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Transfusion with Fibrinogen and Platelet Concentrate: Acute Effects on Platelet Aggregation and Clot Formation

Master of Science Thesis

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Abstract

Cardiac surgery is associated with a risk for excessive blood loss, which increases morbidity and mortality. The increased bleeding after cardiac surgery may be caused by surgical factors, an impaired coagulation, or a combination of both. Transfusion of blood products is used to improve coagulation and platelet function, but transfusions are costly and may have side effects. Therefore, optimizing the use of transfusion products is important. One way to achieve this is to closely monitor the effect of transfusions on coagulation and platelet aggregation. In this thesis, rotational thromboelastometry (TEM) and multiple electrode aggregometry (MEA) were used to examine the *ex vivo* effects of transfusion of fibrinogen and/or platelet concentrate. Increasing doses of fibrinogen and/or platelets was added to blood from healthy volunteers (n=10), cardiac surgery patients (n=10) and patients treated with platelet inhibitors (n=10). The following variables were analyzed: clotting time (CT) and maximum clot firmness (MCF) with TEM and platelet aggregability with MEA, after addition of different initiators and inhibitors. TEM variables improved after treatment with fibrinogen and platelets in all study groups, without intergroup differences. The effect was not potentiated when the products were simultaneously added. Fibrinogen infusion reduced platelet aggregability in healthy volunteers, but did not influence platelet aggregability in cardiac surgery patients and patients treated with platelet inhibitors. Platelet transfusion increased platelet aggregability in all three groups, but markedly more in the two patient groups. The combination of fibrinogen and platelets were less effective than platelets alone. The effect of platelets and/or fibrinogen on platelet aggregation seemed to be enhanced if the baseline aggregation was suppressed by platelet inhibitors or cardiac surgery. In conclusion, a dissociation among different study populations in the *ex vivo* platelet aggregation response to fibrinogen and platelet transfusion was demonstrated. The clot formation response to fibrinogen and platelet transfusion was comparable in all three study populations. These results may have impact on future transfusion protocols.

Keywords: Platelet aggregation, multiple electrode aggregometry, clot formation, thromboelastometry, hemostasis, transfusion, platelet concentrate, fibrinogen concentrate, cardiac surgery, platelet inhibitors

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1. Introduction

Bleeding is a major problem in cardiac surgery [1]. Transfusion of blood products can help to stop the bleeding, or at least improve the coagulation, but not without risks. This thesis was made to examine the effects of the blood products fibrinogen and platelet concentrate on blood clot formation and platelet aggregation, and possibly provide information on how to optimize the use of blood products.

1.1 Background

Cardiac surgery is associated with a risk for excessive blood loss which may and cause increased morbidity and mortality [1]. Events associated with bleeding are renal failure, stroke[1], prolonged mechanical ventilation[2] and stay in critical care, and sepsis[3] [2, 3]. The bleeding could be caused by mechanical damage to the vasculature and/or an affected hemostatic system[4], which result in microvascular bleeding [1, 4]. Microvascular bleeding that may occur after cardiopulmonary bypass (CPB)[5], is most often caused by platelet dysfunction, and may lead to massive blood loss, perioperative blood transfusion, and surgical reexploration [5, 6]. The platelets could be activated or destructed when in contact with the extracorporeal surfaces and because of hemodilution[6] during CPB [5, 7]. The activation is initiated by exposed tissue factor (TF) in the extracorporeal circuit, with release of coagulation factors as a result [1]. Furthermore, the preoperative use of platelet inhibitors, for example aspirin or clopidogrel, and the use of the anticoagulant heparin during the operation may influence on the platelet function [8].

Formation of a blood clot represents the primary hemostasis in bleeding. The first step of this formation is activation of platelets, induced by tissue damage [9]. The activation makes the platelets irregularly shaped and coagulation proteins are released. The coagulation cascade begins, resulting in production of thrombin, which cleaves the protein fibrinogen into fibrin. Fibrin can then attach to platelets and thereby binding them together, and a blood clot is formed [9]. In surgery, low fibrinogen levels have been shown to be associated with increased postoperative bleeding [10]. Experimental and clinical studies have demonstrated that fibrinogen concentrate can improve hemostasis in hemodiluted blood [11], and improve hemostasis in a porcine model with induced platelet deficiency, better than platelet concentrate transfusion [7]. Although interaction between fibrinogen and platelets is a major part of the hemostasis, there is scarce knowledge about the effect of fibrinogen administration on platelet aggregation. In one study, fibrinogen was able to reverse the effect of the platelet inhibiting substances tirofiban and eptifibatide *in vitro* [12]. Furthermore, the immediate effect of platelet transfusion on platelet aggregation has not been studied.

[1] Rapid loss of brain function due to insufficient blood supply
[2] Defined as >21 days of mechanical ventilation for ≥6 hours per day
[3] A whole-body inflammatory state in presence of an infection
[4] Hemostasis is a process which causes bleeding to stop, consisting of platelet aggregation and clot formation
[5] Technique which temporarily takes over the function of heart and lungs during surgery
[6] Decreased concentration of cells and substances in the blood, resulting from gain of fluid

About 490 000 red blood cell transfusions, 115 000 plasma transfusions and 43 000 platelet transfusions are yearly (2009) given in Sweden [13]. The use of blood transfusions is if possible avoided or minimized, because of its costs and side effects. Although the risk of viral transmission is low, the function of stored red blood cells or platelets is not normal and each unit contains activated inflammatory cells and mediators. This result in limited oxygen release, impaired microcirculatory flow, impaired immune response and increased morbidity and mortality [14]. If the coagulation properties are measured, hemostatic abnormalities can be detected early and the perioperative blood loss and need for transfusions can be guided and minimized [5]. Two examples of methods that can be used to assess the coagulation properties are thromboelastometry and impedance aggregometry.

1.2 Hypothesis

It was hypothesized that transfusion with fibrinogen and/or platelets would improve platelet aggregation and clot formation.

1.3 Aims and Objectives

The purpose of this research project was to study the effects of transfusion with fibrinogen and/or platelets on platelet aggregation and clot formation.

