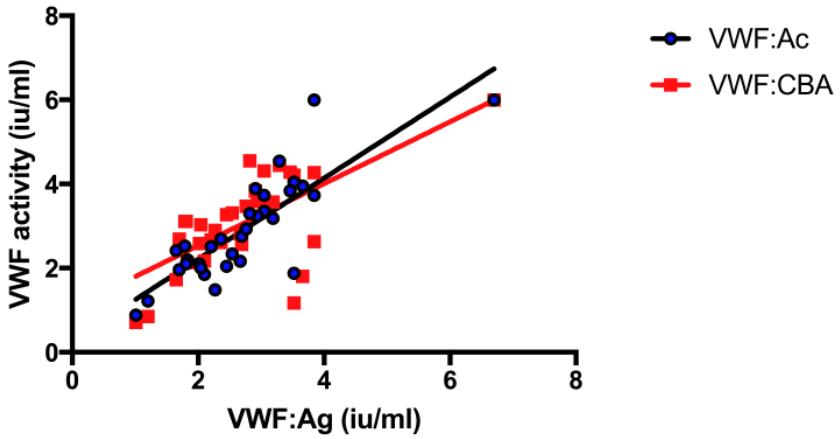


Figure 2.8 Scatter graph of VWF antigen and function. VWF:Ag: Von Willebrand factor antigen, VWF:Ac: Von Willebrand Factor activity (blue circles), VWF:CBA: VWF collagen binding (red squares)

2.3.3.3 Platelet function analyser closure time

PFA closure time results were available for 36 patients from the ATHENA cohort, who had either a VWF:Ag ≤2.0 iu/ml or ≥3.0 iu/ml. Patients with a VWF:Ag ≤2.0 iu/ml had a similar platelet count than those with a VWF:Ag ≥3.0 iu/ml (median 20; IQR 12 to 31 $x10^9$/l vs median 19; IQR 11 to 25 $x10^9$/l; p=0.59). Closure time was not reached in 22/25 (88%) patients with VWF:Ag ≤2.0 iu/ml and 8/11 (73%) patients with VWF:Ag ≥3.0 iu/ml. The lowest platelet count where a closure time was obtained for patients with VWF:Ag ≤2.0 iu/ml was 33 seconds, compared to 22 seconds for those with VWF:Ag ≥3.0 iu/ml (Figure 2.9).

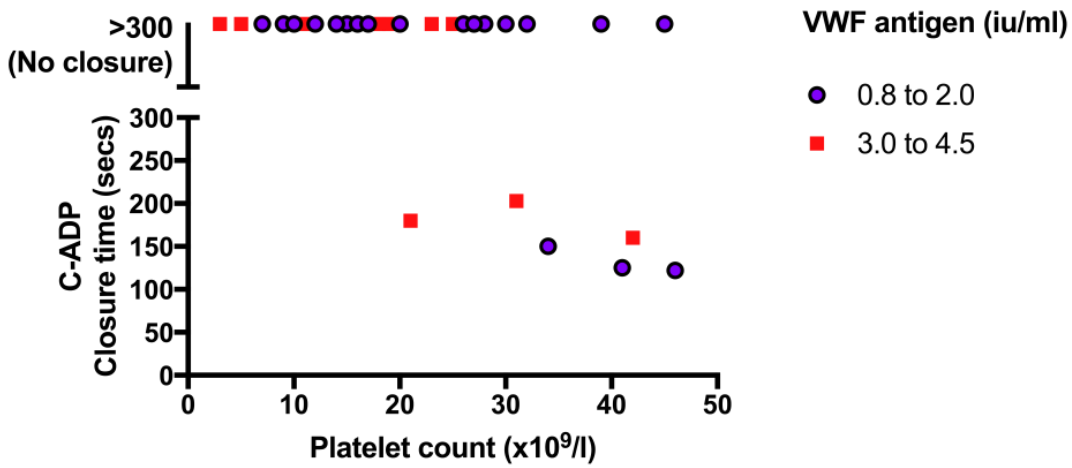


Figure 2.9 Scatter graph of platelet function analyser closure time. Closure time compared for samples with the lowest levels of VWF antigen (≤2.0 iu/ml) and the highest levels of VWF antigen (≥3.0 iu/ml). C-ADP: collagen/adenosine diphosphate cartridge.

2.3.4 Predictors of bleeding

None of the markers consistently predicted bleeding using logistic regression. There was a statistically significant association between platelet count and WHO grade 1+ bleeding on the same day (OR 0.94, 95%CI 0.91 to 0.99; p=0.01) and for VWF:Ag and WHO grade 2+ bleeding on the same day (OR 0.31, 95%CI 0.10 to 0.98; p=0.047; Table 2.11).

Predictor	WHO grade 1+	WHO grade 2+ bleed
	Odds ratio (95% CI)	Odds ratio (95% CI)
VWF (iu/ml)	**0.58** (0.29 to 1.17)	**0.31** (0.10 to 0.98)
	P=0.13	*P=0.047*
Fibrinogen (g/l)	**0.95** (0.72 to 1.26)	**1.03** (0.63 to 1.67)
	P=0.72	*P=0.91*
Platelet count ($x10^9$/l)	**0.94** (0.91 to 0.99)	**0.98** (0.92 to 1.04)
	P=0.01	*P=0.49*
Peak thrombin (nM)	**1.00** (1.00 to 1.01)	**1.00** (0.99 to 1.01)
	P=0.50	*P=0.89*
50% lysis (minutes)	**1.01** (0.99 to 1.03)	**1.02** (0.99 to 1.05)
	P=0.31	*P=0.28*
Bilirubin (mmol/l)	**1.01** (0.97 to 1.03)	**0.96** (0.89 to 1.04)
	P=0.97	*P=0.32*
Creatinine (μmol/l)	**1.01** (0.99 to 1.02)	**1.00** (0.98 to 1.02)
	P=0.28	*P=0.74*
Evidence of infection	**1.64** (0.61 to 4.41)	**1.37** (0.26 to 7.31)
	P=0.33	*P=0.71*

Table 2.11 Multivariable logistic regression: bleeding on same day. Odds ratios for all outcomes except "evidence of infection" are for every one unit increase. The odds ratios for "evidence of infection" are for infection compared to no infection. CI: confidence interval. VWF: Von Willebrand Factor. WHO: World Health Organization.

For bleeding in the 24 hours following the blood test, there was a statistically significant association between clinical evidence of infection and WHO grade 1+ bleeding (OR 4.22, 95%CI 1.41 to 12.66; p=0.01). None of the variables predicted WHO grade 2+ bleeding over the 24 hours following the blood sample (Table 2.12).

Predictor	WHO grade 1+ Odds ratio (95% CI)	WHO grade 2+ bleed Odds ratio (95% CI)
VWF (iu/ml)	**0.60** (0.30 to 1.22) *P=0.16*	**0.48** (0.10 to 2.34) *P=0.36*
Fibrinogen (g/l)	**1.07** (0.81 to 1.41) *P=0.65*	**1.00** (0.62 to 1.62) *P=0.99*
Platelet count ($x10^9$/l)	**1.03** (0.98 to 1.07) *P=0.21*	**1.01** (0.94 to 1.00) *P=0.76*
Peak thrombin (nM)	**1.00** (1.00 to 1.01) *P=0.26*	**1.00** (0.99 to 1.01) *P=0.38*
50% lysis (minutes)	**0.99** (0.96 to 1.01) *P=0.18*	**1.00** (0.96 to 1.05) *P=0.87*
Bilirubin (mmol/l)	**1.01** (0.98 to 1.04) *P=0.63*	**0.88** (0.76 to 1.01) *P=0.06*
Creatinine (μmol/l)	**1.01** (1.00 to 1.03) *P=0.12*	**1.00** (0.96 to 1.03) *P=0.81*
Evidence of infection	**4.22** (1.41 to 12.66) *P=0.01*	**2.76** (0.39 to 19.76) *P=0.31*

Table 2.12 Multivariable logistic regression: bleeding over next 24 hours. Odds ratios for all outcomes except "evidence of infection" are for every one unit increase. The odds ratios for "evidence of infection" are for infection compared to no infection. CI: confidence interval. VWF: Von Willebrand Factor. WHO: World Health Organization.

2.4 Discussion

2.4.1 Summary of results

To my knowledge, this is the first prospective study to examine the relationship between a wide range of haemostatic tests and bleeding in thrombocytopenic patients with haematological malignancies. Previous studies have found a weak relationship between platelet count and bleeding risk (53, 110, 123) and have examined

prothrombin time, aPTT, fibrinogen, rotational thromboelastometry and immature platelet fraction (53, 123, 227). This study incorporated additional tests including VWF:Ag, thrombin generation and clot lysis assays.

Overall, no haemostatic marker added value to the platelet count for prediction of bleeding. Thrombocytopenia was associated with WHO grade 1+ bleeding on the same day but not WHO grade 2+ bleeding, nor was it significant for predicting bleeding over the following 24 hours. Likewise, VWF:Ag was associated with WHO grade 2+ bleeding on the same day, but not WHO grade 1+ bleeding, or bleeding in the 24 hours after the blood test. Clinical evidence of infection was significant by multivariable logistic regression for predicting WHO grade 1+ bleeding over the following 24 hours. However there was not consistent evidence for this as a risk factor. Animal models suggest bleeding risk is increased with infection (111) but clinical data is divided on whether it is a risk factor (112-116).

2.4.1.1 Primary haemostasis

VWF:Ag was elevated in most patients with haematological malignancy undergoing chemotherapy. This is likely to be due to increased Weibel-Palade body release due to inflammation or the action of other agonists (61, 68). This increase in VWF:Ag is predominantly from the endothelium, although platelets (when present) make a small contribution (228). VWF is known to be increased following chemotherapy or stem cell transplant, and this increase is present for several weeks after the transplant (229). I found no significant evidence of VWF dysfunction amongst the patients where VWF:Ac or VWF:CBA were assessed.

2.4.1.2 Thrombin generation

Overall, lag-time, ETP and ttPeak were increased for patients with haematological malignancies undergoing chemotherapy. This is likely to be due to increased endothelial release of FVIII and prolongation of FVIII half-life due to elevated levels of VWF (230).

Lag-time, ETP and ttPeak measure thrombin generation potential, rather than *in vivo* thrombin generation. I suspect that *in vivo* thrombin generation was increased due to elevated levels of d-dimer, PAP complexes and TAFIa in the presence of a 50% lysis time that was within the normal range. This is consistent with the results of previous studies which have demonstrated increased levels of thrombin-anti-thrombin complexes or prothrombin 1+2 fragments following chemotherapy (231-233) or stem cell transplantation (234, 235). Many of the patients in this study had evidence of infection. Severe sepsis is known to change thrombin generation dynamics, with increased lag-time and ttPeak but maintained endogenous thrombin potential (236).

In this study, I did not find PT to be associated with risk of bleeding. However a recent retrospective review of 1077 adults patients (16,320 patient days) who were recruited into a clinical trial suggested that an INR >1.2 (equivalent to a PT of 17 seconds in this study) was associated with an increased risk of WHO grade 2+ bleeding (123). The same study reported that a haematocrit <25% and aPTT >30 seconds might be additional risk factors (123).

2.4.1.3 Fibrinogen

Clauss fibrinogen was elevated at 57% time-points, which is consistent with previous studies (53, 234, 237). Fibrinogen is an acute phase protein and may be released in response to a number of pro-inflammatory cytokines including interleukin-6 (238). Fibrinogen levels were not associated with a change in bleeding risk, which was

consistent with the findings of a recent retrospective study assessing bleeding risk (123).

2.4.1.4 Clot lysis

Clot lysis potential (50% lysis time) was within the normal range at 95% time-points. This technique relies on addition of high levels of exogenous tPA and might not fully take into account of the *in vivo* balance between tPA and PAI-1, its principle inhibitor (102). Both tPA and PAI-1 are released by the endothelium, although PAI-1 is also contained in platelets (239), which might explain why PAI-1 did not rise at the same time as tPA.

By contrast, measures of *in vivo* fibrinolysis such as d-dimer and PAP complexes were elevated. Elevated d-dimers (233, 240, 241) and elevated PAP complexes (235) are common in the setting of haematological malignancy. Given the normal clot lysis time, this is likely to be a response to increased fibrin deposition, rather than representing primary fibrinolysis. In over one third of patients, there was an increase in the two principle promotors of fibrinolysis (tPA and uPA) as would be expected during fibrinolysis. Fibrinolysis is usually held in check by three inhibitors: A2AP, PAI-1 and TAFIa (102). A2AP and PAI-1 were in the normal range in 76% and 80% cases respectively. TAFIa was increased in 55% cases.

The pattern of results is similar to those observed in septic patients, where levels of tPA, uPA and TAFIa are elevated (242, 243). Several studies have also demonstrated elevated tPA and uPA in patients with leukaemia at diagnosis (244) or during treatment (234, 240, 245, 246). Overall, despite the increase in fibrinolysis activators, this did not result in a difference in clot lysis potential.

2.4.2 Strengths and limitations

A wide range of participants with different haematological malignancies and treatment regimens were included in this study. This should make the results relatively generalizable across different types of chemotherapy associated with profound thrombocytopenia. The prospective data collection on bleeding outcomes performed by trained research nurses and using a validated bleeding tool ensured consistency of bleeding assessment.

WHO grade 2+ bleeding rates were low in this study compared to most patient cohorts which limits data on more severe bleeding. In this cohort, 33% had at least one episode of WHO grade 2+ bleeding, which was lower than is reported in most large randomised controlled trials assessing bleeding (8, 209, 237). It is possible that this difference in patient mix might account for differences in bleeding rates, although it might be due to random variation. The proportion of patients undergoing induction chemotherapy, autologous stem cell transplantation and allogeneic stem cell transplantation was similar to the TOPPS trial where the WHO grade 2+ bleeding rates were 43 to 50% (8). However the proportion of patients receiving induction chemotherapy was considerable lower than in the SToP trial (6% vs 83% where the where 49.2 to 51.7% had a WHO grade 2+ bleed (209).

The small number of patients included in this study mean it is underpowered to detect small differences in laboratory parameters that might be associated with bleeding. Large retrospective studies may provide better data for the link between blood tests that are commonly used clinically and bleeding. These are limited by the need to accurately assess bleeding, which is often not recorded accurately in clinical notes. One solution to this is to retrospectively review patients included in clinical trials where

there is good data on bleeding (123). While this approach might be effective for examination of commonly used blood tests, prospective blood samples collection is necessary for examining blood tests such as VWF:Ag, thrombin generation and clot lysis, which are not performed as part of routine clinical care.

I used a wide range of tests in this study. However none of these of these tests provide a complete assessment of haemostasis. Data completeness was high for most laboratory variables but was lower for VWF:Ag. To account for missing data and to provide an overall assessment of each parameter, the multivariable logistic regression model took into account several different tests of haemostasis but still found no consistent predictive value of any individual test.

This is a small study and given the wide range of patients, it is possible that there are subgroups of patients where these laboratory markers might prove predictive that were not detected in these analyses. However overall, these results suggest that there is no individual measure of haemostasis that is strongly predictive of bleeding risk.

2.4.3 Conclusions

Measures of haemostasis, including the platelet count, appear to be poor predictors of bleeding for thrombocytopenic patients with haematological malignancy undergoing intensive chemotherapy. It is possible that clinical factors such as infection contribute to bleeding risk, which would be consistent with several other studies (113, 114). Platelet transfusions are not recommended unless the platelet count is less than 10 $x10^9$/l in this setting (29, 30). VWF is still dependent on platelets for its function in primary haemostasis and at very low platelet counts it might have limited effect. By contrast, the recommended platelet count before procedures is higher with a range of 20 $x10^9$/l recommended for central line insertion, through to 100 $x10^9$/l for procedures involving

the central nervous system or the posterior segment of the eye (29, 30). Increasing VWF levels at the higher platelet transfusion thresholds might be more effective than trying to prevent spontaneous bleeding where the platelet transfusion trigger is usually <10 $x10^9$/l (29, 30). Consequently in Chapter 3, I will examine the benefits of administering VWF to thrombocytopenic blood for patients with higher target platelet counts.

Chapter 3

3. Development of a model to examine whether increasing plasma VWF levels compensates for thrombocytopenia *in vitro*

3.1 Introduction

In order to examine the interaction between platelets and VWF, it was necessary to examine them under conditions where a shear force is generated. *In vivo*, shear force is generated as blood flows through a pressure gradient, and is subjected to tangential forces in the direction of flow. VWF function is dependent on shear forces (247). In its normal circulating state, VWF has low affinity for platelet GP1b receptors (79). However at high shear forces, or when exposed to high levels of drag when immobilised on collagen, VWF unfurls, revealing its GP1b binding sites in its A1 regions (79).

As described in section 1.3.2.1, thrombus formation under flow and PFA closure time can be used to observe the combined action of VWF and platelets. These techniques both exert a shear force on blood, resulting in exposure of VWF binding sites for platelet GP1b and collagen receptors. Desmopressin increases plasma VWF levels by 2 to 6 fold (129), so this series of experiments aimed to replicate these effects using exogenous administration of VWF to thrombocytopenic blood.

3.1.1 Aims

- To derive a method for simulating thrombocytopenia in normal blood without pre-activation
- To assess how thrombocytopenia changes thrombus formation under flow

- To assess the effects of increasing plasma VWF concentrations on thrombus formation under flow over collagen, and PFA closure time in simulated thrombocytopenic blood and in blood from thrombocytopenic patients
- To assess whether surface coverage or mean fluorescence intensity (MFI) could be used as surrogate measures of thrombus volume
- To assess the optimum duration of thrombus formation necessary to be able to demonstrate a difference in thrombus formation between platelet counts.

3.2 Methods

3.2.1 Blood samples

3.2.1.1 Normal donors

Blood was taken from healthy volunteers using procedures approved by the University of Reading Ethics Committee. Blood from normal donors was manipulated to simulate different levels of thrombocytopenia (section 3.2.3).

3.2.1.2 Patient samples

Patient samples were collected from patients with hypoplastic marrow failure and a platelet count <50 $x10^9$/l. Patients with a congenital bleeding disorder, undergoing intensive chemotherapy or taking anti-platelet drugs were excluded. Approval for sample collection from patients was granted by North West - Haydock Research Ethics Committee; 15/NW/0138; ISRCTN74679473.

3.2.2 Blood collection

Blood was drawn into vacutainers (0.109M citrate) with a 21 gauge needle to ensure a smooth blood flow and the first 2 ml was discarded to avoid pre-activation of platelets or inclusion of endothelial tissue (248). Citrate acts by binding calcium and magnesium ions, preventing further activation of coagulation. An anticoagulant is necessary to

prevent spontaneous aggregation, which can compromise experimental integrity, and can cause blockage of a flow cytometer or microfluidic flow circuit (51).

Calcium is important for normal platelet function (60) but is omitted from most platelet function tests, because it activates the coagulation cascade. Re-calcification of whole blood is not commonly used in thrombus formation under flow experiments (84) but is more physiological. The formation of thrombin is calcium-dependent, and given its important role as a platelet activator, it might be expected that platelet activation would be reduced in a hypocalcaemic environment. However overall platelet responses are increased in citrated plasma compared to plasma aspirated into other anticoagulants (51). In hypocalcaemic blood, thromboxane A_2 production is increased which can result in a second wave of aggregation in response to ADP that is not seen in normocalcaemic blood (249, 250). In the absence of calcium, platelets are more active (due to activation of GPIIb/IIIa) and thrombi are denser (251). P-Selectin is also increased, demonstrating that intracellular stores of calcium are sufficient for granule release (252).

Blood samples were gently inverted 6 to 10 times to ensure maximum mixing of blood and anticoagulant. Vigorous shaking was avoided and the samples were kept at room temperature to avoid pre-activation (248). Blood samples were analysed within four hours of collection (248).

3.2.3 Platelet depletion technique

Blood samples were taken from healthy volunteers. PRP, PPP and red cells were separated and reconstituted in different concentrations to simulate different levels of thrombocytopenia (Figure 3.1).

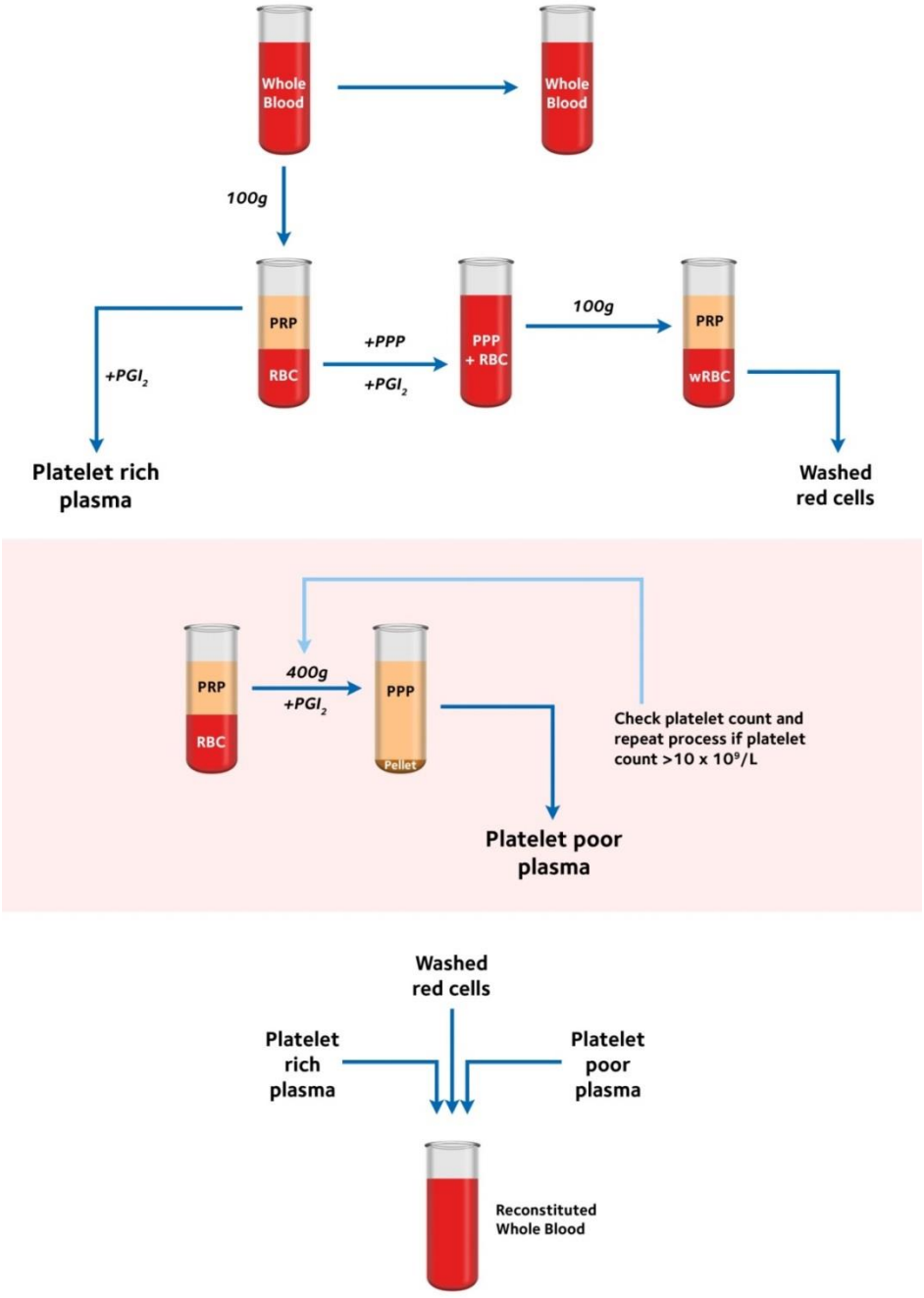


Figure 3.1 Platelet depletion technique. Four millilitres of citrated (0.109M) whole blood was set aside for comparison. Sixteen millilitres citrated (0.109M) whole blood was centrifuged at 100g for 20 minutes to generate platelet rich plasma (PRP). Red blood cells (RBC) are washed with platelet poor plasma at 100g for 20 minutes to form washed RBC (wRBC). Platelet poor plasma is generated by centrifuging PRP at 400g for 20 minutes. At each stage an excess of prostaglandin I_2 (PGI_2) was added to prevent pre-activation of platelets. The blood components were then added together to form reconstituted blood with a controlled platelet count by maintaining a constant haematocrit but adjusting the relative volume of PRP and PPP.

I used fluorescence activated cell sorting (FACS) to check for platelet pre-activation and response to platelet agonists. The reconstituted blood was then used for assessing thrombus formation under flow or PFA closure time.

3.2.3.1 Platelet rich plasma

Whole blood collected into 0.109M citrate was centrifuged at 100g to generate PRP and a red cell fraction. PRP was separated from the red cell fraction and PGI_2 (125ng/ml; Enzo Life Sciences, Exeter, UK) was added to temporarily prevent platelet activation. Preliminary work demonstrated platelet auto-aggregation during the preparation process when PGI_2 was omitted.

3.2.3.2 Platelet poor plasma

PPP was generated from PRP (see above). PRP was centrifuged at 400g, each time for 20 minutes to generate PPP, which was separated from the platelet pellet and set aside. I measured the PPP platelet count and if it ≥10 $x10^9$/l then the process was repeated until the platelet count was <10 $x10^9$/l.

3.2.3.3 Washed red cell fraction

Whole blood collected into 0.109M citrate was centrifuged at 100g to generate PRP and a red cell fraction. The red cell fraction was separated and PGI_2 (125ng/ml, Enzo Life Sciences, Exeter, UK) was added. The red cell fraction was mixed with an equal volume of PPP and centrifuged at 100g for 20 minutes. PRP was aspirated and discarded, leaving a washed red cell fraction. The washing step proved necessary, because preliminary work demonstrated that approximately 40-50 $x10^9$ platelets per litre were present in the unwashed red cell fraction.

3.2.3.4 Reconstituted blood

A platelet count was performed on an unaltered blood sample from the volunteer; on PPP to ensure that the residual platelet count was less than 10 $x10^9$/l; and on PRP to aid calculations for the next step.

To simulate different levels of thrombocytopenia, red cells and differing volumes of PRP and PPP were mixed with final target platelet counts of:

1. Baseline for the donor
2. 100 $x10^9$/l (the highest platelet transfusion recommended before surgery in the guidelines)
3. 50 $x10^9$/l (the threshold for platelet transfusion for most procedures and major surgery)
4. 20 $x10^9$/l (the lowest platelet transfusion threshold in the guidelines) (29, 30)

To assess the effectiveness of the platelet depletion process, a donor blood sample that was not manipulated was set aside for comparison to the reconstituted blood samples. Platelet pre-activation, and activation in response to platelet agonists, was compared between the unmanipulated blood and reconstituted blood at each target platelet count.

3.2.4 Fluorescence activated cell sorting

Fluorescence activated cell sorting (FACS) was used to assess whether platelets had become activated, and retained the capacity to be activated, following the depletion process.

3.2.4.1 Fluorescence activated cell sorting methods

Whole blood FACS was used because it can be used to check for platelet pre-activation on a large number of samples simultaneously and is not affected by thrombocytopenia

(Table 3.1) (253). Platelets were diluted in modified Tyrode's solution to reduce the risk of pre-activation.

Modified Tyrode's solution is a buffer which contains the necessary concentration of ions for platelet activation (51): 134 mM sodium chloride, 2.9 mM potassium chloride, 1.8 mM $CaCl_2$, 0.34 mM sodium phosphate dibasic dodecahydrate, 12 mM sodium bicarbonate, 20 mM HEPES and 1 mM magnesium chloride (all from SIGMA).

Platelet activation was assessed by looking for evidence of alpha granule release (P-Selectin) and GPIIb/IIIa activation (fibrinogen binding). Fibrinogen binding was assessed using anti-fibrinogen antibodies (fluorescein isothiocyanate [FITC] polyclonal rabbit anti-human fibrinogen) (254) and alpha granule release using anti-P-Selectin antibodies (phycoerythrin-cyanine [PE-Cy] mouse anti-human CD62P, BD Pharmingen) (255-257). P-selectin is rapidly shed following alpha granule release (258), so platelets were fixed by adding an excess of 0.2% formyl saline (Sigma) before analysis. I used a dilution of 1:50 for fibrinogen and 1:50 for P-Selectin as this was the optimal dilution to optimise platelet staining without causing non-specific binding (259).

Due to their small size, it is easy to distinguish platelets from other cells in whole blood. A platelet-rich plasma sample was used to form the FACS gating strategy. The gate was placed to get 1-2% positive platelets in the control sample (260).

3.2.4.2 Choice of agonists

I used ADP (10 µmol; Sigma), thrombin receptor-activating peptide (TRAP)-6 (10 µmol; Bachem) and collagen-related peptide (CRP-XL) (3 µg/ml; courtesy of Prof Richard Farndale, University of Cambridge) to assess platelet response to agonists. There are relatively high levels of variation in response to agonists at low concentrations in the normal population (261), so high concentrations of platelet agonists were used to

improve the consistency of results. Control samples, which were only treated with Tyrode's solution, were used to check for platelet pre-activation. Blood samples where more than 15% of platelets in the control sample were above the pre-set threshold were considered to be pre-activated (51).

Advantages	Disadvantages
Many aspects of platelet function can be assessed in a single experiment	Preparation of samples is labour intensive
Low volumes of blood needed	Protocols not validated in large patient cohorts
Whole blood can be used to avoid loss of denser platelet populations	Endpoint measures are only option
Can be used in thrombocytopenic patients	Does not assess shear stress
Easy to test for platelet pre-activation	

Table 3.1 Fluorescence activated cell sorting for platelet function testing. (51).

3.2.5 Thrombus formation under flow

Platelet thrombus formation has three distinct phases: 1. Initiation where platelets adhere to collagen and VWF; 2. Extension which may be autocrine (thromboxane A2 release, or granule release) or due to platelet co-aggregation through GPIIb/IIIa; 3. Perpetuation by thrombin generation and fibrin formation, which result in clot contraction (262).

There is considerable variation in thrombus formation methods used between laboratories and a single approach is yet to be established (84). An advantage of thrombus formation under flow over other tests is that it can provide information on

the totality of clot formation and can provide data in the presence of thrombocytopenia (263).

3.2.5.1 Flow chamber and coating

All experiments used commercially available Vena8+ chips. Each chip contains eight channels with: length 2.8 cm; width 400 μm; and height 100 μm (Cellix Ltd). Maintaining haematocrit is necessary for standardisation of the assay (56) and I maintained it at the same level for samples treated with, or without exogenous VWF. Perfusion was maintained at a constant flow rate with an ExiGo pump (Cellix Ltd).

A number of different surfaces can be used for thrombus formation assays to stimulate aggregation. The most physiological of these are collagen type I and collagen type III, which are exposed *in* vivo when endothelium is damaged (56). For these experiments I used a mixture of collagen I and III diluted in modified Tyrode's solution at a concentration of 100 μg/ml (Collagen Reagens Horm suspension; Takeda). Collagen binds VWF and is a ligand for collagen receptors including GPVI (264). Immobilised VWF bound to collagen binds platelet GPIb-V-IX receptors (69). Binding of platelet GPVI to collagen results in platelet activation, leading to granule secretion; formation of a procoagulant surface on the platelet; and GPIIb/IIIa activation (265). Methods for collagen coating of flow chambers followed the recommendations of the ISTH scientific and standardisation committee (266).

3.2.5.2 Shear forces

Shear forces were calculated in inverse seconds, with a shear rate of $1000s^{-1}$ selected to simulate arterial shear forces. Physiological shear forces in human veins range from 50 to 500 s^{-} and in human arteries from 1000 to 5000 s^{-} (278).

3.2.5.3 Fluorescent marker

A fluorescent platelet membrane stain, 3-3'-dihexyloxacarbocyanine iodide ($DiOC_6$; 2 μM, Life Technologies), was used to label platelets. $DiOC_6$ was selected because it binds to platelets with high affinity and leads to minimal levels of background (plasma) staining (267).

3.2.5.4 Temperature

Experiments in normal blood were run at 37°C. Patient samples were analysed at room temperature due to the need to minimise time delays between venepuncture and analysis. Therefore patient and platelet-depleted blood samples are not compared directly. The majority of laboratories surveyed by the ISTH perform experiments at room temperature. Running experiments at 37°C results in more rapid thrombus formation and inactivates autocrine ADP and thrombin leading to differences in results compared to when samples are analysed at room temperature (56, 84).

3.2.5.5 Microscopy and endpoints

Images were captured using a Nikon Eclipse TE2000-U confocal microscope. Real-time bright-field and fluorescent images were captured in all cases with images taken every 10 seconds for 6 minutes. After 6 minutes, the flow chamber was washed with modified Tyrode's solution and representative bright-field and fluorescent images were captured for calculation of endpoints. Analysis of images was performed with Fiji ImageJ software (268). A z-stack image was also taken at this point to form a 3-D image for estimation of total thrombus volume, which was analysed with NIS-Elements AR 4.60.00 64-bit software (Nikon).

For thrombus formation over time, total surface coverage and fluorescence intensity were plotted every 60 seconds up to 360 seconds. Total surface coverage and MFI at 360 seconds were calculated, which are often considered to be useful surrogate

markers for total platelet deposition. These measures have the potential to overestimate thrombus formation in cases where platelets form a single layer or the aggregates are unstable (56). Total thrombus volume was calculated using z-stack images. This was performed by calculating the sum of the surface coverage of all images in each stack and then multiplying this by the stack distance. This result was divided by the field area to give an estimate of thrombus formation, which reflected the mean height of all thrombi. The three endpoint measures were compared to assess if there were significant differences between these endpoints.

3.2.5.6 Outcomes

Using platelet-depleted normal blood, I examined the relationship between platelet count and thrombus formation endpoints after 6 minutes of perfusion: surface coverage, MFI and thrombus volume. These experiments were performed with/without the addition of Haemate P (section 3.2.7). The absolute and relative increases in thrombus formation parameters following addition of Haemate P were compared to assess if an increase in thrombus formation parameters was consistent at all platelet counts. To assess thrombus formation dynamics and to establish the optimal duration of perfusion for the thrombus formation assay, time-lapse images were captured every 10 seconds so that surface coverage and MFI could be extracted. Each value was normalised, with total thrombus formation after 6 minutes in the baseline samples considered to represent 100% thrombus formation. The different target platelet counts were compared at each time point, as was the difference before and after administration of Haemate P. Lastly, patient samples were analysed using the same technique. However these samples were analysed at room temperature with extraction of a single endpoint measure: surface coverage. These experimental conditions were selected due to the

need to analyse blood samples quickly after they were taken and the logistical difficulties of knowing when patients would be available.

3.2.6 Platelet function analyser

PFA closure time is commonly used as a screening test for platelet disorders (and can be used to detect/monitor von Willebrand disease) (269). This is a simple test but very sensitive to low platelet counts which significantly reduces its accuracy in thrombocytopenic patients (270). This technique is known to be sensitive to the effects of VWF (271) and has previously been used to measure the effectiveness of desmopressin for patients taking antiplatelet drugs (195). For these experiments, whole blood was passed through a 150 µm aperture coated with 2 µg equine type I collagen and 50 µg ADP (C-ADP cartridge, Innovance, Siemens Healthcare Diagnostics GmBH); or a 100 µm aperture coated with 20 µg ADP, 5 ng PGE1 and 459 µg calcium chloride (Innovance PFA P2Y cartridge; Siemens Healthcare Diagnostics GmBH). Analysis was with a PFA200 (Siemens Healthcare Diagnostics GmBH).

3.2.7 Von Willebrand factor

VWF assays were performed as described in section 2.2.6.1. For thrombus formation and PFA experiments, samples were assessed with, or without addition of intermediate purity factor VIII (Haemate P, CSL Behring, UK). Samples where Haemate P was added are marked as (+) Haemate P. One unit of Haemate P contains approximately one VWF ristocetin co-factor unit of factor VWF and 0.4 units factor VIII. To assess if the factor VIII content of Haemate P contributed towards thrombus formation, a matching concentration of recombinant factor VIII (Kogenate, Bayer) was added for comparison (272).

3.2.8 Statistics

All experiments were performed on platelet-depleted blood from normal volunteers, unless otherwise stated. Data were analysed using GraphPad Prism version 7.00 for Mac (GraphPad Software, San Diego, California, USA). Continuous data were inspected for normality. Normally distributed data were expressed as mean ± standard deviation. Non-normal data were expressed as median (range) or median (IQR). Correlations with a Gaussian distribution were analysed using a 2-tailed Pearson's rank. Non-normal data were compared using a 2-tailed Wilcoxon signed rank (for paired data) or 2-tailed Mann-Whitney U tests (for unpaired data). Two-tailed t-tests were used to compare normally-distributed data (paired or unpaired as appropriate). Variance between matched groups was compared with matched groups analysis of variance (ANOVA). All tests were repeated 5 times unless otherwise stated. A p-value of <0.05 was considered to be statistically significant. A linear regression model incorporating the $\log_{10}$[platelet count] and use of Haemate P was used to assess the effect of Haemate P after adjusting for platelet count using the *regress* function in STATA (www.stata.com).

3.3 Results

3.3.1 Patient characteristics

Blood samples were taken from five normal donors for each set of experiments. The normal donors self-reported that they were not taking antiplatelet drugs or non-steroidal anti-inflammatory drugs but no other details were recorded. Patient samples were obtained from five patients with hypoplastic bone marrow failure (Table 3.2).

Patient characteristics		N (%) *or* Median (range)
Age (years)		79.2 ± 6.4
Gender	Male	3 (60)
	Female	2 (40)
Diagnosis	Chronic myelomonocytic leukaemia	3 (60)
	Myelodysplastic syndrome	2 (40)
Bruising or bleeding in preceding 14 days	Yes	2 (40)
	No	3 (60)
Previous chemotherapy	Azacitidine (6 cycles)	2 (40)
	No treatment	3 (60)
Potential confounders of platelet function tests (248)	*Any*	*5 (100)*
	Caffeine	4 (80)
	Furosemide and beta-blocker	1 (20)
Baseline blood results	Haemoglobin (g/l)	106 (77 to 145)
	White cell count (x10^9/l)	3.3 (0.5 to 5.7)
	Platelet count (x10^9/l)	21 (13 to 47)
	Haematocrit (%)	35.2 (25.3 to 43.8)
	VWF antigen (iu/ml)	1.31 (0.71 to 1.53)
	VWF activity (iu/ml)	1.2 (0.70 to 1.51)
ABO blood group	O	4 (80)
	A	1 (20)
	B	0
	AB	0
Total		***5***

Table 3.2 Patient characteristics. VWF: Von Willebrand Factor.

3.3.2 Platelet depletion technique

The platelet depletion technique was reproducible and could be performed within the four hour window necessary for analysis of platelet function. Following platelet depletion, the accuracy for each of the platelet target thresholds was as follows:

- Target 100 x10^9/l: actual 90 ± 19 x10^9/l
- Target 50 x10^9/l: actual 51 ± 10 x10^9/l
- Target 20 x10^9/l: actual 22 ± 2 x10^9/l

Haematocrit was checked before/after every case of platelet depletion. VWF:Ag and VWF:Ac were tested before/after platelet depletion three times. There were no significant differences before and after platelet depletion in haematocrit (42.0 ± 2.8 vs 42.5 ± 5.1; p=0.88), VWF:Ag (0.95 ± 0.21 iu/ml vs 0.94 ± 0.20 iu/ml; p=0.42) or VWF:Ac (0.98 ± 0.27 iu/ml vs 0.96 ± 0.26 iu/ml; p=0.18).

3.3.3 Assessment of platelet pre-activation

There was no evidence of pre-activation in unstimulated samples, measured with both P-Selectin and fibrinogen binding for reconstituted versus unmanipulated blood. This was consistent at all target platelet counts (repeated measures ANOVA P-Selectin p=0.18; fibrinogen binding p=0.09; Figure 3.2).

There was a reduction in response to platelet agonists in reconstituted blood compared to unmanipulated blood measured by both P-Selectin and fibrinogen binding. P-selectin expression in response to platelet agonists in reconstituted blood was reduced compared to unmanipulated blood for TRAP-6 (median fluorescence intensity 2007 ± 2017 units vs 6395 ± 2223 units; p=0.009), ADP (3766 ± 4417 units vs 9631 ± 801 units; p=0.11) and CRP-XL (2534 ± 3663 units vs 8870 ± 1609 units; p=0.04; Figure 3.3), but was consistent between manipulated blood samples at different target platelet counts (repeated measures ANOVA: TRAP-6 p=0.42; ADP p=0.44; CRP-XL p=0.42).

Likewise fibrinogen binding in response to platelet agonists in reconstituted blood was reduced compared to unmanipulated blood for TRAP-6 (769 ± 501 units vs 1404 ± 391 units; p=0.12), ADP (626 ± 412 vs 1602 ± 74; p=0.06) and CRP-XL (334 ± 168 vs 1444 ± 197; p=0.02), but was consistent between manipulated blood samples at different target platelet counts (repeated measures ANOVA: TRAP-6 p=0.35; ADP p=0.34; CRP-XL p=0.69).

