Aspects on bleeding and transfusion in elective orthopaedic surgery

Clinical and experimental studies

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Cover illustration: Anton Carling (4 years) and Oliver Carling (7 years).
Children's view of the circulatory system and blood vessels.

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"The only weapon with which the unconscious patient can immediately retaliate upon the incompetent surgeon is haemorrhage"

William Halsted

(Bulletin of the John Hopkins Hospital 1912; 23: 191)

ABSTRACT

Background: Perioperative bleeding complications are associated with increased morbidity and mortality. One way to minimize perioperative bleeding and transfusion is to identify patients at risk of bleeding. Preoperative fibrinogen plasma concentration and factor XIII (FXIII) activity may be indicators of perioperative bleeding and transfusion. The aim of the thesis was to investigate 1) whether there is a correlation between the levels of preoperative coagulation factors and perioperative bleeding and transfusion in elective orthopaedic surgery patients, 2) transfusion and blood loss in arthroplasty surgery and possible risk factors, and 3) the ability of FXIII to improve clot formation in blood samples from cardiac and scoliosis surgery patients.

Patients and methods: The study described in **Paper I**, involved 82 patients undergoing idiopathic scoliosis surgery. Preoperative fibrinogen plasma concentration was correlated with perioperative bleeding and red blood cell (RBC) transfusion requirements. The studies described in **Papers II** and **III** involved 245 patients undergoing either degenerative spine fusion surgery (52), elective total unilateral primary hip arthroplasty (THA) (114), or knee arthroplasty (TKA) (79). In **Paper II** perioperative bleeding and transfusion requirement in THA and TKA patients were investigated. In **Paper III** preoperative fibrinogen plasma concentration and FXIII activity were correlated with perioperative bleeding and RBC transfusion in the three surgery groups. In **Paper IV**, blood samples from patients undergoing cardiac surgery (9) and scoliosis surgery (10) were supplemented with three increasing doses of FXIII concentrate, alone or in combination with a fixed dose of either fibrinogen concentrate or fresh apheresis platelets. Clot formation was assessed with modified rotational thromboelastometry (ROTEM®).

Results: An association was found between low fibrinogen concentration and large perioperative bleeding and RBC transfusion for scoliosis surgery patients (**Paper I)**. An association was found between low fibrinogen and large perioperative bleeding in spine surgery, but not in THA or TKA patients (**Paper III)**. No association was observed between fibrinogen and RBC transfusion or between FXIII activity and perioperative bleeding and/or RBC transfusion in any of the surgery groups. A lower prevalence of red blood cell transfusion in THA and TKA than previously reported was found (**Paper II)**. Low preoperative hemoglobin levels, low body mass index and long operation time increased the risk for RBC transfusion. In **Paper IV** EXTEM clotting time was shortened and FIBTEM maximum clot firmness

was increased compared to baseline in both surgery groups when FXIII was added. The effect was more pronounced when fibrinogen concentrate or platelets were added.

Conclusions: Preoperative measurement of fibrinogen plasma concentration, but not preoperative FXIII activity, may be useful to identify patients at risk of perioperative bleeding and transfusion in certain types of elective orthopaedic surgery. In THA and TKA patients, RBC transfusions are relatively rarely given today. Risk factors for large bleeding and red blood cell transfusion in unselected elective THA or TKA patients are low preoperative hemoglobin levels, low body mass index and long operation time. Ex vivo supplementation with clinically relevant doses of FXIII dose-dependently improve clot formation in blood samples from cardiac surgery and idiopathic scoliosis surgery patients, both alone and when given in combination with fibrinogen or platelets.

Keywords: orthopaedic surgery, fibrinogen, factor XIII, surgical bleeding, transfusion

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SAMMANFATTNING PÅ SVENSKA

Bakgrund: Blödningskomplikationer i samband med kirurgi är förenade med ökad sjuklighet och dödlighet. Ett sätt att minimera blödning och blodtransfusioner är att identifiera patienter med risk för blödning under och efter operation. Mätning av koagulationsfaktorerna fibrinogen och faktor XIII (FXIII) innan operation har föreslagits kunna ge information om vilka patienter som har ökad risk för större blödning under kirurgi och om det finns risk för behov av blodtransfusion. Studierna i denna avhandling undersöker om det finns ett samband mellan fibrinogen och FXIII, och blödning och blodtransfusion vid planerad ortopedisk kirurgi. Vidare undersöks blödning och transfusionsbehov, samt riskfaktorer för dessa, vid planerad höft- och knäproteskirurgi. I en experimentell studie studeras effekten av FXIII på koagulationsförmågan i blodprover från patienter som genomgått hjärt- och skolioskirurgi.

Patienter och metoder: I artikel I redovisas en studie som inkluderade 82 unga patienter som opererades på grund av skolios. Patienternas fibrinogennivåer före operationen jämfördes med blödning under och efter kirurgin, samt med behovet av blodtransfusion under vårdtiden. I artikel II och III, studerades 245 patienter som genomgick antingen steloperation i ryggen (n=52), ensidig höftproteskirurgi (n=114), eller knäproteskirurgi (n=79). I artikel II undersöktes blödning under kirurgi och behovet av blodtransfusion under vårdtiden samt riskfaktorer för dessa. I artikel III jämfördes patienternas nivåer av fibrinogen och FXIII före operationen med blödning under och efter kirurgi, samt med behovet av blodtransfusion under vårdtiden, hos de tre patientgrupperna. I artikel IV togs blodprover från nio patienter som genomgick hjärtkirurgi och tio patienter som genomgick skolioskirurgi. I blodproverna tillsattes tre olika doser av FXIII-koncentrat, ensamt eller i kombination med en fast dos av antingen fibrinogen-koncentrat eller färska blodplättar (trombocyter). Blodets koagulationsförmåga bedömdes med metoden modifierad rotationstromboelastometri (ROTEM®).

Resultat: Hos unga skoliospatienter (artikel I) sågs ett samband mellan låga nivåer av fibrinogen före operation och större blödning under och efter kirurgi, samt med större behov av blodtransfusion under vårdtiden. I artikel III sågs ett samband mellan låga fibrinogennivåer före operation och större blödning hos ryggkirurgiska patienter som genomgick steloperation. Detta samband sågs ej vid höft- eller knäproteskirurgi. Inget samband mellan fibrinogennivåer och behovet av blodtransfusion, eller mellan FXIII-aktivitet och blödning eller behov av blodtransfusion, kunde påvisas i någon av

patientgrupperna. I artikel II sågs en lägre förekomst av blodtransfusion vid höft- och knäproteskirurgi än vad som tidigare rapporterats. Lågt blodvärde före operationen, lågt body mass index (BMI) och lång operationstid ökade risken för blodtransfusion. I artikel IV förbättrades blodets koagulationsförmåga när FXIII tillsattes, ensamt eller i kombination med fibrinogen-koncentrat eller blodplättar, mätt med ROTEM®, både i blod från hjärt- och från skoliospatienter.

Slutsatser: Fibrinogen-koncentration uppmätt före operation kan identifiera patienter med ökad risk för blödning och behov av blodtransfusioner under och efter operationen vid vissa typer av planerad ortopedisk kirurgi. Däremot är mätning av FXIII-aktivitet före planerad ortopedisk kirurgi inte till hjälp för att identifiera riskpatienter avseende blödning och/eller blodtransfusion.

Antalet patienter som erhåller blodtransfusion vid total höft- och knäledsproteskirurgi är idag lägre än vad som tidigare rapporterats. Riskfaktorer för stor blödning och blodtransfusion är lågt blodvärde före operationen, lågt BMI och lång operationstid.

Vid tillsatts av rimliga behandlingsdoser av FXIII-koncentrat till blodprover från patienter som genomgår hjärtkirurgi eller skolioskirurgi, kan blodets koagulationsförmåga förbättras. Detta gäller både när FXIII-koncentrat ges ensamt och när det ges i kombination med fibrinogen-koncentrat eller blodplättar.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Carling MS, Jeppsson A, Wessberg P, Baghaei F, Brisby H. Preoperative fibrinogen plasma concentration is associated with perioperative bleeding and transfusion requirements in scoliosis surgery. Spine (Phila Pa 1976). 2011 Apr 1;36(7):549-55.

II. Carling MS, Jeppsson A, Eriksson BI, Brisby H. Transfusions and blood loss in total hip and knee arthroplasty: a prospective observational study. J Orthop Surg Res. 2015 Mar 28;10(1):48.

III. Carling MS, Zarhoud J, Jeppsson A, Eriksson BI, Brisby H. Preoperative fibrinogen plasma concentration and FXIII activity and perioperative bleeding and transfusions in elective orthopedic surgery: A prospective observational study. Submitted.

IV. Shams Hakimi C, Carling MS, Brisby H, Jeppsson A. Ex vivo effects of factor XIII supplementation on clot formation in blood samples from cardiac and scoliosis surgery patients. In manuscript.

CONTENT

ABBREVIATIONS

ADP	Adenosin diphosphate
ALI	Acute lung injury
aPTT	Activated partial tromboplastin time
ASA	Acetyl salicylic acid
BMI	Body mass index
cFXIII	Cellular factor XIII
CPB	Cardiopulmonary bypass
CT	Clotting time
EBL	Estimate blood loss
EBV	Estimated blood volume
ESA	European Society of Anaesthesiology
FFP	Fresh frozen plasma
Hb	Hemoglobin
INR	International normalized ratio
ISTH	International Society on Thrombosis and Haemostasis
LMWH	Low molecular weight heparin
MCF	Maximum clot firmness
NOAC	New oral anticoagulant
NSAID	Non-steroidal anti-inflammatory drugs
OR	Odds ratio
PCC	Prothrombin complex concentrate
PCI	Percutaneous coronary intervention
pFXIII	Plasma factor XIII
POC	Point-of-care
PT	Prothrombin time
RBC	Red blood cells
SSRI	Selective seretonin reuptake inhibitors
TF	Tissue factor
THA	Total hip arthroplasty
TIC	Trauma-induced coagulopathy
TKA	Total knee arthroplasty
TRALI	Transfusion-related acute lung injury
TXA	Tranexamic acid
vWF	von Willebrand factor
WBCT	Whole blood clotting time

Introduction

1 INTRODUCTION

Major perioperative bleeding, defined as bleeding during and in relation to the surgical procedure, increases morbidity and mortality. In order to ensure safe and efficient surgical procedures it is important to minimize bleeding and the subsequent need for transfusions but also to optimize the patient's coagulation competence. [204]

Some types of open orthopaedic surgery are associated with large perioperative blood loss and, as a consequence, frequent use of allogeneic transfusion of blood products. Various measures can be taken, pre-, intra, and postoperatively, to reduce bleeding and the need for transfusions; treatment of preoperative anemia, autologous blood transfusion programs, cell saving techniques, hemostatic drugs, transfusion guidelines and protocols. [22, 204] All these measures are based on knowledge on what increases the risk of large perioperative bleeding and for transfusion, *Figure 1*.

This thesis was initiated based on findings in other surgical patient groups. Preoperative measurements of coagulation factors, such as fibrinogen and coagulation factor XIII, have been found to be of importance to minimize perioperative bleeding and transfusion requirements. [50, 116] To investigate this in an orthopaedic surgery setting, studies in patient groups historically known to require transfusions, such as arthroplasty patients and patients undergoing spine surgery procedures, were designed and performed. Due to the many changes in the routines of today a separate study of bleeding and transfusion rates in arthroplasty patients was additionally performed.

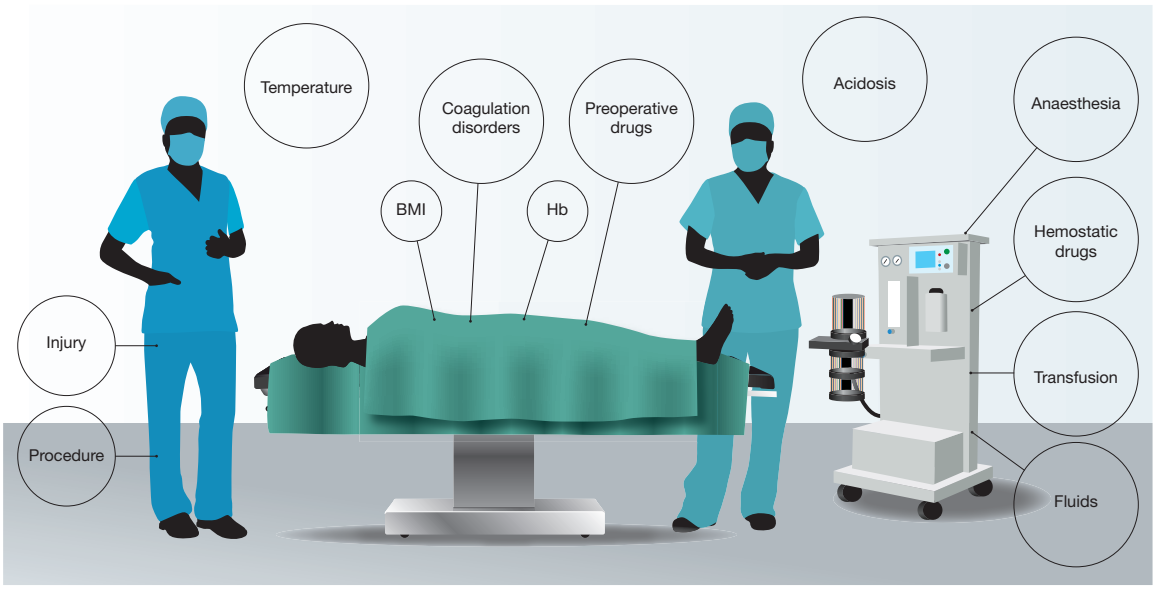

Figure 1. Factors influencing bleeding and transfusion requirements in the surgical setting.

1.1 Bleeding and transfusion in surgery

Bleeding in surgery is inevitable. In order to take the right measures to minimize bleeding it is important to know what factors influence perioperative bleeding.

The International Society on Thrombosis and Haemostasis (ISTH, www.isth.org) has defined major bleeding in surgical patients. [198] Simplified, this definition states that a major bleeding can be a fatal bleeding, bleeding that is symptomatic and occurs in a critical area or organ or surgical site bleeding that requires a secondary intervention. It can also be a bleeding that causes a drop in hemoglobin (Hb) level by 20 g/L or where the patient receives at least two units of red blood cell (RBC) transfusion.

Another definition by the Swedish Society on Thrombosis and Haemostasis (SSTH, www.ssth.se) for massive bleeding is a bleeding requiring transfusion of >10 units of RBC in the previous 24 hours. [23]

Ginzburg and Dujardin studied orthopaedic surgeons' definition of major bleeding in 2011. [75] The study showed that surgeons were more concerned about bleeding that caused re-operation than bleeding that caused a drop in Hb level. Transfusion of >2 units of RBC was considered a relevant threshold for major bleeding.

1.1.1 Surgical factors

Operation time has been shown to affect perioperative bleeding in orthopaedic and cardiac surgery. [144, 169] However, the cause and effect is difficult to establish. During major orthopaedic surgical procedures there is a continuous bleeding from exposed bone marrow and cancellous bone surfaces. Operation time can be increased because of bleeding that obscures the surgery field.

The type of anesthesia affects perioperative bleeding and transfusion in total hip arthroplasty (THA) and total knee arthroplasty (TKA) surgery. [102, 120, 154, 189] Regional anesthesia, most commonly spinal anesthesia, lowers the mean arterial blood pressure and affects the venous return. [161] This gives a lower perfusion pressure in the surgical field with subsequent lower blood loss.

In surgery of the limbs it is easy to minimize intraoperative blood loss by using a tourniquet. Studies on TKA surgery have shown differing results on perioperative bleeding; some studies show lower perioperative bleeding in patients operated in a bloodless field while other studies show no difference. [5, 12, 109, 137] One study by Tetro and Rudan in 2001 even showed a higher perioperative blood loss in the tourniquet group. [228] The same study showed no difference in transfusion requirements between the groups.

The experience of the surgeon might also influence the perioperative bleeding. [251] This relationship is also difficult to establish because of confounding factors.

1.1.2 Patient related factors

Anemia is one of the major patient-related risk factors for transfusion during surgery. [9, 18, 30, 82, 92, 144, 157] A low Hb level affects blood coagulation, described in Section 1.2.2. During surgery the patients with an initial low Hb levels reaches critically low Hb faster because of the bleeding and hemodilution during surgery.

The main function of RBC is to deliver oxygen to the tissues. The body has several mechanisms to optimize this delivery, including respiratory and cardiac adaption as well as increased tissue oxygen extraction in anemia. [205] An Hb concentration of 130 g/L in men, and 120 g/L in women is defined as clinical anemia according to the WHO. [2] Anemia can have a number of causes, such as lack of production of RBCs, destruction of RBCs, or acute or chronic bleeding. [79] Women, on average, have a lower Hb concentration and a smaller blood volume.

Studies show that obese patients bleed more during elective orthopaedic surgery. [32, 88, 110] At the same time surgery in obese patients takes longer, and a long operation time has been shown to increase bleeding. To complicate the matter further, a study in cardiac surgery patients shows an increased risk for reexploration for bleeding in patients with low body mass index (BMI). [118]

Other suggested patient-related risk factors for perioperative bleeding and transfusion are high age and gender. [18, 118, 174, 182] Studies differ, however, and the causality between gender and bleeding is not clear.

1.2 Coagulation

The coagulation system is complex and allows for controlled coagulation at the site of injury without the clot spreading throughout the vascular tree. Blood coagulation interacts with many other bodily systems such as the inflammatory system and angiogenesis, as well as medications, infectious agents and foreign materials. The following section provides an overview of blood coagulation and clinically important factors affecting blood coagulation.