The study objectives for the project were as follow:

- Do fibrinogen or platelet transfusions improve clot formation and platelet aggregation in blood samples from healthy volunteers, cardiac surgery patients and patients treated with platelet inhibiting substances?

- Are the *ex vivo* effects on clot formation and platelet aggregation potentiated if fibrinogen and platelet transfusion are combined?

1.4 Limitations

Fibrinogen and platelet concentrates are not the only blood products that could affect the blood clot formation and the platelet aggregation when transfused in the blood, but this thesis focused on them. Because of time limitations during the analyses of the blood samples with additives of fibrinogen and/or platelets, studying long term effects of the blood products were not possible. Time limitation was also the reason for the small study groups of only ten individuals in each. Larger study groups would have strengthened the statistical analysis. The *in vivo* effects of the clot formation and platelet aggregation affected by fibrinogen and/or platelet transfusion in bleeding patients after cardiac surgery was intended to be studied. Though, because of delayed approval by the Ethics Committee for this study objective, it was excluded from this thesis work.

2. Theory

Thrombosis is about the formation of a blood clot, thromboelastometry about visualization of this process, and aggregometry is about measuring the platelet aggregation during blood clot formation [4, 9, 15]. These concepts and some facts about statistics are described below to enhance the understanding of this thesis. But before going into these subjects, a brief introduction to cell biology and information about the blood will be given.

2.1 Cell Biology

Cell biology is a wide concept. Here, some information about the structure and function of the cell and its components, and its surrounding environment will be given, that is relevant to this thesis.

2.1.1 Cellular Structure and Function

To isolate the cell from the surrounding environment, it possesses a cell membrane. This membrane is bilayered and mainly composed of phospholipids, molecules which have a polar headgroup with a nonpolar tail made up of fatty acids [9]. Phospholipids naturally arranges into a bilayered structure when exposed to an aqueous environment, facing the hydrophilic headgroups and hiding the hydrophobic tails from the water. There are also proteins in the cell membrane. Transmembrane proteins are a type of proteins that have both polar and nonpolar parts, allowing them to traverse the whole membrane [9]. Many of these proteins function as channels or pumps for specific molecules, enabling passage in and out from the cell and maintenance of the intracellular chemistry. The transported molecules are often inorganic ions like Na^+, K^+, Ca^{2+} and Cl^-, and they are either transported down or opposite their concentration gradients. Energy is needed to transport a substance against its concentration gradient [9]. Another type of proteins is anchored in the membrane and projecting into the intracellular or extracellular space, acting as receptors for specific target molecules at the inside or outside of the cell. These target molecules are called ligands [9].

Inside the cell there are cytoskeletal elements, which provide the shape and motility of the cell. As they contract or expand, the cell changes its shape or is able to move. Three types of cytoskeletal elements are actin microfibrils, intermediate filaments and microtubules [9].

Nucleus, endoplasmic reticulum, Golgi apparatus and mitochondria are some organelles which are located in the cytosol[7] of the cell [9]. The nucleus holds the genetic information in the form of DNA which is folded and packed into chromatin. The chromatin is surrounded by phospholipid membranes forming a nuclear envelope, with nuclear pores allowing only specific molecules to pass in and out of the nucleus [9]. The nuclear envelope is continuous with the endoplasmic reticulum (ER). The ER consists of sheets of phospholipid membranes, and is the location at which the syntheses of proteins occur [9]. After being formed at the ER, a protein is transferred to the Golgi apparatus, where it is modified, sorted and packed and then transported to the location where it is meant to go. Mitochondria stand for the energy production in a cell. Pyruvate, that has been formed from glucose via glycolysis, is imported to the inside of the mitochondria, and then adenosine triphosphate (ATP) is formed via oxidative

[7] The fluid of the inside of a cell

phosphorylation of pyruvate [9, 16]. ATP can then be transported to an area in need and be hydrolyzed to adenosine diphosphate (ADP). This ATP hydrolysis can provide energy to reactions that requires energy to occur [9].

2.1.2 Extracellular Matrix

Association between a cell and its surrounding environment, which is called the extracellular matrix (ECM), is essential for the cell. The ECM is made from the fiber-forming molecules collagen and elastin, which are encompassed by space-filling molecules like glycoproteins and proteoglycans [9]. Collagen is the most common among the proteins in mammals and consists of a triple helix made from polypeptide chains, usually forming a fiber. While collagen provides strength to the tissue, elastin is responsible for its elasticity. Elastin fibers are crosslinked by proteins (for example lysyl oxidase), building a three-dimensional network which can reversibly stretch and relax [9]. Glycoproteins and proteoglycans interact to a great extent with water and other elements of the ECM, and thereby influence the extracellular structure. Both of these ECM elements contain protein and carbohydrate components, but in proteoglycans, carbohydrates are the main component, while glycoproteins mainly contain proteins [9]. One type of glycoprotein is called fibronectin. Fibronectin has multiple binding sites for a diversity of ECM molecules which enables attachment of various tissue components to each other. For instance, it possesses a ligand to some integrin receptors, which are transmembrane proteins that are involved in cell-cell and cell-matrix contacts [9]. At the inside of the cell membrane, integrins attach to the intermediate filaments, and at the outside they bind to specific ligands, such as collagen or fibronectin. Fibronectin also contain binding domains to fibrin and heparin and thereby affect the blood coagulation, which will be described below [9].

2.2 The Blood

There are many types of cells in the blood, with various functions. In spite of their different functions, they all originate from a common stem cell[8] in the bone marrow [16]. There are two classes of blood cells; red and white. The red blood cells, also called erythrocytes, supply the tissues in the body with oxygen (O_2) by transporting it along the blood vessels bound to hemoglobin [16]. The tissues then release carbon dioxide (CO_2), which is transferred by the erythrocytes, also bound to hemoglobin, to the lungs for reoxygenation. Erythrocytes do not need to pass the vessel walls to perform their task. However, the white blood cells, or leucocytes, have to pass the wall of small vessels and migrate into the tissues to be able to fulfill their tasks. Leucocytes fight infections, for instance by producing antibodies[9], and some leucocytes phagocytose[10] and digest waste [16]. Besides blood cells, the blood contains a large number of platelets. Platelets are cell fragments which play a major role in the blood clot formation process [16], and they will be further described in the next text section. The blood accounts for 7% of the body weight of a human. Approximately 45% of this volume is taken up by the red blood cells and 1% by white blood cells. The rest is the blood plasma [16].