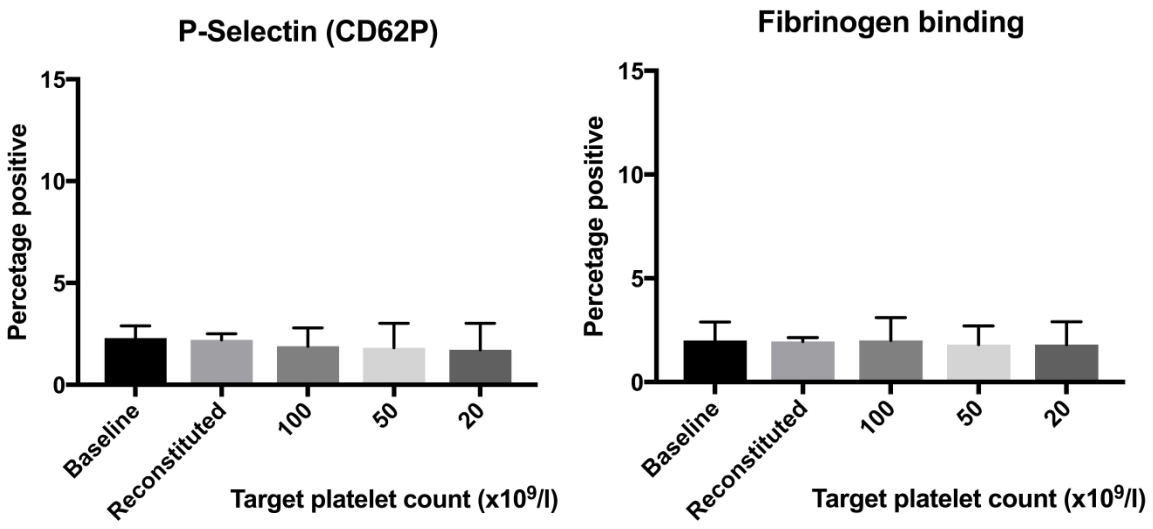


Figure 3.2 Bar chart of platelet pre-activation. Error bars represent standard deviation. Normal donor samples treated with platelet depletion. Resting samples at each simulated platelet count assessed for P-Selectin expression and fibrinogen binding. Baseline represents unmanipulated blood. Reconstituted blood has been through the platelet depletion process but the platelet count has been returned to baseline. A threshold of 15% positivity was used to determine pre-activation (n = 5).

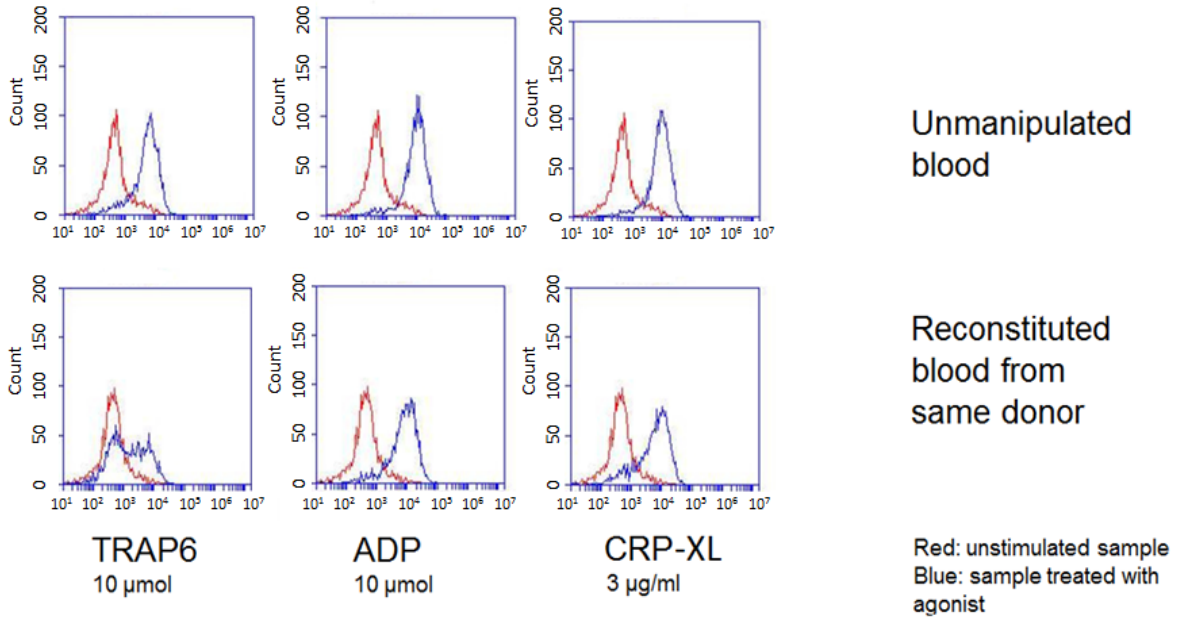


Figure 3.3 Fluorescence activated cell sorting. Representative FACS plots comparing P-Selectin (CD62P) expression in unmanipulated and reconstituted blood from one donor. Red lines are resting (unstimulated) samples and blue lines are samples treated with agonists. TRAP6: thrombin receptor activating peptide 6, ADP: adenosine diphosphate, CRP-XL: collagen-related peptide.

3.3.4 Thrombus formation under flow

Thrombus formation under flow measured by surface coverage, MFI and thrombus volume increased in response to platelet count and Haemate P (Figure 3.4 and 3.5). Log_{10} of the platelet count was more predictive of each thrombus formation parameter than platelet count. Consequently the linear regression models used to calculate each thrombus formation parameter were based on log_{10}[platelet count] as a continuous variable and presence or absence of Haemate P as a dichotomous variable.

On univariate analysis, the regression co-efficient for surface coverage was statistically significant with both log_{10}[platelet count] (co-efficient 12.8, 95%CI 8.5 to 17.2, p<0.0001) and Haemate P (co-efficient 4.9, 95%CI 0.2 to 9.6, p=0.04). The results were similar using multivariable analysis to take account of both log_{10}[platelet count] (co-efficient 12.8, 95%CI 9.0 to 16.7, p<0.0001) and Haemate P (co-efficient 4.9, 95%CI 1.9 to 7.9, p=0.002).

On univariate analysis, the regression co-efficient for MFI was statistically significant for log_{10}[platelet count] (co-efficient 39.6, 95%CI 29.2 to 49.9, p<0.0001) but not Haemate P (co-efficient 10.4, 95%CI -3.0 to 23.8, p=0.13). The regression coefficients for log_{10}[platelet count] (co-efficient 39.6, 95%CI 30.2 to 48.9, p<0.0001) and Haemate P (co-efficient 10.4, 95%CI 3.0 to 17.8, p=0.007) were both statistically significant on multivariable regression.

On univariate analysis, the regression co-efficient for thrombus volume was statistically significant with both log_{10}[platelet count] (co-efficient 59760, 95%CI 35213 to 84306, p<0.0001) and Haemate P (co-efficient 30080, 95%CI 9588 to 50573, p=0.005). The results were similar using multivariable analysis to take account of both log_{10}[platelet

count] (co-efficient 59760, 95%CI 40726 to 78794, p<0.0001) and Haemate P (co-efficient 30080, 95%CI 16668 to 43493, p<0.0001).

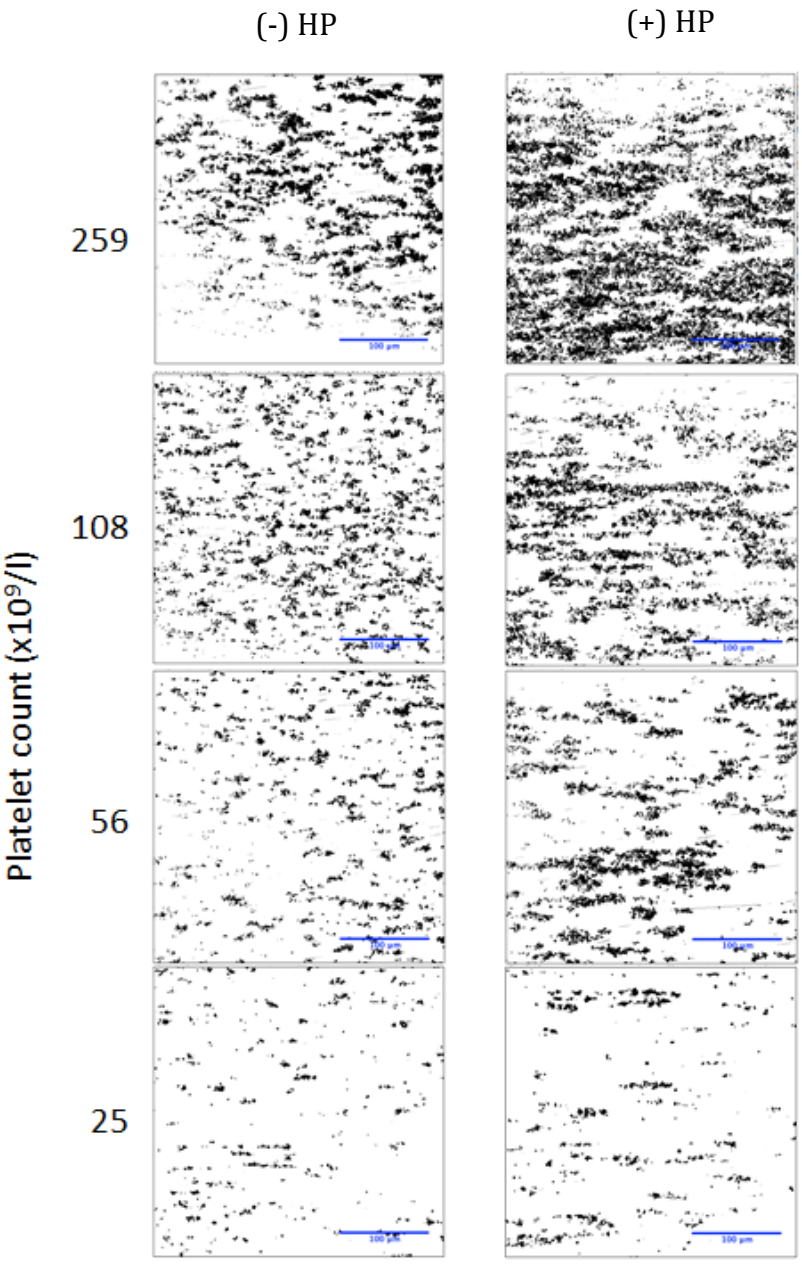


Figure 3.4 Thrombus formation micrographs (representative images). Direction of flow was from right to left. Scale bar represents 100µm. Blood samples taken from normal donors and platelet depleted as described in section 3.2.2. Images taken after 6 minutes of flow over 100 µg/ml collagen. (+)HP indicates addition of 2 units/ml Haemate P. (-)HP indicates samples without addition of Haemate P.

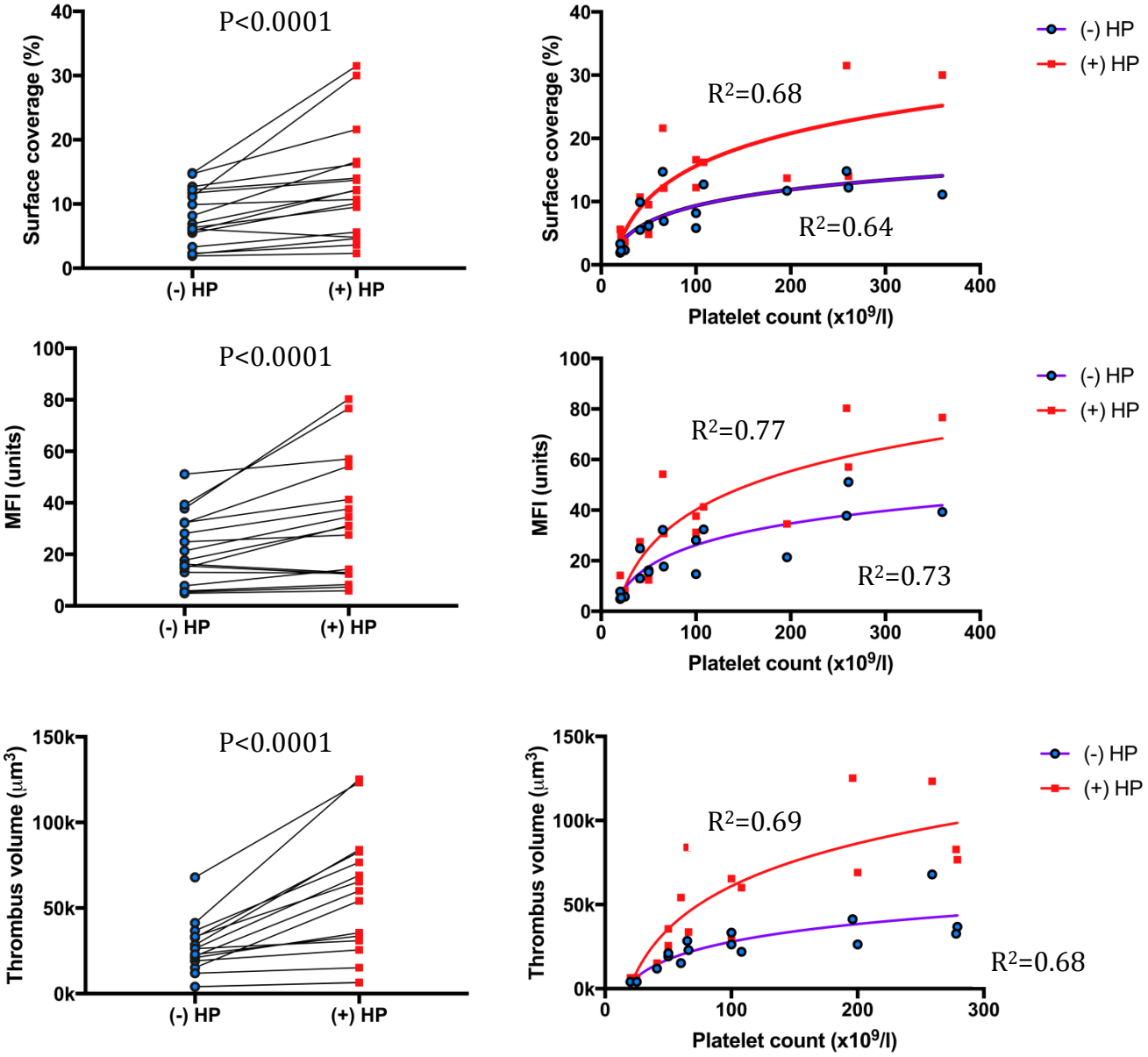


Figure 3.5 Thrombus formation with/without Haemate P. Left-hand column demonstrates change in thrombus formation with/without addition of Haemate P. Right column plots platelet count against thrombus formation with/without VWF with a semilog regression line. Top row: percentage surface coverage. Middle row: mean fluorescence intensity (MFI). Bottom row: thrombus volume. Blood samples taken from normal donors and platelet depleted as described in section 3.2.2. Flow through a collagen-coated Cellix chip at $1000s^{-1}$ for six minutes before analysis. (+) HP indicates addition of 2 units/ml Haemate P. (-)HP indicates samples without addition of Haemate P. Wilcoxon signed rank to compare (-) HP and (+) HP. P-value <0.05 considered statistically significant.

Following addition of Haemate P, the increase in surface coverage was significantly greater when the platelet count was ≥100 $x10^9$/l (median 6.4, range 1.8 to 18.9%) than when the platelet count was ≤50 $x10^9$/l (median 1.8, range 0 to 4.6) (p=0.006). The relative increase in surface coverage compared to baseline was greater for samples with a platelet count ≥100 $x10^9$/l than when the platelet count was ≤50 $x10^9$/l (median 102, range 15 to 170% increase) but this did not reach statistical significance (p=0.23).

Likewise, with addition of Haemate P, the increase in MFI was significantly greater when the platelet count was ≥100 $x10^9$/l (median 13.1, range 5.9 to 42.5 units) than when the platelet count was ≤50 $x10^9$/l (median 1.0, range 0 to 6.4 units) (p=0.0006). The relative increase in MFI compared to baseline was greater for samples with a platelet count ≥100 $x10^9$/l than when the platelet count was ≤50 $x10^9$/l (median 61, range 12 to 113% increase) but this was of borderline statistical significance (p=0.05).

In common with the surface coverage and MFI, addition of Haemate P increased thrombus volume significantly more when the platelet count was ≥100 $x10^9$/l (median 53,000, range 32,000 to 111,000 μm^3) than when the platelet count was ≤50 $x10^9$/l (median 4000, range 2,000 to 6,000 μm^3) (p=0.004). The relative increase in thrombus volume compared to baseline was significantly greater for samples with a platelet count ≥100 $x10^9$/l (median 157.5%, range 81 to 203%) than when the platelet count was ≤50 $x10^9$/l (median 58.5%, range 28 to 258%) (p=0.04) (Figure 3.6).

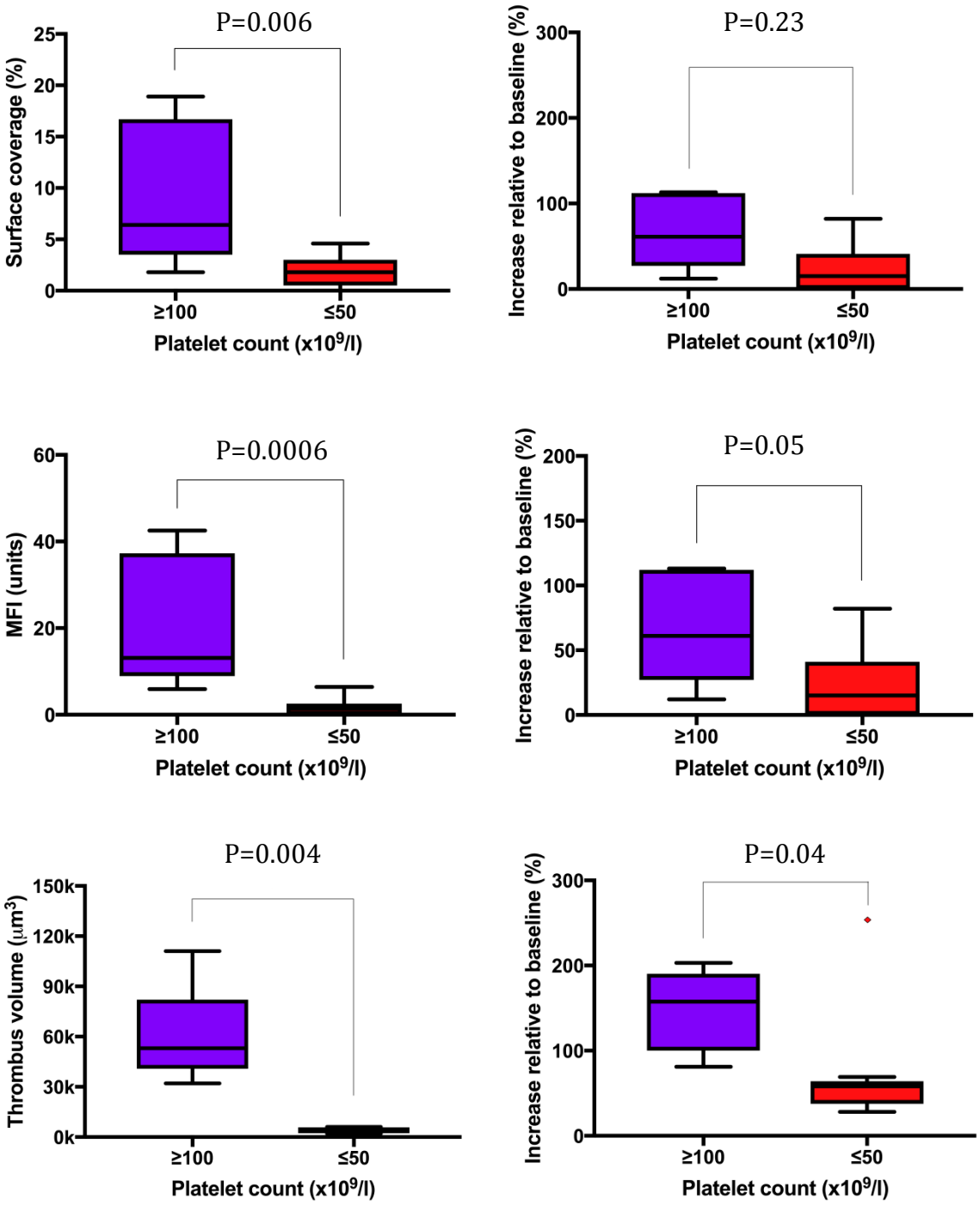


Figure 3.6 Relative change in thrombus formation with Haemate P. Blood samples taken from normal donors and platelet depleted as described in section 3.2.2. Box plots comparing relative increase in thrombus formation for samples with a platelet count ≥100 x100/l (n=8) and those with platelet count ≤50 x10^9/l (n=8). Left-hand columns represent absolute increase after addition of 2 units/ml Haemate P. Right graphs represent percentage increase in thrombus formation relative to untreated samples. Plotted as median, interquartile range, and range.

Statistics: Mann-Whitney U test: $P<0.05$ considered statistically significant. MFI: mean fluorescence intensity.

3.3.5 Thrombus formation over time

Time-lapse images were captured every 10 seconds for estimation of surface coverage and MFI (Figure 3.7). Surface coverage and MFI both increased proportionally with time for all target platelet counts (Figure 3.8). After 300 seconds of perfusion, normalised surface coverage was not significantly different between baseline and samples with target platelet count of 100 $x10^9$/l (92.3 ± 38.6% vs 60.1 ± 24.1%; p=0.23). However normalised surface coverage was significantly different between baseline and target platelet counts of 50 $x10^9$/l (41.4 ± 10.3%; p=0.03) and 20 $x10^9$/l (13.5 ± 1.3%; p=0.008). The difference remained statistically significant down to 180 seconds where baseline surface coverage (61 ± 34.3%), was significantly greater than target platelet counts of 50 $x10^9$/l (22.6 ± 6.3%; p=0.04) and 20 $x10^9$/l (9.7 ± 1.4%; p=0.03).

Likewise, after 300 seconds of perfusion, normalised MFI was not significantly different between baseline and samples with target platelet count of 100 $x10^9$/l (80.1 ± 18.1% vs 63.3 ± 22.4%; p=0.34). However normalised MFI was significantly different between baseline and target platelet counts of 50 $x10^9$/l (45.3 ± 8.0%; p=0.008) and 20 $x10^9$/l (13.7 ± 2.3%; p=0.0007). There was no statistically significant difference in normalised MFI between baseline and target platelet count of 50 $x10^9$/l (58.8 ± 23.2% vs 36.8 ± 7.8%; p=0.09) at 4 minutes (or less). There was no statistically significant difference in normalised MFI between baseline and target platelet count of 20 $x10^9$/l (38.6 ± 33.8% vs 8.4 ± 2.1%; p=0.11) at 3 minutes (or less).

Haemate P increased adhesion at all platelet counts compared to controls and this effect was maintained up to six minutes (Figure 3.9).

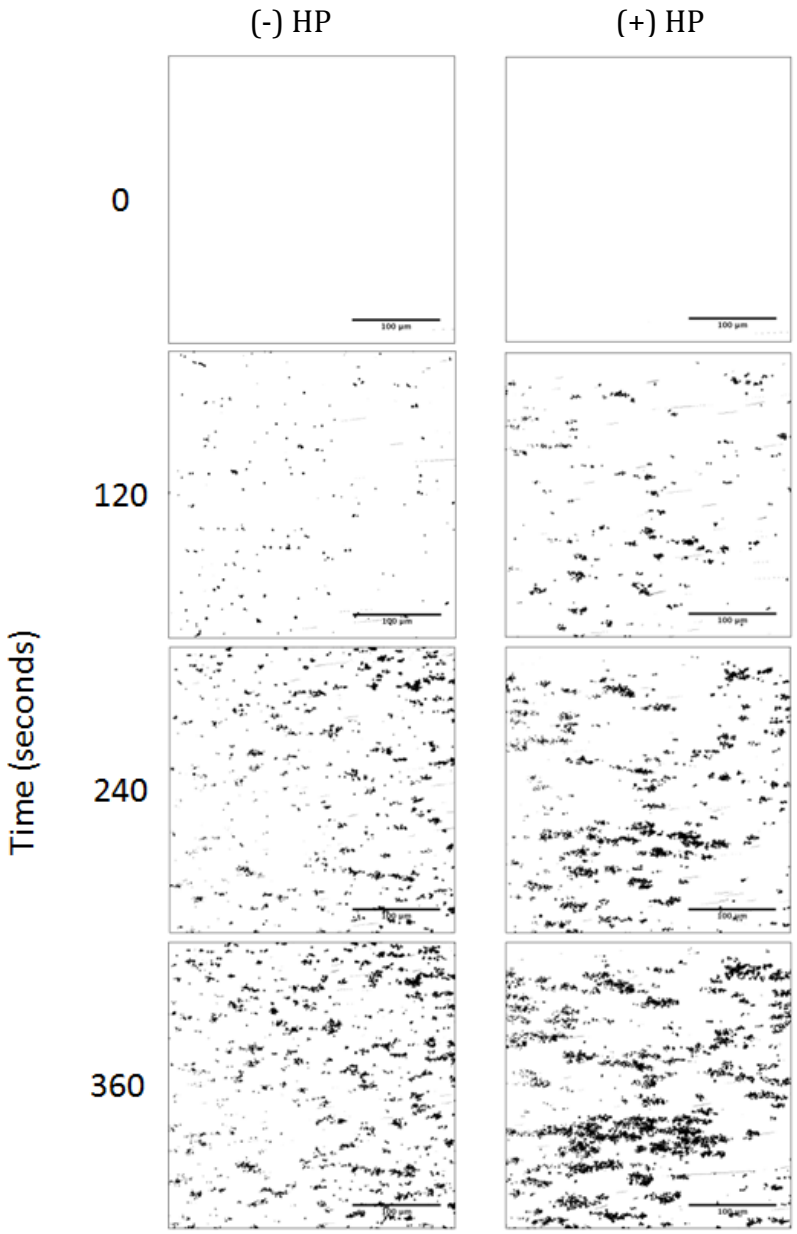


Figure 3.7 Thrombus formation time-lapse micrographs (representative images). Direction of flow was from right to left. Scale bar represents 100µm. Images above taken from experiments on blood from the same donor (platelet count 56 $x10^9$/l) with and without Haemate P. Time-lapse micrographs taken every two seconds during flow over 100 µg/ml collagen (shown every 120 seconds above). (+) HP indicates addition of 2 units/ml Haemate P. (-) HP indicates samples without addition of Haemate P.

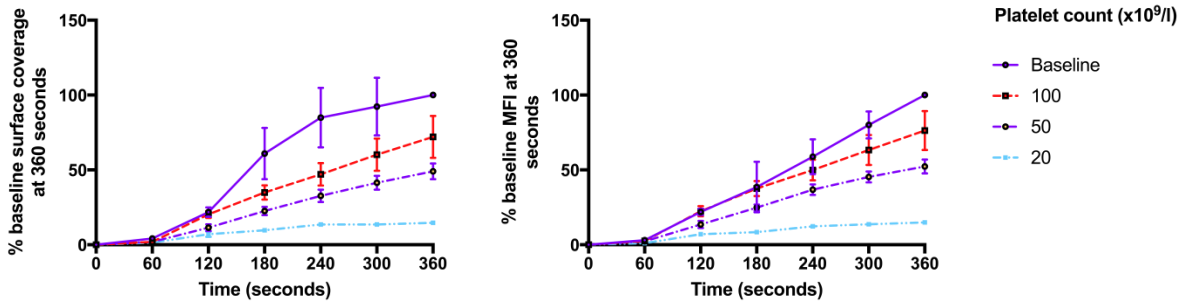


Figure 3.8 Thrombus formation over time by platelet count. Results normalised with 100% equal thrombus formation at 360 seconds in the baseline sample. Percentage surface coverage (left) and mean fluorescence intensity (MFI –right). Error bars represent standard error of the mean.

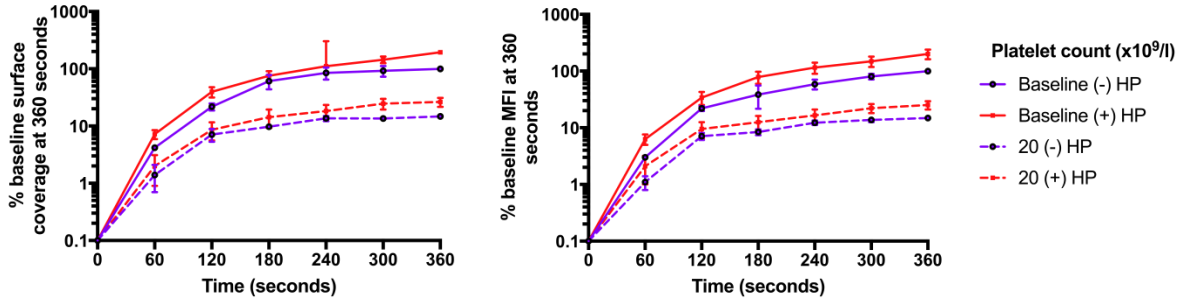


Figure 3.9 Thrombus formation over time with/without Haemate P. Results normalised with 100% equal thrombus formation at 360 seconds in the baseline sample. Percentage surface coverage (left) and mean fluorescence intensity (MFI –right). Solid lines represent baseline blood samples. Dashed lines are those manipulated to a platelet count of 20 x10^9/l. Blue lines are samples not treated with Haemate P. Red lines are samples treated with Haemate P. (+) HP indicates addition of 2 units/ml Haemate P. (-) HP indicates samples without addition of Haemate P. Error bars represent standard error of the mean. Scale is logarithmic.

3.3.6 Comparison of measures of thrombus formation under flow

There was a statistically significant correlation between surface coverage and MFI Surface coverage (Two-tailed Pearson's rank 0.94, 95% CI 0.89 to 0.97, R^2 = 0.88, P<0.0001); surface coverage and thrombus volume (Two-tailed Pearson's rank 0.77, 95% CI 0.60 to 0.88), R^2 = 0.59, P<0.0001) and MFI and thrombus volume (Two-tailed Pearson's rank 0.77, 95% CI 0.59 to 0.88, R^2 = 0.59, P<0.0001) (Figure 3.10).

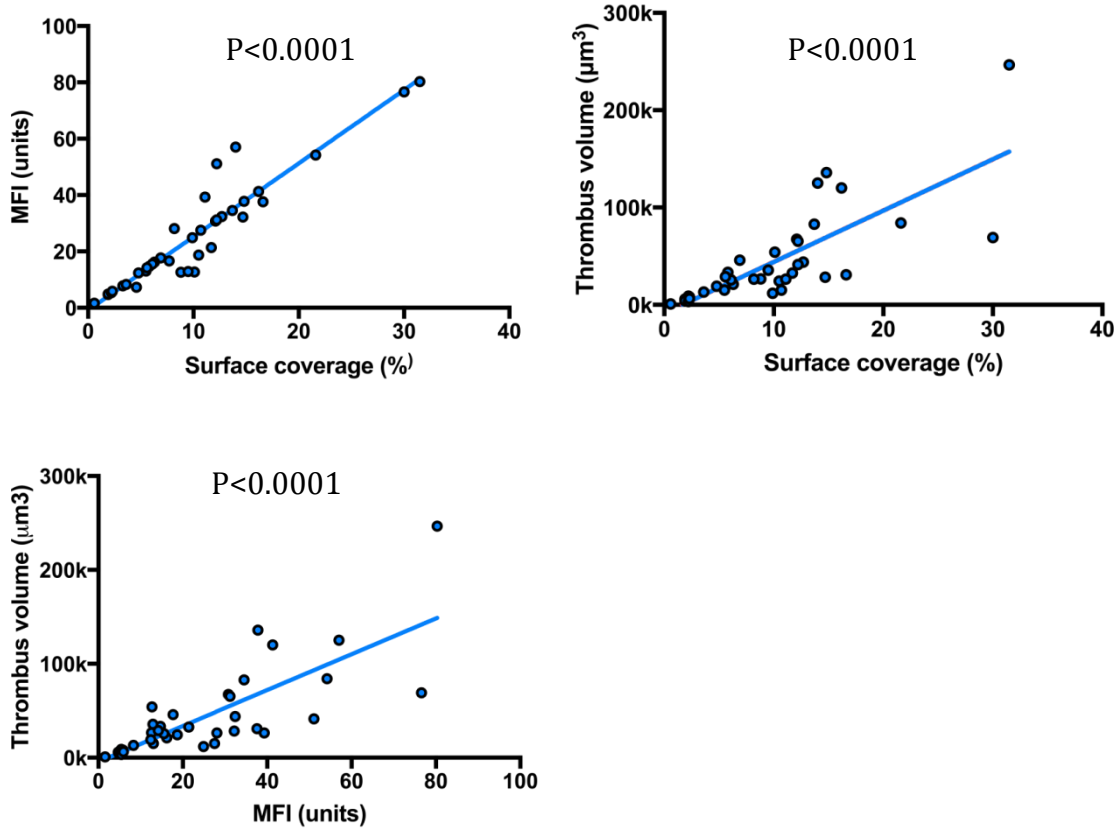


Figure 3.10 Scatter graph of thrombus formation endpoints. MFI: mean fluorescence intensity. 2-tailed Pearson's rank P<0.05 considered statistically significant.

3.3.7 Thrombus formation in thrombocytopenic patients

Surface coverage was not significantly different between untreated thrombocytopenic patient blood (median 4.3, range 1.1 to 5.8%) and blood treated with exogenous Kogenate (median 4.4, range 0.6 to 4.5%; p=0.21). Surface coverage increased in all cases in response to Haemate P (median 4.3, range 1.1 to 5.8% vs median 9.0, range 2.2 to 10.9%; p=0.048) (Figure 3.11).

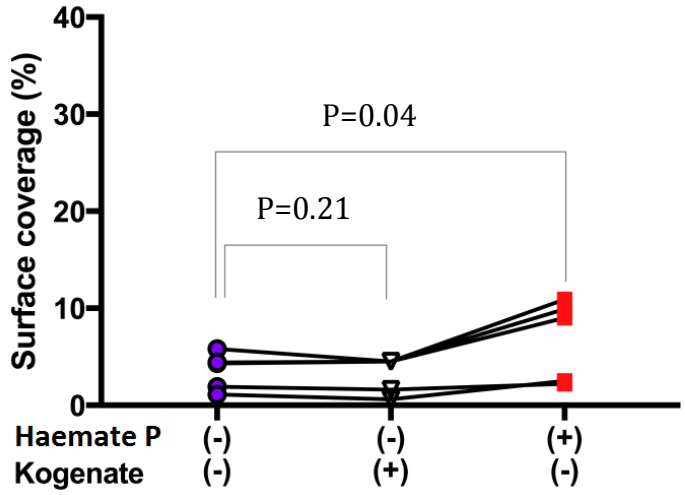


Figure 3.11 Thrombus formation in patient samples with/without Haemate P or Kogenate. Patient samples (n=5). Flow through a collagen-coated Cellix chip at 1000s^{-1} for five minutes before analysis. (+) HP indicates addition of 2 units/ml Haemate P. (+) Kogenate indicates addition of 0.8 iu/ml factor VIII (Kogenate). Wilcoxon signed rank to compare Haemate P(-) Kogenate(-) with Haemate P(-) Kogenate(+); and Haemate P(-) Kogenate(-) with Haemate P(+) Kogenate (-). Statistics: 2-tailed t-test (P<0.05 considered statistically significant).

3.3.8 Platelet function analyser closure time

Patient samples (n=5) and reconstituted blood samples (n=3) were analysed together using the same techniques. PFA closure time with C-ADP cartridges was reduced in 5/8 cases following administration of Haemate P but this did not reach statistical significance (median >300, IQR >300 to >300 seconds vs median 202.5, IQR 155.5 to >300 seconds; p=0.06). PFA closure time with P2Y cartridges was reduced in 6/8 cases following administration of Haemate P and this reduction was statistically significant (median 261.5, IQR 161.8 to >300 seconds vs median 148, IQR 107.8 to >300 seconds; p=0.03) (Figure 3.12).

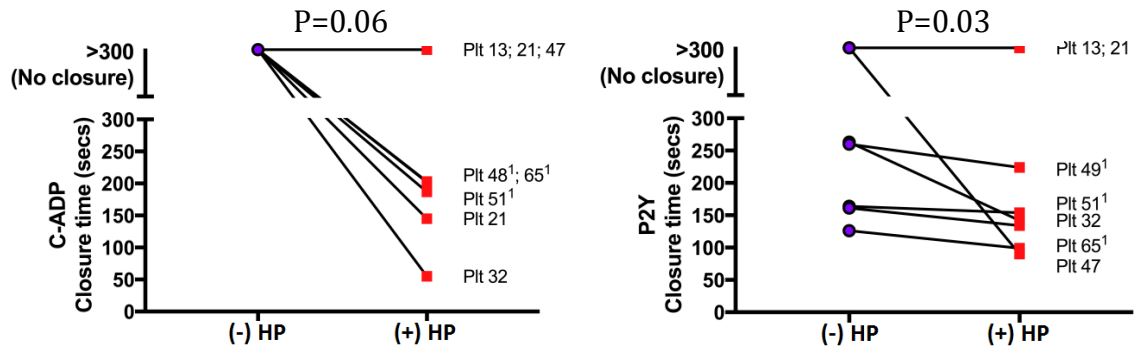


Figure 3.12 Platelet function analyser closure time. Patient samples (n=5); [1]manipulated blood (n=3). Platelet counts of the individual samples are marked on the right side of each graph. (+) HP indicates addition of 2.0 units/ml Haemate P. (-) HP indicates no addition of Haemate P. C-ADP: collagen/adenosine diphosphate cartridge. Statistics: Wilcoxon signed rank ($P<0.05$ considered statistically significant).

3.4 Discussion

3.4.1 Summary of key results

The results of this work showed it was possible to simulate thrombocytopenia in normal blood samples using the technique outlined in section 3.2.3 as demonstrated by the lack of pre-activation using FACS. However this process leads to a reduced response to platelet agonists in manipulated blood. Given that there is no evidence of pre-activation, this is likely to represent loss of larger, more metabolically active platelets (273).

Thrombus formation under flow over collagen was proportional to platelet count. Thrombus formation is known to be reduced in thrombocytopenia (274). Admission of Haemate P to thrombocytopenic blood increased thrombus formation under flow, as measured by total surface coverage, MFI and thrombus volume. These results were corroborated by a decrease in closure time measured by the PFA.

Surface coverage and MFI were positively correlated with the time taken for thrombus formation. For a future trial, a perfusion time of 5 or 6 minutes would be preferable in order to see adequate separation of the thrombus formation results at different platelet counts and VWF concentrations.

The increase in thrombus volume is likely to be due to the VWF content of Haemate P, rather than the factor VIII. Evidence for this is the increase in thrombus formation with Haemate P, which is not present with Kogenate.

Correlation between surface coverage and thrombus volume is reported in normal blood (276) and I have now shown this also operates in thrombocytopenic blood. Surface coverage and MFI are extracted from the same images, which is likely to account for the close correlation between the two values. By contrast, thrombus volume is extracted from z-stack images. A lesson for a future trial, where rapid access to a confocal microscope might be limited, is that surface coverage or MFI provide similar estimates of thrombus formation.

The improvement in thrombus formation with addition of Haemate P is consistent with the results in other studies where an exogenous source of VWF has been added to normal blood (83) or to the blood of patients with type 3 Von Willebrand disease (277). The increase in surface coverage following administration of Haemate P was consistent with the results of a study performed in patients with ITP and normal controls with simulated thrombocytopenia (275). This previous study examined flow through a polystyrene-coated chamber for 2 minutes at $1200s^{-1}$ with/without Haemate P and demonstrated an increase in surface coverage and mean thrombus size.

Surface coverage distinguished different target platelet counts from 180 seconds onwards, although MFI was only able to distinguish a target count of 50 $x10^9$/l after at least 5 minutes of perfusion. Consequently, patient samples were be analysed for 5 minutes and this will be taken forward to a future trial.

Clinical data from the patients in chapter 2 suggests that the PFA closure time is rarely reached using a C-ADP cartridge in thrombocytopenia (section 2.3.3.3). By contrast, closure time could be achieved in the majority of cases treated with Haemate P. It is possible that the narrower aperture of the P2Y PFA cartridge, compared to the C-ADP PFA cartridge, will make evaluation of closure time easier in thrombocytopenic blood.

