Coagulation in paediatric cardiac surgery

– *Clinical studies*

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Abstract

Coagulation in paediatric cardiac surgery

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Background: Surgical correction of congenital heart defects has a profound effect on the coagulation of the patients during and immediately after surgery. The aim of this thesis was to increase the knowledge of this effect, and to add to the work of establishing methods for treating this coagulopathy.

Methods: Study I investigates the platelet response to paediatric cardiac surgery by analysis of platelet count and platelet aggregation measured with multiple electrode aggregation (MEA) before, during and after surgery. Study II measures the *in vitro* potency of the platelet inhibitor ticagrelor by measuring platelet aggregation using light transmission aggregometry and MEA. Study III compares MEA with a vasodilator activated phosphoprotein assay (VASP) before and after cardiac surgery. Study IV examines the coagulation of children with preoperative cardiac failure using rotational thromboelastometry. Study V is a method description of an ongoing study where patients are randomized to platelets or fibrinogen concentrate as the primary treatment of coagulopathy after cardiac surgery.

Results: Platelet count and aggregation fall significantly during cardiac surgery, and impaired aggregation increases the risk of blood transfusion. The potency of ticagrelor *in vitro* does not vary with patient age. The correlation between MEA and VASP is poor. MEA results are similar in children with cardiac defects and healthy children. Preoperative cardiac failure does not have a significant impact on thromboelastometry results.

Conclusions: Cardiac surgery in certain paediatric populations causes significant coagulopathy. Impaired platelet aggregation increases the risk of transfusion. Cardiac failure does not cause coagulopathy in infants. Ticagrelor potency is similar in patients of different ages.

Keywords

Coagulation, platelets, fibrinogen, paediatric cardiac surgery, congenital cardiac defect

Sammanfattning på svenska

Avancerad medicinsk teknik har möjliggjort alltmer invecklade kirurgiska ingrepp, till exempel korrektion av komplicerade medfödda hjärtfel. Dock har dessa metoder avsevärda effekter på patienternas koagulationsförmåga, vilket gör att blödning i ingreppets slutskede har blivit ett av de största problemen inom hjärtkirurgi på barn. Vid större operationer, särskilt på små spädbarn, krävs ofta behandling med olika koagulationsprodukter innan patienten slutar blöda och operationen kan avslutas. Hur man diagnosticerar och behandlar dessa koagulationsrubbningar är den huvudsakliga frågeställningen i den här avhandlingen.

Arbete I är en observationsstudie där effekten av hjärtkirurgi med hjärtlungmaskin på patienternas koagulation, framför allt på blodplättarna, undersöktes.

Arbete III är en observationsstudie som hade två syften. Dels avsåg den att utvärdera hur impedanselektrodaggregometri kan användas under hjärtkirurgi på barn för att få en bättre bild av trombocyternas bidrag till koagulationen. Dels jämfördes resultaten från analyserna på barn med hjärtfel med proverna från friska barn.

Arbete I och III ger en bild av hur koagulationssystemet påverkas av hjärtkirurgi med hjärtlungmaskin på barn, en bild som stämmer ganska väl in på andra studier inom samma område. Man kan se att antalet trombocyter sjunker kraftigt under operationen. Samtidigt försämras också trombocyternas funktion, det vill säga deras förmåga att starta och delta i bildning av ett koagel. Redan morgonen efter operationen avslutats kan man dock se att funktionen hos trombocyterna förbättrats avsevärt. Den vanligaste bilden är till och med att de aktiverats under operationen och har en högre förmåga att bilda koagler än innan operationen började. Detta minskar blödningsrisken men

kan också innebära risker för vissa patienter, eftersom risken för blodproppar på fel ställen ökar. Vidare kunde vi inte se någon skillnad på koagulationen innan operation hos barn med hjärtfel jämfört med friska barn.

Arbete II är en läkemedelsstudie där effekten av ticagrelor, ett blodförtunnande läkemedel, mättes *in vitro* (det vill säga utanför patienten) i blodprov från friska barn och från friska frivilliga vuxna. Vi kunde inte påvisa någon skillnad i effekten av ticagrelor på blod *in vitro* från barn i olika åldrar och vuxna. Detta innebär att man kan gå vidare med studier där man ger läkemedlet till faktiska patienter i behov av behandling för att förebygga blodproppar.

Arbete IV är ett försök att svara på frågan om hjärtsvikt påverkar barns koagulationssystem på ett kliniskt relevant sätt. Barn med hjärtfel som orsakar stort flöde direkt mellan vänster och höger kammare, vilket orsakar hjärtsvikt, inkluderades och deras koagulationskapacitet mättes före och efter man korrigerat deras hjärtfel. Vi kunde inte finna några tecken på en påverkad koagulation hos barn med hjärtsvikt orsakad av medfödda hjärtfel.

Arbete V bygger delvis på det vi lärt oss från arbete I och III, och vi försöker nu ta reda på mer om hur man skall hantera koagulationsproblemen efter hjärtoperationer på barn. För att få bra svar på dessa frågor krävs en annan metodik, vilket förklarar att vi nu gör en randomiserad, kontrollerad studie. Det innebär att två grupper av barn lottas till olika behandlingsstrategier vid blödningsproblem efter hjärtkirurgi. Denna studie jämför om det finns någon skillnad i effekt vid behandling med blodplättar kontra tillförsel av fibrinogen, ett protein som är en av de viktigaste byggstenarna i ett blodkoagel. Detta arbete är pågående; metoden presenteras utförligt i sektionen med originalartiklarna, men några resultat kan inte redovisas än.

List of papers

This thesis is based on the following studies, referred to in the text by their Roman numerals. Söderlund F = Pernbro F (the surname was changed during the PhD studies).

I. Romlin B.S, Söderlund F, Wåhlander H, Nilsson B, Baghaei F, Jeppsson A.

Platelet count and function in paediatric cardiac surgery: a prospective observational study

British Journal of Anaesthesia 2014; 113(5): 847–854.

II. Söderlund F, Asztély AK, Jeppsson A, Nylander S, Berggren A, Nelander K, Castellheim A, Romlin B.S.

In vitro anti-platelet potency of ticagrelor in blood samples from infants and children

Thrombosis Research 2015; 136: 620–624.

III. Pernbro F, Singh S, Wåhlander H, Hansson E.C, Romlin B.S.

Platelet aggregation analysis using multiple electrode aggregometry and VASP assays in surgery for paediatric congenital heart disease

Manuscript (submitted)

IV. Söderlund F, Wåhlander H, Hansson E.C, Romlin B.S.

Preoperative heart failure is not associated with impaired coagulation in paediatric cardiac surgery

Cardiology in the Young 2021; 31: 979–984.

V. Pernbro F, Wåhlander H, Jeppsson A, Romlin B.S.

Fibrinogen or platelet transfusion as first-line treatment of coagulopathy after cardiac surgery in infants: method description of a randomized, controlled study

Method description (manuscript)

Contents

Abbreviations

ACT	Activated clotting time
ADP	Adenosine diphosphate
ASA	Acetyl salicylic acid
APTT	Activated partial thromboplastin time
AT	Antithrombin
AUC	Area under the curve
AVSD	Atrioventricular septal defect
CHD	Congenital heart disease
CPB	Cardiopulmonary bypass
CT	Clotting time
IV	Intravenous
LTA	Light transmission aggregometry
MCF	Maximum clot firmness
MEA	Multiple electrode aggregometry
PRBC	Packed red blood cells
PT-INR	Prothrombin time international normalized ratio
ROTEM	Rotational thromboelastometry
TACO	Transfusion-related acute circulatory overload
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TRALI	Transfusion-related acute lung injury
TRAP	Thrombin receptor activating peptide
t-PA	Tissue plasminogen activator
VASP	Vasodilator stimulated phosphoprotein
VSD	Ventricular septal defect
vWf	von Willebrand factor

Scope of this thesis

Consider the following situation: you are responsible for a patient who just have had surgery where a leaking valve has been replaced with a mechanical one. The patient had surgery before, which means that the procedure has been time-consuming. But the cardiac echo indicates that the surgery has been successful and weaning from cardiopulmonary bypass was uneventful. You can already sense a faint whiff of coffee wafting from the canteen. However, you note that you must keep filling the patient with intravenous volume to maintain a decent blood pressure, and the surgeon complains that there appear to be no clotting going on in the wound. You realize that action is needed. But what action? The patient clearly has a coagulopathy, which needs treatment, but what do you give her, in what doses, and in which order? How much of any given treatment can you give without worrying about the mechanical valve (which is prone to clotting)? Maybe, just maybe, you ought to have anticipated these problems and taken precautionary measures – but which ones?

The paediatric patient who arrives at a hospital for cardiac surgery often follows a rather winding road from when they first arrive at the ward for preparatory talks and procedures, until she leaves the hospital. There are plenty of situations where coagulation issues may occur, but with paediatric patients, a few time points stand out as particularly important. First, we would ideally want to identify the patients at risk of intraoperative coagulopathy before the surgery started, and maybe prepare them with supplementary medications preoperatively. In paediatric patients, this has proven difficult. In study IV, we take a look at one factor which has been said to increase the risk of intraoperative coagulopathy in children, namely heart failure. Secondly, we want to know how the surgery and cardiopulmonary bypass (CPB) affect the patient during the surgery. Preferably, this information would also be not just accurate and useful, but also possible to obtain fast, to enable real-time responses to any coagulopathy which arises. Study I and III are attempts to further this aim. Third, we also would like

to know which treatments to administer based on our analysis of the situation, when to give them, and in which doses. Study V is an attempt to test two initial treatment approaches to the bleeding infant.

Study II is concerned with another field of coagulation, namely the need for less of it in certain circumstances. In paediatric patients, the most common reason for needing anticoagulation treatment is the presence of non-biological materials in the patient, such as a mechanical valve or a synthetic arterial shunt.