[8] Cell that can divide indefinitely giving daughter cells that can differentiate into specific cell types
[9] Protein produced by certain leucocytes in response to a foreign substance or invading microorganism
[10] A cell "eats" a particular material

2.3 Thrombosis

There are hemostatic mechanisms in the body, which function by detaining bleeding in case of injury. Vascular constriction, development of a platelet plug, and blood coagulation (thrombosis) are mechanisms contributing to the hemostasis of the affected area [9]. By locally decreasing the blood flow and temporarily closing the hole in the vessel, further blood loss from the area is restrained. Both proteins and other constituents of the blood, for example platelets, and the endothelial lining of the blood vessels, play a major role in hemostasis [9].

2.3.1 Platelets

Platelets are fragments originating from megakaryocytes in the bone marrow, with a diameter of 3-4 μm. They lack nuclei which make them unable to proliferate[11], and their half-life in the body is only 8-12 days [9]. Platelets contain mitochondria, portions of the endoplasmic reticulum, Golgi apparatus and granules. α granules carry platelet-specific proteins, β-thromboglobulin and plasma proteins like fibrinogen and the coagulation-cascade protein factors V and XIII. Dense granules contain adenosine diphosphate (ADP), calcium ions and serotonin, while hydrolytic enzymes is carried by lysosomal granules [9].

Stimuli that can activate platelets when the vessel wall is injured are mainly the extracellular matrix (ECM) component collagen (that gets exposed in case of an injury), and the blood plasma protein von Willebrand factor (vWF) [9]. The vWF attaches to the exposed collagen, then it binds to the glycoprotein receptor Ib (GPIb) on the platelets, anchoring the platelets to the damaged vessel wall [9, 13]. The first step of activation is stimuli of receptors on the platelets. When activated, the platelets go from a disc-like morphology and swell into an irregular shape while the cytoskeletal proteins contract, with release of granule contents as an outturn. As the platelets are activated, they are able to adhere to ECM proteins, aggregate, secrete various factors that can stimulate other platelets, and display coagulation activity [9]. Upon adhesion, ADP, serotonin, thrombin and thromboxane A_2 (TxA_2) are released from the dense granules, and the synthesis of thrombin and TxA_2 is upregulated [9]. From the α granules, vWF, factor V and XIII, and fibrinogen are released [13]. The substances which are released by the dense granules activate adjacent platelets, attract them to the region and get them to express activated glycoprotein IIb/IIIa (GPIIb/IIIa) receptors on their surfaces. Activated GPIIb/IIIa receptors together with integrin receptors increase aggregation by interacting with collagen, vWF, fibrinogen, and fibronectin ligands [9]. Whereas fibrinogen has two ligands for the platelet receptors GPIIb/IIIa, it enables platelet-platelet binding and stands for a large part of the platelet plug formation. The established plug is quite fragile, thus the platelets promote localized blood coagulation to stabilize it [9].

2.3.2 Coagulation Cascade

Intrinsic and extrinsic pathways are two mechanisms from which blood clot formation may occur and they both end in a common pathway, eventuating in conversion of fibrinogen to fibrin, which is the main component of the blood clot [9].

[11] Grow and increase in number, process called cell division in mammals

2.3.2.1 Intrinsic Pathway

The intrinsic pathway, overviewed in **Figure 2.1**, is initiated by a contact between a negatively charged surface and the blood [9]. This surface could be made up of a damaged vessel wall, exposing ECM molecules, or damaged blood cells. The coagulation contact protein factor XII is then adsorbed to the negatively charged surface, resulting in an activation of this protein, and XIIa (a is for activated) is formed [9]. The coagulation factors prekallikrein and factor XI bind to a cofactor, high molecular weight kininogen (HMWK), which enables them to bind to the charged surface and interact with XIIa, resulting in conversion of prekallikrein and factor XI to the activated kallikrein and XIa, respectively [9]. The kallikrein converts factor XII to form more XIIa, as a positive feedback. After that, XIa converts the factor IX to form IXa. IXa together with factor VIII attach to a phospholipid membrane, in general of a platelet, where they convert factor X to Xa, and the common pathway is reached. This last reaction could occur without the presence of VII or a phospholipid membrane, but would then take much longer [9].

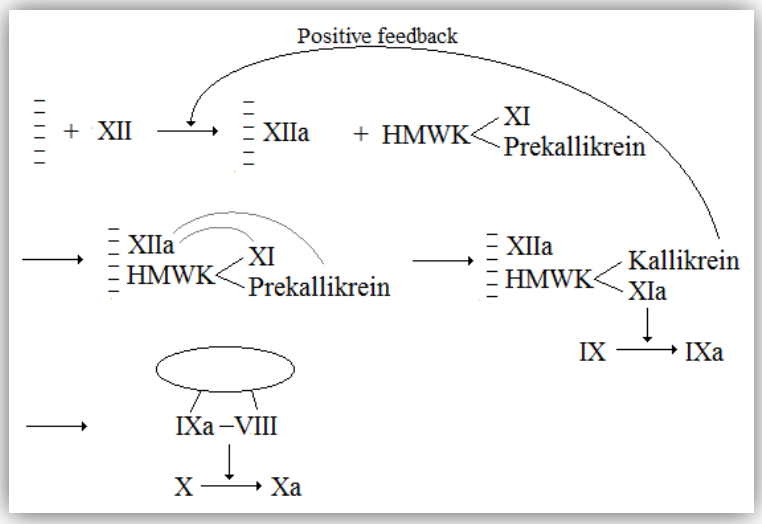

Figure 2.1 Intrinsic pathway. Factor XII adsorbs to a negatively charged surface, which is the initiator of this pathway, and the activated XIIa is formed. Prekallikrein and factor XI bind to HMWK, enabling interaction with factor XIIa and conversion to the activated kallikrein and XIa. Kallikrein produces more XIIa in a positive feedback, while factor XIa converts factor IX into IXa. IXa and factor VIII attach to a phospholipid membrane, where they convert factor X to Xa. Now the common pathway is reached.