3.4.2 Limitations

The platelet-depletion technique did not result in pre-activation but did lead to a decreased response to platelet agonists. Consequently these results are not directly comparable to normal blood. However the reduction in response to agonists was consistent across the different target platelet counts making them comparable to one another. The consistency of these results with those seen in the patient samples suggests that this does not significantly compromise this model. An advantage of the platelet-depletion technique is that haematocrit and VWF antigen can be maintained between samples, making them comparable to one another.

In these experiments, Haemate P has been used to approximate the effects of desmopressin, but there are several differences between the two. It is possible that desmopressin might have a direct effect on platelets. These effects include increasing intracellular calcium signalling and pro-coagulant platelet formation (129, 182). It is unclear if these effects are independent of VWF and they are not found when desmopressin is added to blood *in* vitro. The absolute increase in VWF is variable

between patients treated with desmopressin, with a 2-6-fold rise. Consequently, Haemate P might give a more controlled change in VWF concentration than desmopressin. Although thrombus formation under flow is used extensively in investigation of platelet function in basic science, there is little clinical data to assess whether it provides a good measure of bleeding risk in patients (278).

I did not examine serial VWF concentrations, and I have not used platelets suspended in VWF-deplete plasma as a control. I used the Haemate P concentration which I considered to be most likely to approximate the effects of desmopressin. Future work will include determining the optimum concentrations of VWF necessary to improve thrombus formation (Section 7.8.3).

As previously mentioned, the methods used internationally for thrombus formation under flow are highly variable (84). The method used for these experiments aims to simulate many of the conditions for *in* vivo haemostasis but importantly, does not include the addition of calcium. While this makes the experiments easier to perform, this is less comparable to the environment *in vivo* for haemostasis. In order to address these limitations, it will be necessary to evaluate if thrombus formation under flow can predict bleeding risk in patients.

3.4.3 Conclusions

These results suggest that thrombus formation under flow over collagen is proportional to platelet count when all other factors are equal. Surface coverage, MFI and thrombus volume can be increased by the addition of Haemate P. An hypothesis stemming from these findings is that increasing plasma VWF levels might be a strategy to compensate for thrombocytopenia *in vivo*. However clinical trial data will be necessary in order to assess this. There are no clinical links at present between thrombus formation under

flow and bleeding risk and these clinical correlations will be necessary to determine this. Adding Haemate P provides a consistent level of circulating VWF, but desmopressin produces a more variable response. It should be noted that patients frequently have very high baseline levels of VWF in critical illness, and while studies have shown that desmopressin can still increase VWF levels in these circumstances (83, 200) the effects may be more blunted. Consequently, I next aimed to identify from the relevant literature whether administration of desmopressin results in a reduction in bleeding in surgery (Chapter 4); and whether there is evidence in the setting of thrombocytopenia or platelet dysfunction (Chapter 5).

Chapter 4

4 Meta-analysis of peri-operative desmopressin for unselected patients

4.1 Introduction

In Chapter 3 I showed that thrombus formation under flow is increased when a source of VWF is added to both normal and thrombocytopenic blood. In this chapter I evaluated the evidence for whether desmopressin could be given to unselected patients to minimise peri-operative bleeding and blood transfusion. This chapter has been adapted and published: Desborough MJ et al. Desmopressin for minimising perioperative red cell transfusion. *Cochrane Database of Systematic Reviews* 2017; **7**: CD001884 (142).

Blood loss and anaemia are common following surgery. Anaemia is known to be an independent predictor of poor outcome for surgical patients (279, 280). Correction of anaemia with red cell transfusions does not have a clear benefit in this setting (281, 282) and there is mounting evidence that red cell transfusion may be harmful (283). Consequently there is a need for agents which could reduce blood loss and the need for red cell transfusions. Recent work has demonstrated the importance of alternative agents such as tranexamic acid, which reduces red cell transfusion requirements and mortality, without an increased risk of thrombotic events when administered peri-operatively (155). Desmopressin may also have a role in this setting.

4.1.1 Surgery

In 2014, people undergoing surgical procedures received 26.7% of the red cell units (12,318 units) transfused in the United Kingdom (284). The largest volumes of blood

were transfused in cardiac surgery, trauma, orthopaedic surgery, gastrointestinal surgery and vascular surgery (284). Blood loss tends to be greater with more complex surgery and is associated with an increased risk of death. In routine elective surgery the risk of death is around 0.7% (285), rising to 3.1% for cardiac surgery (286) and up to 30% for high risk emergency vascular surgery (287). In cardiac surgery, both heavy surgical blood loss and the need for a patient to return to theatre to find a source of bleeding are associated with a significant increase in the risk of death (164) and blood loss of more than two litres is associated with an 8-fold increase in the odds of death (288).

4.1.2 Interventional procedures

Bleeding is uncommon following interventional procedures such as liver or kidney biopsies. For example, the risk of bleeding following a trans-jugular liver biopsy is approximately 0.07% with a risk of death of 0.09% (289). However bleeding may still have serious sequelae, especially when it occurs at a site where it cannot be easily arrested where mechanical compression cannot be applied.

4.1.3 Characteristics of patients undergoing surgery which may make them more vulnerable to bleeding

Some patients may be more vulnerable to blood loss due to inherited or acquired defects in haemostasis. Antiplatelet agents and anticoagulants are associated with an increased risk of peri-operative bleeding and mortality. It is important to establish a balance between the need to reduce the risk of haemorrhage for people taking these agents, against the risk of a thrombotic event (290). Other groups who are vulnerable to bleeding include those with thrombocytopenia or a coagulopathy.

4.1.4 Desmopressin

Desmopressin has been described in detail in section 1.5.7.

4.1.5 Aims

The aim of this chapter was to examine the evidence for the efficacy of desmopressin, in reducing perioperative blood loss and the need for red cell transfusion in patients who do not have congenital bleeding disorders using a systematic review and meta-analysis of randomised controlled trials.

4.2 Methods

4.2.1 Criteria for considering studies for this review

4.2.1.1 Types of studies

Only randomised controlled trials were included. Randomisation of participants in studies reduces the risk of imbalances in baseline characteristics between treatment groups (293). When clinicians make a decision about which patient should receive an intervention (rather than using randomisation), many factors may influence the decision, including factors which may be important for determining the outcome (for instance a patient who is bleeding more heavily may be more likely to be prescribed desmopressin). There was no restriction on language or publication status to provide a comprehensive summary and to maximise the generalisability of the findings.

4.2.1.2 Types of participants

I included adults or children undergoing any type of surgery or interventional procedure. I excluded trials in participants with inherited bleeding disorders such as haemophilia A or von Willebrand disease because they are known to respond to desmopressin.

4.2.1.3 Types of interventions

Trials using subcutaneous or intravenous desmopressin were eligible. There is little difference in absorption between subcutaneous and intravenous routes but intranasal desmopressin was not included because it is poorly absorbed by this route (188).

4.2.1.4 Types of comparators

Placebo or no active comparator

4.2.2 Types of outcome measures

Outcomes were selected that were considered to be most relevant to clinicians and patients. These outcomes have been assessed in previous systematic reviews of haemostatic agents that have proved practice changing (155, 294).

4.2.2.1 Primary outcome

Total volume of allogeneic red cells transfused

4.2.2.2 Secondary outcomes:

1. Risk of receiving an allogeneic red cell transfusion
2. Total blood loss
3. Re-operation due to bleeding
4. All-cause mortality
5. Thrombotic events: composite of myocardial infarction, stroke, venous thromboembolism
6. Clinically significant hypotension: hypotension requiring intravenous fluid administration or commencement (or an increase) of inotropes

4.2.3 Search methods for identification of studies

Full details of the searches are outlined in Appendix 1.

4.2.3.1 Electronic searches

The following databases were searched for eligible RCTs:

- CENTRAL (Cochrane Library, 2016 issue 6)
- MEDLINE (OvidSP, 1946 to 07 July 2016)
- PubMed (epublications only, to 07 July 2016)
- Embase (OvidSP, 1974 to 07 July 2016)
- CINAHL (EBSCOhost, 1937 to 07 July 2016)
- UKBTS/SRI Transfusion Evidence Library (www.transfusionevidencelibrary.com) (1950 to 07 July 2016)
- Web of Science: Conference Proceedings Citation Index-Science (CPCI-S) (Thomson Reuters, 1990 to 07 July 2016)
- LILACS (1982 to 07 July 2016)
- IndMed (ICMR-NIC, 1985 to 07 July 2016)
- KoreaMed (KAMJE, 1997 to 07 July 2016)
- PakMediNet (2001 to 07 July 2016)

Searches in MEDLINE, Embase, and CINAHL were combined with adaptations of the Cochrane RCT search filters, as detailed in the *Cochrane Handbook for Systematic Reviews of Interventions* (295). I also searched ClinicalTrials.gov (clinicaltrials.gov), the WHO International Clinical Trials Registry (ICTRP; apps.who.int/trialsearch) and the Hong Kong University Clinical Trials Register (www.hkuctr.com) in order to identify ongoing trials.

A broad search strategy was used to maximise the generalisability of the findings and to create a comprehensive summary.

4.2.3.2. Searching other resources

I searched the bibliographies of eligible trials, review articles, and reports for further potentially relevant studies.

4.2.4 Data collection and analysis

All electronically derived citations and abstracts of papers identified by the review search strategy were screened by two independent reviewers. Risk of bias in the included studies and extracted data was also done in duplicate.

4.2.5 Selection of studies

All electronically derived citations and abstracts of papers identified by the review search strategy were screened for relevance by two independent reviewers. Studies were excluded if they were considered clearly irrelevant at this stage. The full texts of all potentially relevant trials for eligibility against the criteria outlined above by two independent reviewers. All disagreements were resolved by discussion without the need to consult a third review reviewer. An article abstraction form was used to extract information regarding randomisation criteria, study methodology, presence of a transfusion protocol, type of surgery, and treatment outcomes. The reasons why potentially relevant studies failed to meet the eligibility criteria were recorded.

4.2.6 Data extraction and management

Data extraction was performed by two independent reviewers according to the guidelines proposed by The Cochrane Collaboration (296). Any 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. Papers not published in English were translated in their entirety, then data were extracted in the usual way (297-299). Any unclear data were discussed directly with the translators.

Data from studies was extracted in duplicate using an online systematic review management tool (Covidence; www.covidence.org) and then entered the data into Review Manager 5.3.5. The following data were collected:

- Type of study (study design, number of arms, single centre or multicentre)
- Inclusion and exclusion criteria
- Participants (number of participants randomised, number of participants analysed, age, gender, antiplatelet agents, anticoagulants, coagulopathy, thrombocytopenia and use of anti-fibrinolytics).
- Details of surgery (type of surgery, emergency or elective, and use of a transfusion protocol).
- Interventions (desmopressin dose, route of administration, diluent, speed of administration and timing of administration)
- Comparators (Type of comparator, route of administration, speed of administration and timing of administration)
- Outcomes (the number of participants exposed to allogeneic blood; the amount of allogeneic blood transfused (expressed as whole blood or packed red cells); blood loss; re-operation for bleeding. the number of participants experiencing post-operative complications (thrombosis, myocardial infarction, stroke); mortality; and number of participants with and bleeding).
- Risk of bias (see section 4.2.7).

4.2.7 Assessment of risk of bias in included studies

Problems with the design and execution of studies may compromise their validity. One risk to validity is bias, which is the introduction of a systematic error or deviation from the truth (293). It is not possible to know whether bias will result in overestimation or

underestimation, so it is important to assess studies on whether their methodology may place them at *risk* of bias. Inclusion of trials at high risk of bias provides less robust results and reduces the overall confidence in the treatment effect.

It is important to use a risk of bias assessment tool and for the evaluation to be performed by two independent assessors to ensure consistency in assessment of risk of bias. For the work in this chapter, two reviewers worked independently to assess each element of potential bias listed below as 'high', 'low' or 'unclear risk of bias'. Consensus on the degree of risk of bias was assessed by comparison of the review authors' statements. I used the Cochrane Collaboration's tool for assessing the risk of bias, which included the following domains:

- Selection bias: random sequence generation and allocation concealment.
- Performance bias: blinding of participants and personnel.
- Detection bias: blinding of outcome assessment.
- Attrition bias: incomplete outcome data.
- Reporting bias: selective reporting.
- Other bias

4.2.8 Measures of treatment effect

- For continuous outcomes, the mean, standard deviation and total number of participants in both the treatment and control groups were recorded. For dichotomous outcomes, the number of events and the total number of participants in both the treatment and control groups were recorded.
- For continuous outcomes using the same scale, analyses were performed using the mean difference (MD) with 95%CI.

- For dichotomous outcomes, pooled risk ratios (RR) were reported with a 95% CI. Where the number of observed events was small (< 5% of sample per group), and where trials had balanced treatment groups, Peto Odds Ratios (pOR) were reported with 95% CI (300).
- When data allowed, quantitative assessments was undertaken using Review Manager 5.3.5.
- Trials were reported narratively if none of the methods above were suitable

4.2.9 Unit of analysis issues

Trials with three or more arms were treated in accordance with the advice given in Chapter 16 of the *Cochrane Handbook for Systematic Reviews of Interventions* (301). The most appropriate pair of interventions was selected and the other was excluded. There were no cluster randomised trials or crossover trials.

4.2.10 Dealing with missing data

Where data were identified to be missing or unclear in published literature, the study authors were contacted directly. This was limited to contacting authors of studies published in the last ten years. I analysed data on an intention-to-treat basis (300).

4.2.11 Assessment of heterogeneity

When studies were considered sufficiently homogenous in their study design, meta-analysis was performed (300). Statistical heterogeneity of treatment effects between trials was assessed using a Chi^2 test with a significance level at $P < 0.1$. The I^2 statistic was used to quantify possible heterogeneity ($I^2 > 50\%$ moderate heterogeneity; $I^2 > 80\%$ considerable heterogeneity). When necessary, potential causes of heterogeneity were explored by sensitivity and subgroup analyses.

4.2.12 Assessment of reporting biases

Potential publication bias (small trial bias) was explored by generating funnel plots and using a modified Harbord test for dichotomous outcomes (302) and an Egger's test for continuous outcomes (303). I considered a P value of < 0.1 as significant for these tests (304).

4.2.13 Data synthesis

Analyses were performed according to the recommendations of Chapter nine of the *Cochrane Handbook for Systematic Reviews of Interventions* using aggregated data for analysis (300). For statistical analysis, data were entered into the Review Manager 5.3.5 software. Where meta-analysis was feasible, the random-effects model (DerSimonian and Laird method) was used for pooling data (305). The random effects method was used because it was anticipated that studies would include patients from different populations and different time periods. This method takes into account variation between studies that are not due to random chance.

The Mantel-Haenszel method was used for pooling dichotomous outcomes because it performs better than the inverse variance method when study event rates are low and included trials are small (300). In cases where events were rare, and appropriate conditions were satisfied, the pOR was used (301). The pOR works well when events are uncommon and there are similar numbers of participants in the intervention and comparator arms. The inverse variance method was used for continuous outcomes. Transfused blood volume expressed in millilitres was converted to units by assuming 300 ml to be equivalent to one unit of blood to allow meta-analysis to be performed.

4.2.14 Subgroup analysis and investigation of heterogeneity

Statistical heterogeneity of treatment effects between studies was assessed using a Chi^2 test with a significance level at $P<0.1$. The I^2 statistic was used to quantify the degree of potential heterogeneity and classified as moderate if $I^2 > 50\%$, or considerable if $I^2 > 80\%$. The potential causes of heterogeneity were investigated by sensitivity and subgroup analyses (300). Subgroup analyses were performed for each of the following outcomes in order to assess the effect on heterogeneity.

- Type of surgery or procedure.
- Studies where 75% or more participants had platelet dysfunction and/or were taking antiplatelet agents (see chapter 5)
- Studies where 75% or more participants were taking an anti-fibrinolytic agent (see Chapter 5)
- Comparison of patients who received desmopressin at a rate of at least 1.2µg/kg/hour against those who received in at a slower rate.

4.2.15 Sensitivity analysis

The robustness of the findings was investigated by performing the following sensitivity analyses when there were sufficient data.

- Including only studies with a 'low risk of bias' (defined as trials with no high risk of bias assessments and at least half low risk of bias assessments).
- Including only studies with a transfusion protocol
- Including only studies published as full text papers

4.2.16 Summary of findings

The Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach was used to rate the quality of the evidence using the five GRADE considerations (306):

- risk of bias (see section 4.2.7);
- inconsistency: unexplained heterogeneity in the results;
- indirectness: inclusion of studies with the wrong group of participants; not using the intervention of interest; or measuring different outcomes;
- imprecision: insufficient information size to make a judgement (e.g. number of participants is too small, resulting in large confidence intervals and lack of confidence in the true estimate);
- publication bias: systematic underestimation (or overestimation) of treatment effects based on selective publication of studies.

The quality of evidence was ranked as one the following four categories:

- High: I am very confident that the true effect lies close to the estimated effect;
- Moderate: the true effect is likely to lie close to the estimated effect but there is a possibility that it is substantially different;
- Low: the true effect may be substantially different to the estimated effect;
- Very low: I have little confidence in the results and the true effect may be substantially different to the true effect.

Methodology was consistent with the definitions from the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement (307).

4.3 Results

4.3.1 Search results

The database searches identified 1596 records and these were reduced to 1227 after duplicates were removed. 1113 references were excluded as either not a RCT or were clearly outside the scope of this review (Figure 4.1). The full texts of the remaining 114 references were obtained. Thirty-three were excluded (reasons summarised in Figure 1). Six trials were four-arm trials, which were each divided into two separate multi-arm trials (297, 299, 308-317), leaving 62 completed trials (297-299, 308-366); and four ongoing trials (IRCT2015010512642N8; NCT00885924; NCT01982760; NCT02084342).

4.3.2 Trial characteristics

Thirty-nine trials were in the setting of cardiac surgery (297, 299, 308-311, 314, 315, 319-324, 326-328, 332, 333, 336-339, 341, 348-357, 359, 361-364); 12 in orthopaedic surgery (312, 313, 318, 329-331, 334, 340, 344, 346, 358, 365); two each in vascular surgery (325, 345), plastic surgery (316, 317) and interventional procedures (343, 347); one each in hepatic surgery (366), maxillofacial surgery (335), sinus surgery (360). In one trial there were a mix of breast, orthopaedic and abdominal surgery cases (298); and in one trial, the type of surgery was not reported (342). Two cardiac surgery trials included patients undergoing emergency surgery (308, 309) and the remaining trials included only patients undergoing elective surgery.

Trials were published between 1986 and 2016. Four trials were published in abstract form only (308, 309, 324, 338) and the remaining trials were published as full text articles.

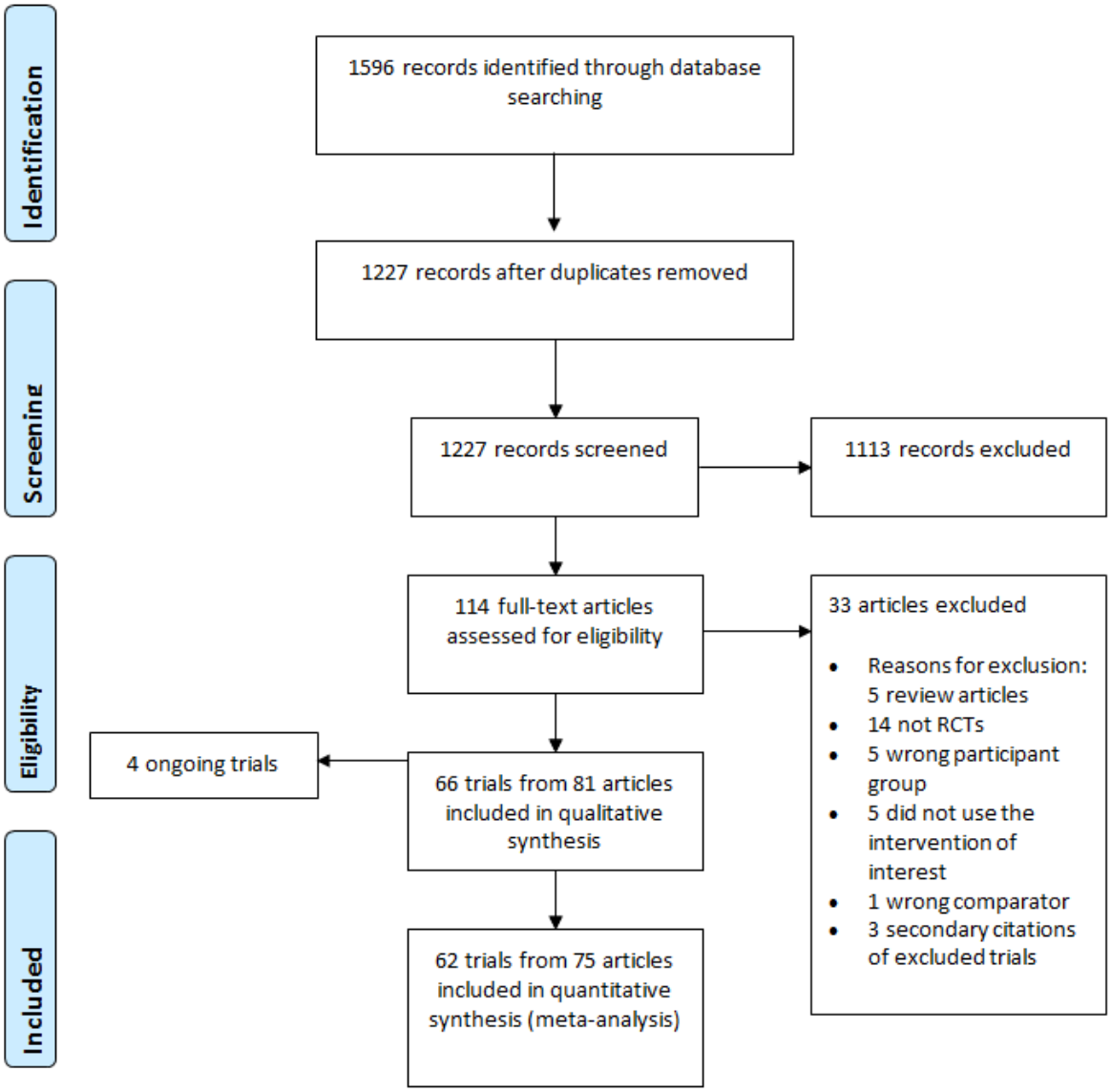


Figure 4.1 Study flow diagram. RCT: randomised controlled trial.

Desmopressin was administered as a single dose of 0.3μg/kg in 50 trials (297, 299, 308-324, 326-329, 332, 333, 336-339, 341-345, 347-357, 359, 360, 362-366); 0.4μg/kg in two trials (327, 344); two doses of 0.3μg/kg in three trials (330, 331, 358); 10μg/m^2 body surface area in four trials (334, 340, 346, 361); a single 20μg in two trials (325, 335); and 15 to 45μg depending on patient weight in one trial (298).

Desmopressin was given pre-operatively in 20 trials (316-318, 329-331, 334, 335, 340, 342-347, 358, 360, 362, 365, 366); intra-operatively in 37 trials (297, 299, 310-315, 319, 320, 322-325, 327, 328, 332, 333, 336-339, 341, 348-357, 359, 361, 363, 364); post-operatively in three trials (308, 309, 326); either intra-operatively or post-operatively in one trial (321); and one trial did not report timing of administration (298). In one trial, desmopressin was administered to treat bleeding (321), and was used prophylactically in the remaining trials.

Three trials were open label and compared desmopressin to standard care (308, 309, 354) and the remaining trials compared desmopressin to a matching 0.9% saline placebo.

Characteristics of included studies are reported in Table 4.1 and Table 4.2.

Trial [Country]	Arm	n	M/F	Age (years)	Operation	Transfusion trigger	High bleeding risk features	EACA or TXA
Cardiac Surgery								
Aida 1991a [Japan]	D P	5 4	2/3 3/1	52 ± 5 57 ± 5	CABG or valve replacement	.	.	.
Aida 1991b [Japan]	D P	5 6	4/1 2/4	60 ± 8 57 ± 17	CABG or valve replacement	.	.	.
Andersson 1990 [Sweden]	D P	10 9	8/2 8/1	57 ± 12 61 ± 5	Primary CABG	.	No	.
Ansell 1992 [USA]	D P	41 42	24/17 21/21	61.9 ± 10.7 60.1 ± 9.2	Valve replacement	.	.	1 EACA None
Bignami 2016 [Italy]	D P	68 67	50/18 50/17	64 ± 13.3 62 ± 13.2	.	Hb <80g/l	25 anti-platelet[7] 29 anti-platelet[8]	All TXA
Brown 1989 [USA]	D P	10 9	8/2 6/3	61.7 ± 8.1 62.5 ± 6.9	CABG	.	6 aspirin 5 aspirin	.
Casas 1995 [Spain]	D P	50 51	33/17 31/20	58 ± 12 54 ± 12	CABG or valve replacement	Hb <80g/l	7 anti-platelet[9] 5 anti-platelet[9]	No
Chuang 1993 [China][1]	D P	48 48	. .	. .	.	.	.	.
de Prost 1992 [France]	D P	47 45	37/10 32/13	60 ± 13 55 ± 16	CABG or valve replacement	Hct <30%	Bleeding time > 10s	2 aprotinin 6 aprotinin
Despotis 1999 [USA]	D P	50 51	30/20 34/17	64 ± 10 66 ± 10	CABG or valve replacement	No	26 aspirin; 3 warfarin; all hemoSTATUS <60% in channel 5 33 aspirin; all hemoSTATUS <60% in channel 5	25 EACA 31 EACA
Dilthey 1993 [Germany]	D P	19 20	19/0 20/0	56 ± 9 58 ± 8	CABG	Hct <30%	All aspirin	No
Frankville 1991 [USA]	D P	20 20	17/3 17/3	59.9 ± 10.7 59.6 ± 11.2	Primary CABG	.	No	.
Gratz 1992 [USA]	D P	29 30	19/10 23/7	62.2 ± 10.4 62.7 ± 12.3	CABG	.	All aspirin	.
Hackmann 1989 [Canada]	D P	74 76	. .	Stratified by age[3]	CABG, valve replacement or ASD repair	.	26 anti-platelet[10] 15 anti-platelet[11]	.

Trial [Country]	Arm	n	M/F	Age (years)	Operation	Transfusion trigger	High bleeding risk features	EACA or TXA
Hajjar 2007[1] [Brazil]	D P	75 75	. .	. .	.	.	.	.
Hedderich 1990 [Canada]	D P	31 31	47/15[2]	61 ± 10 59 ± 9	CABG	.	12 anti-platelet[9] 13 anti-platelet[9]	.
Hemşinli 2012a[1] [Turkey]	D SC	10 10	. .	. .	Emergency CABG	.	All dual anti-platelet therapy[9]	No
Hemşinli 2012b[1] [Turkey]	D SC	16 18	. .	. .	Emergency CABG	.	All dual anti-platelet therapy[9]	All TXA
Horrow 1991a [USA]	D P	38 44	. .	63 ± 11 64 ± 10	ACBG, valve replacement or ASD repair	Hct <21% or ≥ 250ml blood loss; or Hct <24% and unstable	.	No
Horrow 1991b [USA]	D P	40 37	. .	63 ± 9 65 ± 11	ACBG, valve replacement or ASD repair	Hct <21%; ≥ 250ml blood loss; or Hct <24% and unstable	.	All TXA
Jin 2015 [China]	D P	52 50	21/31 21/29	49 ± 10 53 ± 9	Valve replacement	.	No	All TXA
Kuitunen 1992 [Finland]	D P	15 15	14/1 14/1	57 ± 9 59 ± 5	Primary CABG	Hct <20% during CPB; or <30% post-op	No	.
Marquez 1992 [USA]	D P	21 22	. .	59.8 ± 1.2 61.7 ± 1.5	Primary CABG	Hb <100g/l	No	.
Mongan 1992a [USA]	D P	44 42	40/4 36/6	61.5 ± 9.3 60.9 ± 9.7	Primary CABG	Hct <24%	No	.
Mongan 1992b [USA]	D P	13 16	9/4 11/5	58.8 ± 10.7 65.8 ± 10	Primary CABG	Hct <24%	TEG MA ≤50mm	.
Oliver 2000 [USA]	D P	31 29	15/16 11/18	13.9 ± 10 18.1 ± 9.8	Complex congenital heart defect repair	No	3 aspirin; 2 warfarin 1 aspirin; 2 warfarin	.
Ozkisacik 2001 [Turkey]	D P	33 33	24/9 23/10	59.0 ± 11.5 58.1 ± 12.1	CABG	Hct <28%	No	.
Pleym 2004 [Norway]	D P	46 46	40/6 36/10	63.1 ± 8.6 64.4 ± 8	Primary CABG	Hct <25%	All aspirin	3 TXA 2 TXA
Reich 1991 [USA]	D P	14 13	12/2 9/4	60 ± 16 55 ± 15	CABG or valve replacement	.	4 aspirin 5 aspirin	.

Trial [Country]	Arm	n	M/F	Age (years)	Operation	Transfusion trigger	High bleeding risk features	EACA or TXA
Reynolds 1993 [USA]	D P	53 42	29/24 25/17	2.3 ± 3.6 2 ± 2.8	Complex congenital heart disease repair	.	.	.
Rocha 1988 [Spain]	D P	50 50	19/31 25/25	55 ± 13 53 ± 12	CABG, valve replacement or ASD repair	.	.	.
Rocha 1994 [Spain]	D SC	25 28	14/11 22/6	56.6 ± 8.8 56.3 ± 10.1	CABG, valve replacement or ASD repair	.	.	No
Salmenpera 1991 [Finland]	D P	15 15	14/1 14/1	57 ± 9 59 ± 5	Primary CABG	Hct <30%	No	.
Salzman 1986 [USA]	D P	35 35	21/14 21/14	Stratified by age[5]	CABG, valve replacement or ASD repair	.	.	.
Seear 1989 [Canada]	D P	30 30	17/13 18/12	3.8 ± 4.2 5.4 ± 5	Congenital heart defect repair	.	.	.
Sheridan 1994 [Canada]	D P	20 24	20/0 24/0	56.6 (52.9-60.3)[6] 61.6 (58.6-64.6)[6]	Primary CABG	.	All aspirin	.
Spyt 1990 [UK]	D P	49 49	42/7 38/11	58.8 ± 8.8 55 ± 8.4	Primary CABG	Hct <30%	7 aspirin 5 aspirin	.
Steinlechner 2011 [Austria]	D P	20 23	8/12 7/16	72 ± 10 73 ± 8	Valve replacement	Hb <70g/l in operating room; or <80g/l on ward	PFA C-ADP >170s	.
Temeck 1994 [USA]	D P	40 43	. .	. .	.	.	.	8 EACA 13 EACA
Orthopaedic Surgery								
Alanay 1999 [Turkey]	D P	18 22	5/13 6/12	14.8 ± 4 14.5 ± 3	Scoliosis Surgery	.	.	.
Ellis 2001 [Israel]	D P	10 10	2/8 3/7	72 ± 6 72 ± 8	Knee replacement	Hct <27%	.	No
Flordal 1991 [Sweden]	D P	6 6	. .	. .	Hip replacement	.	.	.
Flordal 1992 [Sweden]	D P	25 25	12/13 12/13	64 ± 9 68 ± 9	Hip replacement	.	.	.
Guay 1992	D	15	1/14	13.5 ± 1.9	Spinal fusion	20-30% fall in calculated	No	.

Trial [Country]	Arm	n	M/F	Age (years)	Operation	Transfusion trigger	High bleeding risk features	EACA or TXA
[Canada]	P	15	1/14	15.1 ± 1.9		blood volume		
Karnezis 1994a [USA]	D	17	7/10	65 ± 5.3	Knee replacement	Hct <22-24%	.	.
	P	19	9/10	66 ± 9.3				
Karnezis 1994b [USA]	D	26	12/14	65 ± 7.8	Hip replacement	Hct <22-24%	.	.
	P	30	14/16	67 ± 6.7				
Kobrinsky 1987 [USA]	D	17	8/9	14.8 ± 3.3	Spinal fusion	.	.	.
	P	18	7/11	15.3 ± 1.8				
Leino 2010 [Finland]	D	23	11/12	59 ± 13	Hip replacement	Hb <90g/L	No	.
	P	24	17/7	61 ± 13				
Letts 1998 [Canada]	D	16	9/7	13.4 ± 2.5	Spinal fusion	.	.	.
	P	14	9/5	13.8 ± 2.8				
Schott 1995 [Sweden]	D	39	20/19	71 ± 9	Hip replacement	Hct <27%	No	.
	P	40	15/25	68 ± 7				
Theroux 1997 [USA]	D	10	.	13 (10-19)[6]	Spinal fusion	Hb <100g/l	No	.
	P	11	.	13 (6-18)[6]				
Vascular Surgery								
Clagett 1995 [USA]	D	43	43/0	62 ± 9	Infra-renal aortic aneurysm repair or aorto-femoral bypass	.	No	.
	P	48	48/0	64 ± 8				
Lethagen 1991 [Sweden]	D	25	19/6	.	Aorto-iliac graft surgery	Hct <30%	No	.
	P	25	18/7	.				
Hepatic Surgery								
Wong 2003 [Hong Kong]	D	30	20/10	47.4 ± 11.3	Hepatectomy	Hct <30%	No	.
	P	29	17/13	54.9 ± 11.8				
Plastic Surgery								
Wingate 1992a [USA]	D	14	.	.	Extensive flap reconstruction	.	.	.
	P	9	.	.				
Wingate 1992b [USA]	D	8	.	.	Limited flap reconstruction	.	.	.
	P	13	.	.				
Maxillofacial surgery								
Guyuron 1996 [USA]	D	10	1/9	.	Bimaxillary osteotomy	.	.	.
	P	10	4/6	.				
Sinus surgery								
Shao 2015 [China]	D	45	26/19	45.9 ± 14.9	Endoscopic sinus surgery	.	No	.
	P	45	24/21	40.7 ± 17.4				

Trial [Country]	Arm	n	M/F	Age (years)	Operation	Transfusion trigger	High bleeding risk features	EACA or TXA
Interventional procedures								
Lee 2010 [South Korea]	D	24	16/8	60 (28-93)[6]	Dialysis catheter	.	.	.
	P	24	15/9	57 (27-66)[6]				
Manno 2011 [Italy]	D	80	45/35	39.5 ± 14.2	Kidney Biopsy	.	.	.
	P	82	43/39	41.7 ± 15				
Other								
Lazarchick 1995 [USA]	D	12	.	.	.	.	.	.
	P	11	.	.				
Marczinski 2007 [Netherlands]	D	14	0/14	54.2 ± 14.9	Breast, abdominal & orthopaedic surgery	.	No	.
	P	14	2/12	49.1 ± 12.1				

Table 4.1 Study characteristics. Desmopressin dose 0.3ug/kg intravenously unless stated otherwise. Placebo 0.9% saline in all cases. Age stated as mean ± standard deviation unless otherwise stated. All operations elective unless stated otherwise. Unreported data marked with a full stop. [1]published in abstract form only. [2]Gender specified for combined groups only. [3]Desmopressin arm: 5: <45yrs, 27: 46-60yrs, 39: 61-75yrs, 3: >75yrs; placebo arm: 6: <45yrs; 26: 46-60yrs; 39: 61-75yrs; 5: >75yrs. [4]Age presented as mean (range). [5]Desmopressin arm: 2: 31-40yrs, 3: 41-50yrs, 11: 51-60yrs, 8: 61-70yrs, 9: 71-80yrs, 2: >80yrs; Placebo arm: 31-40yrs, 1: 41-50yrs, 7: 51-60yrs, 11: 61-70yrs, 11: 71-80yrs, 2: >80yrs. [6]Age presented as median (range). [7]Anti-platelet agents (21 aspirin, 4 ticlodipine, 8 clopidogrel, 1 tirofiban). [8]Anti-platelet agents (16 aspirin, 5 ticlodipine, 9 clopidogrel, 1 tirofiban). [9]Anti-platelet agent not specified. [10]Anti-platelet agents (16 aspirin; 10 dipyridamole). [11]Anti-platelet agents (11 aspirin; 4 dipyridamole). ACBG: aorto-coronary bypass graft, ASD: atrial septal defect, CABG: coronary artery bypass graft, CPB: cardiopulmonary bypass, D: desmopressin, EACA: epsilon aminocaproic acid, F: female, Hb: haemoglobin, Hct: haematocrit, M: male, P: placebo, PFA C-ADP: platelet function analyser collagen/adenosine diphosphate cartridge, SC: standard care, TAP: trans-atrial pacing, TEG MA: thromboelastography maximum amplitude.

Trial	Dose (µg/kg)	Diluent	Volume (ml)	Infusion time (min)	Infusion rate (µg/kg/hr)	Timing of administration
Cardiac Surgery						
Aida 1991a	0.3	0.9% saline	50	.	.	Intra-operative
Aida 1991b	0.3	0.9% saline	50	.	.	Intra-operative
Andersson 1990	0.3	0.9% saline	50	15	1.2	Intra-operative
Ansell 1992	0.3	0.9% saline	50	15	1.2	Intra-operative
Bignami 2016	0.3	0.9% saline	50	20	0.9	Intra- or post-operative if bleeding
Brown 1989	0.3	0.9% saline	.	10	.	Intra-operative
Casas 1995	0.3	0.9% saline	50	20-30	0.6-0.9	Intra-operative
Chuang 1993	0.3	.	.	.	.	Intra-operative
de Prost 1992	0.3	0.9% saline	50	30	0.6	Post-operative
Despotis 1999	0.4	0.9% saline	50	30	0.8	Intra-operative
Dilthey 1993	0.3	0.9% saline	.	15	1.2	Intra-operative
Frankville 1991	0.3	0.9% saline	50	15	1.2	Intra-operative
Gratz 1992	0.3	0.9% saline	50	30	0.6	Intra-operative
Hackmann 1989	0.3	0.9% saline	25	15	1.2	Intra-operative
Hajjar 2007	0.3	0.9% saline	50	15	1.2	Intra-operative
Hedderich 1990	0.3	0.9% saline	100	15	1.2	Intra-operative
Hemşinli 2012a	0.3	.	.	20	0.9	Post-operative
Hemşinli 2012b	0.3	.	.	20	0.9	Post-operative
Horrow 1991a	0.3	.	.	15	1.2	Intra-operative
Horrow 1991b	0.3	.	.	15	1.2	Intra-operative
Jin 2015	0.3	0.9% saline	50	10	2.4	Intra-operative
Kuitunen 1992	0.3	0.9% saline	100	15	1.2	Intra-operative
Marquez 1992	0.3	.	.	.	.	Intra-operative
Mongan 1992a	0.3	0.9% saline	50	15	0.9	Intra-operative
Mongan 1992b	0.3	0.9% saline	50	20	0.9	Intra-operative
Oliver 2000	0.3	0.9% saline	50[4]	20-30	0.6-0.9	Intra-operative
Ozkisacik 2001	0.3	0.9% saline	50	20	0.9	Intra-operative

Trial	Dose (µg/kg)	Diluent	Volume (ml)	Infusion time (min)	Infusion rate (µg/kg/hr)	Timing of administration
Pleym 2004	0.3	0.9% saline	20	10	2.4	Intra-operative
Reich 1991	0.3	0.9% saline	.	10	2.4	Intra-operative
Reynolds 1993	0.3	0.9% saline	50	15	1.2	Intra-operative
Rocha 1988	0.3	0.9% saline	50	15	1.2	Intra-operative
Rocha 1994	0.3	0.9% saline	50	20	0.9	Intra-operative
Salmenpera 1991[1]	0.3	0.9% saline	100	15	1.2	Intra-operative
Salzman 1986	0.3	0.9% saline	50	15	1.2	Intra-operative
Seear 1989	0.3	0.9% saline	.	15	1.2	Intra-operative
Sheridan 1994	10 µg/m^2	0.9% saline	20	20	.	Intra-operative
Spyt 1990	0.3	0.9% saline	50	30	0.6	Intra-operative
Steinlechner 2011	0.3	0.9% saline	100	30	0.6	Pre-operative
Temeck 1994	0.3	0.9% saline	50	15	1.2	Intra-operative
Orthopaedic Surgery						
Alanay 1999	0.3	0.9% saline	100	20	0.9	Pre-operative
Ellis 2001	0.3	0.9% saline	.	30	0.6	Pre-operative
Flordal 1991	0.3 (2 doses)	0.9% saline	50	15-20	0.9-1.2	Pre-operative
Flordal 1992	0.3 (2 doses)	0.9% saline	50	15-20	0.9-1.2	Pre-operative
Guay 1992	10 µg/m^2	0.9% saline	100	20	.	Pre-operative
Karnezis 1994a	0.3	0.9% saline	50	20	0.9	Intra-operative
Karnezis 1994b	0.3	0.9% saline	50	20	0.9	Intra-operative
Kobrinsky 1987	10 µg/m^2	.	.	20	.	Pre-operative
Leino 2010	0.4	0.9% saline	50	30	0.8	Pre-operative
Letts 1998	10 µg/m^2	.	.	.	.	Pre-operative
Schott 1995	0.3 (2 doses)	0.9% saline	50	15	1.2	Pre-operative
Theroux 1997	0.3	0.9% saline	100	15-20	0.9-1.2	Pre-operative
Vascular Surgery						

Trial	Dose (µg/kg)	Diluent	Volume (ml)	Infusion time (min)	Infusion rate (µg/kg/hr)	Timing of administration
Clagett 1995	20 µg	0.9% saline	50	15	.	Intra-operative
Lethagen 1991	0.3	0.9% saline	10	10	2.4	Pre-operative
Hepatic Surgery						
Wong 2003	0.3	0.9% saline	50	20	0.9	Pre-operative
Plastic Surgery						
Wingate 1992a	0.3	0.9% saline	50	20	0.9	Pre-operative
Wingate 1992b	0.3	0.9% saline	50	20	0.9	Pre-operative
Maxillofacial surgery						
Guyuron 1996	20 µg	0.9% saline	50	15	.	Pre-operative
Sinus surgery						
Shao 2015	0.3	0.9% saline	100	20	0.9	Pre-operative
Interventional procedures						
Lee 2010	0.3	0.9% saline	50	30	0.6	Pre-operative
Manno 2011[2]	0.3	.	.	.	.	Pre-operative
Other						
Lazarchick 1995	0.3	0.9% saline	10	.	.	Pre-operative
Marczinski 2007[3]	15-45 µg	.	.	.	.	.