Which improvements for our patients can we hope to achieve with this endeavour? Swift diagnosis and correct treatment of coagulation abnormalities shorten the time spent on the operating table, which translates into less instability in the ward after surgery. Less bleeding means less need for blood transfusions, which, although sometimes necessary, are associated with an increased number of adverse events after surgery.[1, 2] Last but not insignificant is the fact that transfusion products are in short supply in many centres. Reducing the need for transfusions of any kind would be of great help.

1. Introduction

1.1 An overview of the coagulation process

The coagulation system is dependent on both molecules (mostly proteins) and cells, which function together to initiate, build up, and eventually dissolve blood clots. The demands on this system are immense. We take for granted that a wound, such as a small cut in a finger, should stop bleeding rather quickly. Yet this requires a system which can immediately muster a large number of proteins and cells in one particular place, to plug the breaches in the blood vessels; to confine it to the area in question, making sure it does not spread too far; and to be able to dissolve the clot and put the healed blood vessel back into service, when the plug is no longer needed. Tuning a system to be able to do this without frequent mishaps of potentially catastrophic clots in inappropriate places is a tall order indeed, and probably explains the need for its complexity.

Haemostasis is conventionally described as having two phases, primary and secondary haemostasis. Sometimes a third phase is added, fibrinolysis, when the clot is dissolved. This phase is not the focus of this thesis. These first two phases can then be further partitioned into the sequence of events described below.[3] As will be obvious from the description below, the primary and secondary haemostasis overlap to a large extent, and while it is convenient to separate them for didactic reasons, it is important to remember that they are physiologically intertwined.

1.1.1. Primary haemostasis

1.1.1.1 Platelet adhesion

In an intact blood vessel, the innermost layer of cells – the endothelial lining – keeps the platelets from adhering to the vessel wall. Furthermore,

endothelial cells release prostacyclin (PGI_2) and nitric oxide, which inhibit platelet activation. The first step when a blood vessel is injured is that tissue which is normally hidden under the endothelial cells in the vessel (the subendothelial tissue) is exposed. This tissue contains molecules which bind receptors on circulating platelets and make them adhere to the vessel wall. The GPVI receptor binds directly to collagen in the subendothelial tissue, whereas the GP1b receptor binds von Willebrand factor (vWf) which in turn binds to subendothelial proteins.[4] See figure 1.

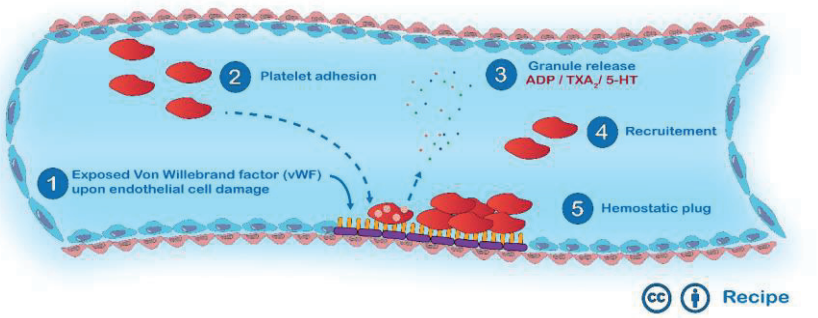

Figure 1. Platelet adhesion. Open-source image.

1.1.1.2 Platelet activation

The adherence of platelets to the vessel wall through the receptors mentioned above also causes the platelets to activate. This activation is brought about by the intracellular parts of the receptors, which cause an increased signalling and a rising calcium concentration within the platelets.

Activated platelets differ in several ways from their inactive state.[5] Their *physical appearance* changes into a flatter, more elongated shape which increases the surface available for binding to fibrinogen and other platelets. In order to further increase the available platelet cell surface area, activated platelets also release tiny, self-contained bubbles of cell wall, so called *microvesiculation*. Activated platelets also undergo *degranulation*, during

which α-granules (which contents include vWf, fibrinogen, factor V and XIII) and dense granules (which contain ADP, $Ca2^{+}$ and serotonin) move to the platelet cell membrane, fuse with it and release their contents.[6] These contents further activate nearby platelets and increase the activity of the coagulation cascade (more of which later). Activation also induces an *increased activity of phospholipase A_2*, which metabolizes phosphatidylcholine to arachidonic acid, which in turn eventually gets converted into thromboxane A_2, a platelet activator and blood vessel constrictor. Finally, signalling within the cytoplasmic matrix of activated platelets changes the conformation of the extracellular part of the *GPIIb/IIIa receptor,*[7] also called integrin $\alpha_{IIb}\beta_3$. The GPIIb/IIIa receptor is normally present on the surface of platelets but does not bind to fibrinogen in its dormant state. This receptor binds fibrinogen in its activated conformation, forming crosslinks between platelets. See figure 2.

Activated platelets bound together with fibrinogen make up the result of primary haemostasis, i.e., a relatively loose blood clot. This clot, in conjunction with vasoconstriction mediated by thromboxane A_2 and ADP, is usually sufficient to plug small injuries in blood vessels, such as skin punctures. However, when the injury is more severe, secondary haemostasis is needed to stop the bleeding.

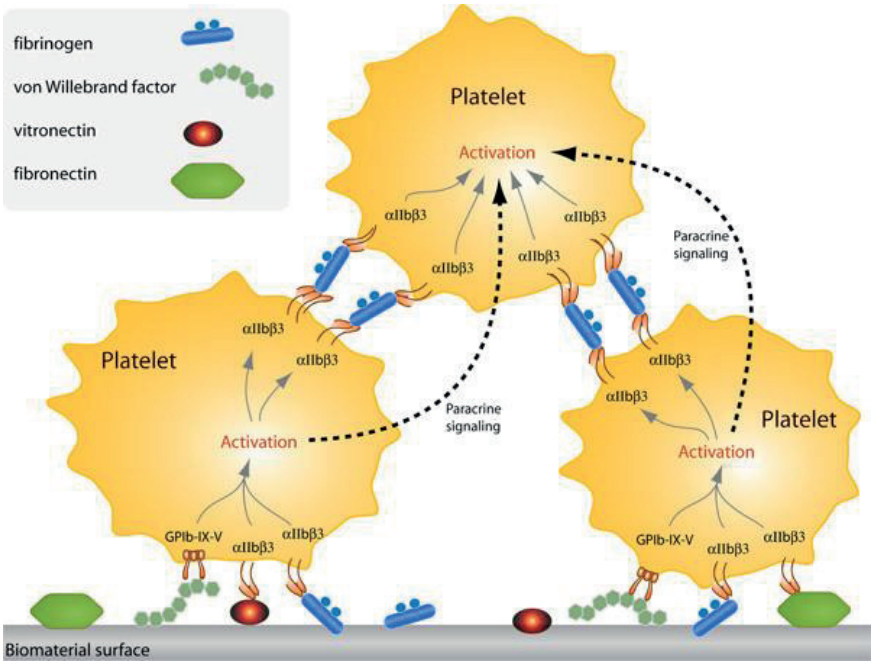

Figure 2. Platelet activation. Open-source image.

1.1.2. Secondary haemostasis

Platelets, interconnected through fibrinogen with the help of GPIIb/IIIa receptors, quickly create a clot which may be enough to stop the bleeding from a vessel injury. In cases where there is damage to larger blood vessels, however, this clot is not stable enough. In order to stabilize the clot, fibrinogen must first be converted to fibrin, and then further cross-linked with other fibrin molecules. To achieve this, the coagulation cascade must be activated. The description below is a simplified summary, since this thesis is not primarily concerned with all its intricacies.

1.1.2.1 Activation of the coagulation cascade

The coagulation cascade is made up of several interconnected chains of enzymatic reactions, which purpose is to amplify and regulate the response to a vessel injury. Conventionally, the cascade is described as having three parts: the intrinsic and the extrinsic pathway, and finally the common pathway, in which the previous two merge and a blood clot is formed. In clinical practice, the two pathways operate in tandem. It is thought, however, that the extrinsic pathway, which begins with exposure to the blood of tissue factor (TF), a cell membrane receptor present on the surface of fibroblasts, macrophages and other cells in the subendothelial tissue, is the most important starting point under physiological (i.e., *in vivo*) circumstances.[8] These cells produce small amounts of thrombin in their physiological resting state. When the endothelial lining is damaged, platelets and vWf encounter this small amount of thrombin and are activated[9] (see the section on primary haemostasis above).

TF interacts with factor VII, and the resulting TF/FVIIa complex activates both factor IX and X.[10] The importance of this reaction is evident from the huge impact of the successful production of recombinant factor VII, both on the patients suffering from factor VII deficiency,[11] but also as a rescue drug for severe bleeding complications.[12]

The final step in the activation phase of the coagulation cascade is the formation of activated factor X, or FXa. (This activated coagulation factor is also the final step of the intrinsic pathway, although the activation in this pathway takes place on the surface of activated platelets, not on fibroblasts.) Initially, a small amount of FXa is produced, during the *initiation phase*. These amounts do not suffice to form a clot, however, for which the level of FXa needs to increase by several orders of magnitude. This *amplification phase* is brought about by the small number of thrombin molecules formed during the initiation phase, which activate factor V and VIII, thus setting off the intrinsic pathway. The FXa enzyme complexes formed through this pathway are significantly more effective at producing thrombin, which in turn converts large amounts of fibrinogen into insoluble fibrin.

The coagulation cascade takes place on the surface of cells expressing phosphatidylserine molecules, chiefly platelets, but also leukocytes, erythrocytes and endothelial cells play a role in providing a platform for coagulation activation. However, platelets are the most crucial cells in this process; not only do they provide a surface for the enzymatic reactions to take place, but they also secrete substrates and activators of the coagulation cascade (see the previous section on primary haemostasis), keep the enzymes of the cascade in a physically small space, increasing concentration and thus enzymatic activity, and finally protect the enzymes from inhibitors and the growing clot from fibrinolysis.For a summary of the coagulation cascade, see figure 3.