2.3.2.2 Extrinsic Pathway

The mechanism of blood clot formation which is initiated by the release of tissue factor (TF) is called the extrinsic pathway [9]. TF is a membrane-associated protein made of a polypeptide chain. In comparison to intrinsic pathway, which initiates clotting in one to six minutes, it only takes up to 15 seconds for clotting to be initiated by this mechanism [9]. Released TF acts as a cofactor and joins with factor VII on the surface of a phospholipid membrane, and factor VII is cleaved by a number of proteases located in the blood and activated into VIIa. The last step before reaching the common pathway is conversion of factor X to Xa

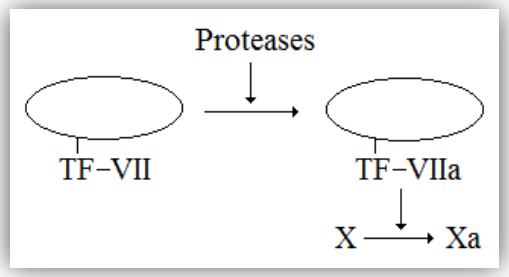

Figure 2.2 Extrinsic pathways. This pathway starts with a release of the TF, which attaches to factor VII and a phospholipid membrane. Factor VII is then cleaved by a number of proteases, and activated VIIa is formed. The TF/VIIa complex converts factor X to Xa, and the common pathway is attained.

by the TF/VIIa complex [9]. This pathway can be viewed in **Figure 2.2**.

2.3.2.3 Common Pathway

The common pathway is induced by secretion of factor V from platelets [9]. As can be seen in **Figure 2.3**, Factor V can bind to certain receptors on the platelets, and while doing so, it can form a complex with factor Xa, that together with calcium ions is called the prothrombin activator complex [9]. When this complex is formed, factor Xa can convert prothrombin to the active enzyme thrombin [9, 17]. Thrombin can then cleave fibrinogen, which is located in granules of platelets and in blood plasma, into fibrin monomers (and fibrinopeptides A and B) [9]. If there is no tissue damage, fibrinogen and prothrombin can coexist in the blood without

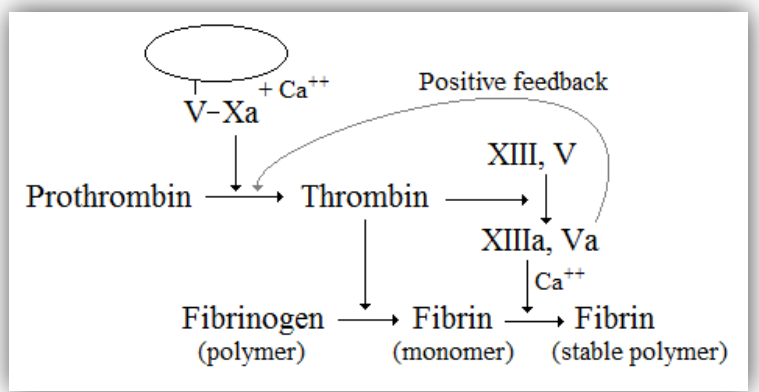

Figure 2.3 Common pathway. Secretion of factor V fromplatelets initiates this pathway. When binding to receptors on platelets, factor V can form a complex with factor Xa. When calcium ions are present Xa can convert prothrombin into thrombin, which then cleave fibrinogen into fibrin monomers. Thrombin also activates factors XIII and V. Va forms more thrombin as a positive feedback, while XIIIa, in the presence of calcium ions, crosslinks fibrin chains into a stable polymer.