1.2.1 The coagulation cascade

Knowledge about the ability of the blood to form clots has been known since ancient times. In the 19th century, scientists began to learn more about the different factors that lay behind the thrombus formation. [195, 237]

Because of the plethora of names used for the different factors in the early days of coagulation research, an International Committee for the Standardization of the Nomenclature of Blood Clotting Factors was established at the International Congress of Thrombosis and Embolism in Basel in 1954. Coagulation factors were given a Roman numeral in the order they were discovered. The first four factors were already known by their names so are not referred to by their standardized number. [246, 247] Coagulation factor XIII was the last factor to be given a roman numeral. Today the committee is called the Scientific and Standardization Committee (SSC) and is part of ISTH.

Table 1. *Coagulation factors*

Factor	Synonym	Factor	Synonym
I	Fibrinogen	**VIII**	Antihemophilic globulin
II	Prothrombin	**IX**	Christmas factor
III	Thromboplastin, Tissue factor	**X**	Stuart-Prower factor
IV	Calcium, Ca^{2+}	**XI**	Plasma thromboplastin antecedent
V	Proaccelerin	**XII**	Hageman factor
VI	Same as factor V	**XIII**	Fibrin stabilizing factor
VII	Proconvertin		

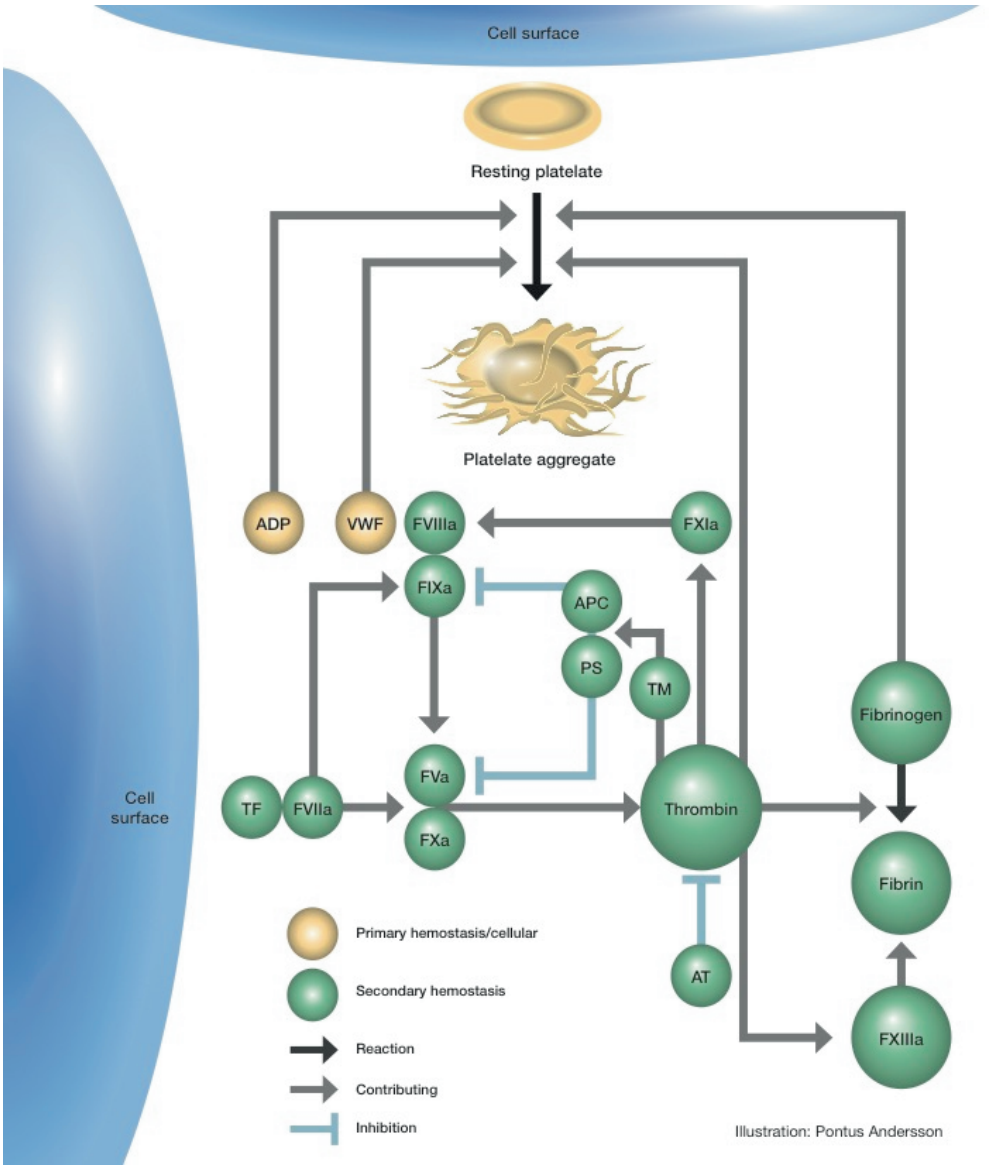

Figure 2. Schematic overview of the coagulation cascade. ADP = Adenosine diphosphate, TF = Tissue factor, vWF = von Willebrand factor, APC = Apoprotein C, PS = Protein S, TM = Thrombomodulin, AT = Antithrombin, FVa = activated Factor V, FVIIa = activated Factor VII, FVIIIa = activated Factor VIII, FIXa = activated Factor IX, FXa = activated Factor X, FXIa = activated Factor XI

Primary hemostasis

The coagulation cascade was previously divided into the intrinsic, platelet derived pathway, and the extrinsic, plasma coagulation factor-driven pathway. Even if this categorization is still in use, for instance when defining coagulation assays, a more explanatory way of describing the coagulation cascade is by dividing it into primary and secondary hemostasis. [15]

The endothelial cells in the blood vessel wall are designed so that they do not spontaneously initiate the coagulation cascade. The negatively charged platelets are repelled by an equally negatively charged glycocalyx on the endothelial cells. The endothelial cells produce a small amount of endogenous heparin that together with circulating antithrombin inactivates coagulation factors.

When a blood vessel injury occurs, the vessel constricts as a response to the injury. The antithrombotic glycocalyx is removed at the injury site and the platelets are not repelled but, instead, are pushed towards the vessel wall by fluid dynamics. Subendothelial collagen is exposed and the platelets are activated. The platelets are also activated by circulating thrombin and adenosine diphosphate (ADP). Von Willebrand factor (vWF) secures the platelets to the vessel wall. The platelets then change form and become irregular and form pseudopods, thereby increasing their surface area, on which platelet procoagulant receptors are exposed. The activated platelets form a monolayer over the injury and the coagulation cascade proliferates on the surface.

The activated platelet releases ADP, Ca^{2+}, serotonin, vWF, factor V, factor XIII and fibrinogen, all substances that help the continuing thrombus formation.

Secondary hemostasis

Injury to the endothelial cells exposes thromboplastin, also called tissue factor (TF). Tissue factor activates factor VII and, together, they form a complex that in turns activates factor V and factor X, which also form a complex at the site of injury. This FVa/FXa complex is called prothrombinase and it activates prothrombin into thrombin.

Thrombin is a strong procoagulant factor and its main role is to activate fibrinogen into fibrin, which forms the fibrin clot. It also activates platelets and other coagulation factors. Only a small amount of thrombin is needed to

initiate coagulation on the surface of the vessel wall. In the next step, the propagation phase, hemostasis takes place on the activated platelets covering the injury.

The thrombin burst, where a large amount of thrombin is activated, and the amplified activation of coagulation factors, is due to enlarged surface of the activated platelets and the large amount of procoagulants expressed on that surface. The coagulation factors link with the surface through Ca^{2+} bonds. The large amount of thrombin can, in turn, convert large amounts of fibrinogen into fibrin, which forms the fibrin clot. Thrombin also activates FXIII, which stabilizes the clot by forming crosslinks with fibrin.

The surplus of activated factors is released to plasma and is inactivated by antithrombin. The anticoagulant properties of the uninjured vessel walls limit coagulation to the site of injury. For instance, circulating thrombin attaches to thrombomodulin on the vessel wall. This complex activates protein C and protein S which inactivates FV and FVIII and stops their activation of thrombin.

1.2.2 What affects coagulation?

Many of the coagulation factors have catalytic functions in the coagulation cascade, and 30% of normal plasma concentration of these factors is assumed to be sufficient to maintain coagulation. Others, like fibrinogen, FXIII and platelets are consumed during coagulation and sufficient levels are critical for clotting. [15]

Patient related factors

The most obvious patient-related factors are different types of bleeding disorders. The most common bleeding disorders are hemophilia A and B and von Willebrand disease, all of which are congenital. In 2012, the prevalence of hemophilia A was 7.1 per 100,000 inhabitants, for hemophilia B 1.7 per 100,000 inhabitants, and for von Willebrand disease one in 100 inhabitants. [16, 221]

Platelet dysfunction and thrombocytopenia also affect coagulation. Congenital platelet dysfunctions are rare and diverse. [113] Examples are Bernard-Soulier syndrome, which affects platelet adhesion to the vessel walls, and Glanzmann thrombasteni, which affects platelet binding with

fibrinogen. Various conditions that affect bone marrow generation and increase consumption of platelets, such as myelodysplastic syndromes, large alcohol consumption or drug-induced thrombocytopenia, cause thrombocytopenia.

In the blood vessel, platelets migrate towards the vessel wall because of hemodynamic and rheological factors. Hemoglobin levels influence this migration, and anemic patients may not have enough red blood cells to push the platelets to the vessel wall, thereby lowering the actual platelet count at the site of injury. [4] The hemoglobin concentration at which coagulation is affected is unclear. [131]

Blood coagulation is also temperature dependent. Even small changes in core temperature (<1°C) increase perioperative blood loss and increases transfusion rates. [187] However, studies are not conclusive and body temperature should be seen as just one of many ways to optimize the patient's coagulation system. [52, 131]

In vitro studies suggest that coagulation is pH dependent as well. The TF-factor VII-complex has diminished activity in an acidotic environment. [156] The combination of low pH and low body temperature is even more detrimental to coagulation. Patients with large bleeding due to trauma are often cold because of the circumstances during and after trauma, and this is often combined with acidosis. In these cases it is important to reverse the low body temperature and acidosis to optimize coagulation before necessary surgery is carried out. [99]

Calcium is an important part of the coagulation cascade. A plasma concentration of 0.9 mmol/L appears to be required to ensure coagulation. [54] In trauma patients it has been shown that hypocalcemia on admission to hospital was associated with increased mortality and a larger risk of massive transfusion. Hypocalcemia may also be the result of massive transfusion since citrate, used in fresh frozen plasma (FFP) and platelet transfusion as an anticoagulant, binds Ca^{2+}. [216]

Diet and other lifestyle factors may also have an impact on hemostasis. [138, 180] This has been widely studied in terms of relation to cardiovascular disease, but not much is known about how this affects bleeding and transfusion in a surgical setting. Fish oil, as a dietary supplementation to prevent atherosclerotic disease, has been studied, but no effect on bleeding or transfusion rates has been demonstrated. [239]

High alcohol consumption can cause thrombocytopenia and liver cirrhosis. Thrombocytopenia in persons with high alcohol consumption both reduces the number of platelets and affects platelet aggregation. [94] Many pro- and anticoagulant factors are produced in the liver, so liver cirrhosis, not only on the basis of alcohol, can cause both bleeding and thrombotic disorders. Even if these patients have a high prothrombin time (PT) they should not be given procoagulants such as prothrombin complex concentrates (PCC) and, in conditions with increased risk of thrombotic events, such as surgery or immobilization, thrombosis prophylaxis should always be considered. [131, 143]

Iatrogenic factors

Different drugs affect the coagulation system. Some are supposed to down-regulate the coagulation cascade by inhibiting coagulation factors or the platelets, while others have this as a side effect. This can cause difficulties if a patient on these medications requires acute or planned surgery or if the patient is subjected to major trauma.

The discovery of heparin in 1916 and the development of low molecular weight heparin (LMWH) were milestones in medicine. Heparin allows physicians to treat hypercoagulant and thrombotic diseases and it is also used to prevent thromboembolism in, for example, patients undergoing surgery and in atrial fibrillation.

Because LMWH are administered as subcutaneous injections, warfarin is preferred for long-term treatment. The disadvantages of warfarin are that it must be monitored regularly and that the drug interacts with other medications as well as different types of food. New oral anticoagulants (NOAC) have been developed that allow safe anticoagulation without monitoring. [199] Because NOACs have not been in use for long; the first NOAC still in use, dabigatran, was released in Sweden in 2008. Consequently, studies are needed of NOACs in combination with surgery, especially emergency surgery. [58, 64]

Platelet inhibitors are mainly used in treating patients with cardio- or cerebrovascular disease. The modern-day inhibitors are very potent, leading to larger perioperative bleeding compared to patients without platelet inhibition. [196] Because of the risk of stent thrombosis in patients having undergone percutaneous coronary intervention (PCI) with stent, discontinuation of antiplatelet therapy must be discussed with a cardiologist.

[37] Acetylsalicylic acid (ASA) is also potent, irreversibly inhibiting the platelets, and there is an effect on perioperative bleeding. [55] Whether this effect is clinically relevant is still debated.

Non-steroidal anti-inflammatory drugs (NSAID) are common over-the-counter drugs to treat conditions such as headaches, fever and pain. Like acetylsalicylic acid they inhibit platelet aggregation and hence reduce blood clotting. Depending on the specific pharmacodynamics properties of the NSAID, their influence on coagulation differs. New NSAID that selectively inhibits cyclo-oxygenase-2 have no impact on platelet aggregation. [158, 191]

Selective serotonin reuptake inhibitors (SSRI) increase the risk of bleeding and transfusion in surgery. [17, 164, 201] Activated platelets expel serotonin into plasma and it is used to help platelet aggregation. Platelets have a similar serotonin transporter to that present on presynaptic neuronal endings, so it is likely that the platelet transporter is inhibited by SSRI in a similar way as in the central nervous system. [150]

In patients experiencing blood loss, the first measure to take is to replace blood with intravenous fluids, colloids or crystalloids. This, together with consumption of coagulation factors caused by bleeding, may result in dilutional coagulopathy. Artificial colloids are known to affect blood clotting more than crystalloids and the reason might be inhibition of fibrin formation since the addition of fibrinogen reverses the effect of colloids on clot formation. [60, 63, 130, 160]

Table 2. *Antithrombotic drugs and their indications, a selection. [3]*

Name	Function	Indication
Heparin	Indirect inactivation of thrombin and FXa	Heparin lock in indwelling venous catheters, anticoagulant during extracorporeal circulation
Low Molecular Weight Heparin	Inhibition of activated factor X	Venous thromboembolism, thrombosis prophylaxis
Warfarin	Inhibition of vitamin K-dependent coagulation factors II, VII, IX, X	Venous thromboembolism, thrombosis prophylaxis, anticoagulation in patients with valve replacement or atrial fibrillation
Dabigatran	Thrombin inhibitor	Venous thromboembolism, thrombosis prophylaxis, anticoagulation in patients with atrial fibrillation
Rivaroxaban	Inhibition of activated factor X	Venous thromboembolism, thrombosis prophylaxis, anticoagulation in patients with atrial fibrillation, prophylaxis after acute coronary syndrome
Apixaban	Inhibition of activated factor X	Venous thromboembolism, thrombosis prophylaxis, anticoagulation in patients with valve replacement or atrial fibrillation
Acetylsalicylic acid	Inhibition of platelet aggregation through cyclo-oxygenase	Acute myocardial infarction, prophylaxis against cardio-vascular complication after acute coronary syndrome, prophylaxis against cerebrovascular disease
Dipyridamol	Inhibition of platelet aggregation	Secondary prevention of cerebrovascular disease
Clopidogrel	Inhibition of platelet aggregation through inhibition of the $P2Y_{12}$ receptor	Prevention of atherothrombotic events in patients with myocardial infarction or acute coronary syndrome, treated with or without PCI, anticoagulation in patients with atrial fibrillation
Prasugrel	Inhibition of platelet aggregation through inhibition of the ADP receptor	Prevention of atherothrombotic events in patients who have had PCI with stent
Ticagrelor	Inhibition of platelet aggregation through inhibition of the ADP receptor	Prevention of atherothrombotic events in patients with myocardial infarction or acute coronary syndrome, treated with or without PCI or coronary bypass surgery

1.2.3 Fibrinogen

The main function of fibrinogen in the coagulation cascade is as the precursor to fibrin, which in turn forms the fibrin clot.