Table 4.2 Summary of desmopressin dose and administration in included trials. 0.9% saline: 0.9% sodium chloride. All desmopressin administered intravenously unless otherwise stated. [1]Desmopressin administered through a pulmonary artery catheter. [2]Subcutaneous desmopressin. [3]Route of desmopressin administration not reported. [4]10 ml if weight less than 10 kg.

4.3.3 Risk of bias

Most trials were at unclear risk of bias because methodological reporting was not adequate to make an accurate assessment of risk. Assessments for individual domains are summarized in Figure 4.2.

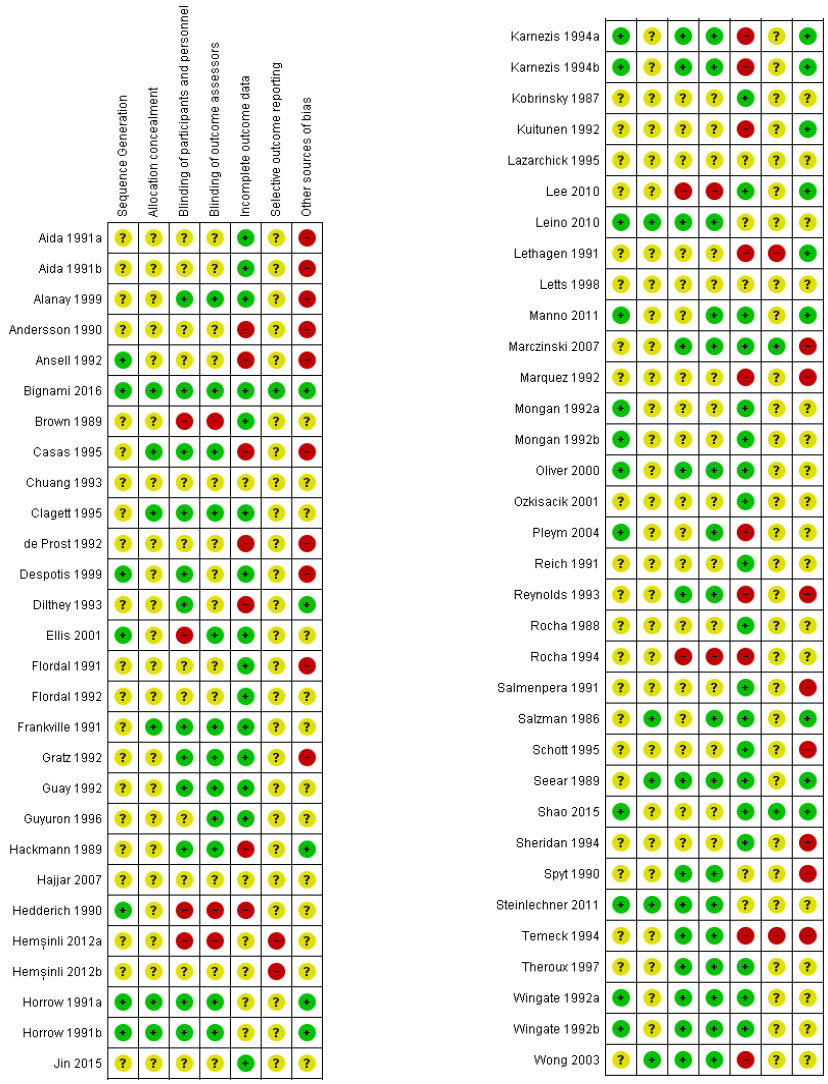


Figure 4.2. Risk of bias summary. High risk of bias for a category is indicated by −, unclear risk of bias by ? and low risk of bias by +.

4.3.3.1 Sequence generation

- Nineteen trials were at low risk of bias for sequence generation because they used either:
  - A table of random numbers or (310-313, 316, 317, 344, 347, 349); or
  - Computer generated random numbers (314, 315, 320, 321, 327, 329, 337, 351, 360, 362).

- The remaining 43 trials were at unclear risk of bias because they did not report details of the randomisation sequence.

4.3.3.2 Concealment of treatment allocation

- Ten trials were at low risk for concealment of treatment allocation all of which used sealed envelopes (310, 311, 321, 325, 332, 344, 357, 359, 362, 366).
- The remaining 42 trials were at unclear risk of bias because they did not report details of treatment allocation.

4.3.3.3 Blinding (performance bias and detection bias)

- Twenty four trials were at low risk of bias with adequate blinding of participants, personnel and analysts because:
    - They used identical vials of desmopressin and placebo (332, 363-365);
    - A pharmacist prepared the desmopressin or matching placebo who was not otherwise involved in the trial (298, 310-313, 316, 317, 323, 325, 333, 344, 349, 353, 359); or
    - A member of the study team not involved in determining outcomes prepared the desmopressin or matching placebo while all other members of the study team and outcome assessors were blinded (318, 321, 334, 336, 362, 366).
- Two trials were at low risk of bias for blinding of participants and personnel but at unclear risk for blinding analysts (327, 328).
- Four trials unclear risk with participants and personnel and low risk with analysts (335, 347, 351, 357)
- One trial was at high risk for blinding of participants and personnel but at low risk of bias for blinding analysts (329).

- Four trials were at high risk of bias for blinding of participants, personnel and study analysts because they were open label (308, 337, 343, 354).
- The remaining 27 trials did not report sufficient information to determine risk of bias from blinding.

4.3.3.4 Incomplete outcome data (attrition bias)

- Thirty-five trials were considered at low risk of bias for incomplete outcome data because participants were analysed on an intention to treat basis (297-299, 314-318, 320-322, 325, 327, 329-335, 339, 340, 343, 347, 349, 350, 352, 355-361, 365).
- Sixteen trials were considered to be at high risk of bias because of:
    - Exclusion of participants with severe bleeding or complications post-randomisation (323, 326, 328, 336, 337, 341, 345, 348, 351, 353, 366); or
    - High or unbalanced drop-out rates (312, 313, 319, 354, 364).
- The remaining 11 trials were considered to be at unclear risk of bias as insufficient information was reported to make a judgement about this outcome.

4.3.3.5 Selective reporting (reporting bias)

- Three trials were considered to be at low risk of bias because they were prospectively registered and reported all pre-specified outcomes (298, 321, 360).
- Four trials were considered to be at high risk of bias because of not reporting a pre-specified outcome (308, 309, 345, 364).
- The remaining 55 trials were considered to be at unclear risk of bias because no protocol or prospective trial registration was published.

4.3.3.6 Other potential sources of bias

- Fourteen trials were considered at low risk of other bias because of detailed methodological reporting (310-313, 321, 336, 341, 343, 345, 347, 357, 359, 360).
- Eighteen trials were considered to be at high risk of bias because they:
    - Were funded by a drug manufacturer or other party with a commercial interest in the outcome of the trial (319, 320, 323, 326, 327, 331, 333, 353, 358, 361, 363);
    - Had an imbalance in baseline characteristics (297, 299);
    - Had an imbalance in co-interventions (364);
    - Included re-randomised participants (318);
    - The study discontinued prematurely without appropriate rationale (298, 348); or
    - Were a subgroup of a larger trial that was not reported (356).
- The remaining 30 trials were considered at unclear risk of other bias.

4.3.3.7 Publication bias

4.3.3.7.1 Total volume of allogeneic red cells transfused

Trials were spread equally around the regression line and did not favour one treatment: Egger's test bias co-efficient 0.93 (P = 0.38). This result was indicative, but not conclusive, of a lack of publication bias for this outcome.

4.3.3.7.2 Risk of red cell transfusion

Trials were spread equally around the regression line and did not favour one treatment: modified Harbord test bias co-efficient -0.26 (P = 0.67). This result is indicative, but not conclusive, of a lack of publication bias for this outcome.

4.3.3.7.3 Total blood loss

Trials were spread unequally around the regression line with bias in favour desmopressin: Egger's test: bias co-efficient 1.49 (P = 0.01). Consequently, I consider this result to demonstrate publication bias for this outcome. Egger's test is known to be sensitive to small study effects but for this outcome, the small p-value suggests publication bias (Figure 4.3).

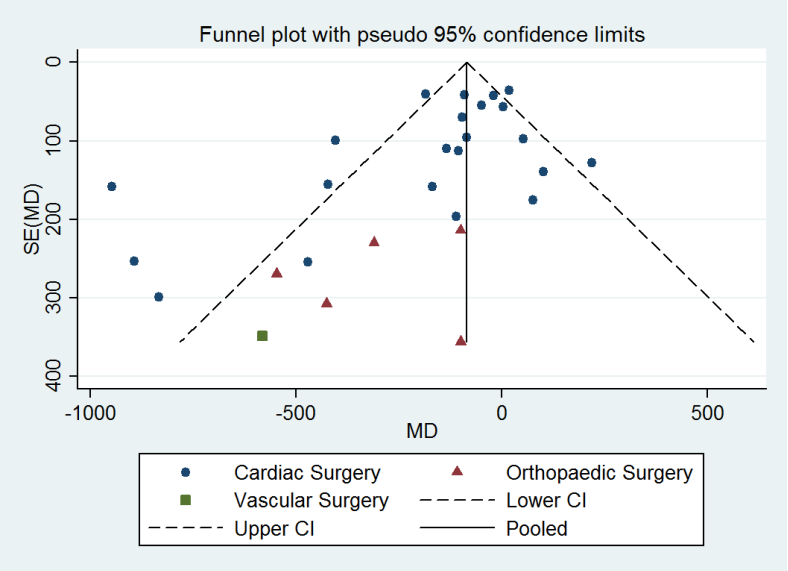


Figure 4.3 Funnel plot of risk of bias for total blood loss. CI: confidence interval. MD: mean difference, SE: standard error.

4.3.4 Effects on outcomes

4.3.4.1 Total volume of allogeneic red cells transfused

Thirty-nine trials (2324 participants) reported the total volume of allogeneic blood transfused (297, 299, 312-315, 318, 320-322, 324-330, 332, 333, 335-338, 340, 341, 344, 345, 348, 350, 352-355, 357, 358, 362, 363, 365, 366). There was considerable variation in volume of transfusion between types of surgery, so this outcome is reported in subgroups only (Figure 4.4). Despite variation in baseline characteristics, there was no evidence of a statistically significant difference between subgroups (Chi^2 = 2.97, df = 3 (P = 0.40), I^2 = 0%).

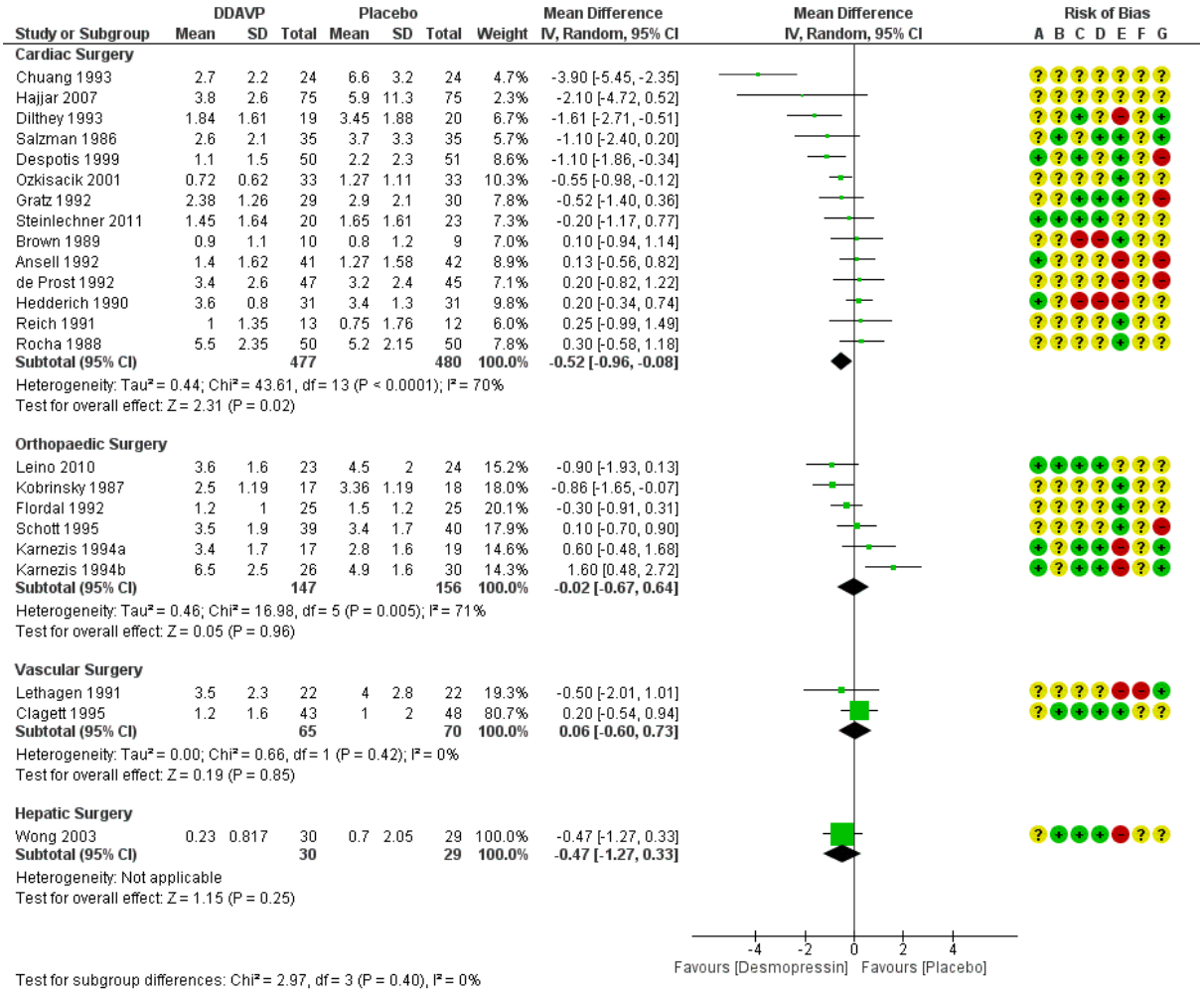


Figure 4.4 Forest plot of volume of red cells transfused. Size of squares for risk ratio reflects weight of trial in pooled analysis. CI: confidence interval, IV: inverse variance. Horizontal bars represent 95% confidence intervals. Risk of bias categories were: (A) Sequence generation, (B) Allocation concealment, (C) Blinding of participants and personnel, (D) Blinding of outcome assessors, (E) Incomplete outcome data, (F) Selective outcome reporting, and (G) Other sources of bias. High risk of bias for a category is indicated by , unclear risk of bias by and low risk of bias by .

4.3.4.1.1 Cardiac Surgery

Twenty six trials (1674 participants) reported total volume of allogeneic red cells transfused for adult cardiac surgery. Thirteen trials did not report this outcome in a way that could be incorporated into meta-analysis and are reported narratively in Appendix 2. The total volume of allogeneic red cells transfused was lower for those treated with

desmopressin compared to placebo (MD -0.52 units, 95% CI -0.96 to -0.08 units, I^2 = 70%, 14 trials, 957 participants) (Figure 4.4). The quality of evidence was judged to be low (Table 4.3).

4.3.4.1.2 Orthopaedic surgery

Eight trials (344 participants) reported total volume of allogeneic red cells transfused for orthopaedic surgery but two trials (41 participants) did not report this outcome in a way that could be incorporated into meta-analysis and are reported narratively in Appendix 2. There was no difference in the total volume of allogeneic red cells transfused for those treated with desmopressin compared to placebo (MD -0.02 units, 95% CI -0.67 to 0.64 units, I^2 = 71%, six trials, 303 participants) (Figure 4.4). The quality of evidence was judged to be very low (Table 4.3).

4.3.4.1.3 Vascular surgery

Due to the small number of participants, it is uncertain if there is a difference in the total volume of allogeneic red cells transfused for participants treated with desmopressin compared to placebo (MD 0.06 units, 95% CI -0.60 to 0.73 units, two trials, 135 participants; Figure 4.4). The quality of evidence was judged to be very low (Table 4.3).

4.3.4.1.4 Hepatic surgery

Due to the small number of participants, it is uncertain if there is a difference in total volume of allogeneic red cells transfused for participants treated with desmopressin compared to placebo (MD -0.47 units, 95% CI -1.27 to 0.33 units, one trial, 59 participants) (Figure 4.4). The quality of evidence was judged to be very low (Table 4.3).

	Number of participants		Effect		
№ of RCTs	**Desmopressin**	**Placebo**	**Relative**	**Absolute**	**GRADE**
Total volume of red cells transfused[1]					
Cardiac 14	477	480	.	**MD 0.52 units less** (0.96 less to 0.08 less)	⊕⊕⊖⊖ LOW [2,3]
Orthopaedic 6	147	156	.	**MD 0.02 units less** (0.67 less to 0.64 more)	⊕⊖⊖⊖ VERY LOW [2,3,4]
Vascular 2	65	70	.	**MD 0.02 units less** (0.67 less to 0.64 more)	⊕⊖⊖⊖ VERY LOW [2,3,4]
Hepatic 1	30	29	.	**MD 0.47 units less** (1.27 less to 0.33 more)	⊕⊖⊖⊖ VERY LOW [2,3,4]
Risk of red cell transfusion					
25	371/895 (41.5%)	393/911 (43.1%)	**RR 0.96** (0.86 to 1.06)	**2 fewer per 100** (from 6 fewer to 3 more)	⊕⊕⊕⊖ MODERATE[2]
Total blood loss[1]					
Cardiac 22	673	685	.	**MD 135 mL less** (211 less to 60 less)	⊕⊖⊖⊖ VERY LOW [2,3,5]
Orthopaedic 5	119	122	.	**MD 286 mL less** (515 less to 57 less)	⊕⊖⊖⊖ VERY LOW [2,3,5]
Vascular 1	22	22	.	**MD 582 mL less** (1264 less to 100 more)	⊕⊖⊖⊖ VERY LOW [2,3,5]
Re-operation due to bleeding					
23	27/886 (3.0%)	40/897 (4.5%)	**pOR 0.66** (0.40 to 1.09)	**2 fewer per 100** (from 3 fewer to 0 more)	⊕⊕◯◯ LOW [2,4]
Risk of bleeding for interventional procedures					
2	13/104 (13%)	32/106 (30%)	**RR 0.42** (0.23 to 0.75)	**18 fewer per 100** (from 23 fewer to 8 fewer)	⊕⊕◯◯ LOW [2,4]
All-cause mortality					
22	14/799 (1.8%)	13/832 (1.6%)	**pOR 1.09** (0.51 to 2.34)	**0 fewer per 100** (from 1 fewer to 2 more)	⊕⊕⊖⊖ LOW [2,4]
Thrombotic events					
29	44/978 (4.5%)	34/1006 (3.4%)	**pOR 1.36** (0.85 to 2.16)	**1 more per 100** (from 0 fewer to 4 more)	⊕⊕⊖⊖ LOW [2,4]
Clinically significant hypotension					
18	46/590 (7.8%)	16/593 (2.7%)	**RR 2.32** (1.37 to 3.91)	**4 more per 100** (from 1 more to 8 more)	⊕⊕◯◯ LOW[2,3]

Table 4.3 Summary of findings. CI: confidence interval, GRADE: Grading of Recommendations, Assessment, Development, and Evaluation guidelines, MD: mean difference, pOR: Peto odds

ratio, RCT: randomised controlled trial, RR: risk ratio. [1]Data not pooled due to significant differences in blood loss between types of surgery, [2]Downgraded one level due to risk of bias: inadequate reporting of blinding and incomplete outcome data, [3]Downgraged one level for inconsistency, [4]Downgraded one level due to imprecision, as confidence intervals include both clinically significant benefit and clinically significant harm, [5]Downgraded one level for suspected publication bias.

4.3.4.1.5 Maxillofacial surgery

One trial (20 participants) reported total volume of red cells transfused in maxillofacial surgery but did not reported this outcome in a way that could be incorporated into meta-analysis and is reported narratively in Appendix 2.

4.3.4.2 Risk of receiving an allogeneic red cell transfusion

Twenty-six trials (1866 participants) reported the total number of participants who were transfused with allogeneic blood (310, 311, 314-317, 320, 321, 323, 325, 328, 329, 332, 333, 335, 336, 339, 347-351, 361, 363, 364, 366). One trial was excluded from the final analysis because every participant in both arms of the trial received a red cell transfusion (333). I found no evidence for a difference in the total number of participants who were transfused with allogeneic blood for those treated with desmopressin compared to placebo (RR 0.96, 95% CI 0.86 to 1.06, I^2 = 17%, 25 trials, 1806 participants) (Figure 4.5). There was no evidence of a difference between subgroups (Chi^2 = 3.02, df = 2 (P = 0.22), I^2 = 33.7%). The quality of evidence was judged to be moderate (Table 4.3).

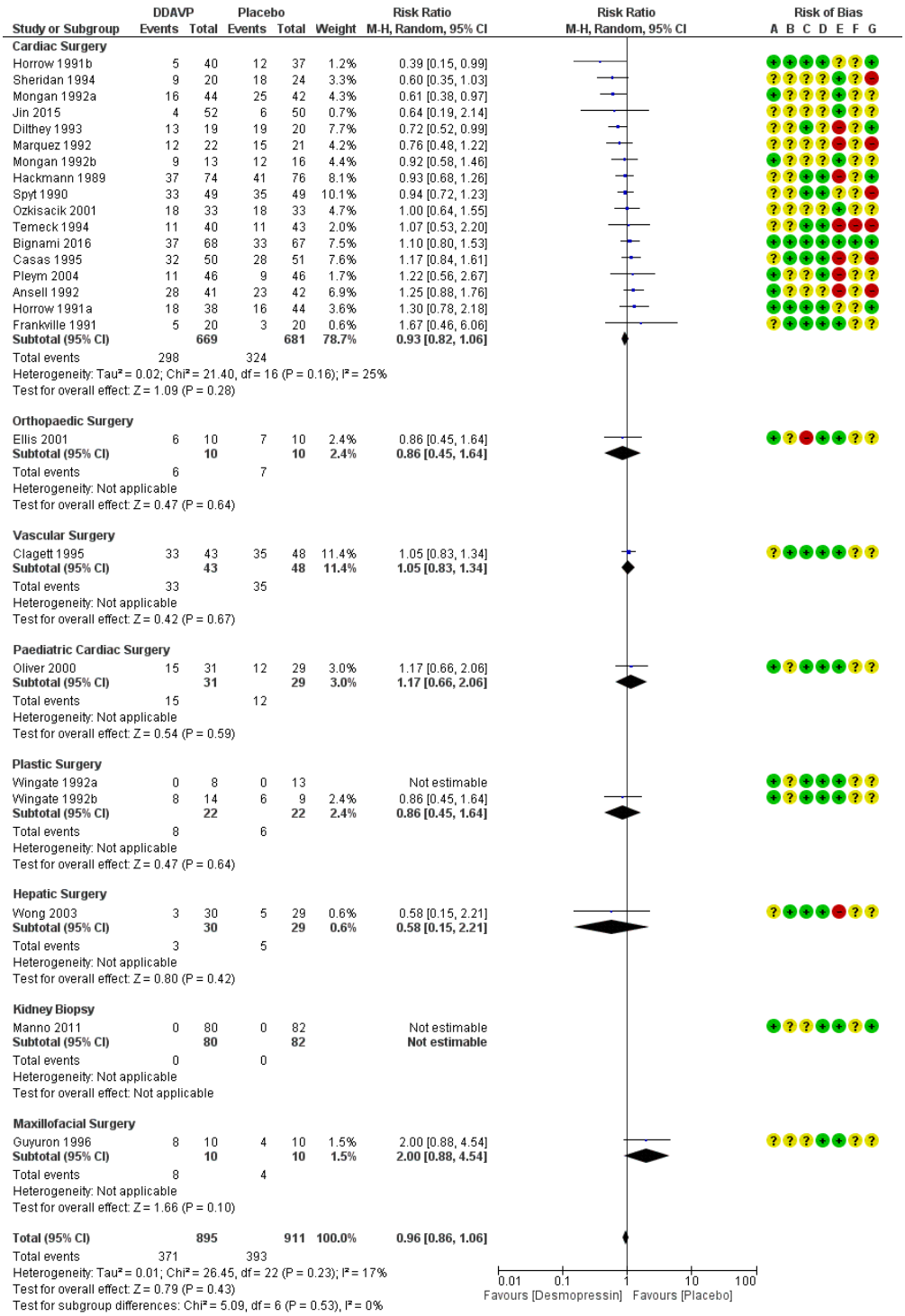


Figure 4.5 Forest plot of risk of red cell transfusion. Size of squares for risk ratio reflects weight of trial in pooled analysis. CI: confidence interval, M-H: Mantel-Haenszel. Horizontal bars represent 95% confidence intervals. Risk of bias categories were: (A) Sequence generation, (B) Allocation concealment, (C) Blinding of participants and personnel, (D) Blinding of outcome assessors, (E) Incomplete outcome data, (F) Selective outcome reporting, and (G) Other sources of bias. High risk of bias for a category is indicated by −, unclear risk of bias by ? and low risk of bias by +.

4.3.4.3 Total blood loss

Forty-eight trials (2808 participants) reported total blood loss (297, 299, 308-311, 314, 315, 318-324, 326-328, 330-341, 344, 345, 348, 350-359, 361-365). Clinical heterogeneity due to differences in blood loss between different operations meant that a pooled estimate for the whole population was not possible and analyses were performed in subgroups according to type of surgery (Figure 4.6). Despite variation in baseline characteristics, there was no evidence of a statistically significant difference between subgroups (Chi^2 = 3.02, df = 2 (P = 0.22), I^2 = 33.7%).

4.3.4.3.1 Cardiac surgery

Thirty-seven trials (2354 participants) reported total blood loss for adult cardiac surgery, but 15 trials (996 participants) did not report this outcome in a way that could be incorporated into meta-analysis and are reported narratively in Appendix 2. Total blood loss for participants undergoing cardiac surgery was lower for participants treated with desmopressin compared to placebo (MD -135.24 mL, 95% CI -210.8 to -59.68 mL, I^2 = 78%, 22 trials, 1358 participants; Figure 4.6). Sensitivity analyses could not account for the level of heterogeneity observed. Heterogeneity was not improved with sensitivity analysis. The quality of evidence was judged to be very low (Table 4.3).

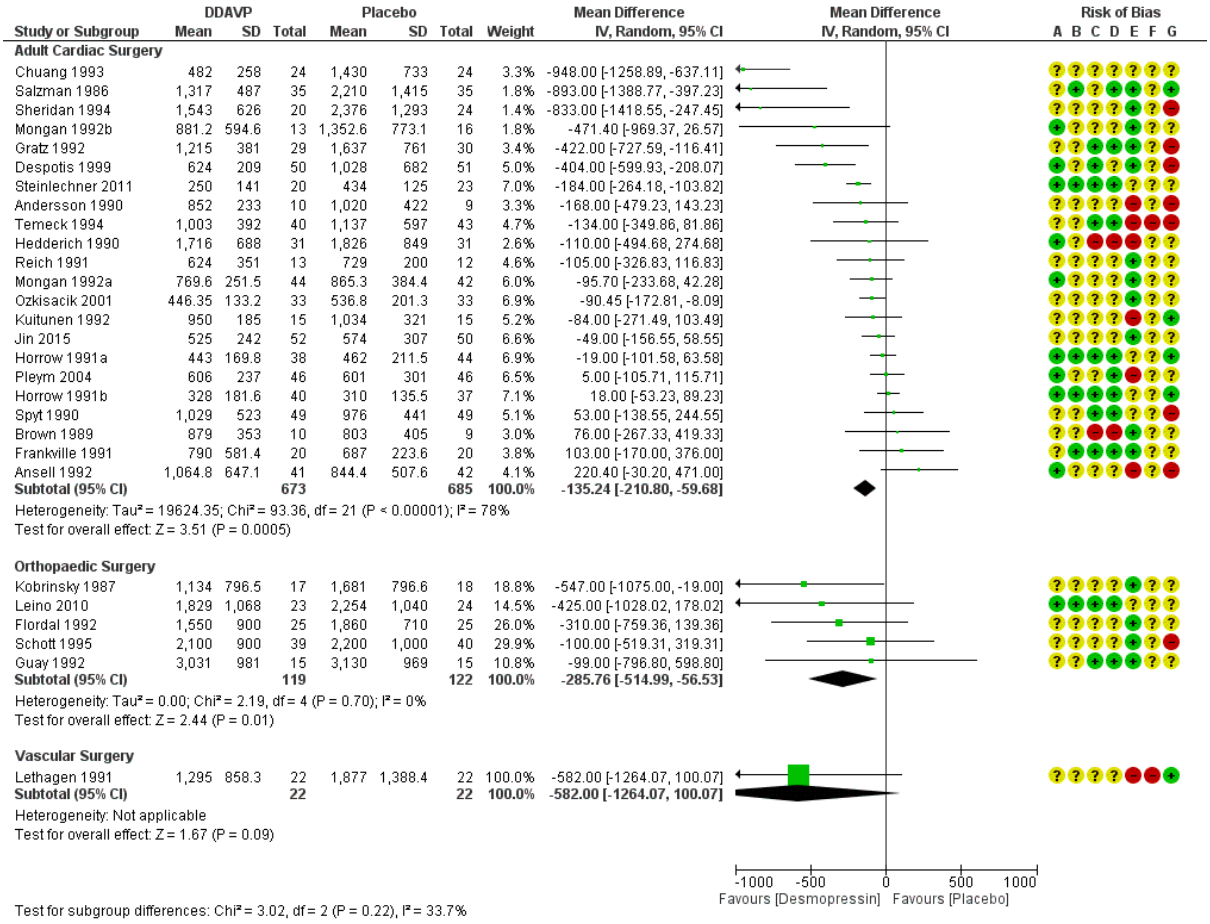


Figure 4.6 Forest plot of total blood loss. Size of squares for risk ratio reflects weight of trial in pooled analysis. CI: confidence interval, IV: inverse variance. Horizontal bars represent 95% confidence intervals. Risk of bias categories were: (A) Sequence generation, (B) Allocation concealment, (C) Blinding of participants and personnel, (D) Blinding of outcome assessors, (E) Incomplete outcome data, (F) Selective outcome reporting, and (G) Other sources of bias. High risk of bias for a category is indicated by −, unclear risk of bias by ? and low risk of bias by +.

4.3.4.3.2 Orthopaedic surgery

Seven trials (274 participants) reported total blood loss for orthopaedic, but two trials (33 participants) did not report this outcome in a way that could be incorporated into meta-analysis and are reported narratively in Appendix 2. Total blood loss for participants undergoing orthopaedic surgery was lower for participants treated with desmopressin compared to placebo (MD -285.76 mL, 95% CI -514.99 mL to -56.53 mL, I^2 = 0%, five trials, 241 participants; Figure 4.6). The quality of evidence was judged to

be very low (Table 4.3).

4.3.4.3.3 Vascular surgery

Total blood loss for participants undergoing vascular surgery was lower for participants treated with desmopressin compared to placebo (MD -582.00 mL, 95% CI -1264.07 to 100.07 mL, one study, 44 participants; Figure 4.6). The quality of evidence was judged to be very low (Table 4.3).

4.3.4.4 Re-operation due to bleeding

Twenty-three trials (1783 participants) reported the number of participants undergoing re-operation due to bleeding and all 23 contributed data towards the final pooled estimate (310, 311, 314, 315, 320-323, 326, 327, 332, 334, 336, 337, 343, 347, 349-351, 354, 355, 357, 362). There was no difference in the number of participants who required re-operation due to bleeding in those treated with desmopressin compared to placebo (pOR 0.66, 95% CI 0.40 to 1.09, I^2 = 33%, 23 trials, 1783 participants; Figure 4.7). There was no evidence of a difference between subgroups (Chi^2 = 1.40, df = 1 (P = 0.24), I^2 = 28.6%). The quality of evidence was judged to be low (Table 4.3).

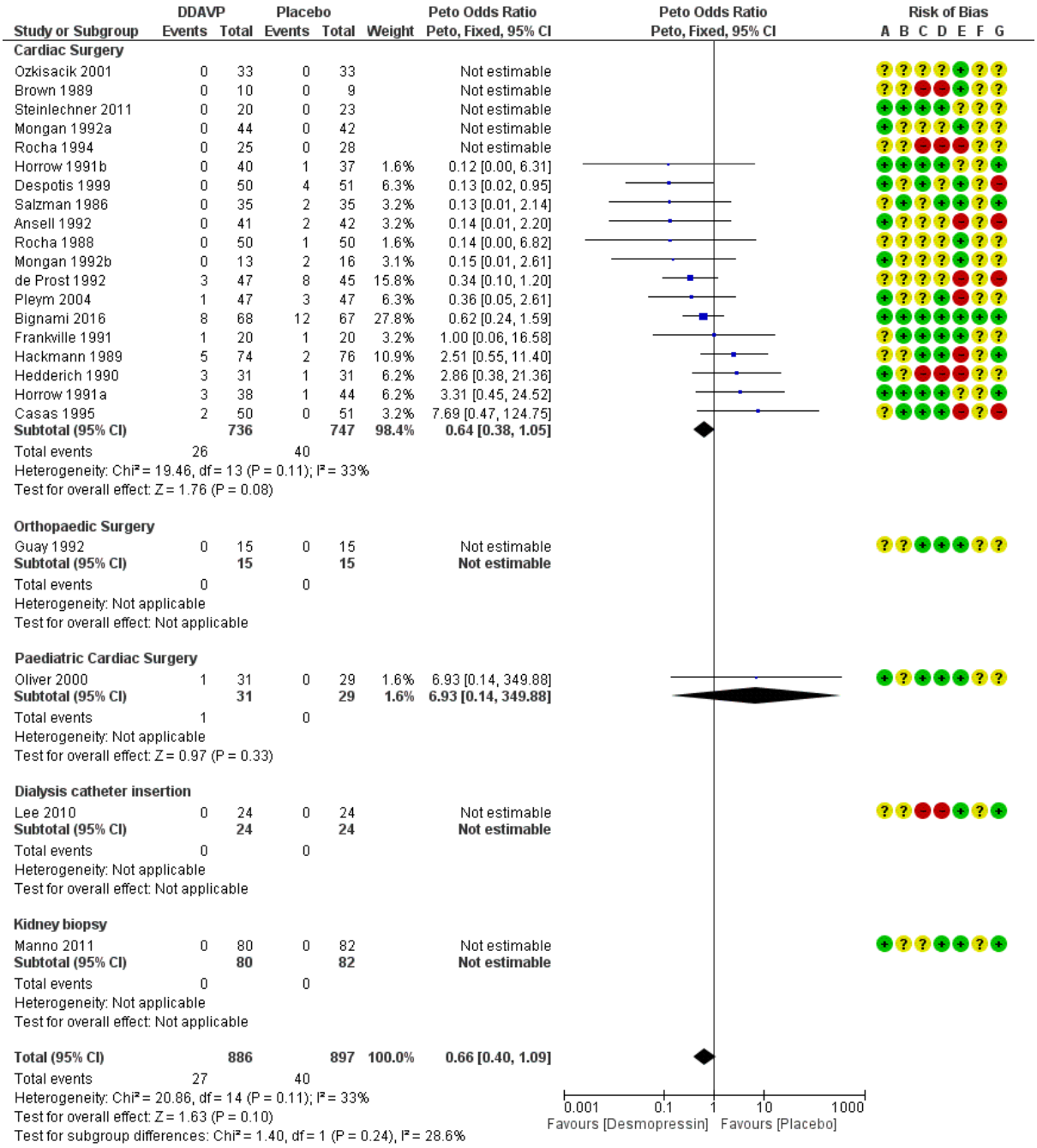


Figure 4.7 Forest plot of reoperation due to bleeding. Size of squares for risk ratio reflects weight of trial in pooled analysis. CI: confidence interval. Horizontal bars represent 95% confidence intervals. Risk of bias categories were: (A) Sequence generation, (B) Allocation concealment, (C) Blinding of participants and personnel, (D) Blinding of outcome assessors, (E) Incomplete outcome data, (F) Selective outcome reporting, and (G) Other sources of bias. High risk of bias for a category is indicated by −, unclear risk of bias by ? and low risk of bias by +.

4.3.4.5 Risk of bleeding for interventional procedures

Two trials (210 participants) report risk of bleeding for interventional procedures and all both contributed data towards the final pooled estimate (343, 347). The risk of bleeding was lower for participants treated with desmopressin than placebo who were undergoing interventional procedures (RR 0.42, 95% CI 0.23 to 0.75, I^2 = 0%, two trials, 210 participants; Figure 4.8). The quality of evidence was judged to be low (Table 4.3).

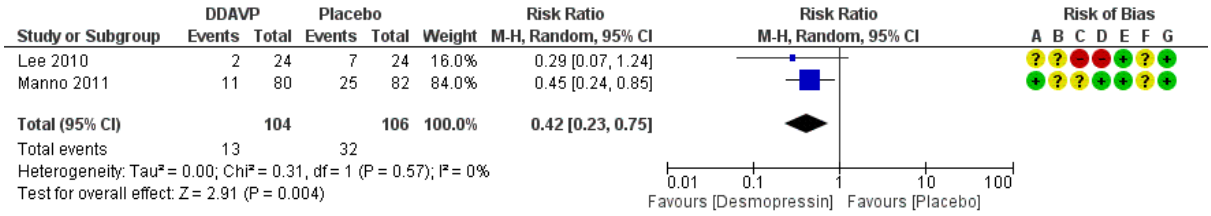


Figure 4.8 Forest plot of risk of bleeding for interventional procedures. Size of squares for risk ratio reflects weight of trial in pooled analysis. CI: confidence interval, M-H: Mantel Haenszel. Horizontal bars represent 95% confidence intervals. Risk of bias categories were: (A) Sequence generation, (B) Allocation concealment, (C) Blinding of participants and personnel, (D) Blinding of outcome assessors, (E) Incomplete outcome data, (F) Selective outcome reporting, and (G) Other sources of bias. High risk of bias for a category is indicated by ⊖, unclear risk of bias by ? and low risk of bias by ⊕.

4.3.4.6 Adverse events

4.3.4.6.1 All-cause mortality

Twenty-two trials (1631 participants) reported all-cause mortality and all 22 contributed data towards the final pooled estimate (312-315, 320, 321, 325, 327, 333, 336, 337, 339, 341, 349, 351, 354, 355, 357-359, 361, 362). I found no evidence for a difference in all-cause mortality for participants treated with desmopressin compared to placebo (pOR 1.09, 95% CI 0.51 to 2.34, I^2 = 3%, 22 trials, 1631 participants; Figure 4.9). There was no evidence of a difference between subgroups (Chi^2 = 4.29, df = 2 (P = 0.12), I^2 = 53.4%). The quality of evidence was judged to be low (Table 4.3).