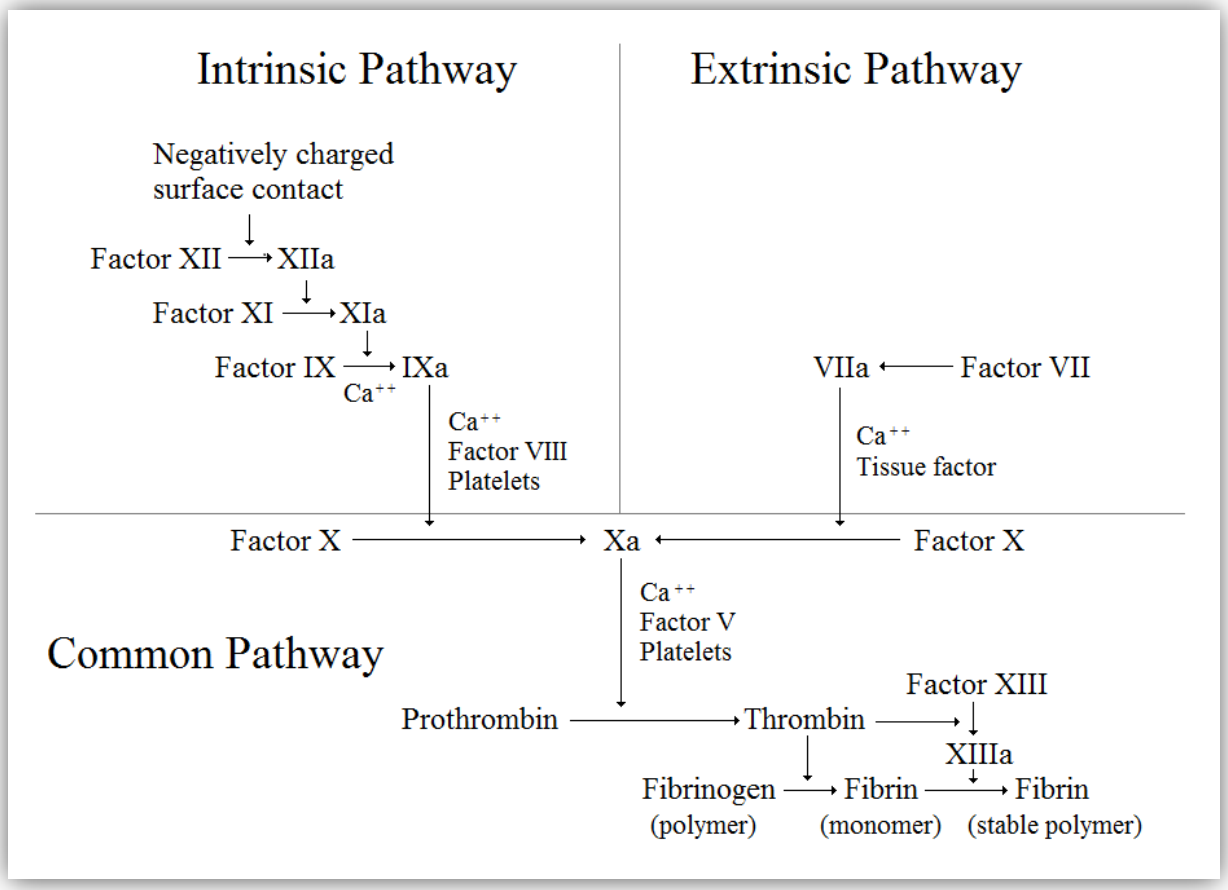

Figure 2.4 Simplified overview of the coagulation cascade.

any occurrence of blood clot formation. Fibrin monomers can polymerize to form long fibrin fibers, the basis of the blood clot. This structure is only held together by hydrogen bonding, therefore, XIIIa, in the presence of calcium ions, stabilizes it by covalently crosslinking the fibrin chains [9]. The calcium ions originate from the dense granules and factor V and XIII from α granules of the platelets. Factor XIIIa and Va are converted by thrombin from XIII and V. Factor Va accelerates the formation of thrombin in a positive feedback [9]. The final result of the coagulation cascade is a meshwork of fibrin that connects platelets, adhesive proteins and other bioactive factors; a mature thrombus. By attaching this clot to the ECM of the blood vessels, further bleeding is prevented [9]. In **Figure 2.4**, a summary of the coagulation cascade can be seen.

2.3.3 Anticoagulant Mechanisms

The interaction between thrombin and fibrinogen is a key phase of the blood clot formation process and the regulation of this process is of high importance. In case of a wound, the clot has to be formed fast enough to prevent bleeding, and regulation has to attend to prevent spontaneous clot formation when not needed [17]. Antithrombin (AT), protein C and tissue factor pathway inhibitor (TFPI) are substances that prevent the clot formation in healthy vessels [13]. Healthy tissues are also surrounded by the platelet activation inhibitors prostacyclin (PGI_2), nitrogen monoxide (NO) and heparin sulphates. When antithrombin binds to a heparin sulphate on the membrane of a healthy endothelial cell, it complex binds to the procoagulatory substances thrombin, factor IXa, Xa, XIa or XIIa, preventing them to spread in the blood [13]. Protein C can be activated by thrombomodulin when attached to it. This activated protein C can inactivate factor Va and VIIa. By inactivation of the TF/factor VIIa complex, a complex composed of TFPI and factor Xa is enable to inhibit the first step of the extrinsic pathway [13].

To enable degradation of a formed blood clot when the wounded area is restored, there is a system called fibrinolysis. This system acts by dissolving the fibrin fibers in a controlled manner. When the clot is mature, endothelial cells start releasing tissue plasminogen activator, which activates plasminogen located in the clot, forming plasmin. Plasmin can then cleave fibrin and dissolve the clot [9].

The thrombosis is not limited only by biochemical factors; also physiological factors play its role. Normal blood flow can prevent initiation of clot formation by diluting the activated substances, and the absence of a coagulation triggering surface beyond the damaged area keeps for example factor XII, which is involved in the intrinsic pathway, from being activated [9]. Also parameters such as temperature and pH can affect the coagulation. Hypothermia and acidosis, especially when combined, could impair the coagulation [18].

2.3.4 Crossovers between Thrombosis and the Inflammatory Response

There are several crossovers between the thrombosis and the inflammatory response [9]. One such is that kallikrein, in the intrinsic pathway, which can act to cleave HMWK, with release of the inflammatory mediatior bradykinin as a result. The inflammatory mediators interleukin-1 and tumor necrosis factor-α can induce macrophages and endothelial cells to produce TF [9], which is initiating the extrinsic pathway. Fibrinopeptides A and B, which evolve in the

common pathway, are chemotactic for neutrophils, which is another example where thrombosis and the inflammatory response overlap [9].

2.4 Platelet Inhibiting and Anticoagulant Substances

There are several types of platelet inhibiting medication, using various approaches to inhibit platelet activation. Activated platelets enhance the production of arachidonic acid, which is converted into prostaglandin by cyclooxygenase-1 (COX-1) and thereafter into TxA_2 [19]. Acetylsalicylic acid (ASA) acts by irreversibly inhibiting COX, and thereby the TxA_2 pathway of platelet activation is inhibited [19, 20]. TxA_2 cannot be formed until new platelets are produced, containing uninhibited COX [19]. By inactivating the ADP receptor of the platelets, clopidogrel irreversibly inhibits binding of ADP to the platelets, and thereby inhibits ADP induced platelet activation [20, 21]. Another substance that inhibits ADP induced platelet activation is called ticagrelor. This is new on the market, and interacts, unlike clopidogrel, reversible with the ADP receptor [22]. Prasugrel is a platelet inhibiting substance intended for simultaneous treatment with ASA. It inhibits platelet aggregation by irreversibly binding to ADP receptors [23]. A substance that restrains platelet aggregation reversibly, by inhibiting binding of fibrinogen, vWF, and other ligands to GPIIb/IIIa receptors, is called eptifibatide [24]. Eptifibatide is a synthetically produced substance, and is intended for simultaneously use with ASA and heparin [24]. Tirofiban is a substance which inhibits platelet aggregation by preventing binding of fibrinogen to the GPIIb/IIIa receptor [25]. The anticoagulants low molecular weight heparins are fractionized heparins which mainly inhibit factor Xa, by enhancing the binding of antithrombin to Xa, but they are also slightly inhibiting thrombin [26]. Warfarin is a synthetic anticoagulant which inhibits coagulation by blocking vitamin K. Thereby, factor VII, IX and X, which are vitamin K dependent, are restrained [27]. Some platelet inhibiting and anticoagulant substances and drugs containing these substances are listed in **Table 2.1**.