1.1.2.2 Fibrin formation and crosslinking

FXa is an active enzyme, which converts circulating prothrombin to active thrombin. Thrombin in turn cleaves off the outermost parts of fibrinogen molecules, rendering them insoluble in plasma, and causing them to aggregate in sheets. Thrombin also activates factor XIII (FXIII), which function is to crosslink individual fibrin molecules, thus greatly increasing the strength of the clot, and inhibits fibrinolysis (the process which eventually will dissolve the clot, once the vessel injury has healed).[13] The result is a blood clot which in mass is mostly made up of trapped erythrocytes, but which draws its strength from the dense network of platelets and fibrin molecules which has formed. We will return to the result when there is a lack of either of these components later in this introduction.

To prevent the clot from spreading to adjacent areas, which have no need of repairing leaks in the vessel walls, the coagulation cascade is inhibited in areas where no subendothelial tissue is exposed. The main endothelial factor is Tissue Factor Pathway Inhibitor (TFPI) which inhibits tissue factor, FVa and FXa. Antithrombin (AT), a circulating thrombin inhibitor, is also an important component in limiting the amount of circulating thrombin.[14] AT also has an important role to play in the pharmacodynamics of the anticoagulant substance heparin, used in CPB, by amplifying its effects by several orders of magnitude.

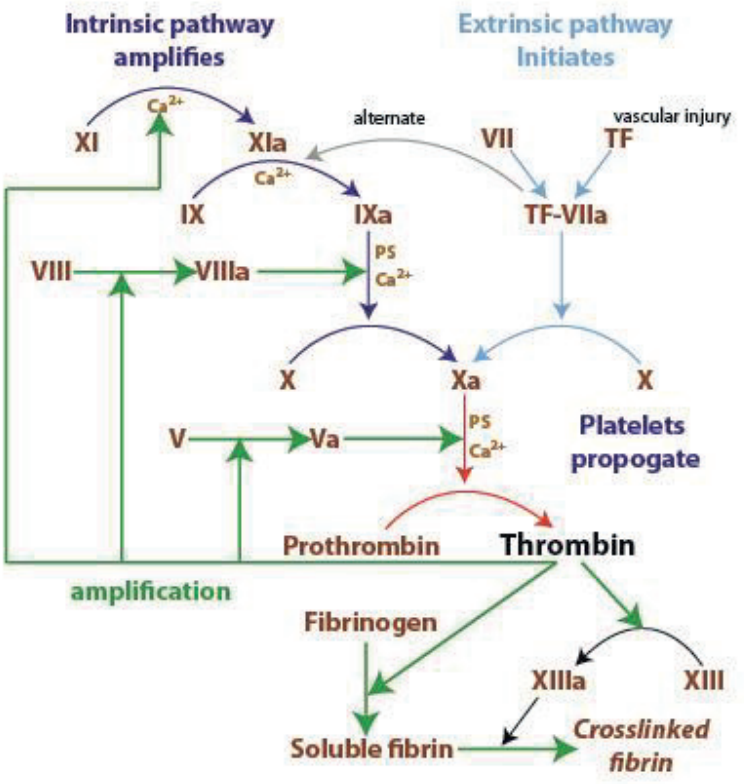

Figure 3. The coagulation cascade. Open-source image.

1.2 The coagulation system in infants and children

Many biological systems and processes in infants and small children are immature at birth, and reach their full, adult capacity at some point during childhood. The coagulation system is no exception, although the extent to which the coagulation system can be said to be immature is a moot question. There are clearly differences in the concentrations and activity of

some coagulation factors between infants and adults, but it remains very unclear whether these differences translate into any clinical impact.

1.2.1. Platelets

Platelets are formed in the bone marrow from large precursor cells known as megakaryocytes.[15] Each megakaryocyte gives rise to many platelets by fracturing its cell membrane into a multitude of small cells without nuclei. It has been shown that these megakaryocytes do not grow to the same size in foetuses and infants as in adults,[16] thus generating fewer platelets when they reach their mature phase.

Once these neonatal platelets reach the circulation, it has been found that they exhibit several characteristics which suggest they may be less efficient than their counterparts in adults. They possess a slightly different ultrastructure than adult platelets, with less glycogen stores and less developed microtubular networks.[17] Neonatal platelets have fewer granules in their cytoplasm, and those they have tend to have lower concentrations of for example ADP. This makes their degranulation phase of the primary haemostasis less efficient. Also, experiments have shown that the intracellular signalling pathways appear to be less active, e.g. with inferior mobilization of plasma calcium ions in response to receptor agonists such as collagen, ADP[18] and adrenaline.[19] Neonatal platelets also produce less TXA_2, and express fewer GPIIb/IIIa receptors, which means that the crosslinking with fibrinogen may be less effective. Intracellular stores of calcium ions are smaller,[20] which also decreases the effectiveness of the neonatal platelets contribution to secondary haemostasis. There are also studies showing that thrombin formation is decreased in neonates.[21] On the other hand, some evidence points to an enhanced aggregation response to agonists (but still deficient degranulation).[22]

Normal childbirth does not appear to activate platelets, while other states of pathological stress seem to do so.[23] However, despite the apparent deficiencies of neonatal platelet function outlined above, neonates show no increased tendency for bleeding problems under normal circumstances. Indeed, bleeding time is usually shorter than in older children and

adults[24] (but prolonged in premature infants[25]). We will note, however, that infants are more effected by major surgery and tend to require more transfusions than older children during heart surgery.[26, 27] The fact that small infants are exposed to a larger area of foreign material than older patients clearly is a major factor. (See also section 1.3.) Whether the disparities between coagulation systems of infants and adult contribute is less certain.

1.2.2. The coagulation cascade

Several differences in the plasma concentration of coagulation factors exist between infants, older children and adults.[28] The most marked (and thus most clinically relevant) differences are present in neonates and infants under six months of age. Infants under six months display decreased levels of coagulation factors II, VI, IX, X, XI and XII. This is probably the reason that activated partial thromboplastin time (APTT) measurements are higher in infants. Prothrombin time international normalized ratio (PT-INR) values are higher in neonates until a few weeks after birth, after which they reach adult levels. Infants also have lower concentrations of coagulation inhibitors such as protein C and S, and antithrombin. Plasminogen and tissue plasminogen activator (t-PA) concentrations are also lower.[28]

Despite these what seem to be aberrations in the coagulation system, neonates and infants are not generally more prone to thromboses or bleeding than older children and adults. It is generally assumed that the coagulation system in small children is balanced throughout its development, with deficiencies in coagulation factors being cancelled out by other deviations elsewhere, such as less coagulation inhibitors and a limited capacity for fibrinolysis.[3]

A feature of the coagulation system in infants which does not affect the children themselves, but certainly makes life slightly more difficult for those inclined to coagulation research, is that most of the analyses mentioned above exhibit much variance in small children and do not always follow a predictable pattern of change as the child gets older.[29] This

sometimes has a confounding effect on attempts to interpret data in these patient groups, particularly since paediatric research cohorts usually are quite small.

1.3 Cardiac surgery and cardiopulmonary bypass

So far, we have mostly discussed how the coagulation system is supposed to function under normal circumstances, be it in children of different ages or in adults. However, most of the research patients in this dissertation find themselves far from normal circumstances. Major surgery and cardiopulmonary bypass (CPB) are two of the medical interventions which have the most profound impact on the coagulation system of any person.[30] Furthermore, the blood transfusions often necessary after major bleeding have been shown to be a risk factor for a plethora of complications, including increased mortality.[31] A brief overview of these effects on the coagulation system is necessary for understanding what we can expect from these patients.

Major surgery is a recognized cause of coagulopathy in adults.[32] Intraoperative bleeding in combination with the stress response of the body to the trauma of surgery are usually blamed for coagulation abnormalities during or after surgery. In non-cardiac paediatric surgery, however, major bleeding during surgery is uncommon, and coagulopathy is rarely an issue for the attending anaesthetist. This is true even for time-consuming surgery of the abdomen and for orthopaedic surgery, for example. (Procedures involving the liver or scoliosis surgery are notable exceptions, and the coagulopathy seen during these procedures is typically related to extensive blood loss, or pre-existing coagulopathy in liver failure.[33]) It is therefore assumed that the coagulopathy often observed during and after paediatric cardiac surgery is in large part caused by the CPB circuit and the anticoagulation necessary to use it.[34]

1.3.1. Mechanisms of the coagulopathy caused by CPB

There are several mechanisms which contribute to disturbances of the coagulation system during cardiac surgery with CPB.[32] These are shared problems for both paediatric patients and adults; however, they tend to be more pronounced the smaller the patient is, with the most serious effects in neonates.

The CPB circuit introduces a large surface area of plastic material, which by the body quite understandably is considered foreign. The parts of modern CPB equipment which are in contact with the patients' blood is coated with heparin, which attenuates this reaction to a certain degree, at least to the extent that it even has been shown to affect clinical outcome.[35] However well designed the equipment may be, though, a number of important reactions take place on the plastic surfaces. An inflammation response is partly triggered by the complement system, which is directly activated by the foreign CPB materials.[36] Furthermore, the complement system is also activated by components of the coagulation cascade, for example FXIIa, which have been formed in the surgical area. Fibrinogen and other plasma proteins also deposits in the CPB circuit, providing an adhesive substrate for platelets and leukocytes to attach themselves to. This process is amplified by circulating pro-inflammatory substances secreted by leukocytes, which make other cells express increased numbers of adhesion molecules, such as integrins and selectins. Platelets which adhere to the CPB circuit are activated and contribute to the pro-thrombotic and pro-inflammatory environment.[37] The deposition of platelets and coagulation factors (most notably fibrinogen) depletes the patients stores of these components of the coagulation system. The activation of platelets also has the paradoxical effect of both increasing thrombogenicity (causing consumption of platelets, and thus thrombocytopenia) and, during the course of the CPB, decreasing their aggregation capacity.[38]

In addition to the effects on the coagulation system outlined above, other factors also play a role in causing coagulopathy. CPB requires priming in order not to cause an acute hypovolaemia in the patient when it is started. Packed red blood cells or albumin is used for this, depending on the size of the patient and the patient's haematocrit. Either way, priming causes

dilution of the components of the coagulation system. Modified ultrafiltration (MUF), which is now commonly used, attenuates but does not entirely counter the dilution effect.[39] Other common phenomena during cardiac surgery, including hypothermia, acidosis, hypercalcemia and low haematocrit also decrease the efficacy of the coagulation system.