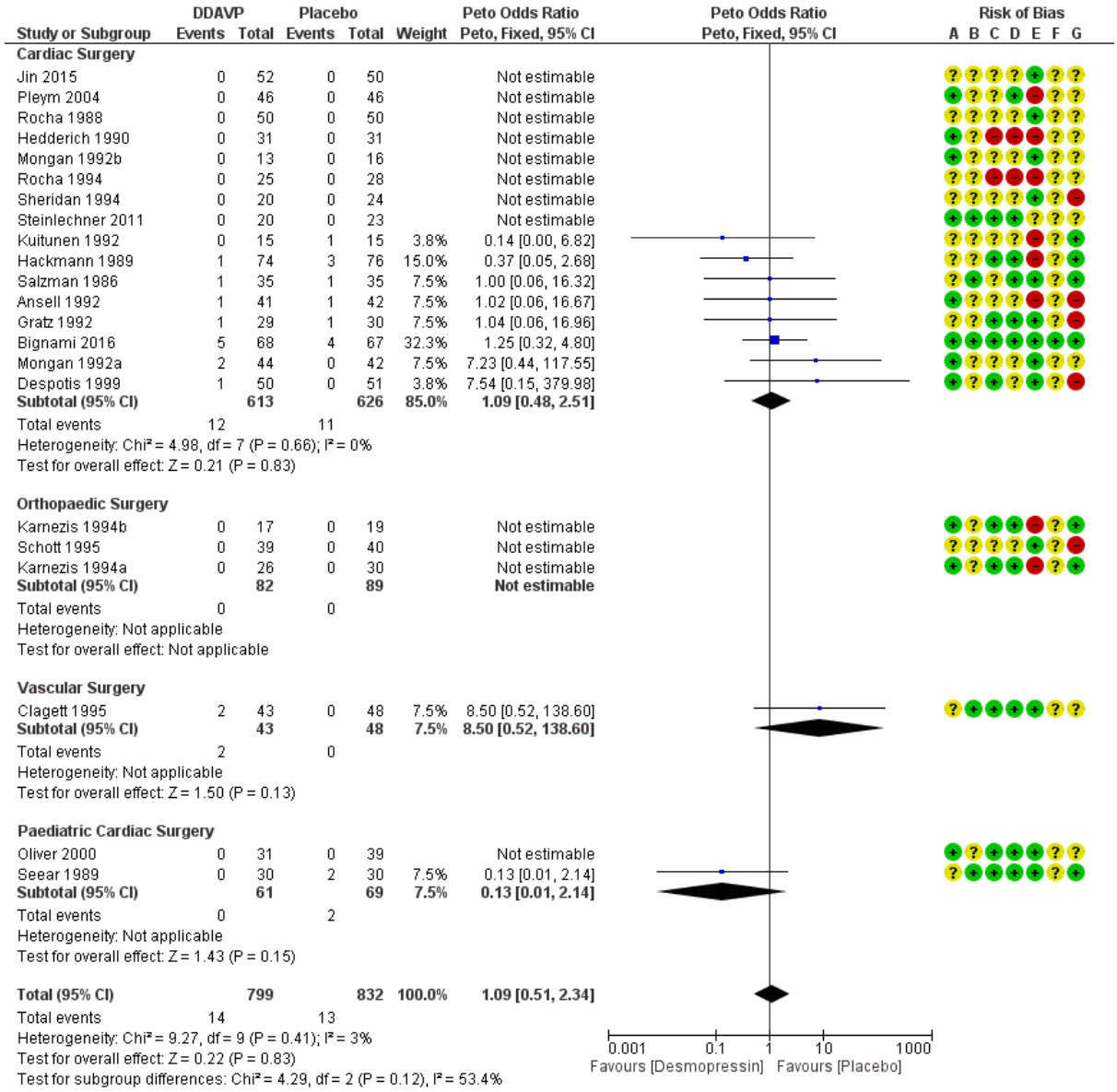


Figure 4.9 Forest plot of all-cause mortality. Size of squares for risk ratio reflects weight of trial in pooled analysis. CI: confidence interval. Horizontal bars represent 95% confidence intervals. Risk of bias categories were: (A) Sequence generation, (B) Allocation concealment, (C) Blinding of participants and personnel, (D) Blinding of outcome assessors, (E) Incomplete outcome data, (F) Selective outcome reporting, and (G) Other sources of bias. High risk of bias for a category is indicated by -, unclear risk of bias by ? and low risk of bias by +.

4.3.4.6.2 Thrombotic events

Twenty-nine trials (1984 participants) reported thrombotic events and all 29 contributed data towards the final pooled estimate (310-315, 320-323, 325, 327, 330, 331, 333, 337, 339, 344, 345, 347, 348, 351, 354, 356-358, 360-362). I found no evidence for a difference in thrombotic events for participants treated with desmopressin compared to placebo (pOR 1.36, 95% CI 0.85 to 2.16, I^2 = 0%, 29 trials, 1984 participants; Figure 4.10). There was no evidence of a difference between subgroups (Chi^2 = 1.60, df = 2 (P = 0.45), I^2 = 0%). The event rate was low for individual causes of thrombotic events with no overall difference in the incidence in any type of thrombotic event: myocardial infarction (pOR 1.32, 95% CI 0.70 to 2.46, I^2 = 0%, 26 trials, 1704 participants); ischemic stroke (pOR 2.95, 95% CI 0.94 to 9.24, I^2 = 0%, 19 trials, 1277 participants); or venous thromboembolism (pOR 0.77, 95% CI 0.17 to 3.38, I^2 = 0%, 20 trials, 1377 participants). The quality of evidence was judged to be low (Table 4.3).

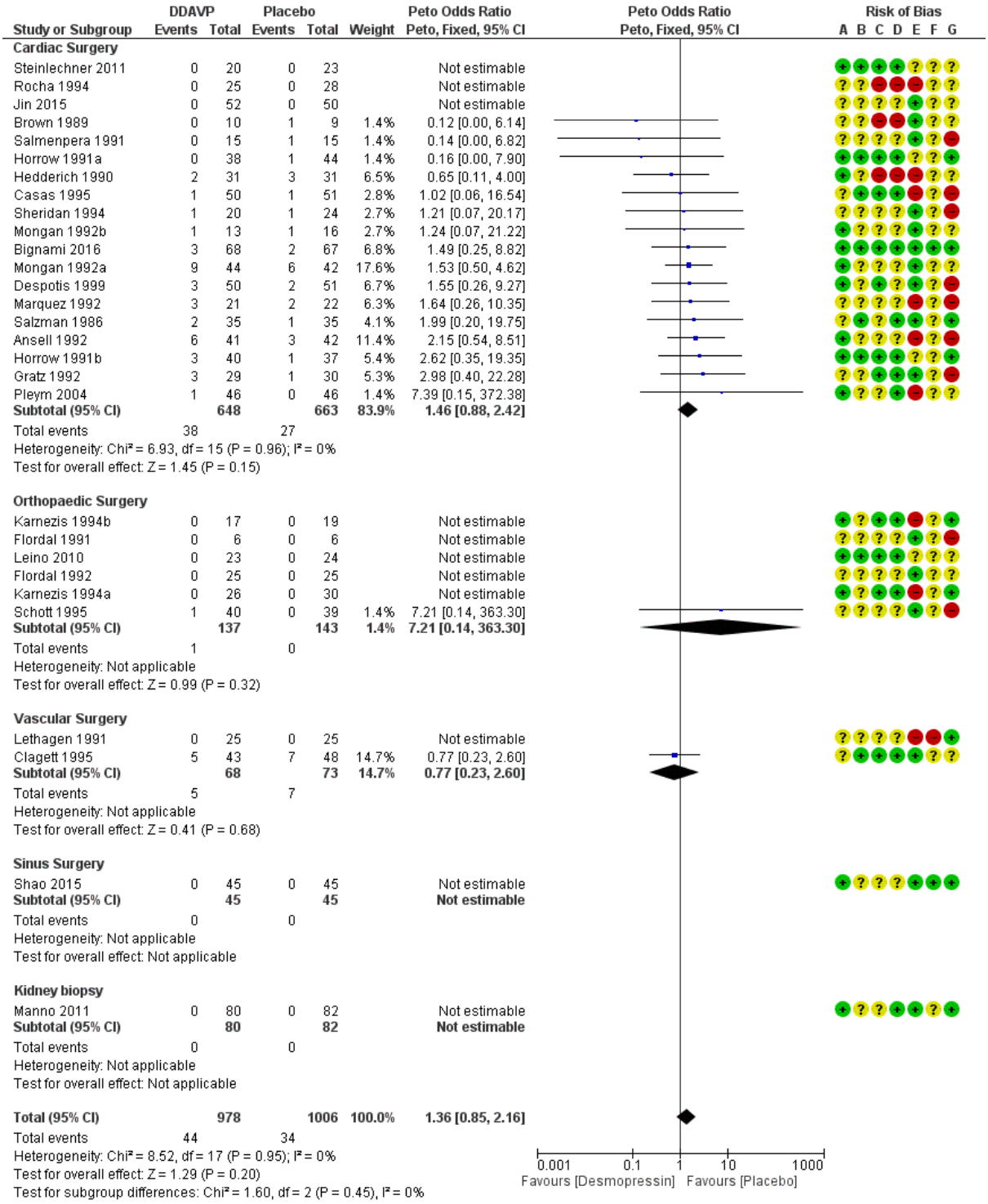


Figure 4.10 Forest plot of thrombotic events. Size of squares for risk ratio reflects weight of trial in pooled analysis. CI: confidence interval. Horizontal bars represent 95% confidence intervals. Risk of bias categories were: (A) Sequence generation, (B) Allocation concealment, (C) Blinding of participants and personnel, (D) Blinding of outcome assessors, (E) Incomplete outcome data, (F) Selective outcome reporting, and (G) Other sources of bias. High risk of bias for a category is indicated by −, unclear risk of bias by ? and low risk of bias by +.

4.3.4.6.3 Clinically significant hypotension

Eighteen trials (1183 participants) reported clinically significant hypotension and all 18 contributed data towards the final pooled estimate (314, 315, 321, 322, 327, 328, 332, 346-349, 351, 352, 354, 356-358, 360). Clinically significant hypotension was more frequent for participants treated with desmopressin compared to placebo: RR 2.32 (95% CI 1.37 to 3.91, I^2 = 0%, 18 trials, 1183 participants; Figure 4.11). There was no evidence of a difference between subgroups (Chi^2 = 0.83, df = 2 (P = 0.66), I^2 = 0%). The quality of evidence was judged to be low (Table 4.3).

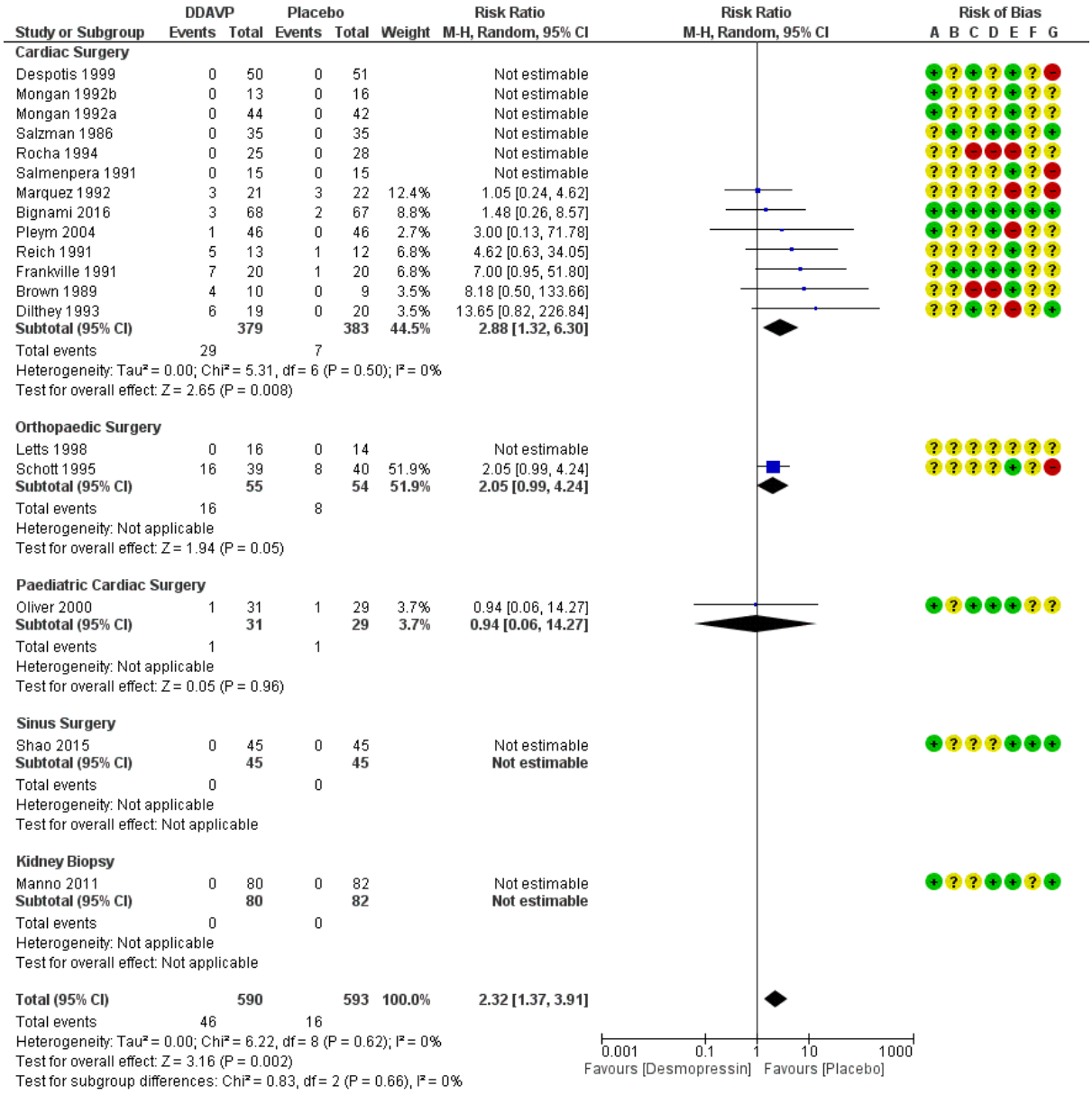


Figure 4.11 Forest plot of clinically significant hypotension. Size of squares for risk ratio reflects weight of trial in pooled analysis. CI: confidence interval, M-H: Mantel Haenszel. Horizontal bars represent 95% confidence intervals. Risk of bias categories were: (A) Sequence generation, (B) Allocation concealment, (C) Blinding of participants and personnel, (D) Blinding of outcome assessors, (E) Incomplete outcome data, (F) Selective outcome reporting, and (G) Other sources of bias. High risk of bias for a category is indicated by −, unclear risk of bias by ? and low risk of bias by +.

4.3.4.6.3.1 Hypotension and rate of desmopressin infusion

In seven trials the rate of desmopressin infusion was ≥1.2μg/kg/hour (328, 332, 351, 352, 356-358). In this subgroup, clinically significant hypotension was more frequent for participants treated with desmopressin compared to placebo: RR 2.77 (95% CI 1.49 to 5.13, I^2 = 0%, seven trials, 375 participants) (Table 4.2; Figure 4.12).

In seven trials the rate of desmopressin infusion was <1.2μg/kg/hour (314, 315, 321, 327, 349, 354, 360). In this subgroup, there was no difference in clinically significant hypotension for participants treated with desmopressin compared to placebo: RR 1.29 (95% CI 0.30 to 5.66, I^2 = 0%, seven trials, 554 participants). Despite the differences in RR between these subgroups, there was no evidence of a difference between subgroups (Chi^2 = 0.87, df = 1 (P = 0.35), I^2 = 0%).

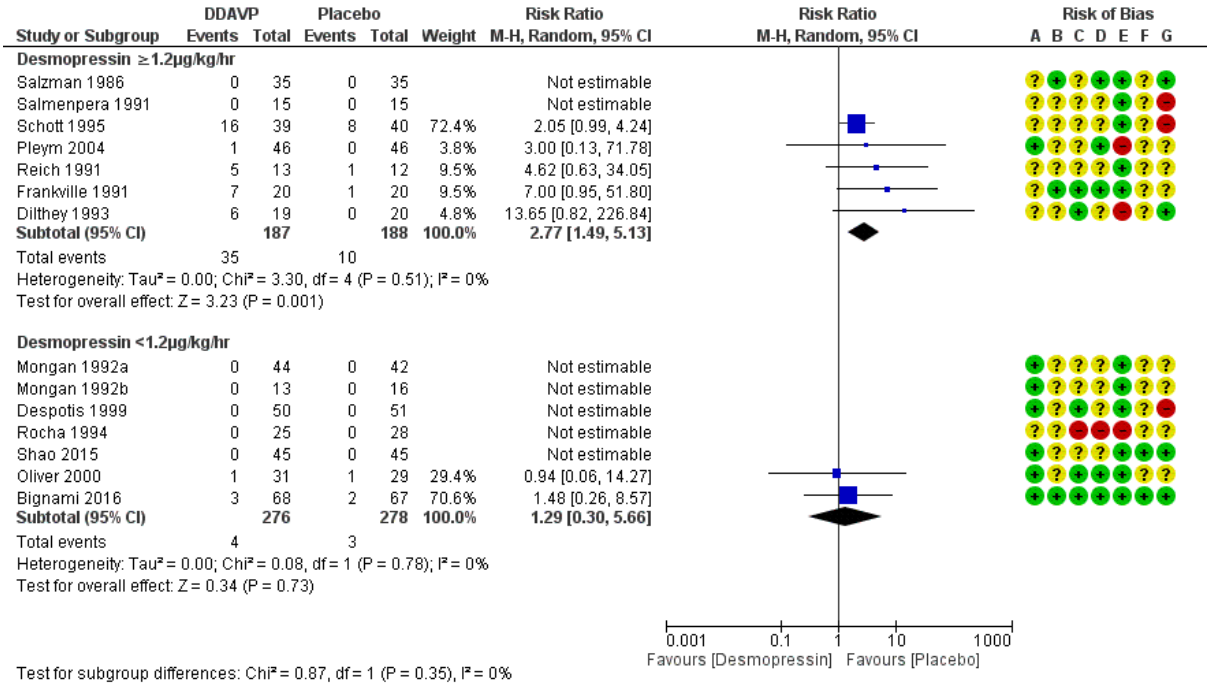


Figure 4.12 Forest plot of hypotension and rate of desmopressin infusion. Size of squares for risk ratio reflects weight of trial in pooled analysis. CI: confidence interval, M-H: Mantel Haenszel. Horizontal bars represent 95% confidence intervals. Risk of bias categories were: (A) Sequence generation, (B) Allocation concealment, (C) Blinding of participants and personnel, (D) Blinding of outcome assessors, (E) Incomplete outcome data, (F) Selective outcome reporting, and (G) Other sources of bias. High risk of bias for a category is indicated by −, unclear risk of bias by ? and low risk of bias by +.

4.3.4.6.4 Other adverse events

Other adverse events, including hyponatraemic seizures, chest tightness, hypertension, abdominal pain, headache, nausea and allergic reactions, were not reported in the included trials.

4.3.5 GRADE quality of the evidence

The overall GRADE quality of the evidence ranged from very low to moderate. All outcomes were downgraded one point for risk of bias. The majority of trials did not report sufficient detail for an assessment of bias to be made. Only one trial was considered at low risk of bias in all domains. Domains where there was a high risk of

bias include blinding of outcome assessors, incomplete outcome data and selective reporting. Heterogeneity was a problem for the continuous outcomes: total volume of blood loss and total number of red cells transfused, due to large differences in baseline blood loss between procedures. Mortality and thrombotic events were rare and consequently at risk of imprecision. Lastly many types of surgery were not represented by the trials in this review, introducing inconsistency (Table 4.3).

4.4 Discussion

4.4.1 Summary of key results

This meta-analysis identified 62 randomised trials with 3661 participants where the effects of desmopressin were assessed. The majority of trials were in the setting of cardiac surgery, where desmopressin was most commonly administered intra-operatively after completion of CPB. However of note, it was also administered before interventional procedures in two trials.

4.4.1.1 Red cell transfusion and blood loss

Due to significant differences in baseline blood loss, subgroups of different procedures were analysed separately for the continuous outcomes: total volume of allogeneic red cells transfused and total blood loss.

In cardiac surgery, desmopressin administration resulted in transfusion of fewer units of red cells (equivalent to a 15% reduction compared to control) and less blood loss (equivalent to a 13% reduction compared to control). However significant publication bias was noted for total blood loss.

In orthopaedic surgery, there was no difference between desmopressin and placebo for reducing allogeneic red cell transfusion. However desmopressin administration did result in less blood loss (equivalent to a 13% reduction compared to control).

No significant differences in allogeneic red cells transfusion or total blood loss were found for other types of surgery, although there were few trials with which to make this estimate.

No significant differences were found between desmopressin and control for the risk of receiving a red cell transfusion for all the trials when pooled, or in any of the subgroups.

For patients undergoing interventional procedures there was a significant reduction in the risk of bleeding for patients treated with desmopressin before the procedure compared to those receiving placebo (equivalent to a 58% reduction in bleeding risk compared to control). However there were few trials with which to make this estimate.

No difference was found overall, or in any of the subgroups in the risk of re-operation due to bleeding.

4.4.1.2 Adverse events

The baseline risk of serious adverse events was low with mortality of 1.5%, thrombotic events 3.4% and clinically significant hypotension 2.8% in the pooled control arms. There were no overall differences reported in the risk of all-cause mortality or thrombotic events (myocardial infarction, ischaemic stroke and venous thromboembolism) for patients treated with desmopressin. Of note, there was no significant difference in the odds of a thrombotic event in the cardiac surgery subgroup where the prevalence of cardiovascular disease is high and side effects may be expected to occur more frequently (368). This offers some reassurance about the safety of desmopressin in relatively high risk groups of patients.

Patients treated with desmopressin had an increased risk of clinically significant hypotension, which is consistent with the results of other reviews (294, 369). This

adverse event is seen commonly when desmopressin is administered rapidly (292), and the results of this meta-analysis suggest it might be less common if administered more slowly. It is unclear whether transient hypotension has more severe outcomes, although no other imbalances of risks were seen across all trials. Other adverse events such as facial flushing, hyponatraemia and seizures were not reported.

4.4.2 Limitations

The majority of trials were in the setting of cardiac surgery and orthopaedic surgery. There were few randomised controlled trials for other surgical procedures, such as interventional procedures. Few trials included children of patients undergoing emergency surgery. In many cases there was inadequate reporting of the clinical characteristics of the included participants.

The majority of trials used 0.3μg/kg intravenous desmopressin, but there was limited information on other desmopressin doses or on subcutaneous administration. Several trials did not report the rate at which desmopressin was administered, or how it was diluted, which is of importance for its safety profile.

Comparators were placebo (0.9% sodium chloride) in almost all cases. No trials manufactured a matching placebo, and relied on desmopressin being reconstituted by a person unrelated to the clinical trial to maintain blinding. The small number of open label trials that were included are open to bias; especially as many of the outcomes are relatively subjective.

Trials were at risk of bias, with a particular risk from incomplete outcomes data. Much of the assessment of risk of bias was limited by inadequate methodological reporting in the original trials.

Meta-analyses of total volume of allogeneic red cells transfused and total blood loss were limited due to inconsistency in baseline levels of red cell transfusion and blood loss. The findings of my review were also limited by data published in a format that could not be incorporated into meta-analysis, which showed inconsistent results.

4.4.3 Conclusion

Desmopressin had little effect compared to placebo in reduction of transfusion requirements or blood loss. The reduction in blood loss and total volume of allogeneic red cells transfused was too small to be of clinical significance, especially considering that tranexamic acid results in a 38% reduction in transfusion requirements and blood loss (155).

There was no overall difference in thrombotic events or mortality. A subgroup that gained significant benefit from desmopressin were patients undergoing interventional procedures where there was a significant reduction in the incidence of bleeding. However there were too few trials too draw firm conclusions. Clinically significant hypotension was significantly more common following desmopressin infusion than placebo. However this effect was most common when desmopressin was administered quickly and there was no difference in the incidence of clinically significant hypotension for those infused with desmopressin more slowly.

Compared to the most recent systematic review of desmopressin for prevention of perioperative transfusion (369), this review identified a further 23 trials with 1346 participants. I have included interventional procedures and children, neither of which were eligible for the original review. The overall findings are similar to those from the previous meta-analysis, which also found small decreases in total volume of red cells

transfused and total blood loss; with no difference found in the number of participants receiving a red cell transfusion, overall mortality or thrombotic events.

I consider there to be little value in further trials of desmopressin in unselected patients. However, there was considerable heterogeneity between the included trials and it is possible that subgroups of patients may benefit from desmopressin. In particular, as desmopressin has a short duration of action (188), patients undergoing procedures where risk of bleeding is for less than two hours may gain particular benefit. It is also possible that patients with thrombocytopenia or platelet dysfunction may gain greater benefit because of the role of VWF in platelet-adhesion to damaged endothelium (77). In addition to stimulating release of VWF, desmopressin also results on endothelial release of tPA (192), which may increase fibrinolysis. It is possible that co-administration of an anti-fibrinolytic agent alongside desmopressin may lead to a synergistic effect (370). The subgroups of patients with platelet dysfunction and who were also taking anti-fibrinolytic agents are examined in more detail in Chapter 5.

Chapter 5

5 Meta-analysis of peri-operative desmopressin for patients with platelet dysfunction or thrombocytopenia

5.1 Introduction

In this chapter, I have used meta-analysis to assess whether there is any evidence for efficacy of desmopressin for patients with platelet dysfunction, thrombocytopenia, or taking antiplatelet agents before surgery or invasive procedures. Parts of this chapter have been published in peer reviewed journals: Desborough et al. Desmopressin for treatment of platelet dysfunction and reversal of antiplatelet agents: a systematic review and meta-analysis of randomised controlled trials. *Journal of Thrombosis and Haemostasis* 2017; **15**(2): 263-72 (141); and Desborough et al. Desmopressin for minimising perioperative red cell transfusion. *Cochrane Database of Systematic Reviews* 2017; **7**: CD001884 (142).

The results of Chapter 4 suggest that desmopressin has little clinical efficacy when used to treat unselected patients. However, given the results of Chapter 3 which suggest that thrombus formation under flow is improved by increased VWF in thrombocytopenia; and previous published data suggesting that desmopressin may improve platelet function for those taking antiplatelet agents or with inherited bleeding disorders (section 1.5.7.2), these subgroups are analysed in more detail in this chapter.

Desmopressin is often used in combination with tranexamic acid (29, 201, 202). The rationale for this is that desmopressin-induces release of endothelial Weibel-Palade bodies. In addition to containing VWF, they also contain tPA, which promotes fibrinolysis (129). It is possible that co-administration of an anti-fibrinolytic agent, such as tranexamic acid, may have a synergistic effect with desmopressin (370).

The aims of this chapter were:

1. To examine the evidence for peri-operative administration of desmopressin to reduce bleeding risk and transfusion requirements for people with platelet dysfunction due to anti-platelet drug therapy or CPB.
2. To assess the effects of desmopressin when co-administered with an anti-fibrinolytic agents such as tranexamic acid

5.2 Methods

This systematic review followed the methods outlined in Chapter 4. Methodology was consistent with the definitions from the PRISMA statement (307).

5.2.1 Eligibility criteria

Only RCTs of participants undergoing any type of elective or emergency surgery were included. Trials were eligible for inclusion if their intervention was intravenous or subcutaneous desmopressin. Comparators were placebo or standard care.

Participants were adults or children taking antiplatelet agents (COX-1 inhibitors, phosphodiesterase inhibitors or $P2Y_{12}$ inhibitors); who had established platelet dysfunction as measured by recognized tests including PFA; or thrombocytopenia were included in the first part of this analysis. Trials were included if at least 75% participants had evidence of platelet dysfunction, or were taking anti-platelet agents, or if it was possible to extract data on a subgroup of participants with platelet dysfunction.

For the second analysis, participants were included if at least 75% of participants in each arm were also taking an anti-fibrinolytic agent such as tranexamic acid or epsilon aminocaproic acid.

5.2.1.1 Primary outcome:

Total volume of allogeneic red cells transfused

5.2.1.2 Secondary outcomes:

- Risk of receiving an allogeneic red cell transfusion
- Total blood loss
- Re-operation due to bleeding
- All-cause mortality
- Thrombotic events: composite of myocardial infarction, stroke, venous thromboembolism
- Clinically significant hypotension: hypotension requiring intravenous fluid administration or commencement (or an increase) of inotropes

5.2.2 Search strategy

The same search strategy was used as in section 4.2.3. However only trials including 75% or more participants with platelet dysfunction or thrombocytopenia; or greater than 75% patients taking an anti-fibrinolytic drug were included in these subgroup analyses.

5.2.3 Data extraction

The same methodology was used as reported in section 4.2.6

5.2.4 Risk of bias assessment and grading the quality of evidence

The same methodology was used as reported in section 4.2.7

5.2.5 Subgroup analysis and investigation of heterogeneity

The same methodology was used for assessment of heterogeneity as reported in section 4.2.11. Sensitivity analyses were performed by assessing results using only trials considered to be at low risk of bias from blinding of study outcome assessors. For the first analysis, trials where platelet dysfunction was due to antiplatelet agents or other causes were included in separate subgroups. All trials identified in this review were in

the same setting (cardiac surgery), so subgroups of different types of surgery were not used.

All trials for the second subgroup analysis used tranexamic acid, so different anti-fibrinolytic agents were not examined in subgroups.

Statistical heterogeneity between subgroups was examined using the I^2 statistic.

5.2.6 Unpublished data

I had access to individual patient data from one trial (321) and data on the subgroup of patients who were taking antiplatelet agents was extracted for incorporation into meta-analysis.

5.3 Results: anti-platelet agents, platelet dysfunction and thrombocytopenia

5.3.1 Search results

Full search results are reported in Figure 5.1. Eleven trials (596 participants) met the inclusion criteria for this chapter and were included in the qualitative synthesis (308, 309, 314, 321, 326-328, 333, 351, 361, 362) and 9 trials were included in the quantitative synthesis (314, 321, 326-328, 333, 351, 361, 362).

5.3.2 Trial characteristics

Although all surgical procedures regardless of specialty were potentially eligible, all 11 relevant trials were in the setting of cardiac surgery. No trials included participants with thrombocytopenia. Seven trials included participants undergoing CABG (308, 309, 314, 328, 333, 351, 361), one trial included aortic valve replacements (362) and three trials included a combination of CABG and valve replacements (321, 326, 327). Nine trials included only elective surgery (314, 321, 326-328, 333, 351, 361, 362) and two trials included only emergency cases (308, 309). Platelet dysfunction was due to antiplatelet

agents in six trials: single agent aspirin in four trials (328, 333, 351, 361); dual antiplatelet therapy in two trials (308, 309); and a combination of single agent aspirin and dual antiplatelet therapy in one trial (321). Platelet dysfunction was due to CPB in four trials (314, 326, 327, 362). Two trials were published in abstract form only (308, 309).

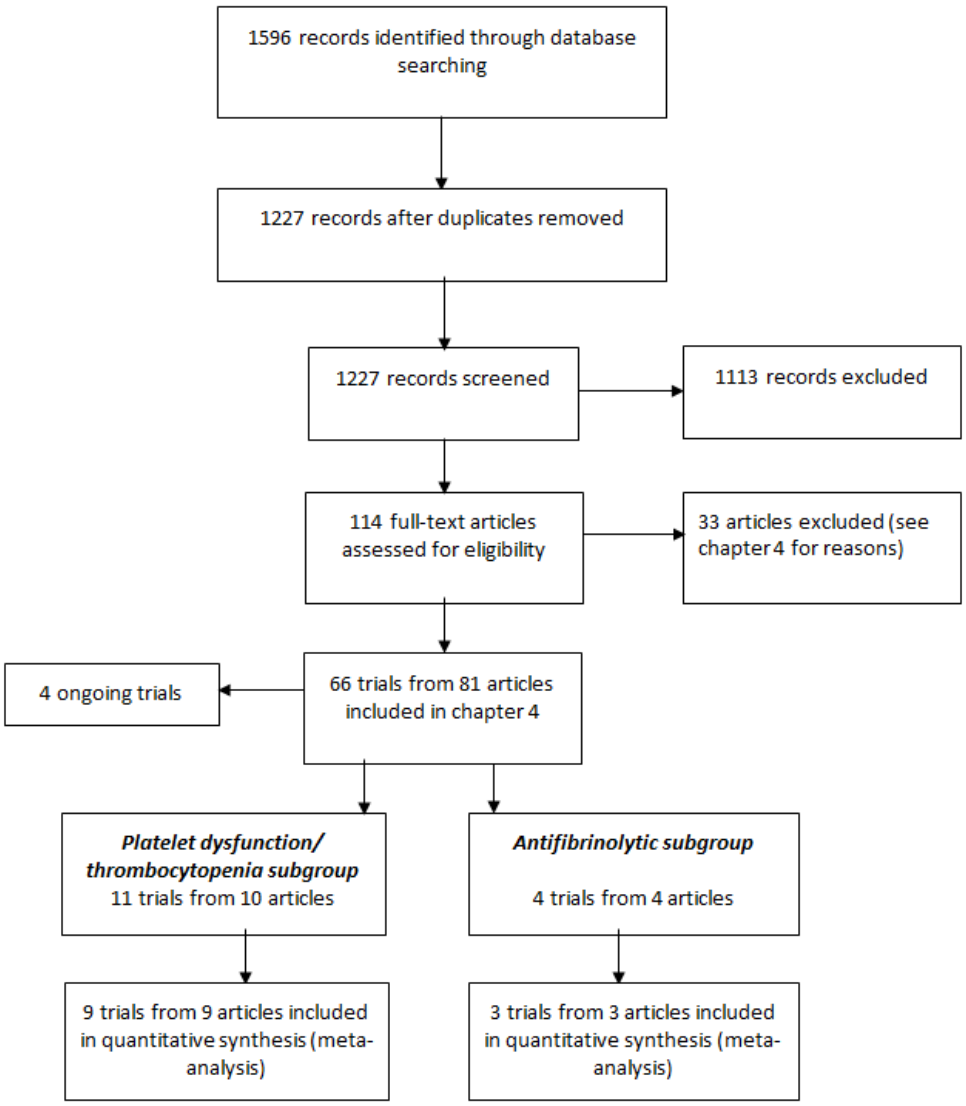


Figure 5.1 Study flow chart.

Desmopressin was administered as a single intravenous dose of 0.3μg/kg in nine trials (308, 309, 314, 321, 326, 328, 333, 351, 362), 0.4μg/kg in one trial (327) and 10μg/m^2 body surface area in one trial (361). Desmopressin was given pre-operatively in one trial (362); intra-operatively but after CPB in five trials (314, 328, 333, 351, 361); post-operatively in four trials (308, 309, 326, 327); and either intra-operatively or post-operatively in one trial (321). In 10 trials desmopressin was administered prophylactically to prevent bleeding (308, 309, 314, 326-328, 333, 351, 361, 362) and in one trial it was administered to treat bleeding (321). Nine trials compared desmopressin to a matching 0.9% saline placebo (314, 321, 326-328, 333, 351, 361, 362) and two trials were open label trial comparing desmopressin to standard care (308, 309). Trials were published between 1992 and 2016. Characteristics of included studies are reported in Table 5.1.

Trial [Country]	Arm	n	Male/ female	Age (years)	Timing	Surgery	Platelet dysfunction
Antiplatelet agents							
Bignami 2016 [Italy]	Desmopressin	25	22/3	67 ± 13	After heparin reversal or post-op	Elective CABG or valve replacement	Aspirin continued throughout (other anti-platelets stopped 5 days before surgery)
	Placebo	29	22/7	66 ± 13			
Dilthey 1993 [Germany]	Desmopressin	19	19/0	56 ± 9	After heparin reversal	Elective CABG	Aspirin within 5 days of surgery
	Placebo	20	20/0	58 ± 8			
Gratz 1992 [USA]	Desmopressin	29	19/10	62 ± 10	After heparin reversal	Elective CABG	Aspirin within 7 days of surgery
	Placebo	30	23/7	63 ± 12			
Hemşinli[1] 2012a [Turkey]	Desmopressin	10	-	-	Post-op	Emergency CABG	Dual antiplatelet therapy
	Standard care	10	-	-			
Hemşinli[1] 2012b [Turkey]	Desmopressin	16	-	-	Post-op	Emergency CABG	Dual antiplatelet therapy
	Standard care	18	-	-			
Pleym 2004 [Norway]	Desmopressin	46	40/6	63 ± 9	After heparin reversal	Elective CABG	Aspirin within 1 day of surgery
	Placebo	46	36/10	64 ± 8			
Sheridan 1994 [Canada]	Desmopressin[2]	20	20/0	57 (53-60)	After heparin reversal	Elective CABG	Aspirin within 7 days of surgery
	Placebo	24	24/0	62 (59-65)			
Platelet dysfunction due to cardiopulmonary bypass							
De Prost 1992 [France]	Desmopressin	47	37/10	60 ± 13	Up to six hours post-op	Elective CABG or valve replacement	Prolonged bleeding time
	Placebo	45	32/13	55 ± 16			
Despotis 1999 [USA]	Desmopressin[3]	50	30/20	64 ± 10	Post-op	Elective CABG or valve replacement	HemoSTATUS <60% in channel 5
	Placebo	51	34/17	66 ± 10			
Mongan 1992 [USA]	Desmopressin	13	9/4	59 ± 11	After heparin reversal	Elective CABG	TEG MA 50mm or less
	Placebo	16	11/5	66 ± 10			
Steinlechner 2011 [Austria]	Desmopressin	20	8/12	72 ± 10	After induction of anaesthesia	Elective aortic valve replacement	Prolonged closure time on PFA
	Placebo	23	7/16	73 ± 8			
Thrombocytopenia							
No randomised controlled trials							

Table 5.1 Study characteristics: platelet dysfunction subgroup. Desmopressin dose 0.3μg/kg intravenously unless stated otherwise. Placebo 0.9% saline in all cases. [1]Published in abstract form only; [2]Desmopressin dose 10μg/m^2 body surface area; [3]Desmopressin dose 0.4μg/kg, CABG: coronary artery bypass graft, PFA: platelet function analyser, TEG MA: thromboelastography maximum amplitude, and TXA: tranexamic acid. Age stated as mean ± standard deviation or median (range).

5.3.3 Risk of bias

Most trials were at unclear risk of bias because methodological reporting was not adequate to make an accurate assessment of risk. Assessments for individual domains are summarized in Figure 5.2.

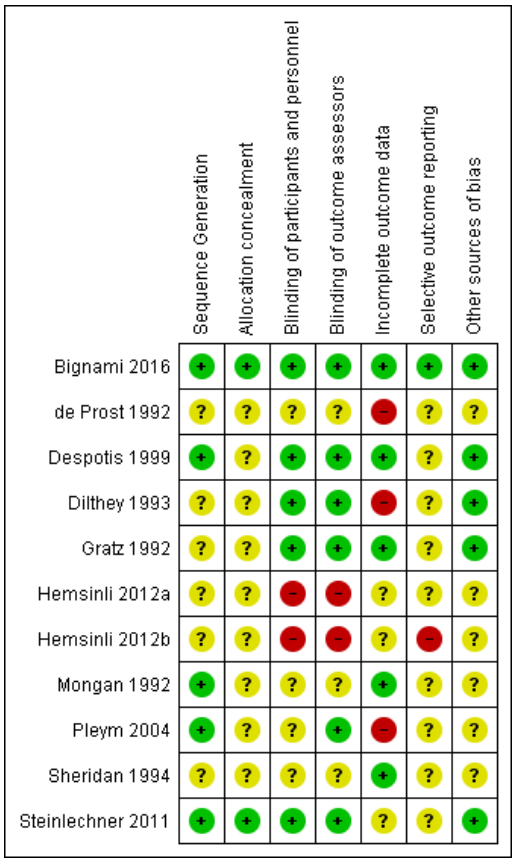


Figure 5.2 Risk of bias summary: platelet dysfunction subgroup. High risk of bias for a category is indicated by −, unclear risk of bias by ? and low risk of bias by +.

5.3.3.1 Sequence generation

- Five trials were at low risk of bias for sequence generation because they used computer generated random numbers (314, 321, 327, 351, 362).
- The remaining six trials were at unclear risk of bias because they did not report details of the randomisation sequence.

5.3.3.2 Concealment of treatment allocation

- Two trials were at low risk for concealment of treatment allocation all of which used sealed envelopes (321, 362).
- The remaining nine trials were at unclear risk of bias because they did not reported details of treatment allocation.

5.3.3.3 Blinding (performance bias and detection bias)

- Five trials were at low risk of bias with adequate blinding of participants, personnel and analysts because:
  - A pharmacist prepared the desmopressin or matching placebo who was not otherwise involved in the trial (333); or
  - A member of the study team not involved in determining outcomes prepared the desmopressin or matching placebo while all other members of the study team and outcome assessors were blinded (321, 327, 328, 362).
- One trial unclear risk with participants and personnel and low risk with analysts (351)
- Two trials were at high risk of bias for blinding of participants, personnel and study analysts because they were open label (308, 309).
- The remaining three trials did not report sufficient information to determine risk of bias from blinding.