Table 2.1 Platelet inhibiting and anticoagulant substances and their brands

Active substance		**Examples of brands**
Acetylsalicylic acid (ASA)		Aspirin®, Trombyl®
Clopidogrel		Plavix®, Grepid®
Ticagrelor		Brilique®
Prasugrel		Efient®
Eptifibatide		Integrilin®
Tirofiban		Aggrastat®
Low molecular heparins*	Fondaparinux	Arixtra®
	Dalteparinnatrium	Fragmin®
	Enoxaparinnatrium	Klexane®
Warfarin		Waran®, Warfarin Orion

*Information brought from FASS.se

2.5 Thromboelastometry

Thrombelastography (TEG) and rotational thromboelastometry (TEM) are two similar methods used for assessment of the hemostasis, and their respective instruments are called thrombelastograph® (TEG®) and thromboelastogram® (ROTEM®, Pentapharm, Munich, Germany) (**Figure 2.5**) [4]. TEG®/ROTEM® have mainly been used to monitor blood component transfusion during surgery. In the field of liver transplantation, the use of TEG® has been described in the literature since 1985 [4], and in cardiac surgery since 1995 [4]. Since ROTEM® is the instrument used in this project, this is the one that is mentioned from here on.

Figure 2.5 The ROTEM® device. Below, the four channels for blood analysis can be seen as blue blocks. To the right, there is an electronic pipette, and on the screen the results from the analysis can be viewed.

In TEM, the viscoelastic changes that arise during the blood clot formation *in vitro*, using whole blood at 37°C, are recorded and a representation of the fibrin polymerization and process, including the polymerization rate, and the overall clot strength are obtained both graphically and numerically [4, 5]. These data provide a complete evaluation of the clot initiation and formation process, clot stability and lysis. The parameters used for analysis in this project are the clotting time (CT), the clot formation time (CFT), alpha angle (α), and the maximum clot firmness (MCF). Definitions of these parameters can be seen in **Table 2.2** and a typical graph evolved from ROTEM® analysis in **Figure 2.6**.

Table 2.2 Some of the parameters which evolve from TEM analysis

Measurement	**Abbreviation**	**Definition**
Clotting time	CT	Period to 2 mm amplitude (seconds)
Clot formation time	CFT	Period from 2 to 20 mm (seconds)
Alpha angle	α	Angle of tangent at 2 mm amplitude (º)
Maximum clot firmness	MCF	Maximum amplitude (mm)

The clot firmness is affected by the platelet count, fibrinogen and coagulation factor XIII. Decreasing levels of these factors normally result in reduced clot firmness [7].

There are different reagents that can be used in ROTEM® analysis depending on which activator/inhibitor that is of interest to examine. Extem and Fibtem are the compositions of reagents that were used in this project. Extem examines the role of TF activation and provides information about the clot formation, fibrin polymerization and fibrinolysis via the extrinsic pathway. In Fibtem, the role of TF activation is examined with exclusion of the platelets by the toxin cytochalasin D, providing information of the fibrinogen status and possible polymerization disorder or deficiency [28]. Intem and Heptem are two other compositions of reagents which examine the intrinsically induced clot formation. The difference between these two is that Heptem also contains heparinase, which neutralizes heparin, providing information

whether there is any heparin effect. However, effects from the platelet inhibitors ASA and clopidogrel are not possible to detect with TEM [29].

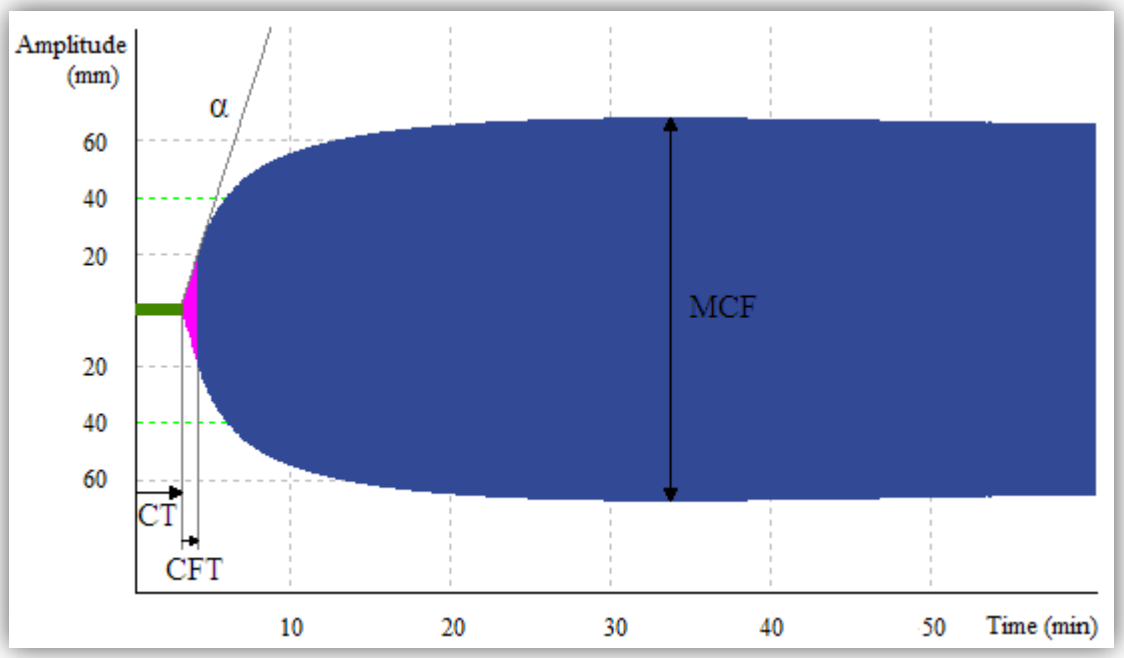

Figure 2.6 Graphical representation of the TEM parameters clot time (CT), clot formation time (CFT), maximum clot firmness (MCF), and alpha angle (α). The curve is reflected along the longitudinal axis.