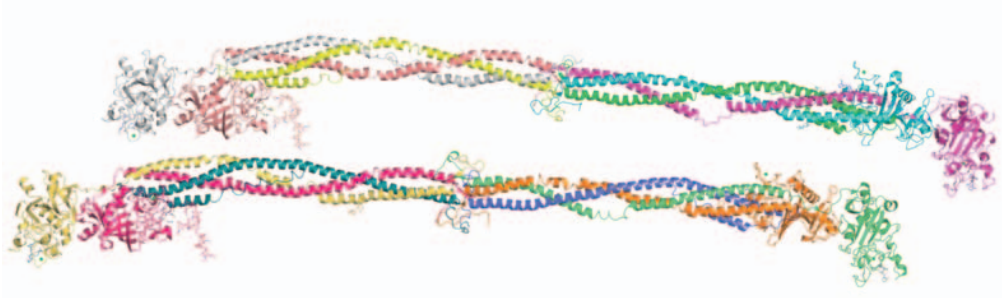

Figure 3. Fibrinogen hexamer. With permission from the Protein Data Bank in Europe. [83]

Fibrinogen is composed of three homologous polypeptide chains – Aα, Bβ and γ – forming two identical sets joined together to make a dimer structure. [163] Fibrinogen is primarily expressed and formed in liver cells and it is secreted in its hexamer form. [190] Cleavage of the Aα-chain by thrombin initiates the formation of the fibrin clot at the site of bleeding. [162] The structure of the fibrin clot formed is affected by the amount of thrombin available – low levels of thrombin create a tighter fibrin clot. [26] The fibrinogen or fibrin γ-chain contains the site for FXIII cross-linking. [209]

The normal fibrinogen plasma concentration is 2.0-4.5 g/L and levels >1.0 g/L are considered enough to maintain hemostasis. [218] In the bleeding patient, recent guidelines suggest that fibrinogen levels should be kept above 1.5-2.0 g/L. [131]

Congenital afibrinogenemia is a rare disease (prevalence 1 in 1,000,000) that usually manifests with umbilical cord bleeding in the neonatal period. [53] In contrast to hemophilia patients, bleeding in different joints is not common in patients with afibrinogenemia. Instead, bleeding occurs in the skin, genitourinary and gastrointestinal tract, and intracranial hemorrhage is a major cause of death in these patients. [178] Fibrinogen is vital for pregnancy and first trimester abortion is common in women with afibrinogenemia. [53]

Patients with hypofibrinogenemia or dysfibrinogenemia are usually asymptomatic. [149, 152] Dysfibrinogenemia patients may experience both bleeding and thromboembolic complications. [152] Defective thrombin

binding, leading to increased levels of thrombin, and an abnormal fibrin clot resistant to fibrinolysis are the probable mechanisms behind this paradoxical clinical presentation. [53]

In the bleeding patient, fibrinogen is consumed and it is the first coagulation factor to reach critically low levels in massive blood loss. [98] Compared to some of the other coagulation factors, fibrinogen is used up once it has been transformed into fibrin and incorporated in the fibrin clot. Sufficient plasma concentration is therefore necessary to maintain hemostasis in the bleeding patient. [131, 216]

Fibrinogen is an acute phase reactant and fibrinogen plasma concentration increases for instance due to trauma, surgery, inflammation or infection. [173, 188, 207, 227] In a study comparing postoperative fibrinogen plasma concentration between patients who did versus did not receive intraoperative fibrinogen supplementation, there was no statistical difference between the two groups. [213]

Fibrinogen derived from human plasma is commercially available with the indication congenital hypo- or afibrinogenemia. Today, fibrinogen concentrate is mainly used in patients with acquired hypofibrinogenemia because of massive blood loss and consumption due to trauma or surgery as described above, and its use is increasing. [212] The development of recombinant fibrinogen is desired and is advancing. Using a human cell line transfected with optimized cDNA encoding the fibrinogen Aα, Bβ and γ chains, fibrinogen can be produced in a controlled setting. [186] In an ex vivo setting, recombinant fibrinogen has been shown to have similar pro-coagulant characteristics as plasma-derived fibrinogen on blood samples from cardiac surgery patients. However, further studies are needed before recombinant fibrinogen can be used in clinical practice.

1.2.4 Factor XIII

Coagulation factor XIII is a plasma pro-transglutaminase with multiple functions not only in coagulation but also in a wide range of physiological and pathological processes. Factor XIII exists in an extracellular form, circulating in plasma, and an intracellular form. The main function of FXIII is to cross-link with fibrin to stabilize the fibrin clot and protect it from fibrinolysis. [209]

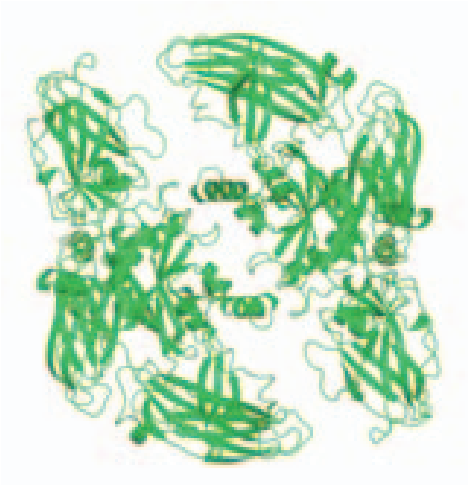

Figure 4. FXIII tetramer. With permission from the Protein Data Bank in Europe. [83]

Plasma FXIII (pFXIII) consists of two potentially active, catalytic A subunits (FXIII-A) and two inhibitory B subunits (FXIII-B), together forming a tetramer FXII-A_2B_2. Factor XIII-A is a transglutaminase consisting of 732 amino acids and is mainly synthesized by cells of bone marrow origin. However, when bone marrow function is impaired cells of unknown origin take over the production of FXIII-A. Factor XIII-B is a glycoprotein consisting of 641 amino acids and is synthesized in the liver. [248] The two subunits form their tetrameric complex in the circulating blood. In plasma, FXIII-A circulates only in its fully complexed form, while approximately 50% of FXIII-B circulates in free, non-complexed form. [252] Factor XIII is effectively activated in plasma only on the surface of newly formed fibrin. [206]

Cellular FXIII (cFXIII) is a dimer consisting of only FXIII-A, FXIII-A_2; it can be found mainly in platelets and monocytes/macrophages but also in chondrocytes, osteoblasts and osteocytes. [6, 171] As opposed to pFXIII, cFXIII is activated by an increase in cellular Ca2+, a non-proteolytic mechanism. [168]

Congenital FXIII deficiency is a rare bleeding disorder (estimated prevalence 1:1,000,000 to1:5,000,000). [72] Mutations occur in the FXIII-A genome, more than 100 are identified so far, causing either absence of FXIII or

dysfunction in the synthesized protein. [197] Patients with congenital FXIII deficiency present with symptoms like severe bleeding, spontaneous intracranial hemorrhages, poor wound healing and spontaneous abortions. [101]

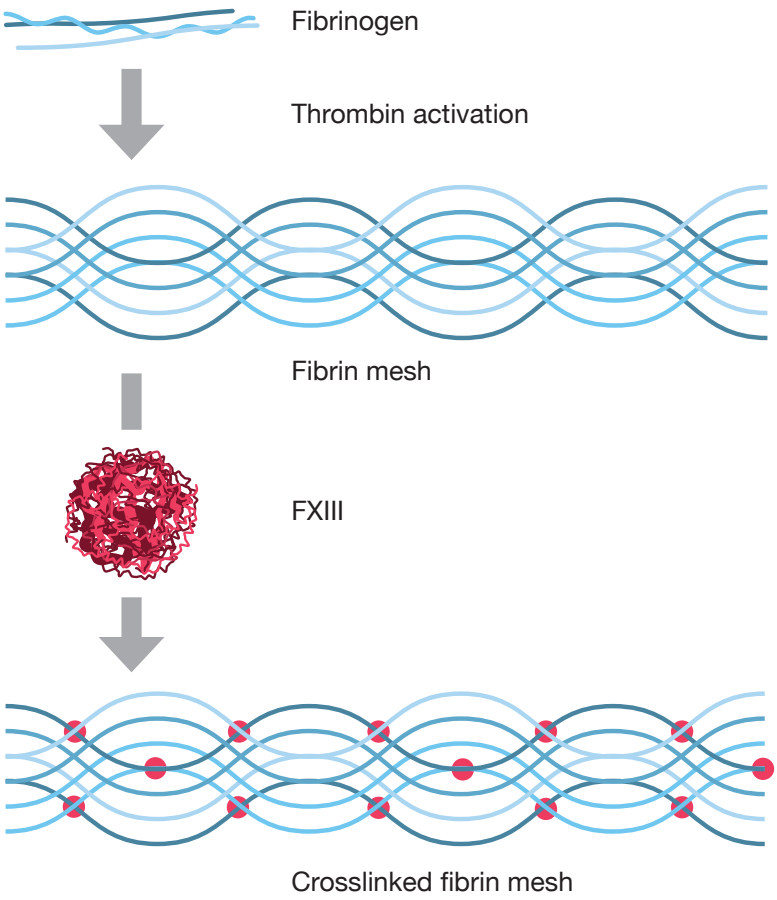

Figure 1. Factor XIII (FXIII) crosslinks with fibrin in the fibrin mesh.

Human plasma-derived FXIII is available and it is used in patients with congenital FXIII deficiency. In study settings, FXIII concentrate has been used in patients with acquired FXIII deficiency due to trauma or perioperative bleeding, with differing results. [77, 115, 128] Recently, recombinant FXIII has been tested in patients with congenital FXIII

deficiency, with promising results. [106, 123] The recombinant FXIII is manufactured in yeast cells and contains no mammalian or human products, thus minimizing the risk of transferring infectious, or other diseases. [146]

1.2.5 Coagulation assays

Various laboratory assays are used to investigate a patient's coagulation status. The most common are PT and activated partial thromboplastin time (aPTT). These two tests are often routinely taken to assess preoperative coagulation status in patients scheduled for elective surgery. [126, 244] Other ways of measuring a patient's coagulation capacity are by measuring individual coagulation factors known to influence perioperative hemostasis, such as fibrinogen, and also by using point-of-care (POC) analyses.

Blood coagulation is a dynamic process and conditions can change quickly. The coagulation assays mentioned above are time consuming and are passé by the time the results arrive in the emergency or operating room. Both PT and aPTT only measure part of the coagulation cascade and the measurement ends at clot formation. Point-of-care analyses, such as viscoelastic tests and platelet aggregation assays, can be used to obtain faster information about a patient's coagulation status, and also provide information on clot quality and fibrinolysis. At the same time, POC analyses require knowledge and experience in how to interpret the results. [69, 245]

Prothrombin time

Prothrombin time measures the extrinsic pathway. The assay is affected by the levels of the vitamin K-dependent coagulation factors II (prothrombin), V, VII and X, as well as very low levels of fibrinogen. [124] The test was first described in 1935 by Quick, therefore initially called Quick's time, but the name was later changed to PT. [184, 185] Different reagents are used when measuring PT, giving different results especially in patients on oral anticoagulation treatment. Prothrombin time is therefore standardized as an International Normalized Ratio (INR). In Scandinavia, laboratories use the method developed by Owren, which only measures levels of factors II, VII and X. [124] Test results for the two tests in the normal range are similar. [100]

A patient with a PT of <1.5 is considered to have a low risk of bleeding during and after surgery. [229] A recent study suggests that the best cut-off point for avoiding major bleeding during surgery is 1.1, which is notably lower than previous recommendations. [223]

Activated partial thromboplastin time

Activated partial thromboplastin time is a test that roughly studies the intrinsic pathway of the coagulation cascade. In 1913, a test designed to confirm the diagnosis of severe hemophilia, called whole blood clotting time (WBCT) was developed. [139] The test was further refined in steps, until it finally became the test used today. [135, 183] Unlike WBCT, aPTT uses platelet-free plasma from blood-samples taken in citrated tubes. Samples are recalcified, and partial thromboplastin is added. The, often automated, photo-optical apparatus then measures the time from when the test is initiated until a clot is formed. In order to speed up the time before the clot forms, an activator is added, hence the name *activated* PTT. Activated partial thromboplastin time is measured in seconds (s) and the reference interval for adults is 30-42 s.

Functional deficits in factors VIII, IX, XI, XII, prekallikrein and high molecular weight kininogen, as well as severe deficits in factors V, X, II and fibrinogen can affect aPTT measurements. [124]

In the latest guidelines, neither PT nor aPTT are recommended for routine preoperative testing of a patient's coagulation status. [131]

Fibrinogen

Fibrinogen plasma concentration can be measured in different ways. The most common is the method described by Clauss in 1957. [49] This method actually measures the clotting time in diluted plasma and compares that time with reference plasma with known fibrinogen concentration. The test sample is saturated with thrombin so that the amount of thrombin in the original sample does not influence the clotting time. The method of Clauss is considered the most frequently used assay.

The PT-derived fibrinogen assay measures the patient's PT in platelet-poor plasma and compares it to the PT in plasma dilutions with known fibrinogen concentration.

To measure protein concentration, various immunological assays, like ELISA or the immune-nephelometric method, may be used. In congenital dysfibrinogenemia there is usually a discrepancy between fibrinogen activity and antigen level. The fibrin clot may also be extracted, cleaned, dried and

then weighed. This is a time consuming test not often used in the clinical setting.

The different assays are not comparable in terms of measured fibrinogen levels. [148] Fibrinogen assays are also influenced by pharmaceuticals, hemodilution and by the level of fibrinogen in the blood sample. [62, 142, 148]

In the acute situation, fibrinogen levels may be estimated using viscoelastic methods such as thromboelastography or thromboelastometry. There is a correlation between fibrinogen plasma concentration, as measured by Clauss, and thromboelastometry/thromboelastography results, but the viscoelastic results are not yet fully standardized. [159, 214, 231]

Factor XIII

The main reasons for analyzing FXIII are to measure FXIII activity in patients with suspected FXIII deficiency and to monitor substitution therapy in patients with congenital FXIII deficiency. [119] There are many different assays, both quantitative and qualitative and, to diagnose a deficiency the assays should be used in combination in order to reach the correct diagnosis and classification. [127]

Quantitative assays activate FXIII with thrombin and Ca^{2+}. In order to eliminate interfering fibrin formation, various measures such as high plasma dilution and fibrin polymerization inhibitory peptide, are used. Amine incorporation assays measure the binding of amine to FXIIIa. This method is highly sensitive and can detect a FXIII activity as low as 1% but the method is time-consuming and, because of the many steps involved the method cannot be automated and standardized. [119]

The recommended screening test for FXIII activity is a quantitative assay based on ammonia release from FXIIIa. It was first described in 1969. [243] Modern assays measure a substrate protein that captures the released ammonia, and the consumption of the protein can be measured with photometric methods. [67, 117] The method is quick, automated and reproducible. Its drawbacks are the low sensitivity, and that it needs a parallel blank sample that can correct for the ammonia produced in the plasma from sources other than FXIIIa. [10]

The original test for the clot stabilizing protein was to create a fibrin clot and then to try to dissolve it in a urea or acid solution. [132] Clot solubility tests are still in use today, but because the test is not standardized – different clot activators, clot formation time, solvent, etc. are used – the test is not recommended for testing for FXIII deficiency testing. [127]

In patients with a suspected congenital or acquired FXIII deficiency a low FXIII activity on a quantitative assay requires further investigation into the type of FXIII deficiency present. [127]

In the bleeding patient, FXIII deficiency can be assumed to result from consumption and a fast evaluation of FXIII may be critical. Unlike measurement of fibrinogen, where there is a correlation between regular fibrinogen plasma concentration assays and viscoelastic tests, no such correlation is seen between viscoelastic tests and regular measurement of FXIII activity. [81]

Viscoelastic tests

Thromboelastography was designed by H Hartet and described in an article in 1948. [89] The idea is that a blood sample is placed in a cup into which a pin is inserted. The cup oscillates and as the blood coagulates the pin starts to move along with the cup, figure 6. This movement increases as the viscosity of the blood sample increases, and this is recorded and analyzed. (Figure 7) Today, two different devices using the same principle of blood viscosity as a means to measure coagulation are in use – the Thromboelastograph® (TEG®) which is based on Hartet's original design, and Thromboelastometry® (ROTEM®), which is a further development using the same principle as thromboelastography. [147]

Sonoclot®, which measures the changes in mechanical impedance using ultrasound, is another bedside device first described by von Kaulla et al. in 1975. [238] The set-up is similar to thromboelastography and thromboelastometry, with the difference that the measuring pin moves up and down in the blood sample.

This section will focus on thromboelastometry, since it is the device used in **Paper IV**. However, thromboelastometry, thromboelastography and Sonoclot® produce largely comparable results.

Blood samples are taken in a citrated tube. Ideally, the sample should rest for 30 minutes before analysis, but the blood sample can be analyzed immediately if time is short. [236] The sample volume in the measuring cup is 340 µL and to that volume, reagents are added. (Table 4) The reagents are chosen depending on what part of the coagulation system is to be assessed. The thromboelastometry device is designed in such a way that the pipetting steps are automated. The cup is placed in a holder and raised so that the measuring pin is inserted into the cup. The analysis starts when the reagent is added to the cup. For a description of test results, see *Table 3.*

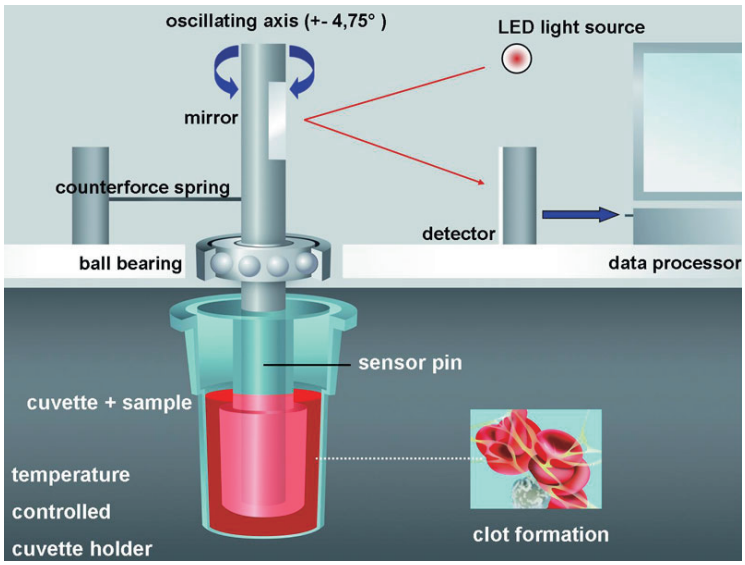

Figure 2. Principle of thromboelastometry. Reproduced with permission from ROTEM®.

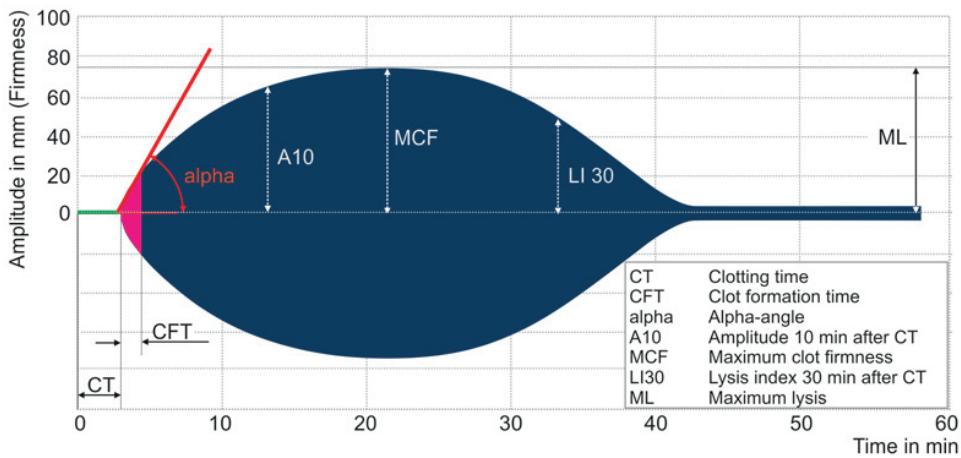

Figure 3. Thromboelastogram. Reproduce with the permission of ROTEM®.