5.3.3.4 Incomplete outcome data (attrition bias)

- Five trials were considered at low risk of bias for incomplete outcome data because participants were analysed on an intention to treat basis (314, 321, 327, 333, 361)
- Three trials were considered to be at high risk of bias because of exclusion of participants with severe bleeding or complications post-randomisation (326, 328, 351)

- The remaining three trials were considered to be at unclear risk of bias as insufficient information was reported to make a judgement about this outcome.

5.3.3.5 Selective reporting (reporting bias)

- One trial was considered to be at low risk of bias because it was prospectively registered and reported all pre-specified outcomes (321).
- One trial was considered to be at high risk of bias because of not reporting a pre-specified outcome (309).
- The remaining nine trials were considered to be at unclear risk of bias because no protocol or prospective trial registration was published.

5.3.3.6 Other potential sources of bias

- Five trials were considered at low risk of other bias because of detailed methodological reporting (321, 327, 328, 333, 362).
- The remaining six trials were considered at unclear risk of other bias.

5.3.3.7 Publication bias

There were too few trials to assess publication bias.

5.3.4 Effects on outcomes

5.3.4.1 Total volume of allogeneic red cells transfused

This outcome was reported in seven trials (314, 321, 327, 328, 333, 351, 362). One trial reported this in a way that could not be included in meta-analysis and is reported separately in Appendix 3. Overall, participants treated with desmopressin required fewer units of red cells than those treated with placebo (MD -0.65 units, 95% CI -1.16 to -0.13, I^2 = 36%, 388 participants, 6 trials, Figure 5.3). The sensitivity analyses supported the results of the main analysis: (MD -0.82 units, 95% CI -1.29 to -0.34, I^2 = 14%, 296 participants, 4 trials). The quality of evidence was judged to be low (Table 5.2).

The effect was similar for the subgroup of participants with platelet dysfunction due to antiplatelet agents (MD -0.90 units, 95% CI -1.62 to -0.18, I^2 = 36%, 152 participants, 3 trials) and with platelet dysfunction due to CPB (MD -0.43 units, 95% CI -1.22 to 0.37, I^2 = 56%, 236 participants, 3 trials). There was no evidence of heterogeneity between the subgroups: I^2 = 0%.

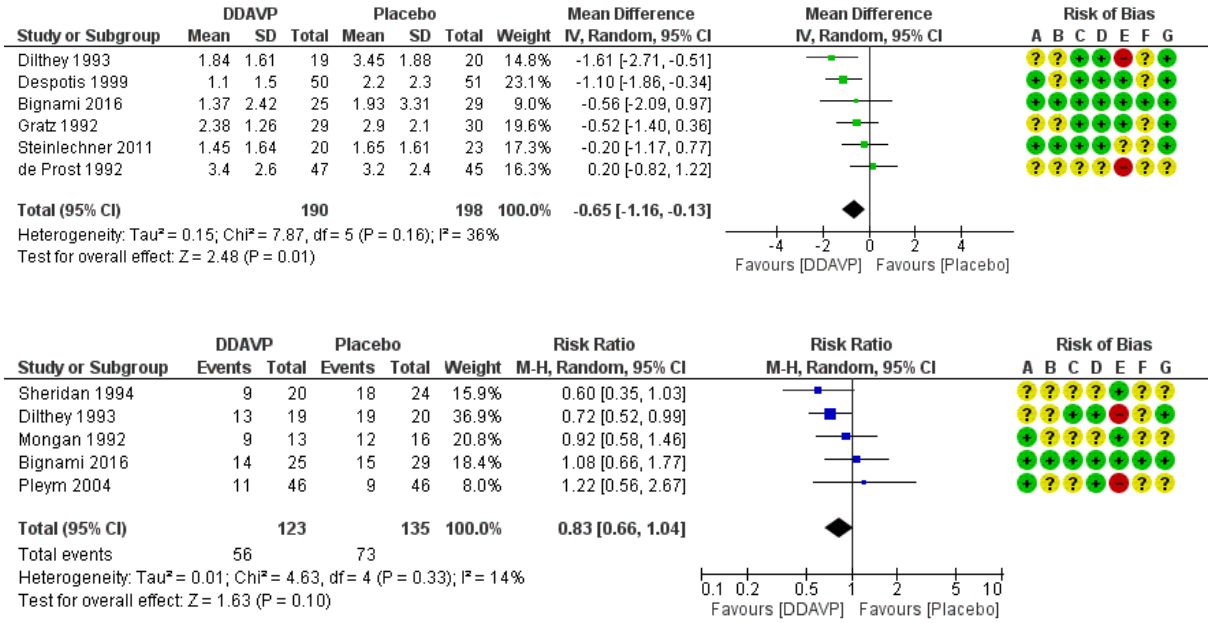


Figure 5.3 Forest plots of red cell transfusion: platelet dysfunction subgroup. Volume of red cells transfused (upper figure) and risk of allogeneic red cell transfusion (lower figure). Size of squares reflects weight of trial in pooled analysis. Horizontal bars represent 95% confidence intervals. CI: confidence interval, df: degrees of freedom, IV: inverse variance, M-H: Mantel-Haenszel, SD: standard deviation. Risk of bias categories were: (A) Sequence generation, (B) Allocation concealment, (C) Blinding of participants and personnel, (D) Blinding of outcome assessors, (E) Incomplete outcome data, (F) Selective outcome reporting, and (G) Other sources of bias. High risk of bias for a category is indicated by −, unclear risk of bias by ? and low risk of bias by +.

№ of RCTs	Number of participants		Effect		
	Desmopressin	Placebo	Relative	Absolute	GRADE
Total volume of allogeneic red cells transfused					
6	190	198	-	**MD 0.65 units less** (1.16 to 0.13 units less)	⊕⊕◯◯ LOW[1,2]
Risk of red cell transfusion					
5	56/123 (45.5%)	73/135 (54.1%)	**RR 0.83** (0.66 to 1.04)	**9 fewer per 100** (from 2 more to 18 fewer)	⊕⊕◯◯ LOW[1,2]
Total blood loss					
7	203	219	-	**MD 253.93 ml less** (408.01 to 99.85 ml less)	⊕⊕◯◯ LOW[1,2]
Re-operation due to bleeding					
6	8/202 (4.0%)	21/211 (10.0%)	**pOR 0.39** (0.18 to 0.84)	**6 fewer per 100** (from 1 fewer to 8 fewer)	⊕⊕⊕◯ MODERATE[1]
All-cause mortality					
7	2/203 (1.0%)	3/219 (1.4%)	**pOR 0.72** (0.12 to 4.22)	**0 fewer per 100** (from 1 fewer to 4 more)	⊕◯◯◯ VERY LOW[1,3]
Thrombotic events					
7	10/203 (4.9%)	7/219 (3.2%)	**pOR 1.58** (0.60 to 4.17)	**2 more per 100** (from 1 fewer to 9 more)	⊕⊕◯◯ LOW[1,4]
Clinically significant hypotension					
5	9/153 (5.9%)	0/162 (0%)	**pOR 9.78** (2.48 to 38.58)	**Not calculable**	⊕⊕◯◯ LOW[1,4]

Table 5.2 Summary of findings: platelet dysfunction subgroup. CI: Confidence interval; GRADE: Grading of Recommendations Assessment, Development and Evaluation; MD: Mean difference; pOR**:** Peto odds ratio; RCT: randomised controlled trial; RR: Risk ratio. [1]Downgraded one point due to risk of bias from blinding of outcome assessors and incomplete outcome data.

[2]Downgraded one point for inconsistency due to variation in baseline level of transfusion and blood loss. [3]Downgraded two points for imprecision, as confidence intervals include clinically significant benefit and clinically significant harm with low background event rate. [4]Downgraded one point for imprecision.

5.3.4.2 Total number of participants receiving an allogeneic red cell transfusion

This outcome was reported in six trials (314, 321, 328, 333, 351, 361). One trial was not included in the analysis because every participant in both arms was transfused (351). No difference was found in the risk of allogeneic red cell transfusion for participants treated with desmopressin compared to placebo (RR 0.83, 95% CI 0.66 to 1.04, I^2 = 14%, 258 participants, 5 trials, Figure 5.3). The sensitivity analyses supported the results of the main analysis: (RR 0.91, 95% CI 0.62 to 1.32, I^2 = 43%, 185 participants, 3 trials). The quality of evidence was judged to be low (Table 5.2).

The effect was similar for the subgroup of participants with platelet dysfunction due to antiplatelet agents (RR 0.82, 95% CI 0.61 to 1.10, I^2 = 32%, 229 participants, 4 trials), but was smaller for those with platelet dysfunction due to CPB (RR 0.92, 95% CI 0.58 to 1.46, 29 participants, 1 trial). There was no evidence of heterogeneity between the subgroups: I^2 = 0%.

5.3.4.3 Total volume of blood loss

This outcome was reported in all trials. Four trials reported this in a way that could not be included in meta-analysis and are reported separately in Appendix 3. Overall, participants treated with desmopressin lost less blood than those treated with placebo (MD -253.93 ml, 95% CI -408.01 to -99.85 ml, I^2 = 75%, 422 participants, 7 trials, Figure 5.4). The sensitivity analyses supported the results of the main analysis: (MD -370.00 ml, 95% CI -556.65 to -183.35, I^2 = 63%, 276 participants, 5 trials). The quality of evidence was judged to be low (Table 5.2).

The effect was similar for the subgroup of participants with platelet dysfunction due to antiplatelet agents (MD -249.90 ml, 95% CI -535.26 to 35.46 ml, I^2 = 78%, 249 participants, 4 trials) and with platelet dysfunction due to CPB (MD -295.73 ml, 95% CI -484.20 to -107.26 ml, I^2 = 61%, 173 participants, 3 trials). There was no evidence of heterogeneity between the subgroups: I^2 = 0%.

5.3.4.4 Re-operation due to bleeding

This outcome was reported in six trials (314, 321, 326, 327, 351, 362). The risk of re-operation due to bleeding was lower for participants treated with desmopressin compared to placebo (pOR 0.39, 95% CI 0.18 to 0.84, I^2 = 0%, 413 participants, 6 trials, Figure 5.4). The number needed to treat to prevent one patient requiring re-operation due to bleeding was 17. The sensitivity analyses supported the results of the main analysis: (pOR 0.48, 95% CI 0.17 to 1.34, I^2 = 37%, 292 participants, 4 trials). The quality of evidence was judged to be moderate (Table 5.2).

There was moderate evidence of heterogeneity between the subgroups: I^2 = 52.5%. The effect was smaller for the subgroup of participants with platelet dysfunction due to antiplatelet agents (pOR 0.77, 95% CI 0.23 to 2.54, 148 participants, 2 trials) than those with platelet dysfunction due to CPB (pOR 0.24, 95% CI 0.09 to 0.66, I^2 = 0%, 265 participants, 4 trials).

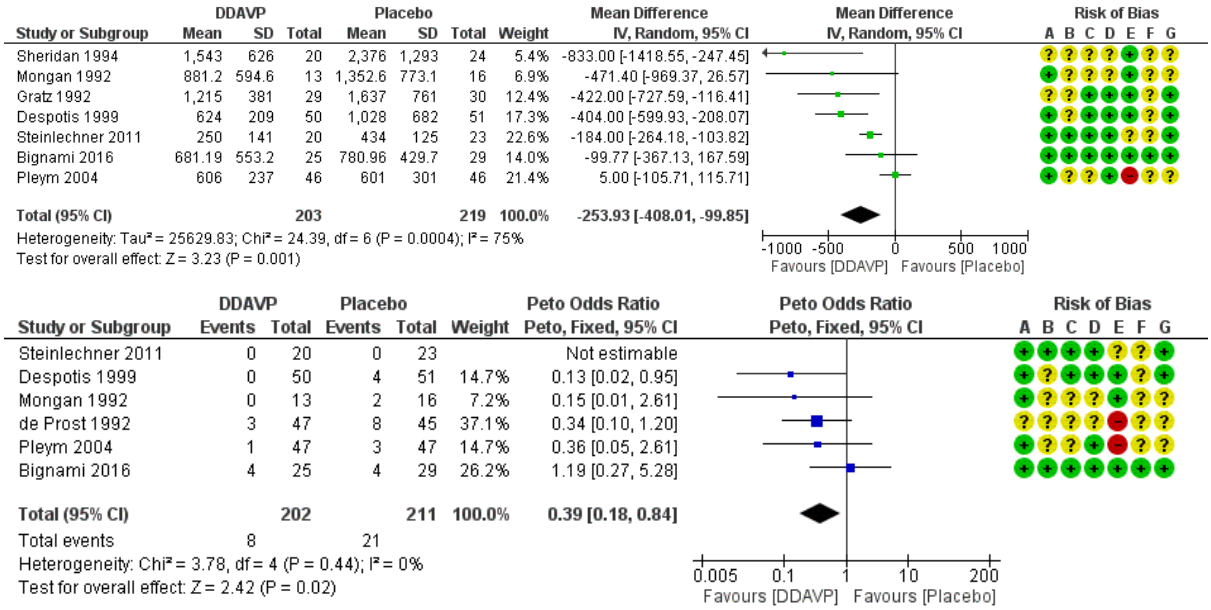


Figure 5.4 Forest plots of bleeding: platelet dysfunction subgroup. Total blood loss (upper figure) and re-operation due to bleeding (lower figure). Size of squares reflects weight of trial in pooled analysis. Horizontal bars represent 95% confidence intervals. CI: confidence interval, df: degrees of freedom, IV: inverse variance, SD: standard deviation. Risk of bias categories were: (A) Sequence generation, (B) Allocation concealment, (C) Blinding of participants and personnel, (D) Blinding of outcome assessors, (E) Incomplete outcome data, (F) Selective outcome reporting, and (G) Other sources of bias. High risk of bias for a category is indicated by -, unclear risk of bias by ? and low risk of bias by +.

5.3.4.5 Adverse events

5.3.4.5.1 All-cause mortality

This outcome was reported in seven trials (314, 321, 327, 333, 351, 361, 362). Four trials reported no deaths in either arm (314, 351, 361, 362). No difference was found in the risk of all-cause mortality for participants treated with desmopressin compared to placebo (pOR 0.72, 95% CI 0.12 to 4.22, I^2 = 24%, 422 participants, 7 trials). The baseline risk of mortality was less than 1%, limiting interpretation of these results due to imprecision. The quality of evidence was judged to be very low (Table 5.2).

5.3.4.5.2 Thrombotic events

This outcome was reported in seven trials (314, 321, 327, 333, 351, 361, 362). No difference was found in the risk of a thrombotic events for participants treated with desmopressin compared to placebo (pOR 1.58, 95% CI 0.60 to 4.17, I^2 = 0%, 422 participants, 7 trials). The event rate was low for individual causes of thrombotic events with no overall difference in the incidence in any type of thrombotic event: myocardial infarction (pOR 2.72, 95% CI 0.60 to 12.37, I^2 = 0%, 277 participants, 5 trials); ischaemic stroke (pOR 1.21, 95% CI 0.07 to 20.17, I^2 = 0%, 157 participants, 3 trials); or venous thromboembolism (pOR 0.56, 95% CI 0.06 to 5.50, 248 participants, 4 trials). The quality of evidence was judged to be low (Table 5.2).

5.3.4.5.3 Clinically significant hypotension

This outcome was reported in five trials (314, 321, 327, 328, 351). No events occurred in either arm of two trials (314, 327). There was an increase in the risk of clinically significant hypotension for participants treated with desmopressin compared to placebo (pOR 9.78, 95% CI 2.48 to 38.58, I^2 = 0%, 315 participants, 5 trials). The number needed to treat for one episode of clinically significant hypotension was 17. The quality of evidence was judged to be low (Table 5.2).

5.3.4.5.4 Other adverse events

Other adverse events, including hyponatraemia, seizures, chest tightness, hypertension, abdominal pain, headache, nausea and allergic reactions, were not reported in the included trials.

5.3.5 GRADE quality of the evidence

Overall the GRADE quality of evidence was very low to moderate suggesting considerable uncertainty over the results and that further clinical trial information may significantly alter the results (Table 5.2). The quality assessment of all outcomes was

downgraded one point for risk of bias. Volume of red cells transfused, risk of red cell transfusion and blood loss were downgraded one point due for inconsistency, as there were significant variations in baseline blood loss and transfusion requirements between studies. All-cause mortality was downgraded two points for imprecision, as the event rate was very low and consequently the confidence interval included both significant benefit and significant harm. Risk of a thrombotic event and clinically significant hypotension were downgraded one point for imprecision.

5.4 Results for co-administration of an anti-fibrinolytic agent

5.4.1 Search results

Full search results are reported in Figure 5.1. Four trials (348 participants) met the inclusion criteria for this chapter and were included in the qualitative synthesis (309, 311, 321, 339) and three trials were included in the quantitative syntheses (311, 321, 339). One trial did not report information in way that could be incorporated into meta-analysis (309).

5.4.2 Trial characteristics

Although all surgical procedures regardless of specialty were potentially eligible, all four relevant trials were in the setting of cardiac surgery. One trial included participants undergoing CABG (309); one trial included valve replacements (339); one trial included a combination of CABG and valve replacements (321); and one trial included a combination of aorto-coronary artery bypass grafts, atrial septal defects repairs and valve repairs (311). Three trials included only elective surgery (311, 321, 339) and one trial included only emergency cases (309). The anti-fibrinolytic agent was tranexamic acid in all cases (regimens summarized in Table 5.3). One trial was published in abstract form only (309).

Trial [Country]	Arm	n	Male/ female	Age (years)	Timing	Surgery	Anti-fibrinolytic
Bignami 2016 [Italy]	Desmopressin	68	50/18	64 ± 13.3	After heparin reversal or post-op	Elective CABG or valve replacement	TXA 1g before surgery followed by either 400 mg/hr infusion, or second 1g bolus
	Placebo	67	50/17	62 ± 13.2			
Hemşinli[1] 2012 [Turkey]	Desmopressin	16	.	.	Post-op	Emergency CABG	TXA 10 mg/kg over 30 min before surgery then 1 mg/kg/hr for 10 hours
	Standard care	18	.	.			
Horrow 1991b [USA]	Desmopressin	40	.	63 ± 9	After heparin reversal	ACBG, valve replacement or ASD repair	TXA 10 mg/kg over 30 min before surgery then 1 mg/kg/hr for 12 hours
	Placebo	37	.	65 ± 11			
Jin 2015 [China]	Desmopressin	52	21/31	49 ± 10	After heparin reversal	Valve replacement	TXA 30 mg/kg intra-operatively
	Placebo	50	21/29	53 ± 9			

Table 5.3 Study characteristics: anti-fibrinolytic subgroup. Desmopressin dose 0.3μg/kg intravenously and placebo 0.9% saline in all cases. [1]Published in abstract form only. ACBG: aorto-coronary bypass graft, ASD: atrial septal defect, CABG: coronary artery bypass graft, TXA: tranexamic acid. Age stated as mean ± standard deviation.

Desmopressin was administered as a single intravenous dose of 0.3μg/kg in all trials. Desmopressin was administered intra-operatively but after CPB in two trials (311, 339); post-operatively in one trial (309); and either intra-operatively or post-operatively in one (321). In three trials desmopressin was administered prophylactically to prevent bleeding (309, 311, 339) and in one trial it was administered to treat bleeding (321). Three trials compared desmopressin to a matching 0.9% saline placebo (311, 321, 339) and one trial was an open label trial comparing desmopressin to standard care (309). Trials were published between 1991 and 2016. Characteristics of included studies are reported in Table 5.3.

5.4.3 Risk of bias

Most trials were at unclear risk of bias because methodological reporting was not adequate to make an accurate assessment of risk. Assessments for individual domains are summarized in Figure 5.5.

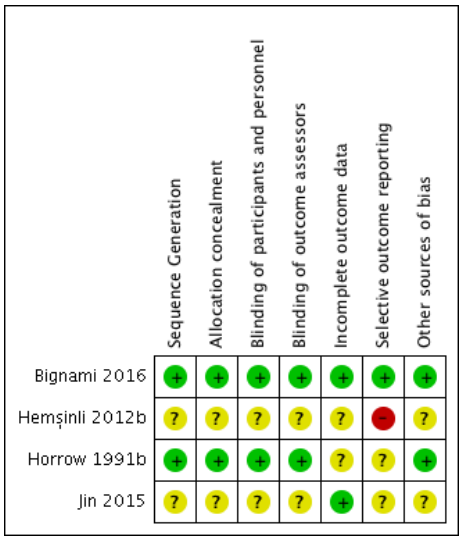


Figure 5.5 Risk of bias summary: anti-fibrinolytic subgroup. High risk of bias for a category is indicated by −, unclear risk of bias by ? and low risk of bias by +.

5.4.3.1 Sequence generation

- Two trials were at low risk of bias for sequence generation because they used computer generated random numbers (321) or a random number table (311)
- The remaining two trials were at unclear risk of bias because they did not report details of the randomisation sequence.

5.4.3.2 Concealment of treatment allocation

- Two trials were at low risk for concealment of treatment allocation both of which used sealed envelopes (311, 321).
- The remaining two trials were at unclear risk of bias because they did not reported details of treatment allocation.

5.4.3.3 Blinding (performance bias and detection bias)

- Two trials were at low risk of bias with adequate blinding of participants, personnel and analysts because:
  - A pharmacist prepared the desmopressin or matching placebo who was not otherwise involved in the trial (311); or
  - A member of the study team not involved in determining outcomes prepared the desmopressin or matching placebo while all other members of the study team and outcome assessors were blinded (321)
- The remaining two trials did not report sufficient information to determine risk of bias from blinding.

5.4.3.4 Incomplete outcome data (attrition bias)

- Two trials were considered at low risk of bias for incomplete outcome data because participants were analysed on an intention to treat basis (321, 339)
- The remaining two trials were considered to be at unclear risk of bias as insufficient information was reported to make a judgement about this outcome.

5.4.3.5 Selective reporting (reporting bias)

- One trial was considered to be at low risk of bias because it was prospectively registered and reported all pre-specified outcomes (321)
- One trial was considered to be at high risk of bias because of not reporting a pre-specified outcome (309)
- The remaining two trials were considered to be at unclear risk of bias because no protocol or prospective trial registration was published.

5.4.3.6 Other potential sources of bias

- Two trials were considered at low risk of other bias because of detailed methodological reporting (311, 321)
- The remaining two trials were considered at unclear risk of other bias.

5.4.3.7 Publication bias

There were too few trials to assess publication bias.

5.4.4 Effects on outcomes

5.4.4.1 Total volume of allogeneic red cells transfused

This outcome was reported in one trial but not in a way that could not be included in meta-analysis and is reported in Appendix 3. No difference was found between desmopressin and placebo in this trial. The quality of evidence was judged to be very low (Table 5.4).

5.4.4.2 Total number of participants receiving an allogeneic red cell transfusion

This outcome was reported in three trials (311, 321, 339). No difference was found in the risk of allogeneic red cell transfusion for participants treated with desmopressin compared to placebo (RR 0.72, 95% CI 0.35 to 1.51, I^2 = 61%, 314 participants, 3 trials, Figure 5.6). The sensitivity analyses supported the results of the main analysis: (RR 0.71, 95% CI 0.25 to 2.04, I^2 = 78%, 212 participants, 2 trials). The quality of evidence was judged to be very low (Table 5.4).

№ of RCTs	Participants		Effect		
	Desmopressin	Placebo	Relative	Absolute	GRADE
Total volume of allogeneic red cells transfused					
No studies contributing towards outcome					
Risk of red cell transfusion					
3	46/160 (28.8%)	51/154 (33.1%)	**RR 0.72** (0.35 to 1.51)	**9 fewer per 100** (from 17 more to 22 fewer)	⊕◯◯◯ VERY LOW[1,2,3]
Total blood loss					
2	92	87	-	**MD 2.88 ml less** (63.71 less to 57.94 ml more)	⊕◯◯◯ VERY LOW[1,2,3]
Re-operation due to bleeding					
2	8/108 (7.4%)	13/104 (12.5%)	**RR 0.63** (0.28 to 1.40)	**0.5 fewer per 100** (from 0.4 more to 0.9 fewer)	⊕⊕◯◯ LOW[1,4]
All-cause mortality					
2	5/120 (4.2%)	4/117 (3.4%)	**pOR 1.25** (0.32 to 4.80)	**1 more per 100** (from 2 fewer to 11 more)	⊕◯◯◯ VERY LOW[1,2,3]
Thrombotic events					
3	6/160 (3.8%)	3/154 (1.9%)	**pOR 1.91** (0.50 to 7.22)	**2 more per 100** (from 1 fewer to 11 more)	⊕◯◯◯ VERY LOW[1,4]
Clinically significant hypotension					
1	3/68 (4.4%)	2/67 (3.0%)	**pOR 1.48** (0.26 to 8.57)	**1 more per 100** (from 1 fewer to 23 more)	⊕◯◯◯ VERY LOW[1,4]

Table 5.4 Summary of findings: anti-fibrinolytic subgroup. CI: Confidence interval; GRADE: Grading of Recommendations Assessment, Development and Evaluation MD: Mean difference; pOR**:** Peto odds ratio; RCT: randomised controlled trial; RR: Risk ratio. [1]Downgraded one point due to risk of bias from blinding of outcome assessors and incomplete outcome data.

[2]Downgraded one point for inconsistency due to variation in baseline level of transfusion and blood loss. [3]Downgraded one points for imprecision, as confidence intervals include clinically significant benefit and clinically significant harm, or sample size too small for accurate estimate. [4]Downgraded two points for imprecision due to small sample side and both considerable harm and considerable benefit being possible within confidence intervals.

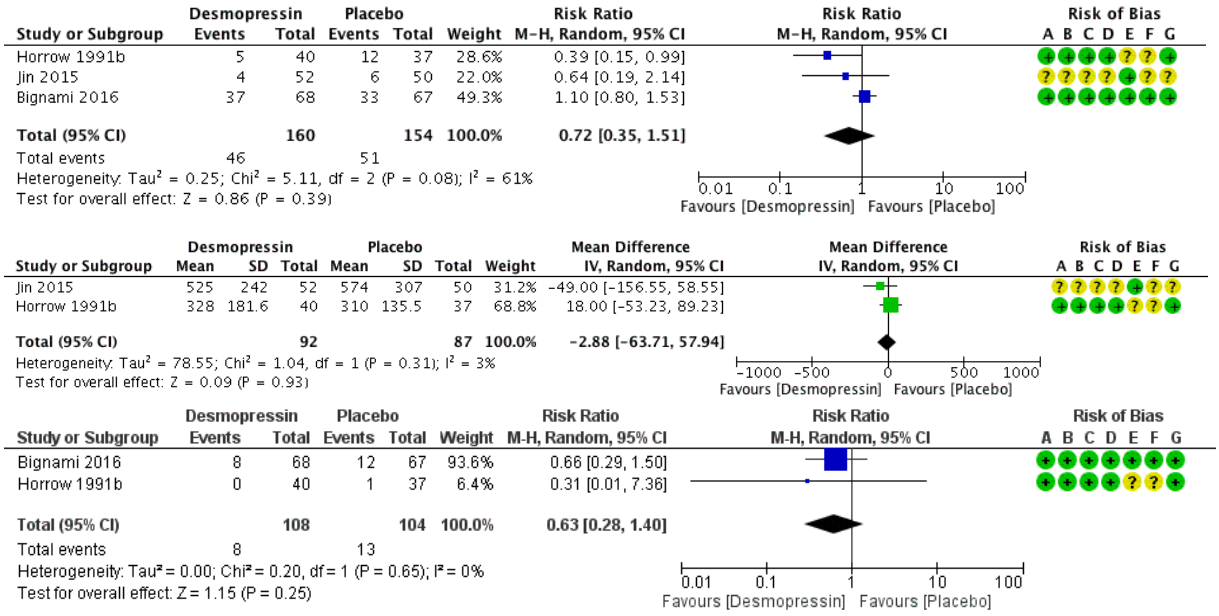


Figure 5.6 Forest plot of red cell transfusion and bleeding: anti-fibrinolytic subgroup. Risk of allogeneic red cell transfusion (upper figure); Total blood loss (middle figure); and risk of re-operation due to bleeding (lower figure). Size of squares reflects weight of trial in pooled analysis. Horizontal bars represent 95% confidence intervals. CI: confidence interval, df: degrees of freedom, IV: inverse variance, M-H: Mantel-Haenszel, SD: standard deviation. Risk of bias categories were: (A) Sequence generation, (B) Allocation concealment, (C) Blinding of participants and personnel, (D) Blinding of outcome assessors, (E) Incomplete outcome data, (F) Selective outcome reporting, and (G) Other sources of bias. High risk of bias for a category is indicated by ⊖, unclear risk of bias by ? and low risk of bias by ⊕.

5.4.4.3 Total volume of blood loss

This outcome was reported in all trials. Two trials reported this in a way that could not be included in meta-analysis and are reported separately in Appendix 3. No difference was found in total blood loss between than those treated with desmopressin and

placebo (MD -2.88 ml, 95% CI -63.71 to -57.94, I^2 = 3%, 179 participants, 2 trials, Figure 5.6). The quality of evidence was judged to be very low (Table 5.4).

5.4.4.4 Re-operation due to bleeding

This outcome was reported in two trials (311, 321). No difference was found in the risk of re-operation due to bleeding for participants treated with desmopressin compared to placebo (RR 0.63, 95% CI 0.28 to 1.40, I^2 = 0%, 212 participants, 2 trials, Figure 5.6). The quality of evidence was judged to be low (Table 5.4).

5.4.4.5 Adverse events

5.4.4.5.1 All-cause mortality

This outcome was reported in two trials (321, 339). One trial reported no deaths in either arm (339). No difference was found in the risk of all-cause mortality for participants treated with desmopressin compared to placebo (pOR 1.25, 95% CI 0.32 to 4.80, 137 participants, 2 trials). The baseline risk of mortality was less than 1%, limiting interpretation of these results due to imprecision. The quality of evidence was judged to be very low (Table 5.4).

5.4.4.5.2 Thrombotic events

This outcome was reported in three trials (311, 321, 339). No thrombotic events occurred in one trial (339). No difference was found in the risk of a thrombotic events for participants treated with desmopressin compared to placebo (pOR 1.91, 95% CI 0.50 to 7.22, I^2 = 0%, 314 participants, 3 trials). The event rate was low for individual causes of thrombotic events with no overall difference in the incidence in any type of thrombotic event: myocardial infarction (no events, 179 participants, 2 trials); ischaemic stroke (pOR 0.92, 95% CI 0.06 to 15.08, I^2 = 0%, 179 participants, 2 trials); or venous thromboembolism (pOR 0.12, 95% CI 0.00 to 6.31, 179 participants, 2 trials). The quality of evidence was judged to be very low (Table 5.4).

5.4.4.5.3 Clinically significant hypotension

This outcome was reported in one trial (321). No difference was found in the risk of clinically significant hypotension for participants treated with desmopressin compared to placebo (pOR 1.48, 95% CI 0.26 to 8.57, 135 participants, 1 trial) (321).

5.4.4.5.4 Other adverse events

Other adverse events, including hyponatraemia, seizures, chest tightness, hypertension, abdominal pain, headache, nausea and allergic reactions, were not reported in the included trials.

5.4.5 GRADE quality of the evidence

Overall the GRADE quality of evidence was very low to low suggesting a lack of certainty over the results (Table 5.4). Volume of red cells transfused was not reported at all. The quality assessments of all other outcomes were downgraded one point for risk of bias. Risk of red cell transfusion, total blood loss and overall mortality were downgraded one point for inconsistency, as there were significant variations in baseline blood loss, transfusion requirements and mortality between studies. Risk of red cell transfusion, total blood loss and overall mortality were also downgraded one point for imprecision, as the event rate was very low and consequently the confidence interval included both significant benefit and significant harm. Re-operation due to bleeding, risk of a thrombotic event and clinically significant hypotension were downgraded two points for imprecision because their confidence intervals included large difference in benefit and harm.

5.5 Discussion

5.5.1 Summary of key results

5.5.1.1 Desmopressin for platelet dysfunction or thrombocytopenia

This meta-analysis identified 11 randomised trials with 596 participants where the effects of desmopressin were assessed in patients with platelet dysfunction. All trials were in the setting of cardiac surgery. Platelet dysfunction was caused by antiplatelet agents in seven trials and CPB in four trials. I found no randomised controlled trials in this setting investigating the desmopressin for the treatment of thrombocytopenia.

Desmopressin administration resulted in transfusion of fewer units of red cells (equivalent to a 25% reduction compared to control), less blood loss (equivalent to a 23% reduction compared to control) and a lower risk of requiring a re-operation due to bleeding. No difference was found in the risk of receiving an allogeneic red cell transfusion. Similar results were found for the subgroups with platelet dysfunction due to antiplatelet agents and due to CPB, with little evidence of statistical heterogeneity between subgroups. For the outcome of re-operation due to bleeding, the odds of returning to theatre with bleeding were lower in the subgroup with platelet dysfunction due to CPB than those taking antiplatelet agents.

Event rates were generally low for adverse events. There were no overall differences reported in the risk of all-cause mortality or thrombotic events (myocardial infarction, stroke and venous thromboembolism) for patients treated with desmopressin. Larger trials are required to confirm or refute any increased thrombotic risk particularly in patients with ischaemic heart disease. Patients treated with desmopressin had an increased risk of clinically significant hypotension, which is consistent with the results of Chapter 4 (section 4.3.4.6.3). Other adverse events such as facial flushing, hyponatraemia and seizures were not reported.

5.5.1.2 Co-administration of desmopressin and an anti-fibrinolytic agent

This meta-analysis identified four randomised trials with 348 participants. All trials were in the setting of cardiac surgery; and the anti-fibrinolytic agent was tranexamic acid in all cases. Interpretation of results was limited by the small number of studies. No difference was found in total volume of red cells transfused, risk of red cell transfusion, total blood loss or risk of returning to theatre with rebleeding but there is considerable uncertainty over the results due to the low quality of the data.

Adverse events including mortality, thrombotic events and clinically significant hypotension were rare and it was not possible to estimate whether the risk increased or is reduced by administration of desmopressin and tranexamic acid compared to tranexamic acid alone. Larger trials would be required to confirm or refute any increased thrombotic risk. Other adverse events such as facial flushing, hyponatraemia and seizures were not reported.

5.5.2 Limitations

5.5.2.1 Desmopressin for platelet dysfunction or thrombocytopenia

All of the trials were in the setting of cardiac surgery. All trials were small and five of the 11 trials were undertaken more than 20 years ago when different standards of practice in cardiac surgery existed. Patients undergoing emergency surgery were included in only one out of 11 trials and desmopressin was used for treatment (rather than prophylaxis) of bleeding in only one of 11 trials. The majority of patients taking antiplatelet agents were taking single agent aspirin, with relatively few patients taking dual antiplatelet therapy or other antiplatelet agents. There was heterogeneity in the nature and timing of desmopressin administration in the trial protocols for the included trials. Many trials were considered to be at risk of bias due to insufficient information on blinding of study outcome assessors and incomplete outcome data. Given these

limitations, and the low quality GRADE levels of evidence, the findings highlight the need for caution to extrapolate the research to areas outside cardiac surgery but also the opportunities for further clinical research.

Meta-analyses of total volume of allogeneic red cells transfused and total blood loss were limited due to inconsistency in baseline levels of red cell transfusion and blood loss. The findings of my review were also limited by data published in a format that could not be incorporated into meta-analysis, which showed inconsistent results. There were few numbers of events for all-cause mortality, thrombotic events and clinically significant hypotension, which limited interpretation of the results due to imprecision.

5.5.2.2 Co-administration of desmopressin and an anti-fibrinolytic agent

Two of the four trials assessing desmopressin versus placebo where all patients received an anti-fibrinolytic agent, were also included in the analysis of those with platelet dysfunction (309, 321). As with the trials in the setting of platelet dysfunction or thrombocytopenia, all the trials were in the setting of cardiac surgery. All four trials were small, with one published more than 20 years ago, and one published in abstract form only. Patients undergoing emergency surgery were included in one out of four trials and desmopressin was used for treatment (rather than prophylaxis) of bleeding in one of four trials. All trials used tranexamic acid and consequently there is no evidence on the use of epsilon aminocaproic acid or aprotinin. The GRADE levels of evidence, was assessed as low or very low, reflecting considerable uncertainty over the results.

The small number of trials and participants limited interpretation, and results in the original papers were often published in a format which could not be incorporated into meta-analysis. There were few numbers of events for all-cause mortality, thrombotic

events and clinically significant hypotension, which limited interpretation of the results due to imprecision.

5.5.3 Conclusion

Overall these meta-analyses provide some evidence for the use of desmopressin to reduce bleeding and transfusion requirements for people with platelet dysfunction or taking antiplatelet agents undergoing surgery. The effect size was considerably larger when treating patients with platelet dysfunction compared to unselected patients (see Chapter 4). However, there remains uncertainty in the size of benefit and the risks in cardiac surgery, including thrombotic events. Significant research gaps exist outside cardiac surgery (including in acute blood loss) and the use of desmopressin for treating antiplatelet agents other than aspirin. There was no randomised controlled trial evidence for its use in thrombocytopenia.

There was insufficient evidence to support the co-administration of tranexamic acid and desmopressin over desmopressin alone. It is possible that tranexamic acid has a large effect which masks the effect of desmopressin. However the quality of evidence was low to very low suggesting considerable uncertainty over these results. No increase in adverse events was noted with the combination of agents but the event rates were too low to provide a definitive answer.

Taken together with the results of Chapter 4, these results suggest the following for future clinical trial design:

- Desmopressin appears more effective for patients with platelet dysfunction than for unselected patients
- At present there is no randomised controlled trial evidence evaluating desmopressin for the treatment of thrombocytopenia

- There is some evidence that desmopressin may prevent bleeding before invasive procedures such as kidney biopsy
- There was no difference in mortality or thrombotic events for those treated with desmopressin or placebo
- The risk of clinically significant hypotension is increased with desmopressin compared to placebo, but this appears to be related to desmopressin infusion rate, with few events below a rate of <1.2 μg/kg/hour.
- There is insufficient evidence for benefit (or harm) from co-administration of tranexamic with desmopressin in this setting.

The results of Chapter 3 suggest that VWF may compensate for the haemostatic deficit in thrombocytopenia. Chapter 6 outlines the design and rationale for a placebo-controlled double-blind randomised feasibility trial of desmopressin in thrombocytopenic patients with critical illness prior to interventional procedures.

Chapter 6

6 The DRIVE trial

6.1 Introduction

This chapter covers the design and justification for a placebo-controlled double-blind randomised feasibility trial of desmopressin in thrombocytopenic patients with critical illness prior to interventional procedures (DRIVE: Desmopressin for procedures of Radiological InterVEntions).

6.1.1 Thrombocytopenia in critical care

Thrombocytopenia is a common clinical problem in Intensive Care, and a large proportion of patients have to undergo interventional procedures during their admission. Data from the multicentre Intensive care Study Of Coagulopathy (ISOC) suggested that 1/3 patients admitted to a critical care unit had a nadir platelet count <100 $x10^9$/l and 1/7 had a platelet count <50 $x10^9$/l (4). Local data from Oxford University Hospitals NHS Foundation Trust from 4200 critical care admissions over three years, demonstrated that 19% of patients admitted to ICU had a nadir platelet count <100 $x10^9$/L. This data also showed that 75% of all ICU patients had a central venous catheter inserted, 40% had a chest or abdominal drain, 15% had an epidural catheter and 5% had a tracheostomy. The bleeding risk with many of these procedures is increased with thrombocytopenia (as summarised in section 1.1).

6.1.2 Desmopressin

Desmopressin's mechanism of action and use in clinical guidelines is summarised in section 1.5.7; evidence for its use in unselected patients undergoing surgery or invasive procedures in Chapter 4; and its use in platelet dysfunction or thrombocytopenia in Chapter 5.

6.1.3 Desmopressin in thrombocytopenia

There are no randomised controlled trial data for the use of desmopressin in thrombocytopenia, although it may be effective in platelet dysfunction (Chapter 5). Laboratory assessment of thrombus formation under flow suggests that increasing VWF levels (an effect of desmopressin) may be an effective way of compensating for thrombocytopenia (Chapter 3). While desmopressin has minimal effectiveness in unselected patients undergoing major surgery, randomised trial evidence suggests it may be more effective for those undergoing interventional procedures (Chapter 4). The risk of adverse events appears to be low, with the lowest risk of clinically significant hypotension when desmopressin is administered at less than 1.2 µg/kg/hour (section 4.3.4.6.3.1). There is no randomised controlled trial evidence for an increase in thrombotic events, even amongst patients at high risk of cardiovascular disease undergoing cardiac surgery (section 4.3.4.6.2). However there is insufficient evidence to exclude this possibility.