When doing an Extem analysis, 20 µL of 0.2 M $CaCl_2$ (Star-tem®), for recalcification of the citrated blood, and 20 µL of the tissue thromboplastin reagent (Extem®) are added to a cuvette (**Figure 2.7**), followed by 300 µL of the citrated blood sample, according to the manufacturer's instructions. The cuvette is placed on the instrument so that a down pointing pin becomes situated in the filled cuvette. Then the pin starts rotating and the instrument measures the viscoelastic changes of the sample. In Fibtem analysis, 20 µL of the Extem® reagent and 20 µL of cytochalasin D (Fibtem®) are added to the cup. Thereafter, the procedure is the same as with Extem analysis [30]. The ROTEM® apparatus consists of four identical channels, allowing for four simultaneous analyses. After a blood sample is taken, the blood must be analyzed within four hours when using this method.

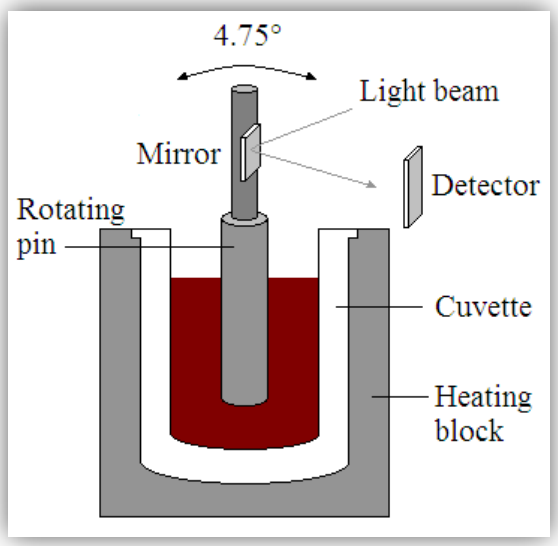

Figure 2.7 Principle of TEM. The blood is inside a cup, situated in a heating block to keep the temperature at 37°C. A pin is rotating in the blood, and the viscoelastic changes are measured using a light beam directed towards a mirror on the pin, which is reflected against a detector. Figure adapted from Lang *et al*.

2.6 Impedance Aggregometry

The most commonly used method for measurement of platelet aggregation is light-transmission aggregometry, which is utilizing citrated or heparinized platelet rich plasma [15]. This technique requires centrifugation to separate other blood cells from platelets, which is both time consuming and affecting the platelet function [15]. The analysis, including centrifugation, takes more than one hour [8]. The centrifugation process could also damage some of the larger platelets, with a nonrepresentative population of platelets as an outcome. Furthermore, some stimuli and drugs depend on interactions with leucocytes and erythrocytes for proper platelet response [31]. Because of these disadvantages, methods allowing measurement of platelet aggregation in whole blood have been developed. One of these methods is called multiple electrode aggregometry (Multiplate®, Multiplate Services, Munich, Germany) and measures platelet aggregation in diluted whole blood by impedance aggregometry, in test cells for single usage with duplicate impedance sensors in the form of four electrodes. There are place for five test cells at the device (**Figure 2.9**) allowing for five analyses at the same time. When platelets attach to the electrodes, the electrical resistance between two adjacent electrodes increases, this change of resistance is called impedance. The impedance is proportional to the amount of platelets attached to the electrodes and is continuously recorded [15]. The electrical resistance is measured in Ohm and transformed to arbitrary aggregation units (AU) which is plotted against time. The parameters area under the curve (AUC) and velocity, which is the steepest slope of the curve, is then calculated and given in AU*min and AU*min^{-1}, respectively. In **Figure 2.8**, a result from such a measurement is to be viewed [32]. All parameters are calculated from the mean of the results from the duplicate sensors.

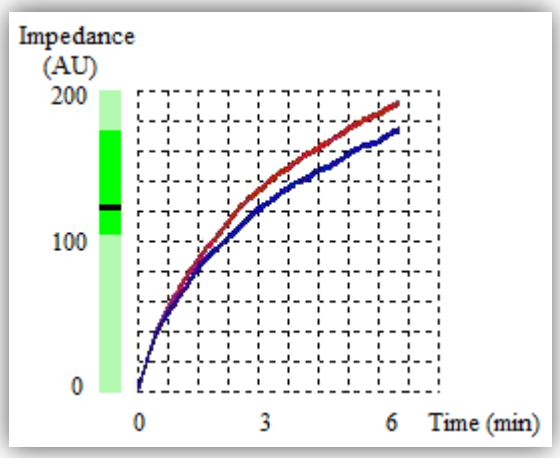

Figure 2.8 Curves derived from Multi-plate® analysis. The two curves represent the results from the two sensor units. The electrical impedance increases as the platelets aggregate on the electrodes of the sensor units. The green area in the vertical axis represents a normal AUC when the TRAPtest is used, and the black line, the actual AUC in this measurement.