1.3.2. Postoperative changes in the coagulation system

At the end of CPB, most variables we use to monitor the patient's coagulation capacity are at their lowest point. After this, an often quite rapid recovery begins. Platelet aggregation capacity often return to preoperative levels or higher the morning after surgery, even though platelet count does not (see article I later in this dissertation for more details on this). Plasma fibrinogen often falls to very low levels at the end of surgery and CPB, but usually recovers rapidly, and may have reached above the preoperative concentration on the first postoperative day, reflecting its property as part of an inflammatory response.[40] Functional tests of global coagulation capacity (such as rotational thromboelastometry, see the next section) most often confirm this picture, since patients rarely are found to have a coagulopathy the day after surgery. This is also reinforced by the fact that paediatric cardiac surgery patients seldom need any coagulation products after the first few hours after the end of the surgery and CPB, and that blood loss from chest tubes and surgery wounds in almost all cases are small, even the first night (even if some patients may lose significant amounts of serous fluid this way, as all paediatric intensive care physicians will recognize).

1.4 Monitoring platelet function and coagulation

Diagnosing and treating disorders of coagulation is of course a fool's errand if there is no way of analysing its components. Ideally, coagulation tests (like all other diagnostic tests) should be accurate, reliable, fast, simple, cheap, with little biological variation and require negligible amounts of blood from the patient. (The last point is seldom made in the realm of adult patients, but when your patient has a total blood volume of about the

size of a coffee cup, analyses which require 20 ml of blood simply do not cut it.) Unfortunately, as all readers working in biological sciences know all too well, no such tests exist today. However, over the last decade a couple of bedside functional tests of coagulation have become available for use in ordinary operating facilities and intensive care units, which have had a huge impact on the treatment and understanding of coagulation issues in clinical practice. Since a large part of the analyses which this thesis is based on rests on these tests, we need to present them in some more detail.

1.4.1. Rotational thromboelastometry

Rotational thromboelastometry (or ROTEM for short) probably got its unwieldy name without the involvement of any marketing department. Nevertheless, it has become an indispensable tool for clinicians in many situations in surgery and intensive care, from severe trauma in the emergency room[41] to long-term monitoring of anticoagulation therapy in patients with ventricular assist devices.[42] In the last couple of years, there has been an interest in the ROTEM profile of patients admitted to hospital with SARS-CoV-2, since one of the main pathological mechanisms of this infection appears to be thrombosis.[43]

ROTEM is a point-of-care, whole-blood functional method for measuring clot formation.[44] 340 µl of blood is inserted into a cylindrical cuvette. An activator is added (more of which later) and a pin is immersed into the blood sample. The cuvette is then rotated every six seconds at a constant force, and as a clot forms in the cuvette, the rotation angle decreases (as the force is constant). The decreasing rotation is detected optically, and the result displayed as a graph, with numerical values derived from this graph. The whole analysis requires about an hour to run its full course, at which point it also can detect pathological fibrinolysis, but in most cases ten minutes is enough to give relevant answers for a clinician.

Several different so-called channels can be used in the ROTEM setup, each which gives insight into a particular part of the coagulation system. Four

of these will be mentioned in this thesis. A ROTEM machine is shown in figure 4, and the graphic output of the analysis in figure 5.

INTEM uses phospholipids as activators and is designed to reflect the intrinsic pathway of the coagulation cascade. It detects deficiencies in factors I, II, V, VIII, IX, X, XI and XII.

EXTEM is activated by tissue factor and represents the extrinsic pathway. It assesses factors I, II, V, VII and X.

HEPTEM is the same analysis as INTEM but with added heparinase, which removes the heparin activity in the sample, if there is any. By comparing INTEM and HEPTEM one may thus infer if there is a heparin effect in the sample.

FIBTEM, like EXTEM, uses tissue factor as activator but also contains cytochalasin D, an actin polymerisation inhibitor which almost entirely removes the contribution of platelets to the clot formation. As expected, this produces a very weak clot, but the results can be interpreted as the effect of the fibrinogen in the sample, and the channel can be used as a substitute for measuring plasma fibrinogen.

It should be noted that even though the INTEM and EXTEM channels are designed to measure the same pathways as APTT and PT-INR, the methods are very different and, as perhaps can be expected, the correlation between them are relatively poor. The exception is FIBTEM, which is quite well correlated to traditional Clauss plasma fibrinogen analysis.[45, 46]

Combining different combinations of these channels, it is possible to get a reasonably correct and detailed overview of the coagulation in a patient, all within roughly 15 minutes. The method is not very sensitive to the handling of the samples (which can be kept at room temperature for at least two hours without detrimental effects on the results) and has a decent precision in the analysis, with a variation coefficient of 3 – 13%, depending on the channel.[47] ROTEM cannot detect low levels of vWf or deficiencies of primary haemostasis, though.

Whether the use of ROTEM during cardiac surgery is of benefit to the patients will be discussed in several of the articles in this thesis. A few remarks will suffice here. It was shown quite a few years ago that ROTEM-guided intraoperative haemostasis management decreased transfusion of packed red blood cells (RBCs) in adult cardiac surgery.[48] However, while there is some data suggesting improved mortality, ICU days and hospital length of stay in adults when ROTEM is used to guide haemostasis intraoperatively,[49] a meta-analysis of the available evidence was not able to conclude that ROTEM had any effect on these endpoints.[50] Available studies in paediatric cardiac surgery show a similar picture, with ROTEM-guided transfusion management tending to decrease transfusions of RBCs while increasing use of platelets and fibrinogen[51] (or plasma, when fibrinogen concentrate is unavailable[52]).

Figure 4. ROTEM analysis setup. Image reprinted with permission from Vingmed AB, Järfälla, Sweden.

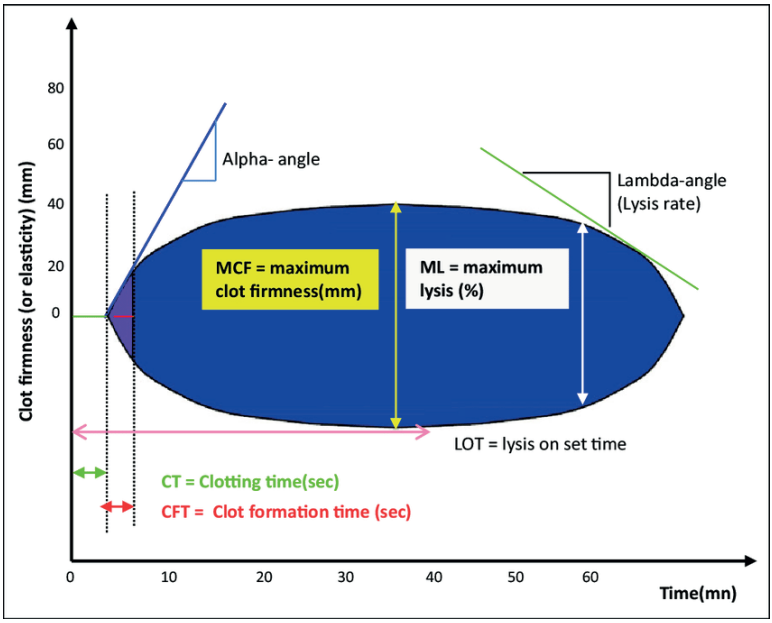

Figure 5. Schematic explanation of a ROTEM output graph. See text for details. Open-source image.

1.4.2. Multiple electrode aggregometry

Another method with a name which suggests it is more easily done than said, multiple electrode aggregometry (or, mercifully, MEA for short) is a test in the same vein as ROTEM, i.e., a point-of-care, whole blood analysis of platelet aggregation. However, when ROTEM was designed to provide a global assessment of secondary haemostasis, with the diverse channels providing different views of this coagulation, MEA is utilized to exclusively test platelet aggregation. Its intended use is chiefly to test responses to platelet inhibitors such as clopidogrel[53] and acetylsalicylic acid[54] (ASA) in order to find and provide alternative treatment regimens for non-responders to these drugs. MEA has subsequently found its way into analysis of platelet aggregation to diagnose inherited platelet disorders[55] and, which is what we are interested in here, acquired defects of platelet aggregation caused by CPB and cardiac surgery.[56]

MEA functions based on the conductivity of an electric current through whole blood samples. (More detailed descriptions have been published.[57]) Whole blood samples are put into cuvettes into which two silver electrodes are inserted. As with ROTEM, different channels are used to test different aspects of platelet aggregation. The channels differ in respect to which agent is added to precipitate aggregation. In the ASPI channel (used to test the response to ASA) arachidonic acid is added. The ADP channel is for evaluating the response to P2Y12-receptor blockers, such as clopidogrel and ticagrelor, and uses ADP as the reagent. In the TRAP channel, aggregation is started with thrombin receptor-activating peptide-6 (TRAP), which is a PAR-1 receptor agonist and a very strong aggregation facilitator. This channel is used as a control of the general aggregation capacity of the platelets in the sample. In each channel, two identical samples are run simultaneously as an internal control. As the platelets aggregate on the silver electrodes, the impedance between them increases, and this is detected and presented as a curve. The output value is the area under the curve (AUC) which is an arbitrary unit. A low AUC indicates a low platelet aggregation in the sample. The MEA analysis equipment we use, Multiplate, is shown in figure 6, and its graphic output in figure 7.