Because of the short time from blood sample to test result, thromboelastometry has become a widely used tool to monitor coagulation in the bleeding patient. In the latest guidelines from the European Society of Anaesthesiology (ESA) on management of severe perioperative bleeding the POC coagulation monitoring with viscoelastic tests is recommended. [131]

Thromboelastometry and thromboelastography are known to be user dependent, results often vary, and test accuracy has not been widely studied. [47, 104] Reference ranges for the different assays have been developed, but results should be analyzed and acted upon based on the combined results from the assays. [134]

Table 3. *Test results*

Result	Description	Reference value
Clotting time – CT	Time from addition of start reagent until clotting begins	INTEM: 137-246 s EXTEM: 42-74 s FIBTEM: 43-69 s
Clot formation time – CFT	Time from CT to when a clot is formed	INTEM: 40-100 s EXTEM: 46-148 s
Amplitude 10 minutes after CT – A10		INTEM: 44-68 s EXTEM: 43-65 s FIBTEM: 9-24 s
Maximum clot firmness – MCF	Maximum vertical amplitude of the graph	INTEM: 52-72 mm EXTEM: 49-71 mm FIBTEM: 9-25 s
Maximum lysis – ML	Amount of lysis	INTEM: 0-12 % EXTEM: 0-18 %

s = seconds, mm = millimeter

Table 4. *List of assays used in thromboelastometry analysis, its reagents and the interpretation of test results.*

Test	Reagent	Interpretation
INTEM	Ca2+, phospholipids, ellagic acid	Coagulation through the intrinsic pathway
EXTEM	Tissue factor	Coagulation through the extrinsic pathway
HEPTEM	Ellagic acid, heparinase	The effect of heparin in heparinazed patients; compare HEPTEM with INTEM
FIBTEM	Cytochalasin D, tissue factor	Coagulation without platelet activation
APTEM	Aprotinin, tissue factor	Faster detection of fibrinolysis

Platelet aggregation

Platelet function is essential for blood coagulation but is difficult to evaluate. The standard platelet count only tells us the amount of circulating platelets but does not give any information on the quality and function of the platelets. As mentioned above, many different factors affect platelet function and, at least in patients prescribed platelet inhibitors, it is important to assess thrombocyte function before surgery.

Various POC devices are available to assess platelet drug inhibition. Impedance aggregometry and light transmission aggregometry are two of the methods to measure platelet function in the clinical setting. Whole blood is used in order to avoid the potential activation of the platelets that may occur when the sample is centrifuged to form platelet-rich plasma. In impedance aggregometry a small electric current is transmitted through the blood sample between two platinum electrodes and, as the platelets aggregate on the electrodes, the electric resistance increases. Cardinal and Flower first described this method in 1979. [41] Light transmission aggregometry measures changes in light as platelets aggregate. Today, there are several POC devices available that are similar in design and test results, such as Multiplate® (Roche Diagnostics, Basel, Switzerland), VerifyNow® (Accumetrics, San Diego, CA, USA) or Chrono-Log® (Chrono-log, Havertown, PA, USA).

1.3 Patient blood management

In 1628, the British physician William Harvey published his book on blood circulation. He described how blood was pumped through the body by the heart and flowed through the vessels. This knowledge formed the foundation for further research on the circulatory system, bleeding and transfusion. There is a debate on who performed the first human-to-human blood transfusion. [136]

Doctor Richard Lower, UK, experimented on dog-to-dog transfusion in 1665, as described in several recordings in the Journal Book of the Royal Society. In 1667 he made a successful dog-to-man blood transfusion. Lamb blood was also used for transfusion but, because of several complications with this practice, the Pope issued a ban on the procedure in 1679. [136]

The first documented human-to-human blood transfusion was performed on 22 December 1818, and was published in 1819 by a British physician and obstetrician named James Blundell. As an obstetrician he saw the consequences of post-partum hemorrhage and saw blood transfusion as a way to treat this condition. [28] From that point on the practice of blood transfusion, although rare at first, became in use.

Two discoveries made blood transfusion feasible and integrated in everyday clinical practice. The first was the discovery of the AB0 blood groups in 1901 by Landsteiner. [133] The second was the introduction of sodium citrate to prevent the blood from clotting thereby enabling storage and blood-banking. [141] Two World Wars led to further development in transfusion practices and how to organize blood-banking and donors so as to ensure a constant supply of blood products. [31]

At the start of transfusion history, whole blood was given. Today fresh whole blood transfusion has seen a revival in war medicine. The harsh conditions in medical facilities close to the battlefield do not provide the infrastructure needed for the modern blood-bank. At the same time, massive hemorrhage is one of the leading causes of death in warfare, and prompt transfusion can be life-saving. Fresh whole blood transfusion has therefor been in practice in several war situations with good results. [20, 217]

Today, blood transfusions are a part of the clinical setting. In Sweden, approximately 100,000 people receive blood transfusion every year and, in 2014, 462,629 blood units were donated. [1] However, the use of blood transfusions is declining, both because of increasing knowledge about

potential risks associated with transfusion but also because of better understanding about when and how to transfuse.

1.3.1 Transfusion and fluid substitution

In modern transfusion medicine, whole blood is divided into three components; erythrocytes, plasma and platelets, and transfusions are based on what the patient needs. Donated whole blood is centrifuged and the blood components are layered and thereafter stored into different storage bags.

Red blood cells

Red blood cells (RBC) is the most common blood component to be transfused. After separation from the other blood components, RBC are stored in an additive solution, in +2° - +6° C for up to 42 days. [1] Over time, the quality of the RBC decreases and changes in RBC takes place. This phenomenon is referred to as storage lesions and there are discussions on how they affect patients. [14, 56]

Plasma

Plasma transfusion has many potential advantages in the bleeding patient. It is a source of coagulation factors, a volume expander and a means to reverse the anticoagulant effect of drugs. Plasma is obtained either from whole blood donors, through separation, or from apheresis with plasma extracted from one donor. [21] Most of the plasma is frozen to -18°C within 6 to 8 hours after donation and is labeled fresh frozen plasma (FFP). When needed, FFP is thawed. Plasma can also be stored as liquid, non-frozen plasma in +2° - +6°C for up to 14 days. [170] However, studies on FFP show that it has little effect on bleeding and transfusion in patients undergoing surgery. [129]

Platelets

Platelets are responsible for the primary hemostasis and are first to arrive at the site of injury to the vessel wall. They are easily activated however and donated platelets, platelet concentrate, are stored at room temperature and in constant agitation. [167] Because of the risk of bacterial growth platelets cannot be stored for more than 5 to 7 days. [234] There are two methods to

prepare platelet concentrate; either pooled buffy coat platelets extracted from 4 to 6 whole blood donors or apheresis platelets extracted from one donor. The advantage with apheresis platelets is that the recipients are exposed to less antigen stimulation. [234]

Fluids

The body is adaptable and can function with a certain amount of anemia as long as there is a sufficient blood pressure to ensure tissue perfusion. Consequently, the recommendation is to start replacing blood loss with crystalloid or colloid fluids. [131, 216] It is important to be aware that colloids can affect blood coagulation. [63]

1.3.2 Hemostatic drugs

There are many different drugs that can be given before, during and after surgery to boost blood coagulation. Depending on the type of surgery and the patient's coagulation status the drugs can reduce perioperative bleeding and transfusion requirements.

Table 5. *List of hemostatic agents, information derived from www.fass.se [3]*

Hemostatic drug	Sales name	Content	Indication
Prothrombin complex concentrate (PCC)	Ocplex®, Confidex®	Human FII, FIX, FVII, FX, Protein C, Protein S	Reversal of anticoagulation with vitamin K inhibitors
Fibrinogen	Riastap®	Human fibrinogen	Congenital fibrinogen dysfunction or hypofibrinogenemia
FXIII	Cluvot®	Human FXIII	Congenital FXIII deficiency
Recombinant FVIIa	Novoseven®	Eptacog alfa	Congenital or acquired hemophilia
Desmopressin	Octostim®	Desmopressin	Congenital or acquired platelet dysfunction
Tranexamic acid	Pilexam®, Cyklokapron®	Tranexamic acid	Prevention and treatment of hyperfibrinolysis
Local hemostatics	For instance Tachosil®, Tiseel®, Evisel®	Fibrinogen, thrombin, aprotinin, FXIII	Local hemostasis

Coagulation factors

Fresh frozen plasma contains a variety of coagulation factors, but their concentration in plasma might not be sufficient to restore coagulation. Concentrated, specific coagulation factors are therefore available. Many of them are primarily produced to treat patients with coagulation disorders, but they are also used to treat acquired coagulation disorders/dysfunctions.

Prothrombin complex concentrate (PCC) contains vitamin K-dependent coagulation factors and is indicated for patients on oral anticoagulation with vitamin K inhibitors. [177] When reversing the anticoagulant effect in trauma or surgery patients, PCC should be administered in combination with vitamin K. The effect of PCC lasts for 6 to 8 hours and by then the reversal with vitamin K should have taken place.

In patients with traumatic or surgical bleeding, the most critical coagulation factor is fibrinogen. Several studies show that fibrinogen substitution in patients with major bleeding and signs of fibrinogen deficit reduces bleeding and transfusion requirements. [61, 232, 249]

Factor XIII is suggested to reduce bleeding and improve coagulation in surgery patients, even though results vary. [85, 115, 128, 203] In vitro studies show that FXIII increases clot firmness, but these results have not been confirmed in a clinical setting. [230, 231]

Recombinant factor VIIa (Novoseven®) has been shown to reduce bleeding and transfusion requirements, but not mortality, in trauma patients. [93] In cardiac surgery patients, recombinant FVIIa reduces bleeding but increases the risk of thrombotic events. [74] Results in studies vary however, and current guidelines do not suggest routine use of recombinant factor VIIa other than as a last resort when other treatments have failed to control the bleeding. [131, 216]

Desmopressin is a synthetic hormone that increases the release of von Willebrand factor on endothelial cells, thereby increasing platelet activation and aggregation. The intended use for desmopressin is in patients with mild hemophilia type A and von Willebrand disease. Studies show a small effect on transfusion requirements in surgery patients treated with desmopressin. [51]

Tranexamic acid

Dr Blundell, who established blood transfusions, was an obstetrician trying to save women from post-partum hemorrhage. [28] Tranexamic acid (TXA) was also developed by obstetricians, but they were looking for a treatment for women with heavy menstrual bleeding. In the 1960s two research groups, in Japan and Sweden, simultaneously found the antifibrinolytic properties of trans-4-aminomethyl-cyclohexanecarboxylic acid, in short tranexamic acid. [155, 175]

Another early use was to reduce bleeding during surgery in patients with bleeding disorders, but soon more and more applications for TXA were uncovered. [225] Today, TXA is used in different surgical settings such as gynecology and obstetrics, orthopaedics and cardiac surgery. It is also used as a local hemostatic in oral extraction and in nose bleeds.

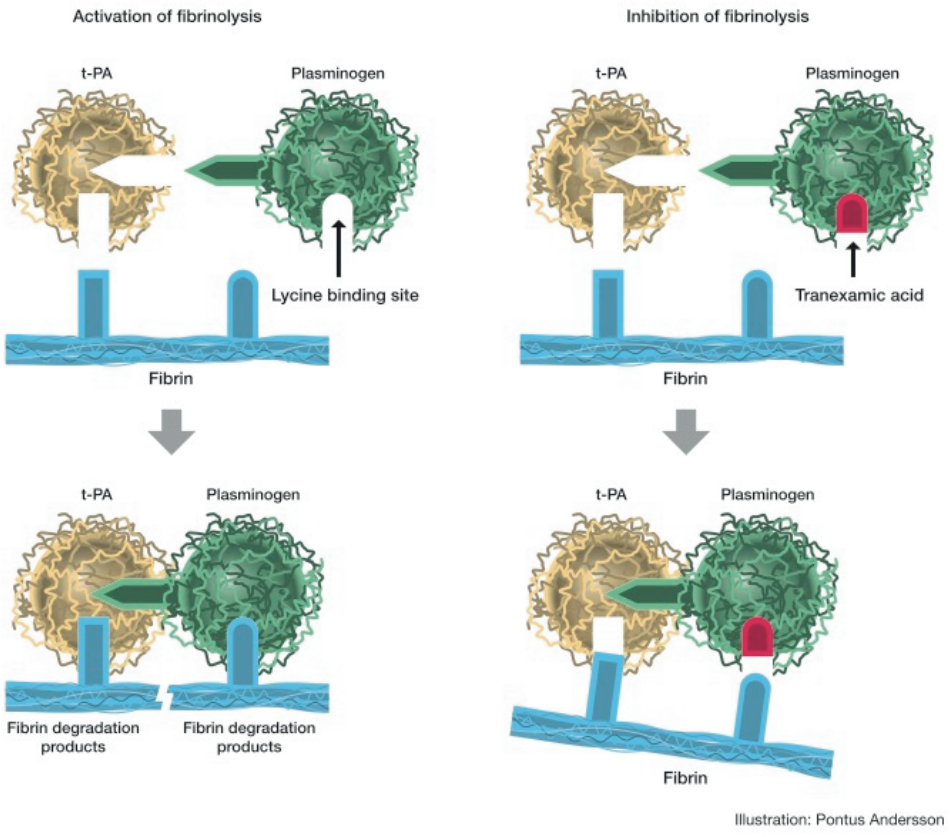

Figure 4. Working mechanism of tranexamic acid. T-PA=tissue plasmin activator.

Tranexamic acid binds to, and blocks, the plasminogen lysine binding site. Plasminogen is the precursor to plasmin, which facilitates the decomposition of the fibrin clot by fibrinolysis. When TXA hinders plasminogen from binding to fibrin at the lysine binding site, plasminogen cannot be activated to plasmin and fibrinolysis is slowed down. [225]

Both intravenous and topical TXA are known to reduce perioperative bleeding and the number of transfusions in many different surgical settings where major blood loss is expected. [38, 73, 122, 225] Tranexamic acid is also a cheap drug, and by reducing the number of transfusions, the use of TXA in surgery is highly cost-effective. [111] The use of TXA has also been evaluated in trauma patients in a large randomized, placebo-controlled study, CRASH-2, including 20,211 patients. The results showed that mortality independent of cause and mortality from hemorrhage was significantly lower in patients receiving TXA compared to placebo. [176]

Questions have been raised on the safety of TXA with regards to thromboembolic events. The CRASH-2 study showed no difference in death from vascular occlusion. [176] A meta-analysis from 2012 on knee arthroplasty patients showed no increase in thromboembolic disease in patients receiving TXA. [250] In patients with ongoing thromboembolic disease and risk factors for thromboembolic events TXA should be given with caution and more studies are required in these patient groups. [131]

Local hemostatic agents

There are different types of agents for obtaining a local hemostatic effect. Some create a larger surface area on which coagulation can occur, while some also contain coagulation factors. [222] The role of local hemostatic agents in orthopaedic surgery is not established and studies differ as to the effect on bleeding and transfusion requirements. [8, 25, 42, 140]

1.3.3 Indications for transfusion

As stated above, RBCs transport oxygen to the tissues and assist somewhat in blood coagulation. Transfusion should therefore be given when the patients lacks sufficient oxygen transportation and is affected by anemia. Transfusions should also be considered as one of many measures to ensure sufficient oxygen transportation.

Not many years ago, RBC transfusions were seen as a way to replenish the patient and also to speed up recovery. Transfusions were given liberally, and apart from rare immunological reactions, transfusions were considered safe and without side effects. In the 1980s, this perception changed with the appearance of HIV and several transfusion-related transmissions of the virus. Even though screening tests for HIV had been developed, the debate about the safety and necessity for transfusion had begun. [204]

It is important to differentiate between the bleeding patient and the non-bleeding patient. In a bleeding patient, indications for transfusion are much more liberal, and in massive-bleeding patients, other methods than Hb levels are used to determine how the patient should be transfused. [131]

The bleeding patient – intraoperative

During surgery, bleeding is ongoing and the blood that is lost contains not only oxygen transporters but also important coagulation factors. The surgical trauma creates a consumption of coagulation factors and triggers fibrinolysis. [179]

Under normal circumstances there is an overcapacity in oxygen transport, which means that the patient can handle a certain amount of bleeding as long as blood is substituted with fluids to maintain sufficient tissue perfusion. [23, 131] Both colloids and crystalloids can be used, but it is important to consider the anticoagulant effect in synthetic colloids.

With continuing bleeding, the blood loss reaches critical levels and transfusions are required to maintain tissue oxygenation. In a bleeding patient recommendations are to maintain the following parameters: [23, 131, 216]

- Hb level > 70-90 g/L
- Platelet count >100 x 10^9
- Fibrinogen >2.0 g/L
- Normothermia
- Normal pH ~7.2
- Calcium concentration, Ca^{2+} > 1 mmol/L

The rule of thumb is to give the patient what is lost through bleeding, which is a mixture of blood products that equals whole blood. In massive bleeding, transfusion packs are used with a predefined ratio of blood products, for instance 4 units of RBC, 4 units of FFP and one unit of platelets, or one unit

each of RBC, FFP and platelets. [131, 216] The optimal relationship between the different blood products is not fully established and more studies are needed.

In a patient with an ongoing large bleeding, fibrinogen is consumed and reaches critical levels. Early supplementation with fibrinogen concentrate is therefore needed, so to avoid compromising formation of the fibrin clot. The recommendation is to give 2-4 g of fibrinogen concentrate to a patient that requires large transfusion. [131, 216] Often fibrinogen is part of a transfusion pack.