6.1.4 Need for a trial

There is no high quality evidence to inform the safety of any thresholds of platelet count for platelet transfusion in critical illness (371). Recent large trials of prophylactic platelet transfusion in thrombocytopenic patients with haematological malignancies have also pointed to a limited role for platelet transfusions to prevent bleeding (8, 372).

Alongside limited evidence of efficacy of platelet transfusions to reduce bleeding risk in non-bleeding critically ill patients pre-procedure, platelet transfusions are associated with risks (infection or transfusion reactions) as biological components (283, 373, 374) (Section 1.2). In addition:

- Platelet increments are variable and relatively poor following transfusion in critical care (375)

- Platelet transfusions may not be immediately available in critical care and must be allocated and delivered from a hospital transfusion laboratory; a potential problem in critical care if time-dependent interventions are required. Studies in trauma have demonstrated long delays with platelet transfusions (46)
- Platelets are a costly resource (376)

6.1.5 Objectives

The primary outcome for this feasibility trial is to derive the proportion of eligible patients who were randomised into the trial and receive the investigational medicinal product (IMP). This will inform the design of a future larger randomised-controlled efficacy trial of desmopressin in this setting.

A secondary aim is to understand baseline measures of platelet function and haemostasis in thrombocytopenic patients admitted to critical care, and to compare haemostatic/platelet function changes before and after desmopressin (vs placebo) in thrombocytopenic patients requiring an interventional procedure (efficacy).

6.2 Methods

The DRIVE trial is still in progress at the time of writing. This methods section will summarise the overall methods for this trial. This leads on to a report on the interim results after five months of recruitment.

6.2.1 Trial design

A placebo-controlled double-blind, randomised feasibility trial of desmopressin in critical illness prior to procedures. The trial process is outlined in Figure 6.1. The trial is presently running on two intensive care units at one NHS trust (Oxford University Hospitals NHS Trust).

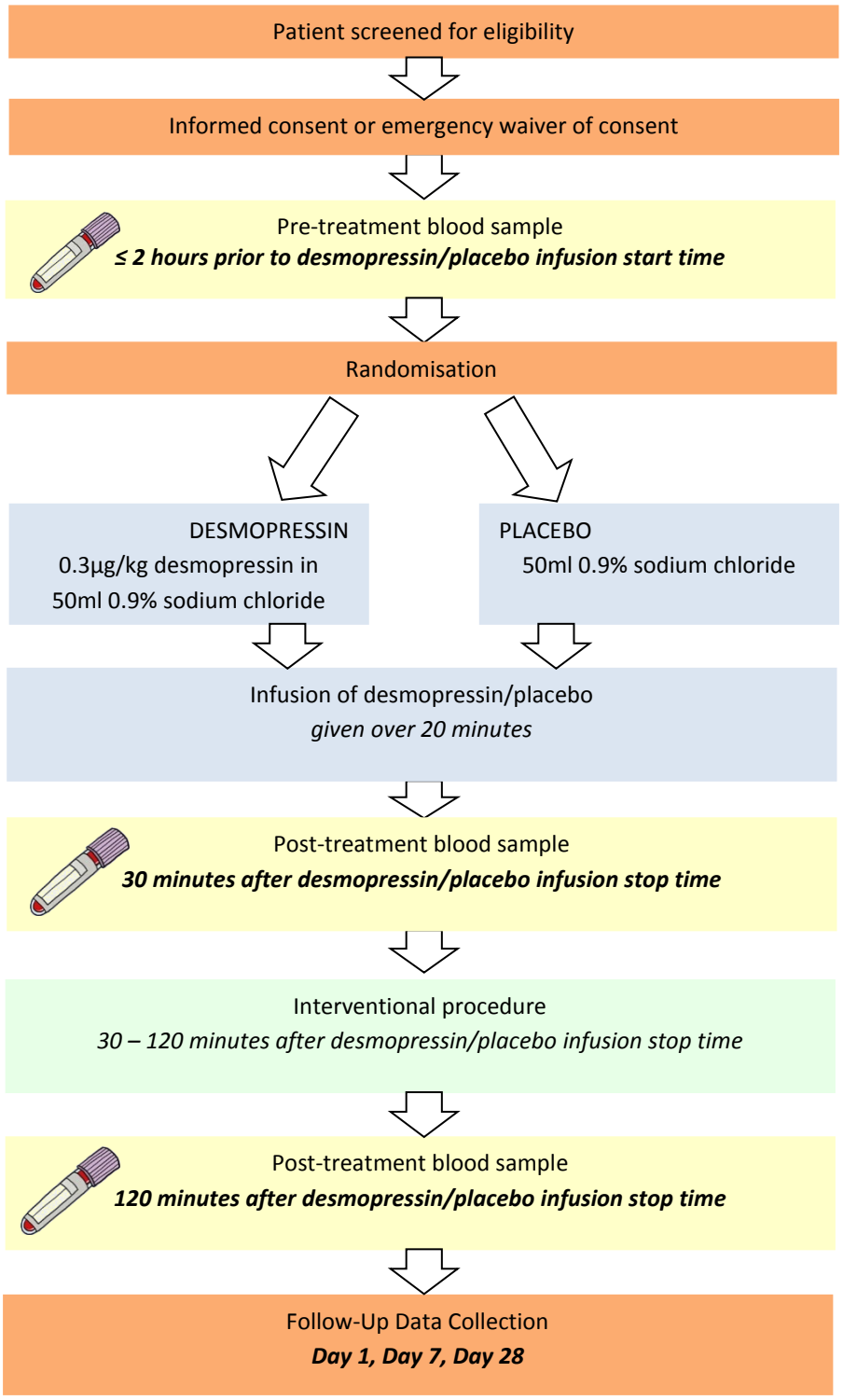


Figure 6.1 DRIVE trial overview.

6.2.2 Ethical and regulatory approval

Ethics approval was granted by the South Central - Oxford C Research Ethics Committee: reference number 16/SC/0524. The trial was approved by the Medicines and Healthcare products Regulatory Agency (MHRA) and Health Research Authority (HRA). It was prospectively registered:

- International Standard Randomised Controlled Trial Number (ISRCTN): ISRCTN12845429;
- European Clinical Trials Database (EudraCT): 2016-001126-33.

The trial is funded by an unrestricted grant from the NHS Blood and Transplant Trust Fund and is sponsored by NHS Blood and Transplant. The trial is supported by the NHS Blood and Transplant clinical trials unit.

6.2.3 Selection of participants

6.2.3.1 Inclusion criteria

- Adult patients (age 18 years or above)
- Platelet count ≤100 x 10^9/L
- Inpatient on a critical care ward
- Due to undergo an interventional procedure

Critical care was selected as the setting for this trial because thrombocytopenia is common and so are interventional procedures (4). The platelet count of 100 x10^9/l was selected because this is the highest threshold recommended in guidelines for transfusion of platelets: usually in the setting of neurosurgery or surgery on the posterior segment of the eye (29, 30). It should be noted that most guidelines

recommend a threshold of around 50 x10^9/l before interventional procedures (29, 30, 49).

Eligible interventional procedures include: central venous catheters; arterial lines; VasCaths; lumbar punctures; epidural catheter insertion or removal; chest, abdominal or pelvic drains; tracheostomy; and endoscopy.

6.2.3.2 Exclusion criteria

- Active bleeding
- History of ischaemic heart disease (myocardial infarction or angina), stroke or transient ischaemic attack (TIA)
- Current deep vein thrombosis or pulmonary embolism
- Patients in whom risk/s of fluid retention associated with desmopressin were judged clinically significant by the attending physician
- Admission to ICU with traumatic brain injury or seizures
- Hyponatraemia (sodium less than site reference range; <135 mmol/l in Oxford University Hospitals NHS Foundation Trust)
- History of anaphylaxis to desmopressin
- Pregnant or breastfeeding women
- Inherited bleeding disorder
- Insufficient understanding of English language (patient or patient's representative)

Desmopressin/placebo is used for prophylaxis rather than treatment of bleeding in this trial, so those with active bleeding are excluded. Patient groups considered to be at potentially heighted risk of thrombotic events, such as those with cardiovascular

disease or current venous thromboembolism are excluded. Likewise, patients considered to be at risk of hyponatraemia such as those with traumatic brain injury, seizures, fluid overload or hyponatraemia are also excluded. Patients with a history of anaphylaxis to desmopressin are excluded because of the risk of recurrence.

Desmopressin is used in pregnancy for women with inherited bleeding disorders (377, 378) with few adverse outcomes. No evidence of fetal malformations has been found when desmopressin has been used in animal studies. There have been reports of fetal malformations in the children of pregnant women taking regular desmopressin for diabetes insipidus although it is unclear if this is related to desmopressin (379). Consequently, women of child-bearing age are only included in the DRIVE trial following a negative pregnancy test.

Desmopressin is excreted into breastmilk, although at low concentrations. There have been no reports of adverse events in the infants of breastfeeding mothers taking desmopressin, and the dose of desmopressin transferred into breastmilk is unlikely to be sufficient to result in haemostatic effects or adverse events (379) but for the purposes of the DRIVE trial they are excluded.

Patients with an inherited bleeding disorder such as haemophilia (201), Von Willebrand disease (202) or an inherited platelet function disorder (29) are excluded because they are already thought to benefit from desmopressin.

6.2.4 Consent procedure

The majority of patients are likely to be incapacitated at the time of screening for this trial (for reasons which included critical illness, mechanical ventilation, and sedation).

The consent process therefore requires careful consideration in accordance with applicable regulatory requirements:

- International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use – Good Clinical Practice (ICH-GCP) (380);
- The Declaration of Helsinki (381);
- Medicines for Human Use (Clinical Trials) Regulations 2004 (382);
- Medicines for Human Use (Clinical Trials) Amendment No.2 Regulations 2006 (383).

Patient and public involvement was used to ensuring that the consent process was acceptable and that the information given to patients and their relatives was easy to understand. I discussed the trial was discussed at the ICU patient forum in Oxford with former patients and their families. They were happy with the consent process and made helpful improvements to the information sheets for patients and their families. There are three approaches by which consent can be obtained for participation in this trial. The approach depends on the urgency of the interventional procedure, and the availability of a suitable personal representative of the patient who is able to make a decision on their behalf. Capacity is assessed by appropriate members of the patient's clinical team, who are trained and have previous experience of assessing mental capacity. The consent process is summarised in Figure 6.2.

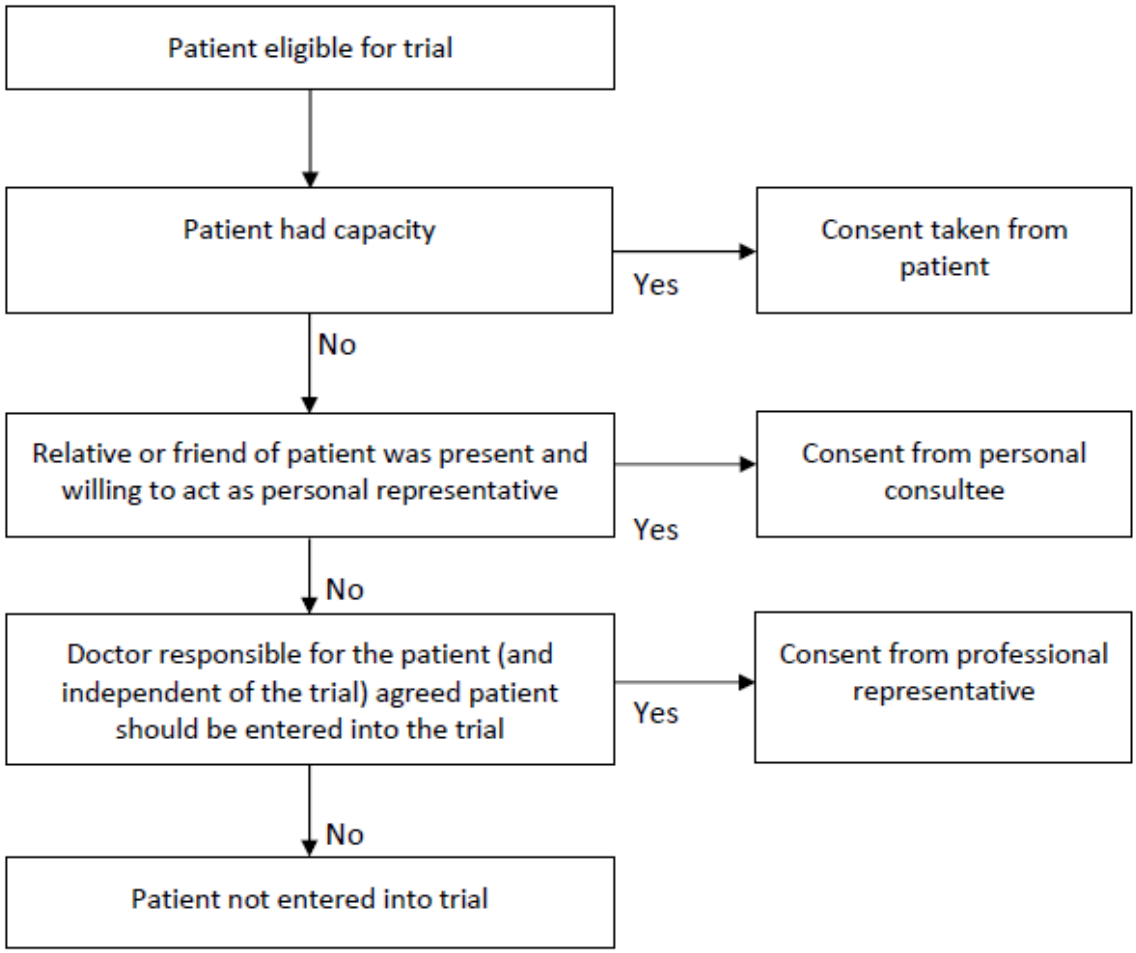


Figure 6.2 Overview of consent process

The approaches by which consent for this trial is obtained are detailed below:

If a patient is considered to have mental capacity they are invited to participate directly. Written informed consent is obtained. If the participant loses capacity during the trial, the consent previously given when capable remained valid. If a patient with capacity refuses participation in the trial and subsequently loses capacity, he/she is not entered into the trial.

If a patient lacks mental capacity, written informed consent is obtained from a representative prior to randomisation (Medicines for Human Use (Clinical Trials)

Regulations 2004) (382). If a relative or friend of the patient is present and willing to act as the patient's personal representative, they are approached and provided with an information sheet. This person has to be knowledgeable about the patient's values and beliefs. The representative is given the opportunity to ask questions, and is provided with a contact point for further details about the trial. The preference for consent is to approach a personal representative, but if there is no personal representative present and willing, a professional representative is approached. This is an independent doctor (not connected with the conduct of the trial) who is responsible for the care of the patient. This approach is only used when no suitable personal representative is available. If the patient regains capacity, the trial is discussed with them and their consent obtained for continuation in the trial. If consent is withheld, the patient is withdrawn from the trial.

If a patient lacks mental capacity, the interventional procedure is required urgently <u>and</u> it is not reasonably practicable to seek written informed consent from a patient representative, the patient can be entered into the trial following the emergency waiver of consent process (Medicines for Human Use (Clinical Trials) Amendment No.2 Regulations 2006) (383). If an eligible patient is identified, agreement for recruitment is sought from an independent doctor (not connected with the conduct of the trial) who is responsible for the care of the patient. Together, the principal investigator and independent doctor ascertain whether the patient is eligible and then decide whether the patient could be enrolled into the trial. This decision takes into account any known views of the patient, or patient's family about trial participation. For patients enrolled under this emergency consent procedure, the patient (if they regain capacity) or his/her personal or professional representative are informed about the

trial as soon as possible. Written informed consent for trial continuation is obtained. If consent is withheld, the patient is withdrawn from the trial. This approach is justified for use in this trial when there is a crucial need to administer desmopressin or placebo rapidly when a decision to perform a procedure is made.

These approaches are in accordance with the Medicines for Human Use (Clinical Trials) Regulations 2004 (382) and the Medicines for Human Use (Clinical Trials) Amendment No.2 Regulations 2006 (383). The requirements of the South Central Oxford – C research ethics committee are adhered to at all times.

The patient / patient representative is informed that they have the right to withdraw from the trial at any time and that this will not affect their / the patient's future treatment in any way. They do not have to provide a reason for withdrawal of consent.

When a participant dies during the study period (from randomisation until 28 days post-trial treatment) or does not regain mental capacity, they remain in the trial based on the informed consent provided by the patient representative (personal or professional), or independent doctor agreeing for trial participation under the emergency waiver of consent process.

6.2.5 Randomisation

Following informed consent (or waiver of consent) patients are randomised to one of two arms, in a 1:1 ratio:

- Desmopressin
- Placebo

The aim of randomisation is to create two groups which are comparable for known, or unknown confounding factors (384). It is important to avoid bias in the selection of

participants. If clinicians are able to anticipate the sequence used to allocate patients, this may lead to imbalances in those that are selected for recruitment. In order to prevent this, it must not be possible to anticipate the sequence used to generate randomisation or to anticipate the treatment allocation before randomisation (385). This is particularly important for outcomes that are relatively subjective, such as assessment of bleeding, but less critical for more robust outcomes such as overall mortality (385) (sections 6.2.5.1, 6.2.5.2 and 6.2.5.3).

6.2.5.1 Sequence generation

The allocation sequence was prepared by an independent statistician using a fixed block size. Allocation and block size were known to the independent statistician but not to any other members of the clinical trial team.

6.2.5.2 Allocation concealment

Patients are allocated to the intervention or placebo arms using sealed opaque envelopes. These envelopes were prepared using a randomisation schedule drawn up by the independent statistician, and are held in a secure location to which the trial team have access. Each envelope is numbered and contains a card, stating the randomisation number and the arm of the trial to which the participant is allocated. Envelopes are opened in sequential numerical order, and the card containing the information about the allocated arm is stored by the site coordinator in a locked drawer or equivalent, so that it cannot be seen by other members of the site staff or the participant.

6.2.5.3 Blinding

In this trial the participants and clinical trial team are blinded to the treatment allocation. The independent statistician who produced the allocation sequence is not blinded. The members of the local team who randomise each participant and prepare and check the desmopressin/placebo are not blinded. One of these staff members

administered the desmopressin/placebo. Staff who are not blinded are not involved in the patient's trial-related care beyond this point, and are asked not to reveal the treatment allocation to other staff members. The rationale for this approach is that the duration of stability of desmopressin once it is suspended in 0.9% sodium chloride is unknown and standard practice is to administer it immediately after it has been diluted. This means that it has to be made on the Intensive Care Unit immediately prior to administration. Infusing the drug over 20 minutes diluted in 50mL 0.9% sodium chloride reduces the risk of participants experiencing facial flushing, which could result in unblinding (as it would not be expected to occur in participants randomised to placebo). For this initial feasibility trial, manufacture of a matching placebo was too costly, but this would offer a solution for a larger future trial.

6.2.6 Intervention

Desmopressin 0.3 μg/kg in 50ml 0.9% sodium chloride, infused intravenously over 20 minutes (rate of 150ml/hr). This desmopressin dose was most commonly used in clinical trials for reversal of antiplatelet agents (Chapter 5) and is the recommended dose for treatment of Von Willebrand disease (202). Increasing the dose beyond 0.3 μg/kg does not result in a greater increase in plasma VWF levels, whereas reduction of the desmopressin dose to 0.2 μg/kg results in lower plasma VWF levels (189). Desmopressin can be administered orally or intranasally but has poor bioavailability through these routes and less consistent plasma levels are achieved (188, 189). It may also be administered in a concentrated form subcutaneously with similar effect to intravenous administration (188). The majority of randomised trials outside the setting of inherited bleeding disorders have used the intravenous route for administration (Chapter 4) and in critically ill patients subcutaneous medicines may be poorly

absorbed, particularly amongst patients receiving vasopressors (386), so I opted for the intravenous route for administration.

6.2.7 Comparator

The comparator is 50 ml 0.9% sodium chloride, infused intravenously over 20 minutes (rate of 150 ml/hr). This was selected to appear identical to desmopressin when it is administered to patients.

6.2.8 Concomitant care

Platelet transfusions are given to participants if clinically indicated, and this is at the discretion of the treating clinician. If a platelet transfusion is given between collection of the pre-desmopressin/placebo (baseline) and post-desmopressin/placebo blood samples, results for thrombus formation under flow and PFA closure time will not be included in the final analysis for these time-points. Transfusion of all other blood components is performed at the discretion of the clinician in charge of the participants' care in concordance with local protocols. All other medications as directed by the attending clinician are permitted. There is no change to any aspect of planned care or treatment whilst a patient is participating in the trial. Patients can receive desmopressin again off-trial at the discretion of the treating physician, but each patient can only be randomised into the study once.

Participants cannot be enrolled into other trials involving platelet transfusions, anti-fibrinolytics, platelet growth factors or other pro-haemostatic agents. Participation in any other type of trial is considered on a case-by-case basis.

6.2.9 Study outcomes

6.2.9.1 Primary outcome

The primary outcome is the proportion of eligible patients who are randomised into the trial and receive desmopressin/placebo. This primary outcome was selected because there are distinct challenges to running a trial in critically care. These include:

- Consent: many participants lack capacity
- Concurrent illness: such as cardiovascular disease
- High number of emergencies and out of hours admissions
- Acceptability to intensive care clinicians

Consequently, before proceeding to a larger trial to determine efficacy, it is necessary to ensure that recruitment to a trial with this design is feasible.

6.2.9.2 Secondary outcomes

1. Adherence to protocol

2. Time taken to administer desmopressin/placebo (from randomisation)

3. Difference in change in thrombus formation under flow before and after infusion between desmopressin and placebo

4. Difference in change in PFA closure time for C-ADP and P2Y cartridges before and after infusion between desmopressin and placebo

5. Difference in change in thrombin generation before and after infusion between desmopressin and placebo

6. Bleeding up to 24 hours after administration of desmopressin/placebo

7. Thromboembolic events up to 28 days after administration of desmopressin/placebo

8. Exposure to blood components (red cells, platelets, fresh frozen plasma or cryoprecipitate transfusion) up to 24 hours after administration of desmopressin/placebo

The overall incidence of bleeding as a result of invasive procedures is relatively low and is confounded by factors such as operator technique and experience (387). Consequently, to obtain initial evidence of efficacy I have used laboratory endpoints. To establish the combined role of VWF and platelets, I am using thrombus formation under flow and PFA closure time (as described in section 1.3.2.1). Although this trial is not powered to detect differences in bleeding rates, these are recorded, as is the incidence of serious adverse events. Bleeding assessments and reporting of thrombotic events are standardised as outlined in section 6.2.10.

6.2.10 Assessments and follow-up

6.2.10.1 Bleeding

Bleeding assessment is performed using the HEmorrhage MEasurement (HEME) bleeding assessment tool (Table 6.1) (388). This tool was developed for use in critical care and has been validated in several subsequent trials including ISOC-2 (ISRCTN50516147). This tool categorizes bleeding by the site where it occurs (for instance the site of a vascular catheter insertion) and by the severity of bleeding (minor bleeding, major bleeding and fatal bleeding).

BLEEDING SITE

- **Vascular catheter or insertion site**
  1. Side
     i. Right ii. Left
  2. Site
     i. Peripheral vein-arm
     ii. Peripheral vein leg
     iii. PiCC line
     iv. Femoral line
     v. Jugular line
     vi. Subclavian line
     vii. Other (specify)

- **Other skin bleeding (not catheter related)**
  1. Bruising
  2. Petechiae
  3. Non-surgical wound

- **Gastrointestinal**
  1. Nasogastric tube blood
  2. Haematemesis
  3. Melaena
  4. Haematochezia

- **Surgical site**
  1. Incision 2. Drain

- **Respiratory**
  1. Tracheostomy
  2. Endotracheal tube aspirate
  3. Haemoptysis
  4. Chest drain

- **Genitourinary**
  1. Gross haematuria
  2. Vaginal bleeding
  3. Bleeding around urinary catheter

- **Retroperitoneal**

- **Intracranial**

- **Intraspinal or epidural**

- **Intraocular** (not subconjunctival)

- **Pericardial**

- **Intraarticular** (non-traumatic)

- **Other** (describe)

BLEEDING SEVERITY (categorised as fatal, major or minor bleeding)

1. Fatal Bleeding

2. Major Bleeding (non-fatal bleeding, associated with ANY of the features below)

- Decrease in haemoglobin of 20 g/l or more
- Transfusion of two or more units of RBCs with no increase in haemoglobin
- Decrease in systolic blood pressure by 10 mmHg or more while patient sitting up
- Spontaneous decrease in systolic blood pressure of 20 mmHg or more
- Increase in heart rate by 20 bpm or more
- Bleeding at ANY of the following critical sites:
  a. Intracranial d. Intraocular (not subconjunctival)
  b. Retroperitoneal e. Pericardial
  c. Intraspinal f. Intraarticular (non-traumatic)
- Wound-related bleeding requiring an intervention

3. Minor bleeding (bleeding not meeting criteria for fatal or major bleeding)

TIMING

1. New 2. Ongoing 3. Recurrent bleed at same site

Start date_____ **Start time**_____
Stop date_____ **Stop time**_____

Table 6.1 HEME bleeding assessment tool. Adapted with permission from Arnold et al. Bleeding during critical illness: A prospective cohort study using a new measurement tool.

Clinical and Investigative Medicine 2007; **30**(2): E93-102 (388). bpm: beats per minute, PiCC: peripherally inserted central catheter, RBC: red blood cell.

6.2.10.2 Thrombotic Events

Myocardial infarction is defined as detection of an elevated cardiac troponin I with at least one value above the upper reference limit in the context of myocardial ischemia ***and*** at least one of the following:

- Symptoms of myocardial ischaemia;
- New electrocardiogram (ECG) changes indicative of ischaemia (for example ST segments changes or new left bundle branch block) or development of new T wave inversion or pathological Q waves
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.
- Autopsy evidence of recent myocardial necrosis (adapted from Carson et al, 2011 (389))

Stroke is defined as evidence of a new haemorrhage or infarction on a magnetic resonance image study, computerised tomography (CT) scan of the head, or autopsy ***and*** new neurologic deficit lasting at least 24 hours (adapted from Carson et al, 2011 (389)).

Venous thromboembolism is defined as deep venous thrombosis confirmed by duplex ultrasound, magnetic resonance venography, venogram, or post-mortem examination; or pulmonary embolism confirmed by high probability ventilation perfusion scan, pulmonary angiogram, magnetic resonance angiography, spiral CT scan, or post-mortem examination (adapted from Carson et al, 2011 (389)).

6.2.11 Laboratory tests

VWF assays (section 2.2.6.1), thrombin generation (section 2.2.6.2), and PFA closure time (section 3.2.6) are performed as previously described. I opted to test thrombin generation in PPP, rather than PRP because I found the variability in results to be too high using platelet rich plasma. Thrombin generation using PPP will be influenced by changes in FVIII due to desmopressin but will not take account activation of platelets or upregulation of phosphatidylserine. Thrombus formation under flow was performed as described in section 3.2.5 but I have adapted the technique to ensure it can be performed rapidly when participants are recruited. Platelets are not labelled with DiOC6 at the time of perfusion through the flow chamber and experiments are performed at room temperature. Following 5 minutes of flow of whole blood through the flow chamber, modified Tyrode's solution is passed through the flow chamber for 1 minute, then platelets are fixed with 10% formylsaline (Sigma). Microscopy is performed within 24 hours of fixing the sample. Thirty minutes prior to microscopy, 2 μM DiOC6 in modified Tyrode's solution is infused through the flow chamber to stain the platelets.

6.2.12 Safety reporting

All serious adverse events, occurring from the start of trial treatment until 28 days post-trial treatment are recorded. This was a condition of MHRA approval of the trial. Non-serious adverse events are not recorded because they are very common in critically ill patients and desmopressin is a drug with a known adverse event profile. In the event that a serious adverse event occurs I will assess whether there is evidence of causality with administration desmopressin/placebo and whether it is an expected or unexpected event.

6.2.13 Statistical analysis

6.2.13.1 Sample size calculation and duration of trial

The primary outcome in this feasibility study is the proportion of eligible patients, meeting all the inclusion criteria and none of the exclusion criteria, who are randomised into the trial and receive desmopressin/placebo. If 50% of eligible patients are randomised, the exact 95% confidence interval for this proportion would be 39% to 61%. So the precision of the estimate would, at worst, be ±11 percentage points, which would be adequate to allow design a larger efficacy trial. The precision of this estimate will improve if the proportion of eligible patients who are randomised is greater than, or less than 50%.

I aim to randomise 40 patients over 15 months to make a future trial feasible. In the *Transfusion of Fresh Frozen Plasma in Non-bleeding Intensive Care Unit Patients* (TOPIC) trial (390), which recruited a similar group of critically-ill patients and randomised them to receive fresh frozen plasma versus no treatment, 29.8% of eligible patients received FFP/no treatment. Applying this to DRIVE, if the 40 patients receiving desmopressin/placebo represented 30% of eligible patients, the exact 95% confidence interval would be 21% to 39% (±9 percentage points).

To calculate the time necessary to recruit this number of patients, I relied on a retrospective analysis of three years of admissions to the critical care units in Oxford. These data indicating that: approximately 1400 patients per year were admitted to ICU in the two hospitals participating in this trial; 80% of these patients underwent an invasive procedure; and 19% had a platelet count $<100 \times 10^9$/l. It was assumed that 50% of ICU patients meeting these two inclusion criteria would meet at least one of the exclusion criteria, so that overall there would be 106 eligible patients each year. If the required 40 patients were to represent 30% of the total, then 133 eligible patients

would be needed. Based on all these assumptions, I expected the target sample size to be achieved in 15 months.

6.2.13.2 Other statistical considerations

This is a feasibility study and the final results of the trial will presented as descriptive statistics only. I have conducted an interim analysis after 5/15 months of the trial (February to June 2017) and feasibility results and baseline data are summarised in section 6.3.

Counts were summarised using N and %. Normally distributed continuous variables were summarised using means ± standard deviations. Non-normally distributed continuous variables were summarised with median (range).

6.3 Interim results

6.3.1 Participants

Over the first five months of the trial there were 510 admissions to intensive care (summarised in Figure 6.3). Thirty seven patients (undergoing 55 procedures) met the inclusion criteria. This was 42% of the expected number. The difference was largely due to interventional procedures performed in the emergency department immediately before admission to ICU in in operating theatres. These procedures were often performed by the intensive care team immediately before admission to the intensive care unit. Eighteen were excluded with the most common reasons being thrombotic risk (history of ischaemic heart disease, cerebrovascular accident or acute venous thromboembolism); or hyponatraemia. This was consistent with the pre-trial estimate of 50% of eligible patients having at least one exclusion criterion. Nineteen patients met all the inclusion and exclusion criteria but 13 were not approached. Eight of these patients underwent procedures at night or during the weekend. Five other patients

were missed by the screening process. Of the six patients who were approached, four were randomised.

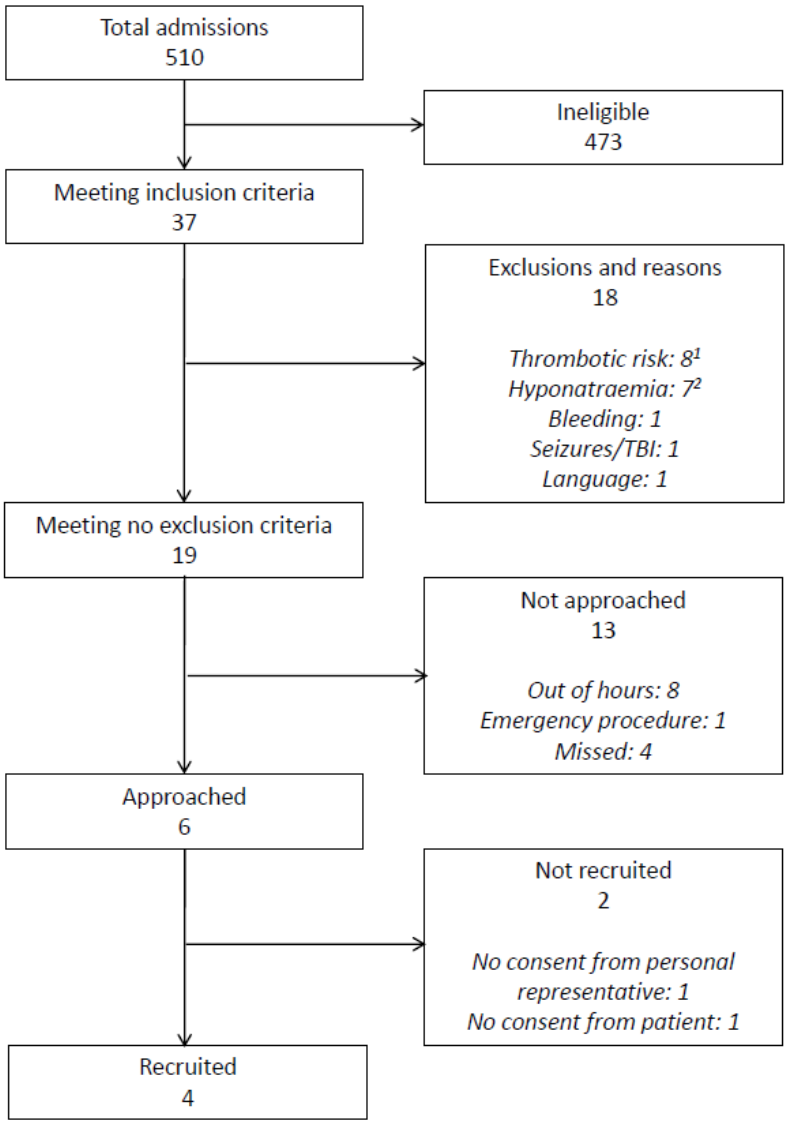


Figure 6.3 DRIVE recruitment over five months. TBI: traumatic brain injury. [1]Two patients excluded for thrombotic risk also presented out of hours. [2]Four patients excluded for hyponatraemia also presented out of hours.

One patient was able to give consent and three lacked capacity. For those that lacked capacity, agreement from a professional representative was initially used for consent. In one case, consent was obtained retrospectively from the patient, and in two cases

retrospective consent was taken from a personal representative. Recruitment was less than the predicted rate of recruitment from before the trial commenced (Figure 6.4).

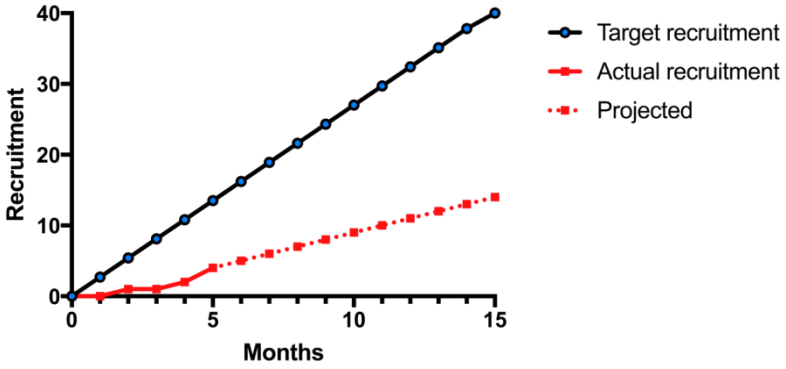


Figure 6.4 DRIVE trial recruitment. Projected recruitment in this graph assumes that no changes are made to the protocol or the number of trial sites.

6.3.2 Baseline characteristics

Baseline characteristics of patients included in the DRIVE trial are reported in Table 6.2. At the time of writing I am still blinded to treatment allocation, so outcome data are not reported by arm to avoid unblinding before completion of the trial.

Baseline characteristics	
Age (years)	56 (42 to 69)
Gender	
Male	2 (50%)
Female	2 (50%)
APACHE II score	14 (8 to 19)
Haemostatic or anticoagulant medications	
Tranexamic acid	2 (50%)
Vitamin K	2 (50%)
Dalteparin (thromboprophylaxis)	1 (25%)
Blood results	
Platelet count ($x10^9$/l)	44.5 (10 to 91)
Prothrombin time ratio	1.16 (1.08 to 1.67)
Fibrinogen (g/l)	3.1 (2.1 to 3.8)
Von Willebrand Factor antigen (iu/ml)	4.33 (2.91 to 6.48)
Creatinine (µmol/l)	50.5 (44 to 72)
Thrombin generation	
Lag time (mins)	2.33 (2.22 to 2.67)
Peak (nM)	269 (145 to 334)
Endogenous thrombin potential (nM.min)	1390 (1186 to 1940)
Time to peak (mins)	4.33 (4.11 to 5.11)
Platelet function analyser closure time	
C-ADP cartridge (secs)	148 (83 to >300 seconds)
P2Y cartridge (secs)	173 (139 to >300 seconds)
Thrombus formation under flow	
Surface coverage (%)	6.5 (4.2 to 9.3)
Mean fluorescence intensity (units)	17 (10.2 to 23.7)

Table 6.2 Baseline characteristics of DRIVE participants: February to June 2017. C-ADP: collagen and adenosine diphosphate. Data expressed as median (range), or n (%).

The 37 screened patients underwent a total of 55 procedures. The most common procedures in thrombocytopenic patients were insertion of arterial lines, central venous catheters and wide-bore central lines used for dialysis (VasCaths) (Table 6.3).

Procedure	Recruited participants	Procedures in participants meeting inclusion criteria	Platelet count ($x10^9$/l)
Arterial line	1 (25)	16 (29)	68 (7 to 97)
Central venous catheter	2 (50)	15 (27)	58 (9 to 97)
VasCath	-	10 (18)	64 (16 to 99)
Removal of wide bore line/drain	1 (25)	5 (9)	82 (59 to 97)
PiCC	-	3 (5)	10 (9 to 83)
Chest drain	-	2 (4)	55.5 (22 to 89)
Other procedures	-	4 (7)	64.5 (6 to 72)
Total	**4**	**55[1]**	**67 (6 to 99)**

Table 6.3 Types of interventional procedures. [1]37 screened patients underwent 55 procedures. PiCC: peripherally inserted central cannula. Results expressed as n (%) or median (range).

The time from randomisation to the end of the interventional procedure took median 109.5 minutes (range 55 to 220 minutes; Table 6.4). The protocol was adhered to in all four cases

Procedures	Time (minutes)
Randomisation to administration of IMP	15.5 (14 to 35)
Administration of IMP to procedure start	84.5 (30 to 165)
Procedure start to procedure end	12.5 (5 to 20)
Randomisation to end of procedure (total time)	*109.5 (55 to 220)*

Table 6.4 Time taken to administer desmopressin/placebo (from randomisation). Results expressed as median (range). IMP: investigative medicinal product (desmopressin or placebo)

No bleeding events occurred in the 24 hours following the procedure for participants recruited between February and June 2017. Eleven serious adverse events were reported from the four participants recruited into the trial. Two of these (clinically significant hypotension and a non-ST-elevation myocardial infarction) were considered to potentially represent serious adverse reactions (Table 6.5).

Clinical outcomes	N
Bleeding[1]	
Major bleeding	0
Minor bleeding	0
Transfusion exposure[1]	
Red blood cells	2
Platelets	2
Fresh frozen plasma	0
Cryoprecipitate	0
Serious adverse events[2]	
Acute kidney injury	2
Atrial fibrillation	1
Clinically significant hypotension[3]	1
Gastrointestinal bleed	1
Non-ST-elevation myocardial infarction[3]	1
Pneumomediastinum	1
Raised intra-abdominal pressure	1
Respiratory failure	1
Ventilator-associated pneumonia	1
Withdrawal of active care	1
Outcome[2]	
Death on intensive care unit	3
Discharge from intensive care unit	1

Table 6.5 Clinical outcome data. DRIVE trial participants from February to June 2017. [1]Up to 24 hours after desmopressin/placebo. [2]Up to 28 days after desmopressin/placebo. [3]Reported as serious adverse reactions.

6.4 Discussion

6.4.1 Summary of key results

Recruitment to the DRIVE trial has proved challenging with a smaller pool of eligible patients than expected. At present, the DRIVE trial is recruiting 11% of potentially eligible patients, rather than 30% as initially calculated. There are many challenges when conducting a clinical trial in a population of critically ill adults. Barriers to

feasibility and potential strategies for overcoming these barriers are described in section 6.4.2.

Intensive care clinicians were happy for patients to be recruited into the trial when their platelet counts were less than 100 $x10^9$/l given uncertainty over the bleeding risk. Given the high prevalence of co-existent coagulopathy, antiplatelet/anticoagulant medications, or renal failure, clinicians considered bleeding risk to be elevated compared to the absolute numbers from standard blood tests (391). The interim results suggest that it is feasible to administer desmopressin/placebo quickly once a patient is randomised. There are no missing clinical data from the participants who have entered the trial up to the point of this interim analysis.

Baseline laboratory results suggest that VWF levels are very high in this patient group and it will be important to assess whether desmopressin can increase VWF levels further given this high baseline. In cardiac surgery, it has proved feasible to raise VWF levels further, even given a high baseline level (83, 200). However, I am not aware of any desmopressin trial with baseline VWF levels as high as those found in the initial four patients recruited into this trial.