MEA is a fast and user-friendly method which needs small amount of blood in the sample compared to the classic platelet aggregation assay, light transmission aggregometry (LTA), which requires specially trained staff and is not very well standardized. Unfortunately, the variation in AUC values from MEA analysis among healthy test subjects is very high.[56] Thus, the method is preferably used when the effect of an intervention (primarily pharmacologic platelet inhibition) is assessed. Nevertheless, we have attempted to find a place for MEA during paediatric cardiac surgery (see article I and III) since we believe there is a use for more detailed information about platelet aggregation in this setting.

Figure 6. Multiplate analysis setup. Image reprinted with permission from Roche Diagnostics Scandinavia AB.

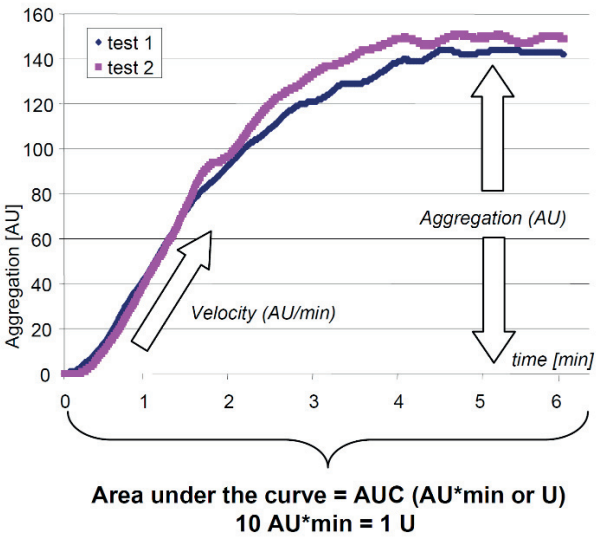

Figure 7. Schematic Multiplate output graph. Note the two analyses of the same blood sample running in tandem. See text for details. Image reprinted with permission from Roche Diagnostics Scandinavia AB.

1.5 Antiplatelet drugs

As mentioned in the beginning of this introduction, the coagulation system is a constant tug-of-war between factors which favour thrombosis, and factors which favour fibrinolysis and bleeding. As clinicians and patients, our interest and fears tend to focus on either of these outcomes in different situations. This thesis is mostly concerned with bleeding issues, i.e., too little coagulation. There are instances, though, when thrombosis (i.e., too much coagulation) is the principal worry. In adult patients, this is very common, and some examples include the situation after a patient has had a cardiovascular incident, such as a cardiac infarction, an ischaemic stroke, or a deep vein thrombosis.[58] In children, these pathological states are rare, and children at risk of thrombotic complications most commonly have foreign materials or devices implanted. These can for example come in the form of synthetic blood vessel replacements[59, 60] (known as shunts),

mechanical heart valves, and different mechanical heart assist devices,[61] used in cardiac failure. Internal surfaces which have been introduced from the outside world are correctly identified by the body as foreign, and invariably trigger an activation of the coagulation system, much like a CPB circuit does (see section 1.3). This can cause life-threatening complications; an example is the patients who are treated with heart assist devices, where thrombosis is one of the most common complications.[62]

Patients at risk of thrombosis need treatment with drugs which prevent the risk of a blood clot forming. There are several types of these drugs; we have previously mentioned heparin, which is used during cardiac surgery with cardiopulmonary bypass. Article II in this thesis concerns another class of drugs, platelet inhibitors. These decreases the risk of thrombosis by decreasing platelet aggregation.

1.5.1. P2Y12 receptor inhibitors

Article II concerns more specifically the substance ticagrelor, which belongs to the group of drugs called P2Y12 receptor inhibitors. These block, as the name suggests, the P2Y12 receptor, which is located on the cell membrane of platelets and normally binds the ligand adenosine.[63] Adenosine, when binding to the P2Y12 receptor, has several downstream effects which promote platelet activation and aggregation, including increasing intracellular Ca2+ concentrations and activation of the GP IIb/IIIa receptor. Inhibiting it consequently decreases the risk of thrombosis. Even though adenosine is considered a weak platelet activator, P2Y12 inhibitors, which apart from ticagrelor also includes the widely used substance clopidogrel and prasugrel, have been shown to be highly effective in preventing thrombosis in adults when used clinically.[58] (The reason for classifying platelet activators as "weak" (adenosine and adrenaline) or "strong" (collagen and thrombin) is based on their effects on the platelet response. Strong agonists are able to cause degranulation and thromboxane A_2 synthesis on their own, while weak agonists are not. Instead, they serve to amplify the effects of the strong agonists.) Their effectiveness in preventing thrombosis also manifests itself in the increased risk of bleeding complications, however. The P2Y12 receptor inhibitors are also subject to variations in their metabolism, with the result that some patients (the proportion varies in different studies) are so-called non-responders.[64]

There have been promising results suggesting that ticagrelor might be superior to the other P2Y12 inhibitors in preventing thrombotic incidents,[65] even though this has been disputed.[66, 67] Other potential advantages of ticagrelor compared to the other P2Y12 antagonists include a faster return to baseline platelet function after cessation of the drug (3 days compared to 5 – 7 days for clopidogrel) which is an advantage when urgent surgery is necessary.[68] Also, the number of non-responders to ticagrelor is much lower compared to clopidogrel.[69] Either way, the fact that some patients respond poorly to any given antiplatelet drug makes it very useful to have several substances to choose between. So far, however, the available data on antiplatelet drugs after paediatric cardiac surgery is limited, and very little has been published on substances other than warfarin, ASA and clopidogrel.[70] The current interest regarding ticagrelor use in paediatrics is actually directed at patients with sickle cell anaemia, where thromboses are common complications.[71]

2. Summaries of the articles

2.1 Platelet count and function in paediatric cardiac surgery: a prospective observational study

Romlin B.S, Söderlund F, Wåhlander H, Nilsson B, Baghaei F, Jeppsson A.

British Journal of Anaesthesia 2014; 113(5): 847–854.

2.1.1. Introduction

A majority of the published research on coagulopathic processes during cardiac surgery with CPB is based on viscoelastic analyses, such as ROTEM.[72, 73] As we have seen in the methods section previously (see 1.4.1), this is a capital method for quickly obtaining an overview of the coagulation status of the patient. Comparing the different channels also allows for teasing out some details of the different components of the coagulation. However, if platelet aggregation is of particular interest, a specific method for measuring this is necessary, and the most feasible technique in a clinical environment at the moment is multiple electrode aggregometry (MEA). We use the apparatus manufactured by Roche, called Multiplate.

2.1.2. Study aims and methods

This study was a prospective, observational study aimed at analysing how platelet count and function changes during cardiac surgery with CPB and modified ultrafiltration (MUF). We also aimed to investigate whether these changes (if any were present) correlated with the need for interventions, such as blood transfusions. Fifty-seven patients, scheduled for elective repair of congenital heart defects, and with a median age of five months were

included. Platelet count, haematocrit and platelet aggregation measured with Multiplate were analysed after induction of anaesthesia, on CPB (but after rewarming), after MUF (but before administration of protamine), after arrival in the paediatric ICU, and on the morning of the first postoperative day.

2.1.3. Results

Both platelet count and aggregation dropped precipitously during surgery, with the aggregation nadir at the end of CPB, and with the most pronounced change in the ADP channel of the Multiplate analysis (-62%). After MUF, aggregation had started to recover slightly, while platelet count continued to fall. On the first postoperative day, platelet count was still significantly (-47%) lower than the preoperative level, while platelet aggregation on the contrary slightly exceeded preoperative values. Age, weight and aortic clamp time were univariately associated with impaired platelet aggregation during CPB.

Impaired platelet aggregation, as measured with Multiplate, was significantly associated with increased transfusion requirements during surgery. In patients with all three Multiplate channels (ADP, AA and TRAP) < 30 U, 81% were transfused with packed red blood cells, whereas in patients with all three channels > 30 U, the transfusion prevalence was 31%. See also figure 8. Platelet aggregation during CBP was not associated with postoperative transfusions, however.

Modified ultrafiltration did not have any effect on platelet count or the ADP and TRAP Multiplate channels. ASPI improved slightly during MUF, from mean 34 to 40 U.

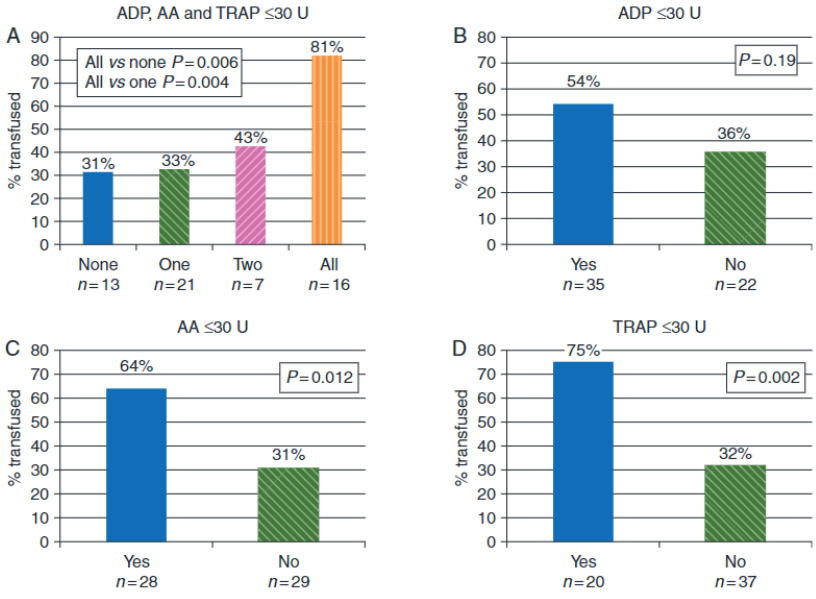

Figure 8. The percentage of patients transfused with packed red blood cells in relation to MEA channels with an AUC < 30.