It is not only critical to transfuse the right blood products; timing is also important. In order to maximize the effect of a transfusion pack, in combination with hemostatic drugs such as fibrinogen, patients need to be given the transfusions all together and as fast as the patient can tolerate. Red blood cells are needed for oxygen transport but also for helping platelets to migrate towards the vessel wall. Fibrinogen concentrate needs the coagulation factors in FFP to become activated to fibrin. Platelets are required to initiate coagulation and as a scaffold for propagation of clot formation. [23]

Trauma induced coagulopathy (TIC), pathophysiology and treatment have attracted increasing interest. [13] In trauma patients, the nature of the injury mechanism affects coagulation. Body temperature is reduced. Acidosis occurs due to tissue damage and compromised tissue perfusion. Fracture and large wounds creates major activation of the coagulation cascade with consumption and a subsequent reduction in coagulation factors. Massive bleeding also reduces coagulation factors and compromise tissue oxygenation through hypotension and anemia. [40]

Non-bleeding patients – postoperative

A patient in a postoperative setting has a different need for coagulation management than in the intraoperative setting. The first large-scale attempt to establish transfusion triggers in critical care was the Transfusion Requirement in Critical Care trial, published in 1999. [96] In the study, 838 patients were either assigned to a restrictive transfusion strategy or to a liberal transfusion strategy. The restrictive transfusion group had an Hb threshold for transfusion of 70 g/L, while the liberal transfusion group had a threshold of 100 g/L. The study showed no difference in mortality and morbidity between

these groups and the number of RBC units transfused were 54% lower in the restrictive transfusion group.

This breakthrough study began a debate on when to transfuse. Several randomized studies have been carried out, reaching similar conclusion. A study on hip fracture patients saw no difference in death or ability to walk across a room 60 days after surgery between a liberal or restrictive transfusion protocol. [45] Similarly, a post-hoc analysis on elective hip or knee arthroplasty patients saw no difference in morbidity and mortality as well as Quality of Life and fatigue scores depending on transfusion regiment. [211] A Cochrane review from 2012 suggests that in non-bleeding patients without acute coronary heart disease, Hb levels of 70-80 g/L do not require RBC transfusion. [44] However, these suggestions were recently challenged by a randomized study in cardiac surgery patients where a more liberal transfusion regimen, Hb <90 g/L compared to Hb <75 g/L, was associated with lower mortality. [165]

1.3.4 Risks associated with transfusions

Transfusion of blood products may be lifesaving but may also be associated with increased morbidity and mortality in patients undergoing surgery. [39, 70] It is however, difficult to distinguish between the risk of the underlying cause, for example excessive bleeding, and the risk of the blood transfusion per se. Known risks associated with transfusion are transmission of infectious microorganisms, immunological reactions and transfusion-related acute lung injury (TRALI). Today, measures are taken to minimize these hazards but patients still experience adverse effects from transfusions.

The transmission of infections through transfusions has been known since the 1940s and many measures, including screening and control of blood donors, have been taken to minimize these risks. [166] In modern transfusion practice, these measures have reduced the risk to extremely low levels.

As part of the procedures relating to transfusions, immunological testing of the recipient and the blood given is performed. Since the discovery of the AB0 blood groups, several other blood groups, like Rhesus antigen, have been revealed. In an acute situation, where there is no time for sufficient blood group testing, group 0 RBC and AB plasma should be used. [179]

Transfusion-related acute lung injury is nowadays considered a major cause of adverse events following transfusion. The first cases were described in

1983, and the connection with HLA antibodies was described, but it was not until 2003 and 2004 that the condition was defined. [151, 181] If acute lung injury (ALI) occurs within six hours of a plasma-containing blood component transfusion in a patient without previous ALI, it is considered to be TRALI. The definition for ALI is new onset hypoxemia (blood oxygen saturation <90% on room air), bilateral pulmonary infiltrates on chest x-rays, and without volume overload.

TRALI is mostly associated with transfusion of FFP or pooled platelets, especially if the blood donor is female. [151]

Studies indicate an increased risk of postoperative infection in patients who have received perioperative transfusion. [224] Other studies indicate an increased risk of recurrence of cancer in patients with perioperative transfusion at the first cancer surgery. The rationale behind these findings could be a transfusion-related immune modulation, but this theory is debated. [46, 235]

1.3.5 Measures to prevent transfusion

Erythropoietin

Erythropoietin is a glycoprotein produced in the kidneys to stimulate RBC proliferation. Production of erythropoietin is oxygen-dependent and hypoxia leads to higher erythropoietin production. Recombinant erythropoietin is available and is used to treat anemia in patients with chronic renal failure, anemia due to certain malignancy, or in preoperative blood management. [68]

Studies have been carried out where patients, preoperatively, are treated with erythropoietin and iron supplementation to stimulate RBC production. In orthopaedic surgery, erythropoietin reduces the need for RBC transfusion when compared to no treatment or iron supplementation alone, preoperatively. [59, 78, 242] Similar results have been seen in other types of surgery as well, and erythropoietin is considered to be of value for patients with mild preoperative anemia where nutritional or other reasons for anemia have been ruled out. [48, 131, 215]

Autologous blood transfusion

Worries about the spread of hepatitis in the 60s and 70s saw an increase in autologous blood transfusion, i.e. the practice of a patient giving blood prior to elective surgery that can be used, if needed, during surgery. This practice was again increased when HIV was discovered. [256]

Today, autologous transfusions should be considered as one of many ways to reduce allogeneic RBC transfusion. Many studies show that the indiscriminate use of preoperative autologous donation may not be cost-effective and that many blood units are wasted. [24, 29, 92] Even though the number of allogeneic transfusions is decreasing, the overall number of transfusions is increasing, probably due to the risk of anemia after donation. [97, 121] However, for a selected group of patients with a high risk of transfusion, autologous transfusion is an option. [153] The recommendation is that these patients should be treated with erythropoietin after blood donation to avoid preoperative anemia. Studies show that the use of autologous transfusion is decreasing. [253, 254] In 2002 only 0.13% of all transfusions in Sweden were autologous. [76]

Cell salvage

Autotransfusion of shed blood during surgery was first described in 1860 during an amputation. [35] Since then, the technique has been refined and is in clinical use in many settings, both intraoperatively and postoperatively. Several studies and meta-analysis have shown that the cell salvage technique reduces the use of allogeneic RBC transfusion. [33, 43, 103] There is a risk, however, that the salvaged RBC hemolyzes and that the suction blood impairs hemostasis. [84, 220] It is also considered that cell salvage should not be used in surgery for malignancy or infection, since there might be a risk of spreading malignant cells or infectious materials to the circulating blood. [57] These considerations are now under scrutiny and, at least for gastrointestinal surgery, one study has showed that intraoperative cell salvage is a safe practice. [34]

1.4 Study objectives

Blood coagulation during surgery has not been widely studied in the orthopaedic surgery setting. This is in spite of the fact that orthopaedic surgery is one of the surgical fields with the highest transfusion rates. As stated above transfusion of blood products is not without complications and there are other measures can be taken to prevent transfusion. Both blood coagulation status as well as other patient- or procedure-related factors could influence perioperative bleeding and transfusion requirements.

In **Paper I**, the goal was to identify preoperative indicators that could predict perioperative bleeding and transfusion requirements in a healthy group of patients undergoing a standardized orthopaedic surgical procedure, scoliosis surgery, known to be associated with relatively large bleeding volumes. Studies in other surgical fields, such as cardiac surgery, had shown that low fibrinogen was a risk factor for large bleeding and transfusion. Similar studies had not been carried out on orthopaedic surgery patients.

Transfusion requirements vary, both between studies but also between different orthopaedic surgical procedures. The trend over recent years has been to minimize surgical trauma by, for example, using smaller incisions and using drainage less frequently. **Paper II** was initiated to study the perioperative bleeding and transfusion requirements, and the associated risk factors, in the everyday surgical setting in total hip and knee arthroplasty patients.

The role of FXIII activity on perioperative bleeding and transfusion in surgery has not been established in orthopaedic surgery. In **Paper III** the objective was to investigate whether preoperative fibrinogen plasma concentration and FXIII activity could be useful tests for identifying patients at risk of perioperative bleeding, and transfusion requirements, in different groups of orthopaedic surgery patients.

The effect of FXIII supplementation on clot formation has been studied but not in blood samples from patients undergoing surgery and not in clinically relevant doses. **Paper IV** was therefore designed to investigate whether ex vivo FXIII concentrate supplementation can improve clot formation and whether the combination with fibrinogen concentrate or platelet supplementation alters the effect of the added FXIII concentrate.

Aim

2 AIM

The overall aim of the thesis was to investigate whether there is a relationship between coagulation factors and perioperative bleeding and transfusion in elective orthopaedic surgery patients.

The specific aims were:

- To study the relationship between preoperative fibrinogen plasma concentration and perioperative bleeding and transfusion requirements in idiopathic scoliosis surgery patients, degenerative spine surgery patients and arthroplasty patients. (**Papers I and III**)

- To describe perioperative bleeding and transfusion in hip and knee arthroplasty patients. (**Paper II**)

- To identify risk factors for excessive bleeding and transfusion in hip and knee arthroplasty patients. (**Paper II**)

- To study the relationship between fibrinogen and coagulation factor XIII activity and perioperative bleeding and transfusion requirements in hip and knee arthroplasty and degenerative spine fusion surgery patients. (**Paper III**)

- To study the ex vivo effect of FXIII supplementation, alone or in combination with fibrinogen or platelets, on clot formation in blood samples from cardiac surgery and idiopathic scoliosis surgery patients. (**Paper IV**)

Patients and methods

3 PATIENTS AND METHODS

3.1 Ethics

All studies were approved by the Research Ethics Committee at the University of Gothenburg.

3.2 Patients

Paper I

Eighty-two otherwise healthy patients with adolescent idiopathic scoliosis, undergoing posterior scoliosis surgery were included in the study. Patients were recruited and underwent surgery at Sahlgrenska University Hospital.

Paper II and III

The studies involved 245 patients undergoing either spine fusion surgery involving two or more spinal segments (n=52), elective total unilateral primary hip arthroplasty (THA) (n=114), or knee arthroplasty (TKA) (n=79). In **Paper II** only the hip and knee arthroplasty patients were investigated. Patients were recruited from a university hospital (Sahlgrenska University Hospital, Gothenburg, Sweden) and a municipal hospital (Kungälv Hospital, Kungälv, Sweden) between October 2009 and January 2011. Hip and knee patients underwent at both hospital, and spine patients solely at the university hospital.

The exclusion criteria were known liver disease and/or coagulation disorder. Patients on acetylsalicylic acid (ASA) or non-steroidal anti-inflammatory drugs (NSAID) were urged to discontinue these medications at least three days before surgery, according to the local guidelines. Patients on warfarin treatment were bridged with low molecular weight heparin (LMWH).

Paper IV

Nine patients undergoing cardiac surgery with cardiopulmonary bypass (CPB) and ten patients undergoing scoliosis surgery for adolescent idiopathic scoliosis were included in the study. Patients were recruited and underwent surgery at Sahlgrenska University Hospital. For cardiac surgery patients, clopidogrel or ticagrelor was discontinued at least five days before surgery. None of the scoliosis surgery patients were on any medication influencing coagulation or platelet function.

Table 6. *Patient characteristics*

	Paper I	Paper II and III			Paper IV	
Type of surgery	Scoliosis surgery n = 82	THA n = 114	TKA n = 79	Spine surgery n = 52	Cardiac surgery n = 9	Scoliosis surgery n = 10
Gender (Female)	70 (85%)	52 (46%)	47 (59%)	27 (52%)	0	8 (80%)
Age (years)	15 ±3	66 ±12	69 ±10	62 ±12	67 ±2	16 ±2
BMI (kg/m^2)	20.8 ±4.4	27.2 ±4.1	30.0 ±4.4	26.8 ±4.4	27.5 ±0.9	18.7 ±1.1

BMI = body mass index, THA = total hip arthroplasty surgery, TKA = Total knee arthroplasty surgery

3.3 Methods

3.3.1 Clinical management

Type of surgery

In **Papers I** and **IV**, all scoliosis surgery patients underwent posterior instrumented fusion. In **Papers II** and **III**, in the THA group, 55 patients (56%) received cemented prostheses, 25 (25%) received uncemented prostheses, 9 (9%) underwent a hybrid procedure (with an uncemented acetabular component and a cemented femoral component), and 10 patients (10%) underwent a reverse hybrid procedure (with a cemented acetabular component and an uncemented femoral component). All the patients in the TKA group received cemented knee prostheses, and all operations were carried out in bloodless field. The spine patients all underwent an instrumented lumbar or thoracolumbar fusion, and the number of segments fused ranged from 2 to 15, median 4.

In **Paper IV**, the cardiac surgery patients underwent coronary artery bypass surgery.

Anesthesia

In **Papers I** and **IV**, scoliosis surgery patients underwent surgery under total intravenous anesthesia. In **Papers II** and **III**, spinal anesthesia was used for 160 of the patients (95 THA and 65 TKA), and general anesthesia was used for the remaining patients, including all the spine patients.

In **Paper IV**, all cardiac surgery patients were operated with CPB. Before cannulation, heparin (Lövens, Ballerup, Denmark), 300 IU/kg, was given and supplemented as required to maintain an activated clotting time of more than 480 seconds. CPB was performed with a hollow-fiber membrane oxygenator. The operations were performed with standard nonpulsatile CPB technique in normothermia and hemodilution (hematocrit 20-30%). A standard flow of 2.4 $L \times min \times m^{-2}$ was used. Cardioprotection was achieved with cold blood cardioplegia. No topical cooling was used. Weaning off CPB was performed at a temperature of at least 36°C. Protamine (1 mg protamine per 100 units of heparin) was given to reverse the effect of heparin.

Tranexamic acid

In **Paper I**, all patients received 1 g of tranexamic acid (TXA) intravenously at the initiation of surgery. In **Papers II** and **III**, all THA and TKA patients received TXA perioperatively according to local routine. Seventeen (33%) of spine patients received TXA either before surgery or when bleeding, according to the individual surgeons preference. In **Paper IV**, all cardiac surgery patients received 2 g of TXA before initiation of surgery and after wound closure. In scoliosis surgery five patients received TXA at the initiation of surgery.

Thrombosis prophylaxis

In **Paper I**, patients received thrombosis prophylaxis with either LMWH or colloid infusion. Fifty-six patients received LMWH for seven days or until mobilized, with the first dose the day before surgery. The remaining 28 patients received Macrodex® 500 mL on the day of surgery and the following five days.

In **Papers II** and **III**, the new oral anticoagulant (NOAC) dabigatran was used as thrombosis prophylaxis in 104 THA patients and 75 TKA patients while the rest of the patients including all spine patients received LMWH. The first dose of dabigatran was administered in the evening after surgery while the first dose of LMWH was given the evening before surgery.

In **Paper IV**, all scoliosis surgery patients received LMWH with the first dose administered the evening before surgery. Cardiac surgery patients received LMWH, the first dose administered the morning after surgery.

Transfusion

In **Paper I**, red blood cell (RBC) transfusion was given when the hemoglobin (Hb) level decreased to <80 g/L. In **Papers II** and **III**, no specific transfusion protocol or transfusion triggers were used. In **Paper I** a cell saver was used for all patients and intraoperative autotransfusion of processed RBC was performed if the cell saver volume exceeded 400 mL.

3.3.2 Blood sampling and analysis

Sampling

In **Paper I**, blood samples were collected within 24 hours before surgery for analysis of Hb, platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen plasma concentration.

In **Papers II** and **III** blood samples were taken from a peripheral vein within 24 hours before surgery. Samples for PT, aPTT, fibrinogen and FXIII were collected in citrated tubes (0.129 M à 2.7 mL). The samples for fibrinogen and FXIII were centrifuged and the plasma was stored in -80°C for further analysis. Samples for Hb analysis and platelet count were collected in EDTA tubes (1.8 g/L).

In **Paper IV** blood samples were collected from an arterial line except in two scoliosis surgery patients where blood was collected from a peripheral venous catheter dedicated for blood sampling. Preoperative blood samples for both cardiac and scoliosis surgery patients were collected after the induction of anesthesia but before surgery started. In cardiac surgery patients, postoperative blood samples were collected when the patient was off CPB and after heparin had been neutralized with protamine. In scoliosis surgery

patients, blood samples were collected immediately on completion of surgery. Samples for ROTEM®, fibrinogen and FXIII analysis were collected in citrated tubes (0.129 M à 2.7 mL). The samples for fibrinogen and FXIII analysis were centrifuged and the plasma was stored in -80°C for further analysis. Samples for Hb analysis and platelet count were collected in EDTA tubes (1.8 g/L).

Analysis

In **Papers I-IV** Hb, platelet count, PT and aPTT were analyzed using clinical standard methods.

In **Papers I, III** and **IV** fibrinogen plasma concentration was measured by the modified method by Clauss (STA®-R; Diagnostica Stago, Asnieres, France), with a reference value of 2.0-4.5 g/L. [49] In **Papers III** and **IV** the FXIII activity was measured using a photometric method with a reference value of 0.7-1.4 kIU/L or 70-140% (Berichrom FXIII/Dade Behring, Marburg, Germany). [67]

In **Paper IV** clot formation was analyzed with thromboelastometry (ROTEM®, Pentapharm GmbH, Munich, Germany).

3.3.3 Definitions and calculation of bleeding

In **Papers I, III** and **IV**, perioperative bleeding in scoliosis and spine surgery patients and cardiac surgery patients was defined as intraoperative bleeding calculated from wound suction plus the estimated amount of blood in the cloths used, and drainage volumes in combination with postoperative bleeding which were drainage volumes during the first 24 h.