6.4.2 Barriers to feasibility and strategies for management

6.4.2.1 Methodology

The most challenging part of the method proved to be getting two members of the clinical team to randomise patients and to draw up the desmopressin/placebo, while maintaining blinding. In Oxford, there is a large pool of clinical staff (approx. 130 intensive care nurses and 30 doctors) and training all of them is difficult. The complexity of this process also means that a member of the trial team needs to be on hand to offer supervision and assistance. In a larger trial a matching placebo could be

manufactured. If this were the case then a research nurse could randomise participants, draw up the desmopressin/placebo and collect outcome data. While this cannot be overcome in this trial, it will be considered if this moves forward to a larger trial.

Meta-analyses examining methods for improving the proportion of patients recruited into trials (392, 393) have found that the following techniques might improve recruitment:

- Using an open label design (394-396). This might improve recruitment but would mean that the validity of subjective outcomes such as the incidence of bleeding would be compromised.
- An "opt-out" approach for recruitment (397). The process of waiver of consent already makes this possible if desmopressin/placebo is considered before emergency procedures (383).

Although these methods have efficacy in other settings, the main barrier to recruitment in the DRIVE trial is identifying eligible participants, rather than consent.

6.4.2.2 Inclusion and exclusion criteria

I have re-evaluated the inclusion and exclusion criteria to see if adjustments could be made to improve recruitment while ensuring safety of trial participants.

6.4.2.2.1 Hyponatraemia

Desmopressin may cause hyponatraemia by causing the syndrome of inappropriate antidiuretic hormone secretion, and consequently all patients with serum sodium below the reference range were excluded in order to minimise this risk. However this resulted in exclusion of a large number of patients with mild hyponatraemia (130 to 134

mmol/l). I have held discussions with specialists in intensive care, biochemistry and endocrinology. We concluded that in the intensive care setting where sodium is monitored closely (often three times daily by venous samples; and every 2 to 3 hours by arterial blood gas sampling) that these precautions might be excessive. We noted that in 60/62 trials of desmopressin versus placebo outlined in Chapter 4, there was no hyponatraemia exclusion criterion. In the two trials which did include hyponatraemia as an exclusion criterion, the threshold was set at <130 mmol/l (298, 366). No trial reported hyponatraemia as an adverse event. Furthermore, this is not listed as an exclusion in the summary of product characteristics for desmopressin (379) but "history of hyponatraemia" is listed as a contraindication in the British National Formulary (398). Reducing the threshold for exclusion from "sodium below the reference range" (135 mmol/l in Oxford) to 130 mmol/l would allow me to have approached four additional participants, although it should be noted that 2/4 underwent procedures outside normal working hours. I plan to make a major amendment to the protocol to adjust the threshold for defining exclusion due to hyponatraemia to a serum sodium of <130 mmol/l.

6.4.2.2.4 Thrombotic events

The most common reason for exclusion was a history of ischaemic heart disease, transient ischaemic attack, stroke or venous thromboembolism. The meta-analysis results summarised in Chapters 4 and 5 suggested no increased risk of thrombotic events. However there were too few patients to be certain of this and it was classified as low quality evidence by GRADE criteria. In the prophylactic setting where the risk of bleeding is relatively low, it is essential to consider whether there is equipoise between the benefits and risks of trial participation. On balance, patients with a potentially elevated risk of thrombotic events will continue to be excluded from the DRIVE trial.

6.4.2.2.3 Out of hours procedures

Procedures occurring out of hours are challenging to manage, especially given the complexity of the randomisation process and drawing up the desmopressin/placebo. Simplification of the trial process might make recruitment easier if the trial team are not present.

6.4.2.3 Number of eligible patients

Recruitment was 30% of the predicted recruitment rate. 11% of patients who met all the inclusion criteria were recruited. In order to reach my target of recruiting 40 patients, I am aiming to set up two further trial sites. The expression of interest summaries from these sites, suggest that it will be possible to recruit an additional 2-3 patients per month if they join the DRIVE trial.

6.4.2.5 Serious adverse event reporting

There were 12 serious adverse events reported between the four patients recruited in the first five months of the trial. In critical care, it is often difficult to assess whether the desmopressin or placebo may have a causal relationship with serious adverse events. Those which plausibly could be associated with desmopressin (clinically significant hypotension and non-ST-elevation myocardial infarction) have been classified as serious adverse reactions. Challenges in assessing causality for thrombotic events are due to the high background incidence of thrombotic events in the critically ill population (399, 400), the majority of which are clinically silent (399). The data monitoring committee for the DRIVE trial continues to review all serious adverse events and advise on the safety of continuing the trial.

6.4.3 Strengths and limitations

To my knowledge, this is the first randomised trial to assess whether desmopressin could compensate for thrombocytopenia. It is designed to be at low risk of bias. The use

of microfluidic thrombus formation under flow and PFA closure time, allow examination of the interaction between VWF and platelets.

Following this interim analysis, clear challenges have been identified for recruitment of participants. Some of these may be overcome through the measures outlined in section 6.4.2 above.

Bleeding events are relatively rare amongst patients with thrombocytopenia undergoing procedures, even though they are at increased risk compared to patients with a normal platelet count. Consequently, it is challenging to power a feasibility/pilot study on the basis of clinical outcomes.

Baseline VWF levels are high in this patient group. This may be due to a combination of factors including: enhanced release of ADP; increased thrombin generation; elevated levels of circulating histones; and administration of vasopressors (adrenaline and vasopressin) (61, 68). An important finding from the final results of this trial will be establishing at baseline the extent that VWF already compensates for thrombocytopenia. Furthermore, the extent to which desmopressin can increase the level of VWF further will be important for assessing the potential efficacy. Desmopressin does not appear to increase thrombotic risk in most patient groups (including patients who would be expected to have high baseline VWF levels, for examples following cardiac surgery) but an important safety outcome will be assessing if there is any increase in thrombotic risk for patients who already have high baseline VWF levels. While there is some evidence that desmopressin might alter intracellular platelet calcium signalling (182), I suspect that the majority of its action is through the increase in VWF levels.

It does appear feasible to perform laboratory tests of platelet function in this patient group. In ¾ recruited patients, it was possible to record a PFA closure time using both C-ADP and P2Y cartridges and this might offer a simple method for monitoring the effects of desmopressin for the treatment of thrombocytopenia in the future. Thrombus formation under flow provides more detailed information on platelet adhesion, platelet aggregation and clot retraction. However it is a complex test to use and is not validated for prediction of bleeding in the clinical setting. Baseline data from patients recruited into this study might enable us to better assess the relationship between thrombus formation and bleeding risk for patients undergoing interventional procedures.

The large proportion of patients who met at least one of the exclusion criteria is another potential barrier to widespread uptake of desmopressin for prevention of bleeding in thrombocytopenia. This is the most important factor that must be addressed in order to proceed to a larger future trial.

6.4.4 Conclusions

The rationale for performing a feasibility trial is to establish whether desmopressin/placebo could be administered in critical care; whether it would be possible to proceed with a larger trial to demonstrate efficacy; and if so, how this would be done (401). I selected recruitment as the key criteria for defining whether it is feasible to progress to a larger trial. Challenges with recruitment for this trial might prove difficult to overcome simply by modifying the protocol. It is possible that more patients could be recruited if the exclusion criterion for hyponatraemia was adjusted and more participants were recruited out of hours. There is a high level of adherence with the trial intervention and collection of outcome data.

Overall, there is a small pool of potentially eligible patients and the best strategy is likely to be increasing this pool of patients by increasing the number of sites. The next steps for the DRIVE trial are recruitment of two additional sites and this process is underway at present. A future larger trial might struggle to recruit sufficient patient numbers to prove a difference in an outcome such as major bleeding, and this is an important finding from the initial analysis of this feasibility study. It is likely that decisions on the use of desmopressin might be based upon laboratory data demonstrating improvements in haemostasis, providing that the adverse event profile is acceptable in this setting.

The objectives of this trial are important and have the potential to improve care for critically ill thrombocytopenic patients. However barriers to feasibility must be overcome first.

Chapter 7

7 Discussion and future work

The overarching objective of the work described in this thesis was to investigate whether desmopressin could be used for treatment of thrombocytopenia or platelet dysfunction. There were five main aims of this research, which are summarised below.

7.1 Aim 1: To determine if levels of VWF or other measures of haemostasis are predictive of bleeding in severe thrombocytopenia.

Patients with haematological malignancy and profound thrombocytopenia due to intensive chemotherapy had many changes in haemostasis including significantly increased VWF antigen, thrombin generation parameters (lag-time, ETP and time-to-peak), Clauss fibrinogen, PAP complexes, d-dimers and CRP. Many of these haemostatic factors rise as part of the acute phase response.

Importantly, none of these measures of haemostasis, including VWF antigen levels, were consistently predictive of bleeding and I found no evidence that they might compensate for thrombocytopenia in this setting. This is the first time that an expanded set of measures of haemostasis have been applied to haematological malignancy and linked with bleeding data. Because the sample size is relatively small, and there is considerable clinical heterogeneity between patients, I consider these results to be exploratory.

Evidence of elevated PAP complexes and d-dimers in patients with haematological malignancy undergoing intensive chemotherapy suggests that there is increased fibrinolysis in this patient group. However it is likely to be due to an increase in fibrin deposition, rather than an increase in vulnerability to fibrinolysis because the clot lysis potential is no different to normal controls. There are four large ongoing trials

investigating the use of anti-fibrinolytic drugs in thrombocytopenia (138) and this provides additional rationale for their use.

VWF is of particular interest due to its role in primary haemostasis but might not be the best target for treatment at the very low platelet transfusion thresholds (<10 $x10^9$/l) used to prevent spontaneous bleeding in profound thrombocytopenia (29, 30). I suspect that VWF might not compensate for thrombocytopenia at very low platelet counts. Consequently, I moved the research focus to the setting of preventing bleeding before invasive procedures or surgery where the target platelet count is higher (20 to 100 $x10^9$/l depending on the procedure) (29).

7.2 Aim 2: To determine whether VWF compensates for thrombocytopenia *in vitro*

It was possible to simulate thrombocytopenia in normal blood using a platelet-depletion technique. This process did not result in pre-activation of platelets but did reduce their response to platelet agonists (TRAP-6, ADP and CRP-XL). This technique could be used in the future for research into conditions where thrombocytopenia is a feature.

Thrombus formation under flow could be used to distinguish between different levels of thrombocytopenia and was responsive to VWF levels. Further work in Chapter 2 evaluated the best way to use this technique.

These results were corroborated by increased surface coverage and decreased PFA closure time for thrombocytopenic patients when Haemate P was added. This provides a technique that could potentially monitor the effects of desmopressin in a clinical trial by evaluating the combined role of VWF and platelets in thrombus formation. It also has the potential to assess how changes in VWF levels might compensate for the effects of

antiplatelet agents. The PFA was insensitive at low platelet counts but might provide a simple method for monitoring the effects which could be used in clinical practice.

7.3 Aim 3: To assess the evidence for the efficacy of desmopressin in all patients undergoing surgery or invasive procedures

The simplest approach to a trial evaluating desmopressin before procedures would be to include unselected patients, rather than only those with platelet dysfunction or thrombocytopenia. Meta-analysis of randomised controlled trials of desmopressin versus placebo or no treatment before surgery demonstrated that for unselected patients, desmopressin might be ineffective. Amongst unselected patients, it did not reduce the number of patients receiving a red cell transfusion or the number returning to theatre with bleeding. There was evidence that outcomes such as total blood loss, which have a subjective element, were reduced with desmopressin. However there was evidence of considerable publication bias, which might account for these results.

Close scrutiny of the published evidence revealed several gaps in the evidence base. Desmopressin appeared more effective when used before interventional procedures than before major surgery. In the setting of interventional procedures, the risk of bleeding was significantly reduced. It is possible that this effect is present because desmopressin has a relatively short half-life (189) and the increase in VWF that occurs independently of desmopressin due to surgery is likely to be greater than the increase with more minor procedures.

While there was no evidence of a clinically significant benefit with desmopressin in unselected patients, there was also no significant evidence of harm. Even amongst patients undergoing cardiac surgery for coronary artery bypass grafts, who might be considered to be at high risk of thrombotic events, there was no significant difference in

thrombotic risk between desmopressin and placebo. Clinically significant hypotension (which required treatment with intravenous fluid or inotropes) was increased in patients taking desmopressin although this appeared more common amongst those administered desmopressin at a faster infusion rate. This provided some reassurance that desmopressin appears safe outside the setting of inherited bleeding disorders.

This is the largest meta-analysis of the peri-operative use of desmopressin and provides the most up to date and largest analysis of desmopressin in this setting.

7.4 Aim 4: To assess the evidence for desmopressin for platelet dysfunction or thrombocytopenia

By contrast to desmopressin for unselected patients, desmopressin did appear to be effective for reducing perioperative bleeding for patients taking antiplatelet agents or with confirmed platelet dysfunction. The risk of returning to theatre with bleeding was significantly reduced, as was the total volume of red cells transfused and total blood loss. Serious adverse events were rare in these trials but once against there was no evidence of an increase in the risk of thrombotic events.

Although desmopressin and tranexamic acid are often given together for patients with inherited bleeding disorders, I found no evidence of a benefit for desmopressin amongst patients who received both agents, although the evidence was of low quality.

Overall, desmopressin appeared considerably more effective for patients with evidence of platelet dysfunction than for unselected patients.

There is a gap in the evidence, with no randomised controlled trials of peri-operative desmopressin for thrombocytopenic patients. I identified two case series examining the use of desmopressin for treatment of bleeding patients with haematological

malignancies (198, 199). These studies concluded that bleeding was stopped in all cases following desmopressin administration, but without a control group, it was not possible to determine if this was due to the effects of desmopressin. The paucity of evidence in the setting of thrombocytopenia and potential for desmopressin to compensate for deficits in primary haemostasis led to the design of the DRIVE trial.

7.5 Aim 5: To determine if it is feasible to use desmopressin to treat critically ill thrombocytopenic patients in a clinical trial.

The DRIVE trial is the first randomised trial of desmopressin versus placebo before interventional procedures in patients with thrombocytopenia. This trial is still actively recruiting at the time of writing.

Critical care was selected as the setting for this trial because both thrombocytopenia and invasive procedures are relatively common. However clinical trials in this environment are associated with challenges such as consent procedures for patients who lack capacity, severe comorbidities and out of hours procedures.

The primary aim of this trial is to assess the feasibility of recruiting patients in this clinical setting. Overall, this has been more challenging than predicted with recruitment running at one third of the predicted level before the trial commenced. Barriers to recruitment have been identified and where possible have been overcome. The main challenge with this trial is that the pool of potential participants is smaller than anticipated. Consequently, I aim to expand the trial to two more sites to improve patient recruitment.

If this trial is successful, it has the potential to improve patient care. Desmopressin can potentially be administered more rapidly than platelet transfusions, which must be

delivered from a hospital blood bank. It is also considerably cheaper than platelet transfusion and will have a different adverse event profile.

7.6 Implications for practice

The evidence base for platelet transfusion remains limited and there is also limited evidence for alternatives or adjuncts to platelet transfusion. I have found no evidence that other measures of haemostasis could be used to supplement the platelet count as a predictor of bleeding.

Much like tranexamic acid, which was in use for many decades before being shown to be life-saving in trauma (108), desmopressin may be an undervalued drug for which there are new potential applications. The evidence for desmopressin remains limited outside the setting of inherited bleeding disorders. While large numbers of trials have been performed, they have been small and of low quality. Many of these trials have focused on outcomes which are not clinically meaningful and are prone to bias. There remains uncertainty over the balance of benefits and harms for desmopressin. While there is insufficient evidence to recommend more widespread use of desmopressin at present, further research might start to identify areas where it could be of benefit.

7.7 Implications for research

Several alternatives to platelet transfusion are being trialled at present. Much of the clinical trial focus at present is on the use of anti-fibrinolytic agents to prevent bleeding in thrombocytopenia. This is under investigation in four major trials (138). However another potential future strategy is to use desmopressin alone, or in combination with other agents.

The techniques used in Chapter 3 to analyse the combined contribution of VWF and platelets to thrombus formation could be used more widely to investigate the potential benefit of increasing VWF for conditions where patients might be prone to bleeding. In addition to evaluating the role of VWF in thrombocytopenia, this could be used to investigate whether increasing VWF levels might bypass, or ameliorate the effects of antiplatelet drugs. Although desmopressin is widely used for treatment of inherited bleeding disorders, there are no randomised controlled trials assessing its efficacy. When desmopressin is used for the treatment of Von Willebrand Disease or Haemophilia A, its efficacy can be assessed using VWF levels and FVIII respectively. However for inherited platelet function disorders there is no established test to demonstrate the efficacy of desmopressin and it is possible that some of the techniques used in this thesis could be of use.

The DRIVE trial will provide information on the feasibility of using desmopressin in thrombocytopenia. If this is feasible, then larger trials to establish the efficacy of desmopressin in thrombocytopenia will be warranted. Because desmopressin is inexpensive and could be stored easily on an intensive care unit it could be rapidly incorporated into clinical guidelines.

Antiplatelet drugs are associated with an increased risk of intracerebral haemorrhage along with worse outcomes compared to patients not taking antiplatelet agents (13, 18). Clinical conditions like intracerebral haemorrhage, where mortality from bleeding is very high, are likely to be ideal areas for future research into the value of reversing or reducing the effects of antiplatelet drugs. An intervention such as desmopressin, which might reduce the effect of antiplatelet drugs, could lead to a reduction in overall mortality.

7.8 Further work arising from this thesis

The work outlined in this thesis has given rise to several further research projects, which are outlined below. While the majority of this thesis has focused on the use of desmopressin in thrombocytopenia, there is potential benefit for the reversal of antiplatelet agents or in the treatment of platelet dysfunction.

7.8.1 Completion of the DRIVE trial

The DRIVE trial is not due to be completed until April 2018. In the first five months of recruitment, four participants were recruited for DRIVE. Final results will be assessed in April 2018 using the analysis plans outlined in Chapter 6.

7.8.2 DASH trial

I have secured funding for the DASH (desmopressin for reversal of antiplatelet drugs in stroke due to haemorrhage) trial from the National Institute for Health Research, Research for Patient Benefit funding stream as co-lead applicant with Prof Nikola Sprigg, University of Nottingham. We aim run this trial at ten stroke centres, starting in April 2018.

7.8.2.1 Trial design

Multicentre, double-blind, randomised, placebo-controlled feasibility trial.

7.8.2.2 Study participants

7.8.2.2.1 Inclusion criteria

- Adults (>17 years)
- Confirmed intracerebral haemorrhage on imaging
- Less than 12 hours from onset of symptoms [or from when last seen healthy]

- Prescribed and thought to be taking a daily oral antiplatelet drug in the preceding seven days (cyclooxygenase inhibitors, phosphodiesterase inhibitors or $P2Y_{12}$ inhibitors)
- Signed consent (or waiver of consent)

7.8.2.2.2 Exclusion criteria

- Aneurysmal subarachnoid haemorrhage
- Haemorrhage known to be due to transformation of infarction
- Haemorrhage known to be due to thrombolytic drug
- Haemorrhage known to be due to venous thrombosis
- Significant hypotension (systolic blood pressure <90 mmHg)
- Known drug eluting stent in preceding three months
- Allergy to desmopressin
- Pregnant or breast-feeding
- Life expectancy less than four hours, or planned for palliative care only
- Glasgow coma scale less than 5
- Participation in another concurrent drug trial

7.8.2.3 Intervention

Desmopressin acetate 0.4 µg/kg in 50ml 0.9% sodium chloride administered over 20 minutes. All participants will receive standard care in addition to desmopressin/placebo.

7.8.2.4 Comparator

50ml 0.9% sodium chloride administered over 20 minutes.

7.8.2.5Primary objective

Feasibility

- Proportion of eligible patients randomised and reasons for non-randomisation – feasibility will be confirmed if 50 participants are randomised in a 12 month period.
- Proportion of participants followed-up to 90 days and reasons for loss to follow-up
- Proportion of randomised participants with the following outcome data (which is to be collected for a definitive study)

7.8.2.6 Primary outcome for the definitive study

Death or dependency at Day 90 (modified Rankin scale, shift analysis)

7.8.2.7 Secondary outcomes for the definitive study

- *Safety:* Hyponatraemia at 24 hours, early mortality <28 days, mortality up to day 90, serious adverse events (including thromboembolic events) up to day 90
- *Radiological*: Change in intracerebral haemorrhage volume at 24 hours
- *Functional/other*: discharge destination, disability (Barthel index, day 90), quality of life (EuroQol, day 90), cognition (telephone mini-mental state examination day 90) and length of hospital stay.
- *Health economics*: Health economic assessment (EQ-5D), length of hospital stay, discharge destination.
- *Laboratory*: Assessment of baseline platelet dysfunction and correlation with response to desmopressin, change in factor VIII, VWF antigen and VWF activity, one hour after administration of desmopressin.

7.8.3 *In vitro* reversal of antiplatelet drug effects with VWF

In collaboration with Dr Alex Bye and Prof Jon Gibbins at the University of Reading, I have developed a protocol for experiments examining whether administration of an exogenous source of VWF could reverse the effects of antiplatelet drugs *in vitro*. This will lead on from the thrombus formation work described in Chapter 3. Dr Alex Bye will perform the laboratory analysis and will lead the laboratory work.

7.8.3.1 Objective

The overarching objective will be to obtain laboratory data which could be used to inform a future clinical trial using a VWF concentrate to reverse the effects of antiplatelet drugs in intracerebral haemorrhage.

7.8.3.2 Experimental plan

Aspirin or cangrelor (a $P2Y_{12}$ inhibitor) will be added *in vitro* to whole blood from normal donors. We will assess thrombus formation under flow over collagen and PFA closure time using C-ADP, collagen/epinephrine (C-EPI) or P2Y cartridges as appropriate using the techniques outlined in Chapter 3. The concentration of VWF will be titrated to find the optimum level for reducing the effects of antiplatelet drugs. Leading on from this, we will aim to replicate these findings by adding VWF to the blood of patients taking antiplatelet drugs.

7.9 Conclusion

The first manuscript describing the synthesis of desmopressin was published 50 years before the submission of this thesis (172), and the first trial of desmopressin as a haemostatic agents was published 40 years ago (174). Despite this, there is a paucity of evidence for its use outside inherited bleeding disorders. The work in this thesis describes potential uses of desmopressin in the setting of thrombocytopenia or platelet

dysfunction. The programme of work arising from this research has the potential to benefit large numbers of patients by preventing bleeding and reducing expose to allogeneic blood components such as platelets. The results presented in this thesis are exploratory but are an important step on a path towards using desmopressin as an alternative, or adjunct to platelet transfusion.

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*original reference has been split into two separate references, with each treated as a separate study.

Appendices

Appendix 1: Systematic review search strategy

CENTRAL, *The Cochrane Library*

#1 MeSH descriptor: [Deamino Arginine Vasopressin] this term only
#2 desmopressin* or deamino* or desamino* or adiuretin* or stimate or desmotabs or "D-void" or octim or octostim or minurin* or minirin* or minrin or desurin or desmospray or defirin or concentraid or desmotab* or desmogalen or presinex or nocutil or noctisson
#3 DDAVP or DDVAP
#4 #1 or #2 or #3

MEDLINE (OvidSP)

1. Deamino Arginine Vasopressin/

2. (desmopressin* or deamino* or desamino* or adiuretin* or stimate or desmotabs or D-void or octim or octostim or minurin* or minirin* or minrin or desurin or desmospray or defirin or concentraid or desmotab* or desmogalen or presinex or nocutil or noctisson).tw.

3. (DDAVP or DDVAP).tw.

4. 1 or 2 or 3

5. Meta-Analysis.pt.

6. ((meta analy* or metaanaly*) and (trials or studies)).ab.

7. (meta analy* or metaanaly* or evidence-based).ti.

8. ((systematic* or evidence-based) adj2 (review* or overview*)).tw.

9. (cochrane or embase or cinahl or cinhal or lilacs or citation index or psyclit or psychlit or psycinfo or psychinfo or "web of science" or scopus).ab.

10. Cochrane Database of systematic reviews.jn.

11. ((literature or systematic* or comprehensive* or electronic*) adj2 search*).ab.

12. (additional adj (papers or articles or sources)).ab.

13. (bibliograph* or handsearch* or hand search* or manual* search* or searched or reference list*).ab.

14. (relevant adj (journals or articles)).ab.

15. or/5-14

16. Review.pt.

17. RANDOMIZED CONTROLLED TRIALS AS TOPIC/

18. selection criteria.ab. or critical appraisal.ti.

19. (data adj (extraction or analys*)).ab.

20. RANDOMIZED CONTROLLED TRIALS/

21. or/17-20

22. 16 and 21

23. 15 or 22

24. randomized controlled trial.pt.

25. controlled clinical trial.pt.

26. randomi*.tw.

27. placebo.ab.

28. clinical trials as topic.sh.

29. randomly.ab.

30. groups.ab.

31. trial.tw.

32. or/24-31

33. 23 or 32

34. (ANIMALS/ or exp ANIMAL EXPERIMENTATION/ or exp MODELS, ANIMAL/) not HUMANS/

35. (Comment or Editorial).pt.

36. 34 or 35

37. 33 not 36

38. 4 and 37

PubMed (epublications only)

(desmopressin* OR deamino* OR desamino* OR adiuretin* OR stimate OR desmotabs OR "D-void" OR octim OR octostim OR minurin* OR minirin* OR minrin OR desurin OR desmospray OR defirin OR concentraid OR desmotab* OR desmogalen OR presinex OR nocutil OR noctisson OR DDAVP OR DDVAP) AND (random* OR blind* OR "control group" OR placebo* OR controlled OR groups OR trial* OR "systematic review" OR "meta-analysis" OR metaanalysis OR "literature search" OR medline OR cochrane OR embase) AND (publisher[sb] OR inprocess[sb] OR pubmednotmedline[sb])

EMBASE (OvidSP)

1. desmopressin acetate/ or desmopressin/ or desmopressin diacetate/ or "argipressin[1 deamino]"/

2. (desmopressin* or deamino* or desamino* or adiuretin* or stimate or desmotabs or D-void or octim or octostim or minurin* or minirin* or minrin or desurin or desmospray or defirin or concentraid or desmotab* or desmogalen or presinex or nocutil or noctisson).tw.

3. (DDAVP or DDVAP).tw.

4. 1 or 2 or 3

5. Meta Analysis/

6. Systematic Review/

7. (meta analy* or metaanalys*).tw.

8. (systematic adj2 (review* or overview* or search*)).tw.

9. (literature adj2 (review* or overview* or search*)).ti,ab.

10. (cochrane or embase or cinahl or cinhal or lilacs or BIDS or science citation index or psyclit or psychlit or psycinfo or psychinfo or cancerlit).ti,ab.

11. (electronic* adj (sources or resources or databases)).ab.

12. reference lists.ab.

13. (bibliograph* or handsearch* or hand search* or manual* search*).ab.

14. (hand-search* or handsearch*).ab.

15. (additional adj (papers or articles or sources)).ab.

16. (relevant adj (journals or articles)).ab.

17. (search term* or published articles or search strateg*).ab.

18. or/5-17

19. data extraction.ab.

20. selection criteria.ab.

21. or/19-20

22. review.pt.

23. 21 and 22

24. editorial.pt.

25. 18 or 23

26. 25 not 24

27. Controlled Clinical Trial/ or Phase 3 Clinical Trial/ or Phase 4 Clinical Trial/

28. Randomized Controlled Trial/

29. Randomization/

30. Single Blind Procedure/

31. Double Blind Procedure/

32. Crossover Procedure/

33. Placebo/

34. (randomized or randomised).tw.

35. RCT.tw.

36. (random* adj5 (allocat* or assign* or divid* or receiv*)).tw.

37. single blind*.tw.

38. double blind*.tw.

39. ((treble or triple) adj blind*).tw.

40. (phase III or phase three or "phase 3").ti,ab.

41. (crossover* or cross over* or cross-over* or placebo*).tw.

42. Prospective Study/

43. or/27-42

44. Case Study/

45. case report*.tw.

46. (note or editorial).pt.

47. or/44-46

48. 43 not 47

49. 26 or 48

50. limit 49 to embase

51. 4 and 50

CINAHL (ESBSCOHost)

S1 (MH "Desmopressin")

S2 TI (desmopressin* or deamino* or desamino* or adiuretin* or stimate or desmotabs or D-void or octim or octostim or minurin* or minirin* or minrin or desurin or desmospray or defirin or concentraid or desmotab* or desmogalen or presinex or nocutil or noctisson or DDVAP or DDAVP) OR AB (desmopressin* or deamino* or desamino* or adiuretin* or stimate or desmotabs or D-void or octim or octostim or minurin* or minirin* or minrin or desurin or desmospray or defirin or concentraid or desmotab* or desmogalen or presinex or nocutil or noctisson or DDVAP or DDAVP)

S3 S1 OR S2

S4 (MH Clinical Trials+)

S5 PT Clinical Trial

S6 TI ((controlled trial*) or (clinical trial*)) OR AB ((controlled trial*) or (clinical trial*))

S7 TI ((singl* blind*) OR (doubl* blind*) OR (trebl* blind*) OR (tripl* blind*) OR (singl* mask*) OR (doubl* mask*) OR (tripl* mask*)) OR AB ((singl* blind*) OR (doubl* blind*) OR (trebl* blind*) OR (tripl* blind*) OR (singl* mask*) OR (doubl* mask*) OR (tripl* mask*))

S8 TI randomi* OR AB randomi*

S9 MH RANDOM ASSIGNMENT

S10 TI ((phase three) or (phase III) or (phase three)) or AB ((phase three) or (phase III) or (phase three))

S11 (TI (random* N2 (assign* or allocat*))) OR (AB (random* N2 (assign* or allocat*)))

S12 MH PLACEBOS

S13 MH META ANALYSIS

S14 MH SYSTEMATIC REVIEW

S15 TI ("meta analys*" OR metaanalys* OR "systematic review" OR "systematic overview" OR "systematic search*") OR AB ("meta analys*" OR metaanalys* OR "systematic review" OR "systematic overview" OR "systematic search*")

S16 TI ("literature review" OR "literature overview" OR "literature search*") OR AB ("literature review" OR "literature overview" OR "literature search*")

S17 TI (cochrane OR embase OR cinahl OR cinhal OR lilacs OR BIDS OR science AND citation AND index OR cancerlit) OR AB (cochrane OR embase OR cinahl OR cinhal OR lilacs OR BIDS OR science AND citation AND index OR cancerlit)

S18 TI placebo* OR AB placebo*

S19 MH QUANTITATIVE STUDIES

S20 S4 or S5 or S6 or S7 or S8 or S9 or S10 or S11 or S12 or S13 or S14 or S15 or S16 or S17 or S18 or S19

S21 S3 AND S20

TRANSFUSION EVIDENCE LIBRARY
Clinical Specialty: Surgery

Search: desmopressin OR deamino OR desamino OR adiuretin OR stimate OR desmotabs OR D-void OR octim OR octostim OR minurin OR minirin OR minrin OR desurin OR desmospray OR defirin OR concentraid OR desmotab OR desmogalen OR presinex OR nocutil OR noctisson OR DDAVP or DDVAP

WEB OF SCIENCE CPCI-S

TOPIC: (desmopressin OR deamino OR desamino OR adiuretin OR stimate OR desmotabs OR D-void OR octim OR octostim OR minurin OR minirin OR desurin OR desmospray OR defirin OR concentraid OR desmotab OR desmogalen OR presinex OR nocutil OR noctisson OR DDAVP

AND

TOPIC: (randomi* OR randomly OR “random assignment” OR “random allocation” OR blind* OR “control group*” OR “controlled trial” OR “controlled study”

LILACS

tw:((desmopressin* OR deamino* OR desamino* OR adiuretin* OR stimate OR desmotabs OR "D-void" OR octim OR octostim OR minurin* OR minirin* OR minrin OR desurin OR desmospray OR defirin OR concentraid OR desmotab* OR desmogalen OR

presinex OR nocutil OR noctisson OR ddavp OR ddvap)) AND (instance:"regional") AND (db:("LILACS") AND type_of_study:("clinical_trials"))

IndMed

(desmopressin OR deamino OR desamino OR adiuretin OR stimate OR desmotabs OR octim OR octostim OR minurin OR minirin OR minrin OR desurin OR desmospray OR defirin OR concentraid OR desmotab OR desmogalen OR presinex OR nocutil OR noctisson OR DDAVP) AND (randomized OR randomised OR randomly OR random allocation OR random assignment OR blinded OR controlled trial OR controlled study OR control group)

KoreaMed

"Deamino Arginine Vasopressin" [MH] "Randomized Controlled Trial" [PT] OR desmopressin [TI] "Randomized Controlled Trial" [PT] OR DDAVP [TI] "Randomized Controlled Trial" [PT] OR desmopressin randomized

PakMediNet

Desmopressin OR DDAVP

ClinicalTrials.gov

desmopressin OR DDAVP

WHO ICTRP

Interventions OR Title: desmopressin OR deamino OR desamino OR adiuretin OR stimate OR desmotabs OR DDAVP

HKU Clinical Trials Registry

Search Terms: Desmopressin

Study Type: Interventional

Appendix 2. Trials not incorporated into meta-analysis in Chapter 4

Trial	Reason not included in meta-analysis	Desmopressin arm	Placebo arm
Cardiac Surgery			
Aida 1991a	Reported as ml/kg (mean ± SD)	8.3 ± 5.6 ml/kg (n = 5)	10.8 ± 6.3 ml/kg (n = 4)
Aida 1991b	Reported as ml/kg (mean ± SD)	10.2 ± 6.4 ml/kg (n = 5)	13.2 ± 6.6 ml/kg (n = 6)
Alanay 1999	Reported as median (IQR)	1.7 (2.3) units (n = 18)	0.6 (1.3) units (n = 22)
Bignami 2016	Reported as median (IQR)	2 (1 to 4) units (n = 68)	2 (1 to 3) units (n = 67)
Frankville 1991	Reported as mean (no SD)	2.4 units (n = 15)	2 units (n = 15)
Hackmann 1989	Reported as median (90% CI)	2 (1 to 8.5) units (n = 74)	2 (1 to 9.8) units (n = 76)
Kuitunen 1992	Reported as mean (range)	1.3 (0 to 2) units (n = 15)	1.1 (0 to 3) units (n = 15)
Marquez 1992	Reported as median only	2 units (n = 21)	2 units (n = 22)
Mongan 1992a	Reported as mean only	0.86 units (n = 44)	1.79 units (n = 42)
Mongan 1992b	Reported as mean only	2.4 units (n = 13)	2.2 units (n = 16)
Reynolds 1993	Reported as ml/m^2 (mean ± SD)	65 ± 43 ml/m^2 (n = 53)	64 ± 46 ml/m^2 (n = 42)
Rocha 1994	Reported as ml/m^2 (mean ± SD)	740.4 ± 416.3 ml/m^2 (n = 25)	662.8 ± 380.7 ml/m^2 (n = 28)
Spyt 1990	Reported as mean only	1.38 units (n = 49)	1.30 units (n = 49)
Orthopaedic Surgery			
Ellis 2001	Reported as mean only	0.7 units (n = 10)	1.1 units (n = 10)
Theroux 1997	Reported as median and range	64.8 (30.3 to 124.6) ml/kg (n = 10)	64.9 (33.8 to 110) ml/kg (n = 11)
Maxillofacial Surgery			
Guyuron 1996	Reported as mean (no SD)	0.6 units (n = 10)	0.9 units (n = 10)

Trials excluded from total volume of allogeneic red cells transfused meta-analysis in section 4.3.4.1. CI: confidence interval, IQR: interquartile range, SD: standard deviation.

Trial	Reason not included in meta-analysis	Desmopressin arm	Placebo arm
Cardiac Surgery			
Aida 1991a	Reported as ml/kg (mean ± SD)	8.0 ± 1.4 ml/kg (n = 5)	5.9 ± 1.5 ml/kg (n = 4)
Aida 1991b	Reported as ml/kg (mean ± SD)	11.3 ± 10 ml/kg (n = 5)	7.5 ± 4 ml/kg (n = 6)
Alanay 1999	Reported as median (IQR)	950 (950) ml (n = 18)	975 (811) ml (n = 22)
Bignami 2016	Reported as median (IQR)	575 (422.5 to 770) ml (n = 68)	590 (476 to 1014) ml (n = 67)
Casas 1995	Reported as ml/m² BSA (mean ± SD)	400 ± 192 ml/m² (n = 50)	489 ± 361 ml/m² (n = 51)
De Prost 1992	Reported as ml/m² BSA (mean ± SD)	582 ± 410 ml/m² (n = 44)	465 ± 303 ml/m² (n = 37)
Dilthey 1993	Reported as median (range)	1000 (600 to 1800) ml (n = 19)	1075 (400 to 1740) ml (n = 20)
Hackmann 1989	Reported as median (90% CI)	865 (358 to 2495) ml (n = 74)	783 (300 to 2219) ml (n = 76)
Hajjar 2007	Reported as ml/m² BSA (mean ± SD)	258 ± 106 ml/m² (n = 75)	526 ± 314 ml/m² (n = 75)
Hemşinli 2012a	Reported as mean (no SD)	1430 ml (n = 10)	1767 ml (n = 10)
Hemşinli 2012b	Reported as mean (no SD)	574 ml (n = 16)	535 ml (n = 18)
Kuitunen 1992	Reported as median only	1157 ml (n = 21)	1180 ml (n = 22)
Marquez 1992	Reported as ml/m² BSA (mean ± SD)	458 ± 206 ml/m² (n = 50)	536 ± 304 ml/m² (n = 50)
Reynolds 1993	Reported as ml/m² BSA (mean ± SD)	30 ± 33 ml/m² (n = 53)	35 ± 36 ml/m² (n = 42)
Rocha 1988	Reported as ml/m² BSA (mean ± SD)	551.8 ± 324.1 ml/m² (n = 28)	438.7 ± 228.1 ml/m² (n = 25)
Rocha 1994	Reported as median (range)	1020 (530 to 1155) ml (n = 15)	1100 (425 to 1720) ml (n = 15)
Salmenpera 1991	Reported as ml/kg (mean ± SD)	8.0 ± 1.4 ml/kg (n = 5)	5.9 ± 1.5 ml/kg (n = 4)
Seear 1989	Reported as ml/m² BSA (mean ± SD)	40 ± 33.1 ml/m² (n = 30)	30.5 ± 37.9 ml/m² (n = 30)
Orthopaedic Surgery			
Flordal 1991	Reported as mean (no SD)	1320 ml (n = 6)	1380 ml (n = 6)
Theroux 1997	"Percentage blood loss": median (range)	147.8% (57 to 428.8%) (n = 10)	111.2% (65 to 239.5%) (n = 11)
Maxillofacial Surgery			
Guyuron 1996	Reported as mean (range)	675 (380 to 1330) ml (n = 10)	819 (200 to 1600) ml (n = 10)

Trials excluded from total blood loss meta-analysis in section 4.3.4.3. BSA: body surface area, CI: confidence interval, IQR: interquartile range, SD: standard deviation.

Appendix 3. Trials not incorporated into meta-analysis in Chapter 5

Trial	Reason not included in meta-analysis	Desmopressin	Placebo
Total volume of allogeneic red cells transfused			
Mongan 1992	Reported as mean (no SD)	2.4 units (n = 13)	2.3 units (n = 16)
Total blood loss			
De Prost 1992	Reported as ml/m^2 BSA (mean ± SD)	582 ± 410 ml/m^2 (n = 44)	465 ± 303 ml/m^2 (n = 37)
Dilthey 1993	Reported as median (range)	1000 (600 to 1800) ml (n = 19)	1075 (400 to 1740) ml (n = 20)
Hemşinli 2012a	Reported as mean (no SD)	1430 ml (n = 10)	1767 ml (n = 10)
Hemşinli 2012b	Reported as mean (no SD)	574 ml (n = 16)	535 ml (n = 18)

Summary of data excluded from meta-analysis for platelet dysfunction subgroup in section 5.3.4. BSA: body surface area, SD: standard deviation

Trial	Reason not included in meta-analysis	Desmopressin	Placebo
Volume of allogeneic red cells transfused			
Bignami 2016	Reported as median (IQR)	2 (1 to 4) units (n = 68)	2 (1 to 3) units (n = 67)
Total blood loss			
Bignami 2016	Reported as median (IQR)	575 (423 to 770) ml (n = 68)	590 (476 to 1014) ml (n = 67)
Hemşinli 2012b	Reported as mean (no SD)	574 ml (n = 16)	535 ml (n = 18)

Summary of data excluded from meta-analysis for anti-fibrinolytic subgroup in section 5.4.4. IQR: interquartile range, SD: standard deviation.