2.2 In vitro potency of ticagrelor in blood samples from infants and children

Söderlund F, Asztély AK, Jeppsson A, Nylander S, Berggren A, Nelander K, Castellheim A, Romlin B.S.

Thrombosis Research 2015; 136: 620–624.

2.2.1. Introduction

Platelet inhibition is sometimes necessary in selected paediatric patients to decrease the risk of thrombosis.[74] In children, the most common reasons for platelet inhibition are implantation of prosthetic heart valves or systemic-to-pulmonary shunts, or during treatment with ventricular assist devices. The most common drugs used to this end are acetylsalicylic acid (ASA) and clopidogrel, an irreversibly binding $P2Y_{12}$ receptor antagonist.

Ticagrelor is a new treatment option for these patients, which in studies on adult patients has proven to provide a more consistent platelet inhibition with a faster onset of action, and also with a faster decrease in platelet inhibition when the drug is discontinued.[65] However, the potency of ticagrelor in children has not been tested.

2.2.2. Study aims and methods

Thirty-six healthy children, 0 – 12 years old, scheduled for minor outpatient surgery, and 13 healthy adult controls were enrolled in a prospective, observational study. In vitro platelet aggregation was measured using light transmission aggregometry (LTA) and multiple electrode aggregometry (MEA). In the LTA analysis, ADP was used as an aggregation activator, and in MEA ADP, ASA and thrombin receptor-activating peptide-6 (TRAP) were used. Platelet aggregation was measured at baseline and at increasing concentrations of ticagrelor, which was added to the blood samples.

The aims were 1) to compare the in vitro potency of ticagrelor in children of different ages and adult controls, and 2) to establish the difference in baseline ADP-dependent platelet aggregation, if any, between children and adults.

2.2.3. Results

No significant differences in the potency of ticagrelor (mean IC_{50}) was detected between the children in the different age groups, or when comparing children to the adult controls. See also figure 9.

Baseline (without ticagrelor) ADP-dependent platelet aggregation did not differ significantly between the children and the adult controls when measured with both LTA and MEA, except for the age group 2 – 6 years old, which had a minimally decreased aggregation response to ADP when compared to adults.

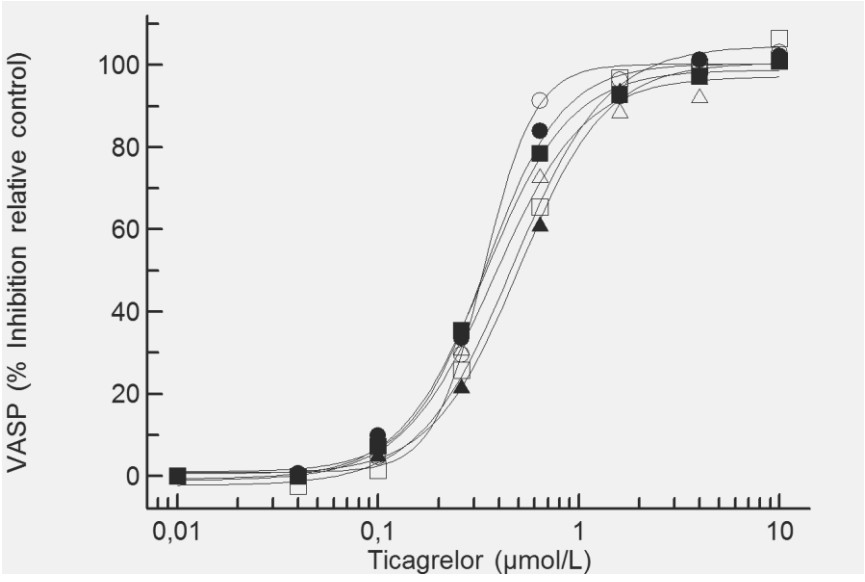

Figure 9. Inhibition of platelet aggregation measured using VASP at increasing concentrations of ticagrelor. The different curves represent the different age groups. See the original article in this publication for details.

2.3 Platelet aggregation analysis using multiple electrode aggregometry and VASP assays in surgery for paediatric congenital heart disease

Pernbro F, Singh S, Wåhlander H, Hansson E.C, Romlin B.S.

Manuscript (submitted)

2.3.1. Introduction

Platelets are an integral part of a functioning coagulation system, both as structural components in a blood clot, but more importantly as a source of pro-coagulant substances and as a surface for coagulation reactions to take place.[5] Platelets are also severely affected by cardiac surgery with CPB, with both decreases in platelet count and function.[38] Monitoring of platelet function during paediatric cardiac surgery is thus an attractive concept.

Multiple electrode aggregometry (MEA) is a quick bedside method for measuring platelet aggregation. In adults, several studies have shown promising results when incorporating MEA into existing coagulation treatment routines during cardiac surgery.[75-77] In paediatric patients, there is evidence that platelet dysfunction, detected with MEA, affects transfusion requirements intraoperatively.[38] There are, however, questions about the reliability of MEA testing during cardiac surgery. To assess this, we decided to test MEA in a setting of paediatric cardiac surgery alongside another test of ADP-induced platelet aggregation, vasodilator stimulated phosphoprotein assay (VASP). In addition, we would compare the results of the MEA analysis to data from previously collected samples from healthy children.

2.3.2. Study aims and methods

Thirty-six children under the age of ten years, scheduled for elective repair of a congenital cardiac malformation, were included in the study. The same

number of healthy children, with samples collected previously during scheduled anaesthesia for minor surgery in an outpatient unit, served as a comparison group.

In the study group, blood samples for analysis of platelet count, MEA and VASP were collected before the start of surgery, during CPB after warming, after the end of CPB, after arrival in the intensive care unit, and on the morning of the first postoperative day. In the control group, samples for the same analyses were drawn before the start of surgery.

The aims were to 1) evaluate MEA as an analysis of platelet aggregation during cardiac surgery by comparing it to a VASP assay and 2) to compare MEA results before surgery in children with cardiac malformations with results from healthy children.

2.3.3. Results

Both the ADP and TRAP tests in the MEA analysis were sensitive to the effect of surgery and CPB on platelet function. However, the correlation between VASP and ADP-induced aggregation in MEA was poor and the trends in the results were very disparate. The MEA results dropped precipitously during CPB, only to recover or overshoot the preoperative level on the first postoperative day. VASP, on the other hand, declined during the study period.

There was no significant difference in MEA analysis results between our study group of children with CHD and the control group of healthy children.

2.4 Preoperative heart failure is not associated with impaired coagulation in paediatric cardiac surgery

Söderlund F, Wåhlander H, Hansson E.C, Romlin B.S.

Cardiology in the Young 2021; 31: 979–954.

2.4.1. Introduction

Coagulopathy is a well-known complication to major cardiac surgery, particularly in young infants.[26] Finding out which patients are at the most risk of developing a coagulopathy in need of treatment, ideally before surgery is started, would be of great help to the staff in charge of the patient in the operating theatre. While some data suggests that adult patients prone to excessive bleeding may be identified preoperatively using analyses of their coagulation function,[78] the same cannot be said of paediatric patients.[79] Another approach would be to establish patient characteristics which increases the risk of postoperative coagulopathy. Some of these characteristics are already known, such as low weight and lengthy surgical procedures.[27, 80]

One patient group which has traditionally been considered a risk group due to a perceived coagulopathy preoperatively is patients with cardiac failure. Suggestions as to the mechanism of this coagulopathy include an excessively activated inflammation system with endothelial dysfunction and platelet activation,[81] and a lack of coagulation factors due to liver impairment, which in turn could be caused by an elevated venous pressure in the liver, transmitted from an increased pressure in the failing right ventricle.[82] There has been no previous attempt to test the hypothesis that children with heart failure have an impaired coagulation response, however.

2.4.2. Study aims and methods

Forty children scheduled for surgical repair of a ventricular septal defect (VSD), or an atrio-ventricular septal defect (AVSD) was enrolled in the study. Additional inclusion criteria were the presence of a non-restrictive

shunt in the septal defect, diagnosed using echocardiography, and a Ross score (a clinical scoring system for heart failure)[83] of at least 3. 24 of the patients had Down's syndrome, and the mean body weight was 5.6 kg.

Platelet count, plasma fibrinogen and rotational thromboelastometry (ROTEM) was measured preoperatively, 8 hours after surgery, and 18 hours after the end of surgery. The ROTEM channels analysed were INTEM, HEPTEM and FIBTEM. To quantify the degree of heart failure in the patients, plasma brain natriuretic peptide (BNP), a hormone clinically used to quantify heart failure,[84] was analysed preoperatively.

The aims were to 1) explore if there was any correlation between the degree of heart failure, measured with BNP and the Ross score, and coagulation function, both pre- and postoperatively, and 2) to see if the presence of Down's syndrome effected the coagulation of the patients either before or after surgery.

2.4.3. Results

All coagulation parameters were within the published normal age-adjusted ranges preoperatively. There was a statistically significant correlation between BNP and plasma fibrinogen before surgery. Apart from that, there were no significant correlations between the Ross score or BNP and any of the tests of coagulation function, neither pre- nor postoperatively. Consequently, there was no convincing evidence of heart failure having any effect on the coagulation function of the patients in this study.

There were subtle differences in the coagulation measurements before surgery between the patients with and without Down's syndrome. See table 1. After surgery, however, no significant differences between the two patient categories could be detected.