In **Papers II** and **III**, THA and TKA patients did not receive any postoperative drains and, for the TKA patients, bloodless field was also used, which meant that total bleeding could not be measured as described above. In these patients, perioperative bleeding was estimated instead based on the drop in Hb between the preoperative measurements and the measurements 24–48 h postoperatively, according to a formula developed by Brecher. [36] The formula used was as follows:

Estimated blood loss (EBL) = ((‘estimated blood volume (EBV)’ × ‘hematocrit preoperatively’ - EBV × ‘hematocrit postoperatively’) + (‘RBC transfusion’ × 200 + ‘intraoperative cell salvage’ × 0.55)) / 0.35.

For EBV, the formula is as follows: EBV = (0.0235 × (height in cm)$^{0.42246}$ × (weight in kg)$^{0.51456}$) × k, where k = 2.430 for women and 2.530 for men (12).

In **Paper I**, extensive bleeding was defined as bleeding in the upper quartile (>1920 mL), and extensive transfusion was defined as >2 units of RBC. Patients with a bleeding of ≤1920 mL were defined as normal bleeders.

In **Paper II** patients in the upper 75th percentile of EBL/kg in the THA and TKA group respectively, were defined as excessive bleeders while all other patients were considered to be non-excessive bleeders.

In **Paper III** large bleeding was defined as >2,000 mL and large transfusion was defined as RBC >2 units and/or transfusion of ≥500 mL cell saver-processed blood. [19, 200]

3.3.4 Preparation of samples

In **Paper IV**, preoperative clot formation was analyzed in whole blood without additives. Postoperatively, ten different samples were prepared for each study subject; one baseline and three with increasing doses of FXIII concentrate (Fibrogammin®, CSL Behring, Marburg, Germany) (+20, +40 and +60%) alone or in combination with a fixed dose of fibrinogen concentrate (+1.0 g/L) (Riastap®, CSL Behring) or freshly made apheresis platelets (+92 × 109 × L^{-1}) from the institutional blood bank. Phosphate-buffered saline (PBS), 140 mM NaCl, 10 mM Na3PO4, pH 7.4) was used in different volumes to keep the same hemodilution in the samples. All samples for clot formation analysis had a total volume of 987 µL thereof 760 µL citrated whole blood.

3.3.5 Statistical analysis

In **Papers I-IV**, statistical significance was defined as a p-value of <0.05. Data are presented as mean and standard deviation, median and range, or numbers and percentage where appropriate. Continuous variables were compared with Student t-test or Mann-Whitney U-test, depending on

distribution, and categorical variables were compared with Chi-square test. In the statistical analyses PT was considered a continuous variable.

In **Paper I**, correlation between laboratory variables and bleeding were calculated with Pearson test.

In **Paper II**, statistical models for identification of predictors of blood loss and transfusion were made with logistic regression analysis. For multiple regression analysis, stepwise logistic regression analysis was used.

In **Paper III**, baseline and perioperative variables in the three surgery groups were compared using an ANOVA or Kruskal-Wallis test, depending on distribution. Changes within a group were compared with paired t-test or Wilcoxon rank-sum test, as appropriate.

In **Paper IV**, factor analysis was used to identify the difference in ROTEM® results between the different blood samples prepared. Calculations were done on logarithmic data in order to produce a normal distribution. Factor XIII dose was used as a fixed factor while patient and treatment, FXIII alone or in combination with fibrinogen or platelets was used as random factors.

For statistical calculations, SPSS software version 18.0 was used for **Paper I** and version 20.0 for **Papers II-IV**. (IBM Corp., Armonk, NY, USA)

Results

4 RESULTS

4.1 Paper I

Patient characteristics, hemostatic variables and bleeding and transfusion data are presented in *Table 7*. Patients defined as bleeders (perioperative bleeding >1920 mL) had a longer operation time, were more likely to be on colloid prophylaxis and had a lower preoperative fibrinogen concentration compared to normal bleeders (perioperative bleeding ≤1920 mL), *Table 8*. Patients needing extensive transfusion, defined as >2 units of RBC transfusion, had a higher total bleeding volume, a lower preoperative fibrinogen concentration, were more likely to be on colloid prophylaxis, were more likely female, and had a lower body mass index (BMI).

The positive and negative predicting values for extensive total bleeding for a preoperative fibrinogen concentration cut-off level of 2.8 g/L, were 47% and 94%, respectively. The corresponding values for extensive transfusion were 30% and 88%, respectively.

Total bleeding volume correlated significantly with preoperative fibrinogen concentration (r= -0.31, p=0.005) but not with platelet count, activated partial thromboplastin time (aPTT) nor prothrombin time (PT) (p=0.61, 0.46 and 0.57, respectively), *Figure 9*.

Table 7. *Patient hemostatic variables, and bleeding and transfusion data for patients in* ***Papers I-III****.*

	Paper I	**Paper II and III**		
Type of surgery	**Scoliosis surgery n = 82**	**THA n = 114**	**TKA n = 79**	**Spine surgery n = 52**
Hb (g/L)	137 ±12	136 ±12.7	133 ±12.3	124 ±14.0
Platelet count (10^9/L)	308 ±70	283 ±64.7	280 ±72.0	263 ±70.2
PT (INR)	1.1 ±0.1	1.0 ±0.1	1.0 ±0.1	1.1 ±0.093
aPTT (s)	35 ±3	34 ±3.8	33 ±3.3	36 ±4.7
Fibrinogen (g/L)	3.0 ±0.7	3.3 ±0.67	3.4 ±0.58	3.0 ±0.78
Factor XIII (kIU/L)	-	1.17 ±0.24	1.25 ±0.24	1.06 ±0.23
Operation time (min)	194 ±50	125 ±28.0	113 ±28.2	247 ±77.5
Total bleeding (mL)	1552 ±1019	984 (0-4,751)	758 (0-1,927)	1,715 (400-7,420)
Perioperative RBC transfusion	30 (37%)	21 (18%)	9 (11%)	35 (67%)
Extensive transfusion (> 2 RBC Units)	13 (16%)	2 (2%)	1 (1%)	18 (35%)
Large transfusion (> 2 RBC Units or > 500 ml cell salvage)	-	4 (3%)	1 (1%)	25 (48%)

THA = total hip arthroplasty surgery, TKA = total knee arthroplasty surgery, Hb = hemoglobin level, PT = prothrombin time, aPTT = activated partial thromboplastin time, RBC transfusion = red blood cell transfusion

Table 8. *Pre- and perioperative variables in bleeders (>1920 mL) vs. normal bleeders (≤1920 mL) and patients with extensive transfusion (>2 units of RBC) vs. patients with no or limited transfusion.*

	Bleeders n = 20	**Normal bleeders n = 62**	**Extensive RBC transfusion n = 13**	**No or limited RBC transfusion n= 69**
Age (years)	15 ±3	15 ±3	15 ±2	16 ±3
Female (n)	15 (75%)	55 (89%)	12 (92%)***	58 (84%)***
BMI (kg/m^2)	20 ±3	21 ±5	18 ±1*	21 ±5*
Operation time (s)	221 ±56**	156 ±45**	205 ±54	192 ±49
Colloid prophylaxis	13 (65%)**	15 (24%)**	9 (69%)**	19 (28%)**
Fibrinogen (g/L)	2.6 ±0.6***	3.1 ±0.6*	2.5 ±0.5**	3.1 ±0.6**
PT (INR)	1.1 ±0.1	1.1 ±0.1	1.1 ±0.1	1.1 ±0.1
aPTT (s)	36 ±3	36 ±3	36 ±4	36 ±3
Platelet count (10^9/L)	301 ±63	310 ±71	285 ±62	312 ±70
Hb (g/L)	138 ±9	136 ±13	136 ±8	137 ±12
RBC transfusion	3.0 ±2.4***	0.4 ±0.9***	-	-
Total bleeding (ml)	-	-	2964 ±1382**	1286 ±669**

*** p < 0.05, **p < 0.01, ***p < 0.001** vs. excessive bleeding or extensive transfusion. BMI = body mass index, Hb = hemoglobin level, PT = prothrombin time, aPTT = activated partial thromboplastin time, RBC transfusion = red blood cell transfusion

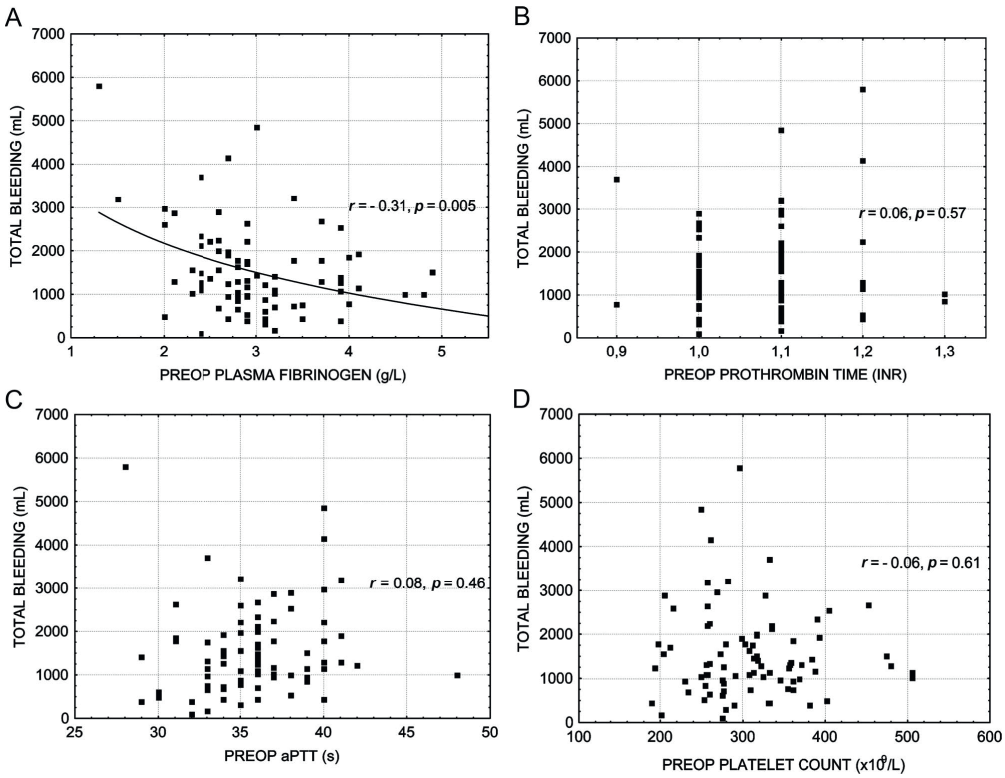

*Figure 5. Correlation between total bleeding volume and preoperative fibrinogen plasma concentration (A), prothrombin time (B), activated thromboplastin time (C), and platelet count (D). The equation for the logarithmic fit line in (A) is y = 3319 - 3807*log10(x).*

4.2 Paper II

Patient characteristics are presented in *Table 7*, page 48. All patients survived the perioperative period and there were no major complications except in one total hip arthroplasty (THA) patient who was re-operated because of inappropriate stem placement, and one total knee arthroplasty (TKA) patient who was re-operated because of early, deep infection.

Observed median intraoperative bleeding for THA patients was 450 mL (150-3,000 mL) and for TKA patients 0 mL (0-600 mL). Median estimated blood loss (EBL) was 984 and 758 mL for THA and TKA patients, respectively. There was a significant difference between observed perioperative bleeding and EBL for both THA and TKA patients, *Figure 10*.

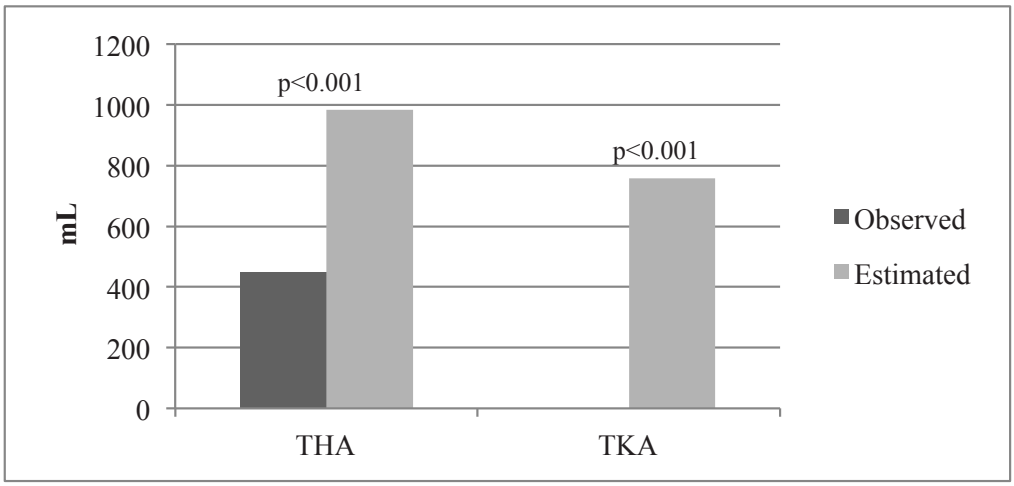

Figure 6. Observed and estimated median perioperative bleeding in patients undergoing total hip arthroplasty (THA) or total knee arthroplasty (TKA).

Univariate regression analysis results are presented in *Table 9* and *Table 10* for THA and TKA patients, respectively.

For THA patients a multiple regression analysis, female gender (odds ratio (OR) 3.91, 95% CI 1.41-10.88 per kg, p=0.009), low BMI (OR 0.82, 95% CI 0.71-0.94 per unit, p=0.005), and long operation time (OR 1.03, 95% CI 1.01-1.05 per min, p=0.004) increased the risk for excessive bleeding.

Preoperative Hb was the only factor significantly predictive of allogeneic RBC transfusion (OR 0.87, 95% CI 0.82-0.93 per g/L, p<0.001).

For TKA patients a multiple regression analysis, low BMI (OR 1.1, 95% CI 0.64-0.93 per unit, p=0.006) and high preoperative Hb (OR 1.11, 95% CI 1.04-1.17 per g/L, p=0.001) increased the risk of excessive bleeding. In multiple regression analysis, low BMI (OR 0.71 95% CI 0.51-0.98 per unit, p=0.034), low preoperative Hb (OR 0.71 95% CI 0.51-0.98 per g/L, p=0.015), and long operation time (OR 1.04 95% CI 1.00-1.07 per minute, p=0.036), increased the risk for RBC transfusion.

Table 9. *Univariate risk factor analysis in THA patients for excessive bleeding and transfusion*

	Excessive bleeding (EBL/kg >17.1 ml/kg)			**Allogeneic RBC transfusion**		
	OR	95% CI	P value	OR	95% CI	P value
Age (years)	1.00	0.99 - 1.07	0.11	1.05	1.01 - 1.10	*0.022*
Sex(F)	3.39	1.37 - 8.38	*0.008*	7.04	2.19 - 22.63	*0.001*
BMI (kg/m^2)	0.83	0.73 - 0.95	*0.006*	0.90	0.79 - 1.03	0.13
Weight (kg)	0.92	0.89 - 0.96	*<0.001*	0.94	0.91 - 0.98	*0.003*
NSAID (No)	1.42	0.51 - 3.93	0.50	0.66	0.24 - 1.83	0.42
ASA (No)	1.17	0.35 - 3.89	0.80	0.75	0.22 - 2.57	0.65
SSRI (No)	0.45	0.12 - 1.73	0.24	0.29	0.08 - 1.15	0.079
Hemoglobin (g/L)	0.97	0.94 - 1.01	0.12	0.87	0.82 - 0.93	*<0.001*
Platelet count (10^9/L)	1.01	1.00 - 1.01	0.17	1.00	1.00 - 1.01	0.52
PT (INR)	0.76	0.001 - 70.9	0.90	0.03	0.00 - 7.71	0.21
aPTT (s)	1.01	0.90 - 1.13	0.87	1.05	0.93 - 1.17	0.45
Operation time (minutes)	1.02	1.00 - 1.04	*0.017*	1.02	1.00 - 1.03	0.065
Anesthesia (Spinal)	0.89	0.29 - 2.75	0.85	0.82	0.24-2.77	0.75
EBL/kg		n.a.		1.17	1.09 - 1.26	*<0.001*

aPTT = activated partial thromboplastin time, BMI = body mass index, EBL = estimated blood loss, LMWH = low molecular weight heparin, NSAID = non-steroidal anti-inflammatory drugs, PT = prothrombin time, RBC transfusion = red blood cell transfusion, SSRI = selective serotonin receptor inhibitor

Table 10. *Univariate risk factor analysis in TKA patients for excessive bleeding and transfusion*

	Excessive bleeding (EBL/kg >12.7 ml/kg)			**Allogeneic RBC transfusion**		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Age (years)	1.02	0.97 - 1.08	0.45	0.99	0.92 - 1.06	0.79
Sex (F)	0.69	0.24 - 1.95	0.49	0.29	0.068 - 1.28	0.10
BMI (kg/m^2)	0.81	0.69 - 0.95	*0.010*	0.78	0.62 - 0.98	*0.034*
Weight (kg)	0.96	0.92 - 1.0	*0.046*	0.97	0.92 - 1.02	0.26
NSAID (No)	0.94	0.26 - 3.34	0.92	0.88	0.16 - 4.68	0.88
ASA (No)	1.14	0.32 - 4.0	0.84	2.57	0.3 - 22.02	0.39
SSRI (No)	1.12	0.21 - 5.93	0.89	1.03	0.11 - 9.37	0.98
Hemoglobin (g/l)	1.09	1.03 - 1.16	*0.001*	0.91	0.84 - 0.97	*0.007*
Platelet count (10^9/L)	1.0	0.99 - 1.01	0.56	1.001	0.99 - 1.01	0.88
PT (INR)	0.001	0.00 - 1.03	0.051	0.036	0 - 220.4	0.46
aPTT (s)	0.91	0.78 - 1.07	0.26	1.34	1.03 - 1.74	*0.028*
Operation time (minutes)	1.00	0.98 - 1.02	0.87	1.03	1.00 - 1.06	*0.034*
Anesthesia (Spinal)	2.12	0.43 - 10.48	0.35	0.72	0.13 - 3.92	0.71
EBL/kg		n.a.		1.020	0.88 – 1.18	0.795

aPTT = activated partial thromboplastin time, BMI = body mass index, EBL = estimated blood loss, LMWH = low molecular weight heparin, NSAID = non-steroidal anti-inflammatory drugs, PT = prothrombin time, RBC transfusion = red blood cell transfusion, SSRI = selective serotonin receptor inhibitor

4.3 Paper III

The three groups of patients, total hip arthroplasty (THA), total knee arthroplasty (TKA) and spine fusion surgery patients, differed in several baseline variables and coagulation parameters; age, BMI, Hb, PT, aPTT, fibrinogen and FXIII. Based on these findings the three surgical groups were analyzed separately. Preoperative fibrinogen plasma concentration and FXIII activity was higher in women than in men (3.4 ±0.7 vs. 3.1 ±0.7, p=0.001 and 1.24 ±0.23 vs. 1.10 ±0.24 kIU/L, p<0.001, respectively). One THA, one TKA and two spine patients were reoperated, none because of bleeding complications.

Information on perioperative bleeding and transfusion is presented in *Table 7*, page 48. Ten (9%) of THA patients, no TKA patients and 33 (64%) spine patients received intraoperative cell saver autotransfusion. No THA or TKA patients and 49 (94%) had postoperative drainage. There was a significant difference in bleeding volumes between the three study groups, p<0.001. In spine patients, there was no direct correlation between preoperative fibrinogen concentration and perioperative bleeding (r=-0.15, p=0.29) but a fibrinogen concentration ≤2.5 g/L was associated with a significantly larger perioperative bleeding (2,430 (400-6,560) mL vs. 1,390 (400-7,240) mL, *Table 11*. Accordingly, the proportion of spine surgery patients with a bleeding volume of >2,000 mL was significantly higher in patients with low fibrinogen concentration (61% vs. 32%, p=0.046). No such correlation could be seen for THA or TKA surgery patients.

Spine patients with fibrinogen ≤2.5 g/L were younger and had a lower BMI and preoperative FXIII activity while PT and aPTT were marginally longer. Number of segments fused and operation time did not differ significantly, *Table 13*. In a univariate analysis comparing patients with or without large bleeding, the only preoperative variables significantly associated with large bleeding were fibrinogen concentration >2.5 g/L (41% vs. 20%, p=0.046), and number of segments fused (5.5 (2-13) vs. 3 (2-15), p<0.001).

There was no correlation between preoperative fibrinogen plasma concentration and transfusion requirements for any of the surgery groups.

Low preoperative FXIII activity (<1.0 kIU/L) tended to correlate with large bleeding in spine surgery patients (p=0.055). There was no correlation between large bleeding and FXIII activity in THA and TKA patients. There was no correlation between FXIII activity and transfusion requirements in any of the surgery groups, *Table 12*.

Table 11. *Bleeding and transfusions in THA, TKA and spine surgery patients with preoperative fibrinogen concentration ≤2.5 or >2.5 g/L. Median and range or numbers (%).*

	THA		TKA		Spine surgery	
Fibrinogen (g/L)	≤2.5 n = 14	>2.5 n = 100	≤2.5 n = 4	>2.5 n = 75	≤2.5 n = 18	>2.5 n = 34
Bleeding (mL)	1,036 (91-2,393)	972 (0-4,571)	922 (620-1,313)	758 (0-1,927)	2,430 (400-6,560)	1,390 (400-7,420)
	p = 0.56		p = 0.61		*p = 0.029*	
Large bleeding (n)	6 (6%)	2 (14%)	0	0	11 (61%)	11 (32%)
	p = 0.26				*p = 0.046*	
Large transfusion (n)	0	4 (28%)	0	1 (1%)	11 (61%)	14 (41%)
	p = 0.45		p = 0.82		p = 0.17	

Large bleeding = >2,000 mL, large transfusion = RBC transfusion >2 units and/or transfusion of ≥500 mL cell saver-processed blood

Table 12. *Bleeding and transfusion in THA, TKA and spine surgery patients with FXIII concentration ≤1.0 or >1.0 kIE/L. Median and range or numbers (%).*

	THA		TKA		Spine surgery	
FXIII (kIU/L)	≤1.0 n = 28	>1.0 n = 86	≤1.0 n = 12	>1.0 n = 67	≤1.0 n = 21	>1.0 n = 31
Bleeding (mL)	1,011 (34-3,191)	964 (0-4,571)	626 (368-1,393)	782 (0-1,927)	2,040 (400-7,420)	1,380 (400-4,100)
	p = 0.88		p = 0.11		p = 0.055	
Large bleeding (n)	3 (11%)	5 (6%)	0	0	11 (52%)	11 (35%)
	p = 0.40		n.a.		p = 0.26	
Large transfusion (n)	2 (11%)	2 (2%)	0	1 (1 %)	10 (48%)	15 (48%)
	p = 0.23		p = 0.67		p = 0.96	

Large bleeding = >2,000 mL, large transfusion = RBC transfusion >2 units and/or transfusion of ≥500 mL cell saver-processed blood

Table 13. *Pre- and intraoperative variables in spine surgery patients with fibrinogen concentration ≤2.5g/L or >2.5g/L. Mean and standard deviation, median and range or number (%).*

	Fibrinogen ≤2.5g/L (n=18)	**Fibrinogen >2.5g/L (n=34)**	**p-value**
Female gender (n)	6 (33%)	13 (38%)	0.73
Age (years)	56 SD 12	65 SD 10	0.004
BMI (kg/m^2)	24.7 SD 3.9	27.9 SD 4.3	0.011
Hb (g/L)	127 SD 11	122 SD 15	0.81
Platelet count (10^9/L)	250 SD 64	270 SD 73	0.33
PT (INR)	1.05 SD 0.09	1.12 SD 0.09	*0.004*
aPTT (s)	38 SD 5.9	35 SD 3.7	*0.028*
Factor XIII (kIU/L)	0.91 SD 0.19	1.13 SD 0.21	*<0.001*
Tranexamic acid (n)	8 (44%)	9 (26%)	0.19
Number of segments (n)	5 (2-13)	4 (3-15)	0.60
Operation time (min)	254 (124-516)	217 (146-362)	0.20
Proportion of patients with drain (n)	17(94%)	32 (94%)	0.96

BMI = body mass index, Hb = hemoglobin level, PT = prothrombin time, aPTT = activated partial thromboplastin time

4.4 Paper IV

Patient characteristics are presented in *Table 7*, page 48. There was a significant difference in age, gender and BMI ($p<0.001$) for cardiac and scoliosis surgery patients. Perioperative blood analysis is presented in *Table 14*.

In both cardiac and scoliosis surgery patients, supplementation with increasing doses of FXIII shortened clotting time (CT) and increased maximum clot firmness (MCF). Supplementation with FXIII in combination with a fixed dose of fibrinogen or platelets shortened CT and increased MCF more compared with only FXIII, *Figure 11* and *Figure 12*.

In a factorial analysis the dose-dependent effect of increasing doses of FXIII on CT and MCF was unaffected by the supplementation of fibrinogen or platelets in both cardiac and scoliosis surgery patients. In cardiac surgery patients there was a significant decrease in CT between the lowest and the highest dose of FXIII (-4.2%, 95% CI -0.4% - -8.1%, $p=0.030$) and a significant increase in MCF between the lowest and the highest dose of FXIII (+7.5%, 95% CI 2.2% - 12.5%, $p=0.007$). In scoliosis surgery patients there was a significant increase in MCF between the lowest and the highest dose of FXIII (+9.3%, 95% CI 4.2% - 14.1%, $p<0.001$). There was no significant decrease in CT between the lowest and the highest dose of FXIII (-1.5%, 95% CI -6.6% - 3.4%, $p=0.55$).

Table 14. *Perioperative blood analysis*

	Cardiac surgery n=9	**Scoliosis surgery n=10**	**p-value between groups**
Hemoglobin (g/L)			
Preoperative	128 ± 3.3	118 ± 2.8	*0.032*
Postoperative	110 ± 4.3***	107 ± 3.0**	0.64
Platelet count (×10^9/L)			
Preoperative	215 ± 18	256 ± 18	0.13
Postoperative	145 ± 9.7**	236 ± 14	*<0.001*
Fibrinogen (g/L)			
Preoperative	3.1 ± 0.16	2.4 ± 0.15	*0.004*
Postoperative	2.7 ± 0.20*	2.0 ± 0.14**	*0.011*
Factor XIII (%)			
Preoperative	90 ± 5	76 ± 4	*0.035*
Postoperative	78 ± 5*	64 ± 4*	*0.036*
EXTEM-CT (s)			
Preoperative	45 (42-68)	55 (41-67)	0.079
Postoperative[#]	77 (60-101)**	69 (48-89)**	0.18
FIBTEM-MCF (mm)			
Preoperative	17 (14-26)	12 (10-19)	*0.004*
Postoperative[#]	12 (8-18)**	7 (6-14)**	*0.020*

EXTEM-CT = EXTEM clotting time; FIBTEM-MCF = FIBTEM maximum clot firmness
***=p<0.05, **=p<0.01, ***p<0.001,** # = After dilution to maintain the same hemodilution in all samples.

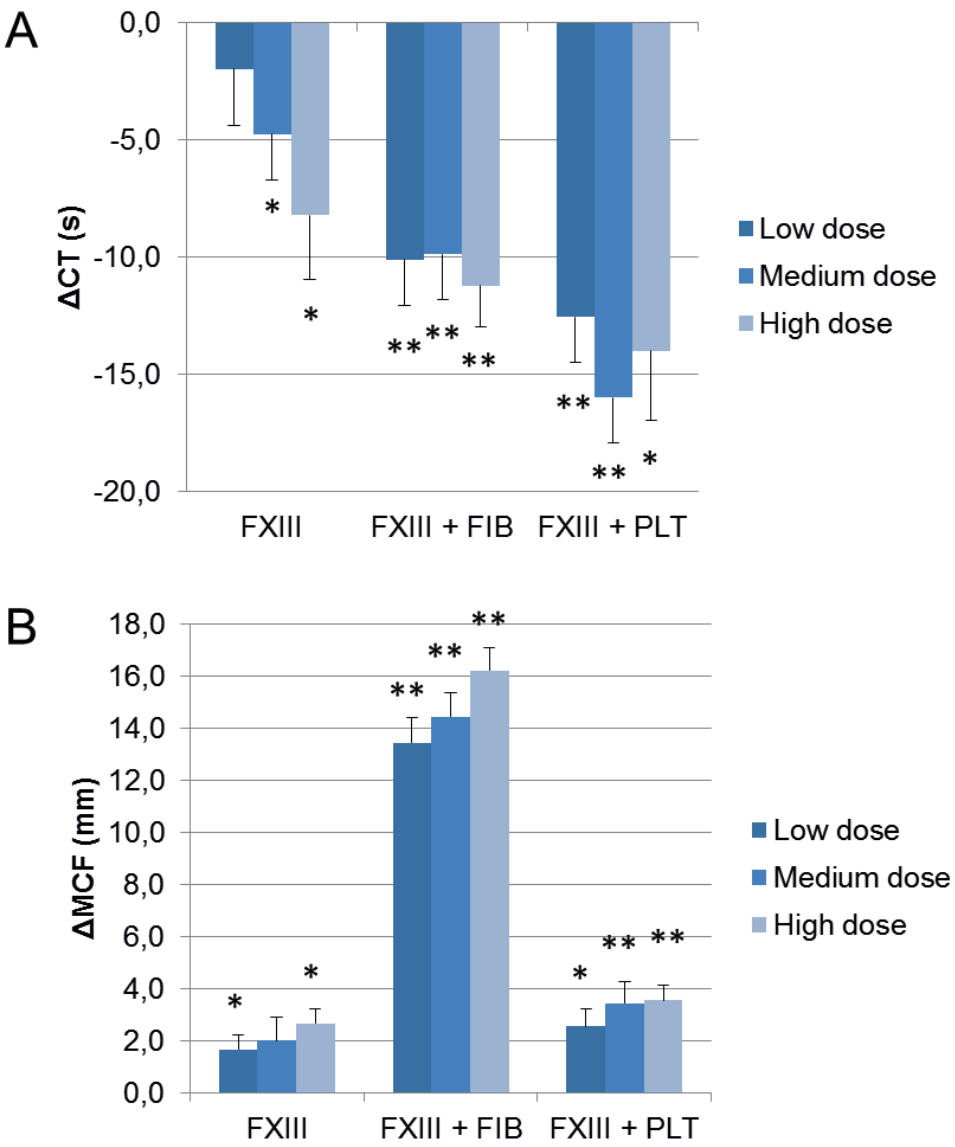

*Figure 7. Cardiac surgery patients. Absolute changes in EXTEM-clotting time (Panel A) and FIBTEM-maximum clot firmness (Panel B) after addition of increasing doses of Factor XIII (+20, +40 and +60%, respectively) alone or in combination with a fixed dose of fibrinogen or platelets.. * = p<0.05,**=p<0.01 in comparison to baseline.*

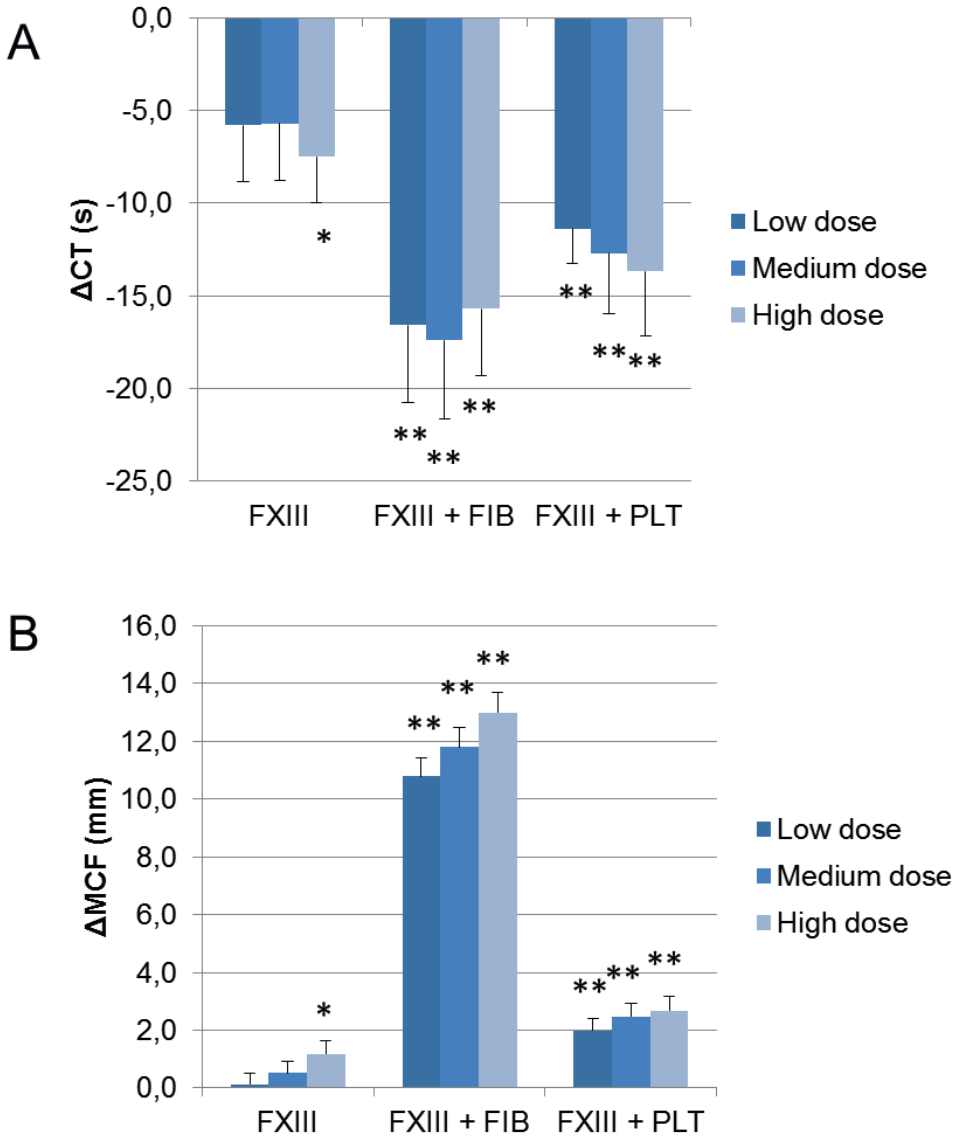

*Figure 8. Scoliosis surgery patients. Absolute changes in EXTEM-clotting time (Panel A) and FIBTEM-maximum clot firmness (Panel B) after addition of increasing doses of Factor XIII (+20, +40 and +60%, respectively) alone or in combination with a fixed dose of fibrinogen or platelets. See Methods for details. * = p<0.05,**=p<0.01 in comparison to baseline.*

Discussion

5 DISCUSSION

In modern orthopaedic surgery, many surgical procedures are carried out with minimal invasive methods, thereby minimizing bleeding and subsequently transfusion requirements. There are however, surgical procedures that require large exposures, like spine surgery, hip and knee arthroplasty surgery or trauma surgery. A large number of different factors can influence bleeding volumes and transfusion requirement and in order to minimize perioperative bleeding and transfusion requirements it is always important to make a plan to optimize the patients coagulation status and take appropriate measures.

5.1 Bleeding and transfusion in orthopaedic surgery

In **Papers I** and **III** perioperative blood loss was similar when comparing idiopathic scoliosis surgery, mean 1,552 mL ±1019 mL, and degenerative surgery patients, median 1,715 mL (range 400-7,420 mL). These numbers are similar with findings in other studies on bleeding in spine surgery. [241, 255] There was a large variation in bleeding in **Paper I** despite the similarity in surgical procedure, indicating that both patient-related and procedure-related factors influence perioperative blood loss. It is important to preoperatively identify the patients and procedures at risk of large bleeding in order minimize bleeding.