	Mean difference between Down's syndrome group and non-Down's syndrome group	p values
BNP (ng/L)	63.5 (95% CI 109, 236)	0.59
Ross score	−1.27 (95% CI -2.59, 0.54)	0.053
Platelet count	−50 (95% CI -117, 50)	0.12
Plasma fibrinogen (g/L)	−0.16 (95% CI -0.43, 0.11)	0.25
APTT	−3.8 (95% CI -7.2, -0.40)	0.030
PT	0.047 (95% CI 0.040, -0.035)	0.29
Intem-MCF (mm)	−5.1 (95% CI -9.4, -0.79)	0.022
Fibtem-MCF (mm)	1.9 (95% CI -0.7, 4.5)	0.14

APTT = activated partial thromboplastin time; BNP = brain natriuretic peptide, CI = confidence interval; PT = prothrombin time, MCF = maximum clot firmness.

Table 1.

2.5 Fibrinogen or platelet transfusion as first-line treatment of coagulopathy after cardiac surgery in infants: method description of a randomized, controlled study

Pernbro F, Wåhlander H, Jeppsson A, Romlin B.S.

Method description manuscript

2.5.1. Introduction

Major cardiac surgery in children using cardiopulmonary bypass (CPB) often causes a significant coagulopathy after weaning from CPB.[26] In most cases, this coagulopathy requires intervention in the form of supplementation of coagulation components. Research and clinical experience have shown that the principal constituents of a blood clot, namely platelets and fibrinogen, are the most common missing coagulation components.[85] To aid the clinician in choosing the correct treatment, rotational thromboelastometry (ROTEM) is often used. However, often the analysis cannot help in choosing the proper therapy, mainly because in many cases the picture is one of global insufficient coagulation. In these instances, it remains unclear whether the correct treatment is a transfusion of platelets, administration of fibrinogen concentrate, or indeed both. The anaesthetist in charge of the patient may be tempted safeguard by administering both components. However, platelet transfusions carry similar risks as do packed red blood cells: immunological (platelets being biological material from a donor), infectious (bacterial or viral contamination), administrative (units given to the wrong patient), and specifically to platelets, the risk of a transfusion-related acute lung injury (TRALI)[86] or transfusion-related circulatory overload (TACO).[87] Furthermore, platelets are often in short supply at blood banks. Fibrinogen is less problematic but avoiding unnecessary drug administration is still important. Both treatments are also expensive. Finding out whether one of them is sufficient, or providing evidence for the need to use both, would be of great importance for paediatric cardiac surgery.

2.4.2. Study aims and methods

This is a randomized, controlled, single-blind study which compares two study groups: one which, as the first treatment for cogulopathy after CPB, receives a transfusion of platelets, and one which receives a dose of fibrinogen concentrate. The patients are infants under one year of age and below 10 kg in weight, scheduled for elective repair of cardiac malformations requiring complex surgery. The patients are randomized to either group if the clinical situation at the end of CPB demands replacement of coagulation components, while ROTEM analysis simultaneously cannot provide a clear answer to which treatment is the most appropriate. The groups are as the first treatment given either a platelet transfusion (20 ml/kg body weight) or fibrinogen concentrate (300 mg/kg body weight). After the intervention, a new ROTEM analysis is made. The primary endpoint of the study is the change in HEPTEM-MCF after the intervention.

2.4.3. Results

The data collection is still ongoing, and the results have not yet been reviewed. The study is included in the dissertation as a method paper. For details, see the articles section.

3. Main results

Study I describes the effect of cardiac surgery with CPB on platelets. During surgery, the platelet count dropped significantly, as did platelet aggregation. On the first day after surgery, the platelet count was rising but had not reached preoperative levels yet, whereas the platelet aggregation had reached and even surpassed the preoperative level. The platelet aggregation, as analysed immediately after the end of CPB using the Multiplate assay, had a significant impact on the need to transfuse packed red blood cells.

In **study II**, we showed that the in vitro potency of ticagrelor, a P2Y12 antagonist, was similar in children of different ages and in adult controls. This result is a necessary step to eventually be able to prescribe this drug to paediatric patients.

Study III picked up where study I left off, by concentrating on platelet aggregation during cardiac surgery and trying to evaluate the use of intraoperative Multiplate analysis of platelet aggregation. In order to test how well Multiplate performs in this setting, we ran it side by side with VASP, a more precise and limited assay for measuring ADP receptor activity in platelets. The results were unfortunately discouraging, with very limited correlation between the two methods. We also compared preoperative Multiplate results from the children with heart defects with healthy children of different ages and found no differences between the groups.

Study IV examined the coagulation capacity of children with congenital heart defects and preoperative heart failure. The degree of heart failure was quantified using Ross scores and brain natriuretic peptide (BNP). No correlations between these measures and coagulation were found when analysing platelet count, plasma fibrinogen, APTT, PT and ROTEM. A little over half of the patients in the study had Down's syndrome; when comparing their coagulation tests with those of the children without Down's

syndrome, only subtle differences were found, which are unlikely to have any clinical impact.

Finally, **study V** is an attempt to build on our previous research and put the knowledge generated mainly in study I and III to work in a treatment trial. In this randomized, controlled, single-blind study we compare two treatment strategies when our ROTEM analysis yields no clear answer. In this study, we include only young infants undergoing major surgery, and test whether a bolus dose of fibrinogen or a transfusion of platelets is superior in raising HEPTEM-MCF after the end of CPB. Since we are in the process of recruiting the last patients when this is printed, the results are not available in this thesis.

4. Discussion

Any discussion of coagulation matters inevitably increases in complexity as the arguments become more nuanced. Since the coagulation system sits deep within a huge network of interrelated homeostatic systems, complications and complexities seem to close in from all angles. What about the inflammatory system? What about complement? What about the age-specific peculiarities of the coagulation maturity of this particular child? It is easy to despair and consider switching to a less complex scientific field, such as the structure of the court bureaucracy of the later Byzantine empire. However, we believe we have been able to spread a degree of light on some of the current problems in coagulation management of infants and children, and we will now summarize and discuss this in the last part of the thesis.

While all of us who are involved in this research are interested in the theoretical underpinnings of the coagulation system, we remain committed to having the theory work for us in the operating room and in the intensive care unit. For this reason, it is difficult not to measure the success of our studies based on whether they have any effect on our decision-making in our day-to-day clinical practice. When viewed through this prism, this thesis offers both hits and misses.

Coagulopathy after cardiac surgery with CPB

Study I is focused on analysing and describing the effect of cardiac surgery on platelets and fibrinogen. The findings are well in line with other studies, with a significant decrease in platelet count and platelet aggregation after the end of CPB,[85] followed by a rapid recovery of platelet aggregation to preoperative levels or above, while platelet count remains lower than before surgery. Today this is common knowledge; when this study was published, these facts were less obvious. One of the most interesting results of study I, though, is the striking relation between platelet aggregation and transfusion of RBCs (see figure 8). Our study thus indicates that platelet

aggregation is an important factor in the postoperative coagulation capacity of the patient, something which other research has not found to be the case in paediatric patients.[88, 89] The question these conflicting results poses is whether the disparate results in the different studies are a consequence of the variation in patient selection, other methodological issues, or simply reflects that platelet aggregation is a minor item in the postoperative coagulopathy of paediatric cardiac surgery patients. We are still inclined to believe that platelet aggregation is an important factor in how well the patient handles postoperative bleeding, but the question remains how a deficient aggregation, if detected, ought to be addressed.

As a very important aside, study I also found that modified ultrafiltration (MUF) did not affect the platelet count or Multiplate results. When added to previously published data which show that MUF also does not distort ROTEM analysis,[90, 91] it can be safely assumed that an assessment of the coagulation of a patient during cardiac surgery can be started towards the end of CPB, before MUF has been completed. This is very significant for the clinical workflow during cardiac surgery, since it allows the anaesthetist time to consider and prepare treatment of any observed coagulopathy.

Platelet aggregation after paediatric cardiac surgery

In study III, we attempted to get a better grip on the reliability of the whole blood Multiplate assay by comparing it to an established method, VASP, which also tests for a more limited mechanism of platelet aggregation capacity (ADP receptor signalling).[92] However, we found very limited correlation between the Multiplate and VASP results, which is in line with other results which have also noted that VASP measurements do not correlate well with other methods for assessing ADP-stimulated platelet aggregation, such as LTA.[93]

As we have seen in study I, there was a clear and significant correlation between low platelet aggregation and the need for transfusions of packed red blood cells. Another result from study I, that low patient age, low

weight, and long aortic clamp time were significantly correlated with decreased platelet aggregation, mirrors other studies which have found that the same patient characteristics are risk factors for increased bleeding.[26] Other authors have also presented evidence that deficient platelet aggregation is a risk factor for the need for transfusions of packed red blood cells or platelets, albeit in adult patients.[49, 94, 95] As we discussed in the previous section, this is a motivation for finding a way of incorporating platelet aggregation analysis into the clinical decision making process during paediatric cardiac surgery. It remains to be seen how this could be done. One recent study by Dieu concerning paediatric cardiac surgery patients found no added value of using Multiplate in addition to ROTEM during surgery.[89] Others have used aggregation analysis in neurointensive care of patients with traumatic brain injury.[96] In a similar way to Dieu, patients who were not treated with antiplatelet drugs before the injury had no correlation between Multiplate results and outcome or lesion progression. Available guidelines do not comment on the use of aggregometry for detecting low platelet aggregation in the setting of paediatric cardiac surgery.[97]

In study III, we also compared preoperative Multiplate analyses of children with CHD with those of healthy children. There were no significant differences between the groups. Perhaps this should come as no surprise, since analyses of the baseline coagulation of paediatric cardiac surgery patients have not been shown to be of any use for predicting blood loss or transfusion need postoperatively,[98] as opposed to adult cardiac surgery.[75, 99] Our contribution to these analyses of baseline factors, study IV, similarly failed to show that the condition in question – preoperative heart failure – increased the risk for intraoperative coagulopathy.