In **Paper II**, total hip arthroplasty (THA) patients had a median visual perioperative bleeding of 450 mL (150-3,000 mL) and in total knee arthroplasty (TKA) patients 0 mL (0-600 mL). However, the calculated perioperative bleeding was larger and there was a significant difference between the two types of bleeding measurements. This can easily be understood in TKA patients since the surgery was done in bloodless field. However, a substantial hidden blood loss also occurred in THA patients and the reason could be that no patients in the present study received drainage postoperatively. The extent of occult bleeding or hidden blood loss in THA and TKA surgery has previously been described and should be taken into consideration in perioperative blood management. [145, 202]

A study by Smorgick et al. shows hidden blood loss also occurs in spine surgery. [210] **Paper III** showed a good correlation and no significant difference between calculated blood loss and observed bleeding for spine surgery patients. A majority of the patients had postoperative drainage and

this regimen made it easier to assess the observed bleeding. At the same time drainage could increase perioperative bleeding by removing tissue pressure and allow for bleeding to continue.

Previous studies show a wide range of transfusion requirements in arthroplasty patients, between 21-70%, with the majority of studies reporting numbers in the middle range. [39, 108, 193, 219] The main finding in **Paper II** was that the rate of transfusion was lower than previously reported in unselected patients undergoing THA or TKA surgery. In the study, 18% of THA and 11% of TKA patients received perioperative red blood cell (RBC) transfusion. These transfusion rates are similar to studies where a transfusion protocol was used. [30, 114, 153, 192, 211] Some studies that use preoperative autologous blood donation programs still describe allogeneic transfusion rates of 9.6% and 13% in arthroplasty patients. [30, 153] Transfusion requirements have been decreasing the last decades and this seems to be a global trend.

The reasons behind the low transfusion rate in the present study can only be speculative, since indications for the transfusions given have not been studied. One reason could be that the patients had a normal preoperative hemoglobin (Hb) level which is known to decrease the risk of transfusion. Mean preoperative Hb levels were 136 ±12.7 g/L in THA patients and 133 ±12.3 g/L in TKA patients. Another reason could be the use of tranexamic acid (TXA), which is known to lower transfusion rates in orthopaedic surgery.

In **Papers I** and **III**, spine surgery patients had a high transfusion rate, 37% in idiopathic scoliosis surgery patients and 67% in degenerative spine surgery patients. Similar transfusion requirements, 18-60%, can be seen in other studies on spine surgery patients. [33, 255] In the present studies, the higher transfusion rate in spine surgery, compared to THA and TKA surgery, is reflected in a lower perioperative bleeding. At the same time, TXA was not used in all spine surgery patients in **Paper III** compared to the patients in **Paper I** and THA and TKA surgery patients, which could also explain the high transfusion rate in these patients.

There can be many reasons why there is a large difference in transfusion practice in general. One simple reason could be that some attending physicians are not aware of the risks of transfusion, thinking more about the risk of a postoperative low Hb level. Better knowledge about the disadvantages of transfusion – spread of infectious disease, transfusion related acute lung injury (TRALI) and immunosuppression – might reduce

transfusion rates. There are studies suggesting changes in attitudes towards lower transfusion thresholds. [95, 172] More studies need to be made on how both hospital staff, but also patients, perceive transfusion of blood products.

Fast-track surgery with perioperative protocols has reduced the length of hospital stay in arthroplasty surgery. In order to get the patient home quicker it might be tempting to treat general discomfort, dizziness and tiredness after surgery with a RBC transfusion. Studies on fast-track THA and TKA surgery indicate that RBC transfusion in fact increases length of stay. [105, 107] Since RBC transfusion is strongly correlated with pre- and postoperative anemia, which also is associated with prolonged hospital stay, it is however difficult to determine cause and effect. [205]

A transfusion protocol should include predefined Hb levels for transfusion as well as pre- and intraoperative measures to minimize bleeding and transfusion. Another important measure is to inform all the staff involved in patient care about the transfusion guidelines and about why measures are taken to minimize transfusions. [226] This training needs to be continuous. [233]

In **Paper II**, the low transfusion rates were not accompanied with any high complication rates when compared to the literature. Since the large study on transfusion requirements in critical care in 1999, several other studies in the surgical setting have been made to identify transfusion triggers for different patients. [96] In 2011 a randomized, prospective study on patients with a history of or risk factors for cardiovascular disease, undergoing hip fracture surgery a transfusion trigger of Hb level <80 g/L or <100 g/L had no impact on death or ability to walk independently 60 days after surgery. [45] Another retrospective study on patients who underwent major surgical procedures showed no increased risk of complications in patients with a restrictive transfusion trigger (Hb level <70 g/L) compared to a liberal transfusion trigger. [125] However, there is presently an ongoing discussion in this field. [66]

Using a low Hb level as a transfusion trigger in all patients is not an end in itself. In a recent study examining frail elderly patients undergoing hip fracture surgery there was an increased 90-day mortality for nursing home residents in the restrictive transfusion group compared to the liberal transfusion group. [80] The Hb levels used in this study were higher than in the studies previously mentioned; <97 g/L in the restrictive group and <113 g/L in the liberal group. In order to maintain a high acceptance for the

decided transfusion guidelines, a constant evaluation of the transfusion thresholds and their consequences in clinical practice is needed. [233]

In patients who cannot receive RBC transfusions, for religious or medical reasons, blood management protocols enable safe and successful arthroplasty or spine surgery without the need for transfusions. [90, 91, 112] With the known risks following transfusion, a suggestion worth to consider ought to be that all patients should be treated as if they refused transfusion and transfusion should only be used when all other measures – preoperative, intraoperative and postoperative – fail to sustain sufficient oxygen delivery.

The idiopathic scoliosis surgery patients studied in **Paper I** showed a correlation between perioperative bleeding and operation time and also with type of thrombosis prophylaxis; longer operation time gave a larger bleeding and patients with colloid prophylaxis bleed more. These risk factors for bleeding have been previously presented. [130, 255]

The causality between bleeding and operation time is not clear. Operation time may increase because the patient is bleeding which can make it difficult to proceed with the surgery. On the other hand, a more complex operation takes longer time and can also be expected to cause more bleeding. Of course the operation time should not be prolonged more than is necessary for a safe procedure to be carried out. Preoperative planning, for instance the approach, the reduction method, the choice of implant, may reduce operation time and this could thereby, indirectly, reduce the bleeding.

Artificial colloid infusions are known to impair hemostasis and this anticoagulant effect can be used as thrombosis prophylaxis. [63] Because of the increased bleeding risk, also described in **Paper I**, colloids should not routinely be used as thrombosis prophylaxis, especially since safer alternatives are now available. [71] Colloids also need to be distributed intravenously, which may extend the length of hospital stay.

Paper I did not show a correlation between preoperative Hb levels and extensive transfusion. This correlation has been shown in previous studies in different surgical settings. [9, 18, 30, 82, 92, 144, 157] The reason could be that the patients had a sufficient preoperative Hb level, 137 ±12 g/L. Since there was no correlation with bleeding, another reason could be that the patients with low Hb bleed less and therefore did not acquire a low enough Hb to be subject for transfusion. On the other hand, in **Paper II**, low preoperative Hb levels were a risk factor for transfusion in both THA and TKA patients. The difference in results could be explained by a difference in

patient management and the lack of a standardized transfusion protocol. There are many risk factors for perioperative bleeding and transfusion requirements. In order to minimize bleeding and transfusion, each patient should be addressed according to their individual needs.

Low body mass index (BMI) was associated with RBC transfusion in both idiopathic scoliosis surgery patients as well as in THA and TKA surgery patients in **Papers I** and **II**. The association between bleeding and transfusion, and BMI and obesity differs in studies. [32, 88, 110, 118] A patient with a low BMI has a smaller blood volume and is therefore more vulnerable to bleeding. Blood loss in a person with a higher BMI and a larger blood volume may not require transfusion but in a smaller person the same numeral blood loss might be enough to lower the Hb level below the transfusion threshold. On the other hand, surgery in obese patients could be a challenge, with the need for larger incisions with larger wound surfaces increasing the bleeding. Operation time is also known to be longer in obese patients compared to normal-weight patients undergoing similar surgical procedures. [88, 110] These factors combined could lead to an increased risk of large perioperative bleeding and thereby increased risk for transfusion in obese patients whereas factors unrelated to bleeding per se can increase the risk for transfusion in patients with low BMI as described above.

It is well known that TXA reduces perioperative blood loss and RBC transfusion. [38, 73, 122, 225] However, TXA is not always a routine in major elective orthopaedic surgery, although there are convincing data demonstrating that a correct regimen of TXA does not lead to an increased risk of thromboembolic events. [38, 250]

5.2 Fibrinogen; bleeding and transfusion

Many studies have shown the correlation between preoperative fibrinogen concentration and perioperative bleeding in other surgical settings such as cardiac surgery, neurosurgery and in liver transplant surgery. [7, 11, 116, 240] **Papers I** and **III** show a similar association in spine surgery patients. No such association could be seen in THA and TKA patients. The reason could be that THA and TKA patients did not have a sufficiently large perioperative bleeding for fibrinogen to reach critically low levels. The median perioperative bleeding for THA and TKA patients was 984 mL and

758 mL respectively, compared to 1,715 mL in spine surgery patients. Mean perioperative bleeding in scoliosis surgery patients was 1,552 mL.

As with fibrinogen and bleeding, a correlation between preoperative fibrinogen plasma concentration and perioperative transfusion requirements has been demonstrated in different types of surgery, for instance cardiac surgery and liver transplant surgery. [50, 116] These are surgical procedures considered to be coupled with large transfusion requirements. In both **Papers I** and **III** there was a considerable amount of perioperative transfusions, 37% in scoliosis surgery patients and 67% in degenerative spine surgery patients. Nevertheless, a correlation was only found in idiopathic scoliosis surgery patients. This difference in results could be caused by the fact that fibrinogen concentration is only one of many factors that influence transfusion requirements in surgery.

Another reason for the diverging results could be the lack of a standardized transfusion protocol during the study period. In **Paper I**, RBC transfusion was prescribed when the Hb level was <80 g/L. In **Paper III**, RBC transfusion was prescribed at the discretion of the attending physician. The recommendation today is to use transfusion protocols as a tool in prescribing transfusions. [86, 131]

Even though the results in **Papers I** and **III** are not conclusive, they indicate that preoperative fibrinogen concentration should be one factor to consider in the care of these patients. In patients scheduled for surgery with a high risk of large bleeding, preoperative fibrinogen concentration gives more information on the patient's coagulation status than prothrombin time (PT) and activated partial thromboplastin time (aPTT) does. [131] Adding fibrinogen concentration to the routine blood analysis in these patient groups could identify patients with low fibrinogen concentration and in the event of large intraoperative bleeding, supplementation with fibrinogen concentrate could be initiated.

If fibrinogen concentration is not measured preoperatively, it is still important to bear in mind that fibrinogen is always consumed in the bleeding patient. In a situation with large intraoperative blood loss, the surgeon could be the first to observe if the patient's coagulation is impaired and should notify the anesthesia staff so they can take measures. During a surgical procedure with large bleeding, the use of viscoelastic methods to evaluate the coagulation system is a fast tool for guidance on what type of blood products or hemostatic drugs should be given to the patient in that specific situation.

5.3 Factor XIII; bleeding, transfusion and coagulation capacity

In **Paper III** no correlation could be seen between bleeding and preoperative FXIII activity for any of the patient groups. Studies on the effect of FXIII activity on perioperative bleeding have previously demonstrated diverse results. [7, 27, 203, 227] For instance, Blome et al. showed no correlation between preoperative FXIII activity and postoperative drain volume in cardiac surgery patients. [27] In contrast, Ternström et al. showed a correlation between both pre- and postoperative FXIII activity and perioperative blood loss in cardiac surgery patients. [227] These results in combination with the present study indicate a need for more research to be done on FXIII and perioperative bleeding and blood loss.

It could be that FXIII activity would be more valuable as a postoperative marker to give an impression of how much FXIII is available to stabilize postoperative clot formation, as is indicated in the study by Ternström et al. [227] Factor XIII also influences wound healing and, if FXIII has been consumed during surgery and has reached critically low levels, supplementation might be important to improve wound healing.

In **Paper IV** the main finding was that FXIII supplementation independently decreased clotting time (CT) and increased maximum clot firmness (MCF) in both cardiac and idiopathic scoliosis surgery patients in an ex vivo setting. The dose-dependent effect of FXIII on MCF was independent of the addition of fibrinogen or platelets.

The study in **Paper IV** was made with clinically relevant doses of FXIII, fibrinogen and platelets, all of which may be used in the clinical setting to treat acquired coagulation disorders. [65, 131] Previous studies on ex vivo FXIII supplementation have used supraphysiological doses of FXIII concentrate and blood samples from intensive care patients or diluted samples from healthy volunteers. [87, 194, 208, 230]

In **Paper IV** none of the cardiac surgery patients and only one of the scoliosis surgery patients had a postoperative FXIII activity below 0.50 kIU/L. Despite almost normal FXIII activity, supplementation with FXIII alone decreased CT and increased MCF. The effect was however limited. Compared to baseline CT decreased approximately 10% and MCF increased about 20% for

both surgery groups. The effect of fibrinogen or platelet supplementation on CT and MCF were markedly higher than the effect of FXIII alone. This finding is in line with current guidelines suggesting that FXIII supplementation should only be considered in patients with adequate fibrinogen levels or when fibrinogen supplementation is not adequate to restore coagulation. [65, 131] It is fibrinogen that is the critical coagulation factor in bleeding patients, and supplementation should focus on restoring fibrinogen concentration.

In the only large scale, randomized, clinical trial with FXIII supplementation in cardiac surgery patients no effect was seen on bleeding and transfusion with recombinant FXIII. [115] When combining the results from **Papers III** and **IV** and also previous ex vivo studies on the effect of fibrinogen on CT and MCF, the effect of FXIII concentrations seems to be low compared with the effect of fibrinogen. [60] Consequently, the indication to use measurement of FXIII in clinical practice in patients undergoing orthopaedic surgery does not seem to be relevant in patients without coagulation disorders.

5.4 Limitations

The strength of **Papers I-III** is also a major limitation; the studies are made on consecutive patients in an everyday clinical setting without selection bias. In **Paper III**, there is a large variation in the types of spine surgery performed. In **Papers II** and **III**, the studied patients are restricted to hip and knee arthroplasty but a number of different surgeons performed the surgeries and different types of implants were used in the hip arthroplasty patients.

Another limitation is that the definitions for large bleeding are based on the upper quartile of perioperative bleeding in **Paper I** and **II**. This makes comparison with other studies, using other definitions, difficult.

Because of few studies on preoperative fibrinogen and FXIII in correlation with perioperative bleeding and transfusion requirements, and also the large variation in bleeding and transfusion requirements in the literature, power calculations have been difficult to make. The study population in **Paper III** might be too small to detect a correlation and also makes subgroup analysis difficult.

In **Papers I**, **II** and **III**, a limitation is that even though different risk factors for large perioperative bleeding and transfusion are presented, the causality is

not proven. The results may be caused by other factors that covary with, for instance, fibrinogen.

Paper IV examined clot formation in an ex vivo setting, and it is not clear if the results could be related to the clinical setting. The ex vivo model does not include the influence of the vascular wall and the endothelium and blood flow on clot formation.

Conclusions

6 CONCLUSIONS

- There was a correlation between preoperative fibrinogen plasma concentration and perioperative bleeding and red blood cell transfusion requirements in idiopathic scoliosis surgery patients. (**Paper I**)

- Red blood cell transfusions in unselected elective total hip and knee arthroplasty patients were found to be lower than previously reported. (**Paper II**)

- Risk factors for excessive bleeding and red blood cell transfusion in arthroplasty patients were low preoperative hemoglobin levels, low body mass index and long operation time. (**Paper II**)

- There was an association between preoperative fibrinogen plasma concentration and perioperative bleeding but not with red blood cell transfusion in degenerative spine fusion surgery. No such correlation could be seen in hip or knee arthroplasty surgery patients. (**Paper III**)

- There was no correlation between preoperative FXIII activity and perioperative bleeding or red blood cell transfusion in spine surgery, nor in hip or knee arthroplasty surgery. (**Paper III**)

- Ex vivo supplementation with clinically relevant doses of FXIII dose-dependently improved clot formation in blood samples from cardiac surgery and idiopathic scoliosis surgery patients, both alone, and when given in combination with fibrinogen or platelets. (**Paper IV**)

Future perspectives

7 FUTURE PERSPECTIVES

Large perioperative bleeding, and subsequent transfusion of blood products, is now known to be a risk factor in surgery. Measures to minimize bleeding needs to be studied further, both from an efficacy and a safety perspective. We need to find out more about which patients are at risk of large bleeding during and after surgery, and focus our effort on these patients. Since the perioperative care of the patient involves many different categories of staff and also different medical specialties, communication about and consensus on the patient's treatment needs to be established and reviewed.

The role of fibrinogen, appropriate preoperative plasma concentration for safe surgery as well as indications for perioperative supplementation, is not fully established in orthopaedic surgery. Studies with greater patient numbers could give more information on fibrinogen and its role in perioperative coagulation management. It is also of interest to study fibrinogen in the orthopaedic trauma setting such as for hip fracture patients, who are often frail and need thorough perioperative care to survive both the surgical procedure and the postoperative rehabilitation.

Since this is the first study on the effect of preoperative FXIII activity on bleeding and transfusion requirements in orthopaedic surgery more studies ought to be performed, preferably on larger study populations. Another orthopaedic aspect of FXIII is its role in bone and cartilage formation. Intracellular FXIII is present in chondrocytes, osteoblasts and osteocytes and FXIII is also involved in wound healing. Could some form of FXIII supplementation, systemically or locally, improve bone healing and cartilage regeneration?

Bearing in mind the risks of transfusion, it is important to know when this, sometimes lifesaving, treatment should be given and which patients benefit the most. More and larger studies need to be carried out. Larger studies are also required to establish the relationship between transfusion and postoperative infection, an issue that is very important in, particularly, arthroplasty surgery.

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"Can't you tell I got news for you

Sun is shining and so are you!" Axwell ∧ Ingrosso

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