The reason it is possible to find preoperative risk factors in adult patients is probably that these patients often are treated with antiplatelet agents, which despite being timely withheld may have a residual effect which increases the postoperative bleeding risk. Paediatric patients are much less likely to be prescribed these drugs, due to their very different panorama of diseases. Multiplate and other MEA methods are tailored to measuring the effects of antiplatelet drugs and fulfil this role admirably, including in in-

fants and children (something which figure 9 from study II nicely illustrates, see above), and perhaps it should come as no surprise that they may not be precise enough to assess a patient's native coagulation capacity, as it were. The inherently wide normal interval of Multiplate, particularly in infants less than one year of age, is certainly of no help.[100] Controlled studies comparing different haemostasis protocols, incorporating Multiplate in a role which for example has it decide whether to transfuse platelet concentrates or not could perhaps be one way forward.

The effect of cardiac failure and CHD on the coagulation in infants

Cardiac failure has traditionally been considered a risk factor for coagulopathy in children who are subject to surgical correction of congenital heart disease. This has been attributed to mechanisms such as liver congestion caused by an elevated venous pressure,[101] and platelet activation caused by inflammatory mechanisms.[81] Study IV tested this assumption by analysing the coagulation of a group of infants with cardiac ventricular shunts, where the flow through the shunts were non-restrictive, causing heart failure preoperatively. The degree of heart failure was assessed by BNP levels and by the patients' Ross scores, and this was compared to the results of traditional coagulation tests and to their ROTEM values pre- and postoperatively. Except a modest positive correlation between preoperative plasma fibrinogen and BNP, no signs of an effect on the coagulation by the presence of heart failure could be detected. Also, all coagulation parameters, including ROTEM values, were within the age-adjusted normal ranges for healthy children. This result conflicts with one previous study by Osthaus,[102] which found that children with CHD had significantly lower ROTEM values preoperatively, notably FIBTEM-MCF, than children without heart conditions, although all patients in the study were still within the age-adjusted normal ranges. However, we believe the apparent discrepancy between our study and Osthaus is caused by their control group, which had a rather elevated mean FIBTEM-MCF, while their CHD group had a mean FIBTEM-MCF similar to our patients. Another study by Longchamp [103] found that children with CHD had either similar ROTEM values as healthy children, or slightly increased coagulation

capacity, in the form of a shorter CT and higher MCF in most ROTEM channels. Our results are also in line with another viscoelastic study by Haizinger on children with CHD,[104] where thromboleastography (TEG) was used. They found that the preoperative coagulation capacity of children with CHD was lower than that of a control group of healthy children, but within the normal range. They speculate that children with CHD have a coagulation system within normal limits, but with a reduced reserve for events which put a strain on their coagulation capacity. Testing this hypothesis is unfortunately not straightforward, since we believe that the main effect on the coagulation system during cardiac surgery is from the CPB, and a control group of children without heart conditions in need of surgery with CPB is for obvious reasons not easy to find.

Ticagrelor and paediatric patients

Platelet inhibition is an important treatment for a small group of paediatric patients, mostly involving children with ventricular assist devices (VAD)[105], mechanical heart valves,[106] or vascular shunts made out of synthetic materials.[107, 108] The most common treatment option today is still ASA,[70], even though clopidogrel is also used.[109] The problematic issue of patients who are non-responders unfortunately applies to both ASA[110] and clopidogrel.[64]

Ticagrelor, a relatively new platelet inhibitor, seems to have less problems with non-responding patients,[69] and could be a useful addition to the treatment toolbox for paediatric cardiac patients. In study II, we measured the potency of ticagrelor *in vitro* in children of different ages and in healthy adult controls. The fact that one cannot assume that ticagrelor dosing would be the same in children as in adults are illustrated by the case of the close relative of ticagrelor, clopidogrel, which is given at 30 – 50% of the adult dose.[111] The potency of ticagrelor, however, did not differ between adult controls and children of different ages, at least not *in vitro*. This means that further research involving actual patients can get under way.

Treatment protocols for postoperative bleeding after paediatric cardiac surgery

The discussion above about the complexities the coagulation system during heart surgery brings us to our rationale for study V, which unfortunately could not be completed before the publication of this text. Randomized, controlled trials are complex and time-consuming (as this very study has illustrated), but in our opinion sorely needed to complement the plethora of observational studies which are produced (to which we of course have contributed a few ourselves). Regardless of the direction the results of this study will be pointing, we are hoping to gain several insights upon its completion. Not only will we hopefully be able to answer the null hypothesis – that the choice between and order of administration of platelets and fibrinogen concentrate is of no consequence – but also whether platelets and/or fibrinogen are enough for most patients, or if many of them will need additional supplements, such as factor concentrates or plasma, and whether the doses of platelets and fibrinogen appears to be adequate.

One factor in the study design which we hope will be an advantage is the relative homogeneity of the study population in terms of age and surgical procedure (all patients are under one year of age, weigh less than 10 kg, and are all scheduled for complex procedures with long CPB runs). We suspect that many studies, including our own, may suffer in precision by including a too wide array of patients. Restricting the inclusion criteria does obviously limit the applicability of the results, but we believe that this is a price worth paying for focusing on the patients with the most bleeding complications. These patients are the ones with the most need for an effective treatment algorithm. There has been data in adult patients [112] which indicate that selecting suitable patients (i.e., those at risk of bleeding after weaning from CPB) for inclusion in a treatment algorithm is very important for the algorithm to be of any benefit to the patient. It is also a common view that applying a coagulation algorithm on all patients will cause overtreatment of those with a low risk of bleeding complications after CPB.[113]

Using a ROTEM-based algorithm for the treatment of coagulopathy after cardiac surgery has been shown to decrease the need for transfusions in

both adults [114-116] and children.[52] A study in adults by Tanaka[117] also compared platelet transfusion and fibrinogen as a first treatment in adult patients undergoing valve replacement surgery. This study did not find any difference in postoperative blood loss or transfusion requirements, but the patients who received fibrinogen concentrate were subject to less donor exposure, due to the lower incidence of platelet transfusions.

The study protocol of this ongoing RCT details how patients are included in the randomization process. In accordance with the previously mentioned arguments about only including patients who actually are at risk of problematic bleeding in coagulation algorithms, only patients with a clinically obvious bleeding problem after reversal of heparin are included. Whether significant bleeding is present or not is at the discretion of the surgeon. Subsequently, the ROTEM analysis (taken while on CPB) is inspected, and if the HEPTEM-A10 is < 32 and FIBTEM-A10 is < 5, the patient is randomized. We have previously shown that A10 values are excellently correlated to MCF,[90] which means there is no need to wait for the MCF result to appear during the ROTEM analysis. The cut-off values chosen are a combination of the lower end of the normal range for published ROTEM values,[47] and a clinical assessment of at which point the patient might be at risk of bleeding complications. This point which we have chosen is below the lower end of the normal range. There is also a study by Nakayama,[52] where EXTEM-A10 and FIBTEM-A10 were found to be the variables most correlated to postoperative bleeding in a study of CHD surgery. They also developed a bleeding algorithm, where they calculated cut-off points for intervention to EXTEM-A10 < 30 mm and FIBTEM-A10 < 5 mm. It must be said that postoperative bleeding in this study was defined as chest tube output during the first 24 hours, a definition which might be challenged on the grounds that most of that fluid normally is serous, in our experience. Faraoni [27] in their suggested bleeding algorithm chose a lower cut-off point of FIBTEM-A10 $\leq$ 3 mm, albeit in a patient population significantly older than ours, and hence less sensitive to coagulopathy. We consider our cut-off points a decent compromise between available data and clinical experience.

Another obvious point of discussion when a transfusion algorithm is developed is the dosing of the chosen treatments. It may be argued that the

doses of fibrinogen (300 mg/kg body weight) and platelets (20 ml platelet concentrate /kg body weight) are rather generous. In the previously mentioned study by Faraoni et al,[27] a treatment algorithm for bleeding after protamine administration in paediatric cardiac patients was developed based on a retrospective analysis of 150 cases. In this algorithm, 25 mg fibrinogen concentrate/kg body weight was suggested when FIBTEM-A10 was < 3 mm. The population this algorithm was based on, however, had a mean age of 14 months, with no child younger than 5 months. In our current study, all children are small infants. From clinical experience in our institution, we know that lower amounts of both fibrinogen and platelets than the doses we use in this randomized study are unlikely be of much use in the situation one finds oneself in at the end of CPB. When Fibtem-A10 is < 5 in a small infant after cardiac surgery, the dose used in our study is usually adequate to simply reach slightly above the lower end of the normal age-adjusted range of this ROTEM channel. In adults, of course, substantially lower doses are needed.[118] In another recently published RCT by Tirotta,[119] the intervention group received 70 mg fibrinogen concentrate per kg body weight, which improved FIBTEM-MCF but no other ROTEM parameters, and did not influence total blood loss, transfusion requirements or other clinical endpoints. In our opinion, this also reflects an inadequate dose of fibrinogen. Finally, in yet another RCT by Siemens,[120] infants with a mean age of 6 months, with a FIBTEM-MCF ≤ 6 mm during CPB, were randomized to placebo or fibrinogen concentrate calculated using FIBTEM-MCF values. This individualized regimen yielded a dosage of 51 – 218 mg fibrinogen/kg body weight. Again, however, these patients were older than those in our study, and had CHD diagnoses which we would consider low risk for bleeding (mostly septal defects and tetralogy of Fallot). In our patient sample, randomizing between treatment or placebo is a medical impossibility, since they all will need a coagulation intervention of some kind.

The volume of platelets transfused as one of the study interventions similarly reflects standard practice in this patient group in our hospital. One might of course worry that excessive administration of coagulation products would put the patients in harm's way by exposing them to an increased risk of thrombotic complications. In another study, also by Faraoni et

al,[121] an attempt to find risk factors for thrombosis after paediatric cardiac surgery was made. The most important risk factor was administration of activated factor VII. ROC analysis further suggested that a FIBTEM-MCF value of > 22 mm after arrival in the intensive care unit offered the best predictive value for thrombotic complications. None of the patients in our randomized study will come nowhere close to this level.

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