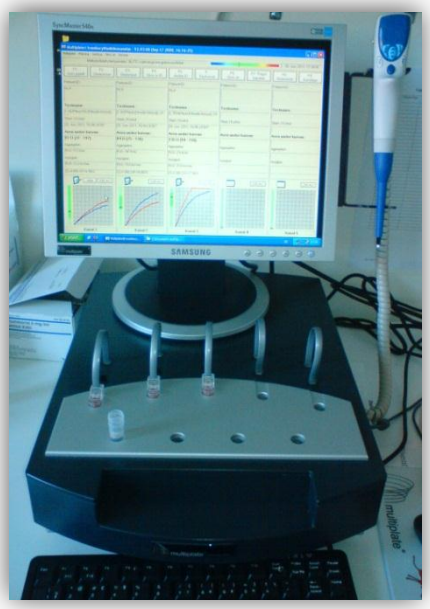

Figure 2.9 The Multiplate® instrument. There are five channels for analysis, in the picture three of them are in use and test cells are placed there. The test cells are connected to the computer with sensor cables which enable the computer to measure the impedance in the blood sample.

As in ROTEM®, different tests with various reagents are available for Multiplate® analysis. The reagents use

different approaches to activate the platelets. ADPtest High Sensitivity (HS) is used for analysis of ADP induced aggregation with prostaglandin E1 for increased sensitivity. Prostaglandin is a natural inhibitor of ADP dependent aggregation [33] and thereby enhances the sensitivity for detection of clopidogrel, prasugrel and other ADP receptor antagonists [34]. ASPItest is used to study COX dependent aggregation with arachidonic acid as a trigger. This test is sensitive to ASA and other COX inhibitors. To examine aggregation caused by induced thrombin receptor, TRAPtest is used. The activation is triggered by TRAP-6 (thrombin receptor activating peptide), which imitates the action of the platelet activator thrombin. Eptifibatid is a substance that can be detected with TRAPtest [34]. In accordance with the manufacturer's instructions, 300 µL of 0.9 mass-percent NaCl is added to a cup connected to the instrument by electrodes, followed by 300 µL of the blood sample. After three minutes of incubation at 37°C, 20 µL of ASPI reagent, TRAP reagent, or 20 µL prostaglandin solution and 20 µL ADP reagent is added. Then the impedance between the electrodes is measured for six minutes [35]. After taken a blood sample, the blood must rest for 30 minutes before analyzing it, but it must not take more than three hours before the analysis is made.

2.7 Statistics

Here some statistical concepts will be described, that were used in the thesis.

2.7.1 The Mean

A usual way to summarize data is to calculate its mean. It is made by summarizing all the values and dividing this sum with the number of values. In mathematical terms, the set of observations of the parameter x is written as x_1, x_2, x_3, …, x_n. The equation for the mean is:

$$\bar{x} = \frac{\sum_{i=1}^{n} x_i}{n}$$

where Σ stand for "the sum of", *i* is any of the observed values, "*i=1* and *n*" means that the first to the nth value are summarized and the sum is then divided by the total numbers of values, *n* [36].

2.7.2 The Standard Deviation

To get a picture of how spread the values are from the mean, the standard deviation can be used. The standard deviation can be seen as a type of average of the deviations of the observations from the mean [36]. It has the same unit as the raw data and is calculated as follows:

$$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$$

2.7.3 Hypothesis Testing

In hypothesis testing, a null hypothesis (H_0) is usually tested. A null hypothesis is assuming that there is no difference in means of a variable among two populations. An alternative hypothesis (H_1) is also stated, which assumes that the means are different. When a so called two-tailed test is used, the direction of the differences of the means is not stated. It means that, if H_0 is rejected in advantage of H_1, it is so either if the mean of the first or the second population is the largest [36].

2.7.4 P value

The P value is the probability of obtaining a certain result. A significance level is chosen, and when the P value evolved is below this level, the H_0 is rejected. If the significance level is set to 5%, H_0 is rejected if the P value is below 0.05. In contrast, a P value greater than 0.05 do not have to mean that the H_0 is correct, rather that there is not sufficient evidence to reject it [36].

2.7.5 Correlation

Correlation analysis provides information about the degree of association between two variables; x and y. If there is one numerical value of both x and y for each of the individuals in the sample, a point for each individual can be plotted in a scatter diagram. In this diagram, the x variable is on the horizontal axis, and y on the vertical axis. If the scatter of points forms a line, where the x variable seems to increase as the y variable increase or decrease, there may be a relationship between the variables. A P value is obtained, and such a value beneath the chosen significance suggests that the correlation is significant. Spearman's correlation coefficient is a way to calculate correlation. This method is used when for example the sample size is small, as the case with the study groups in this thesis [36].

3. Materials and Methods

In this study, the effects of platelet and/or fibrinogen concentrate on platelet aggregation and blood clot formation were studied *ex vivo*. Increasing doses of platelets and/or fibrinogen were added to blood samples from healthy volunteers, cardiac surgery patients and patients treated with platelet inhibiting substances. Impedance aggregometry and thromboelastometry were used to evaluate the effects on platelet aggregation and clot formation, respectively.

3.1 Inclusion Criteria and Study Design

After approval by the Ethics Committee at Sahlgrenska University Hospital and written informed consent, healthy volunteers (n=10), cardiac surgery patients (n=10) and patients treated with platelet inhibiting substances (n=10) were participating in the study. Cardiac surgery patients were treated with ASA, while the platelet inhibited patients were treated with both ASA and clopidogrel. Patients with known bleeding disorder, liver disease, or ongoing treatment with drugs influencing bleeding and coagulation, other than platelet inhibitors, were excluded. To prevent any reaction due to alloimmunization[12] when platelet concentrate was added, only men without previous history of transfusion recipients were included in the *ex vivo* study. To prevent unreliable result for Multiplate® analysis, blood samples with a preoperative platelet count below $150*10^9$ L^{-1} were also excluded in the study.

3.2 Blood Sampling

The blood samples were taken from the healthy volunteers and the patients treated with platelet inhibiting substances by venipuncture in the brachial vein, whereas the blood samples from cardiac surgery patients were taken from previously inserted venous catheters. For the ROTEM® analysis, citrated tubes (0.129 M, á 2.7 mL) were used, and hirudin tubes (0.25 $\mu g*L^{-1}$, á 3 mL) were used for the Multiplate® analysis. At the same time, an EDTA tube (K2EDTA; REF 454411, 3 mL) was filled to enable measurement of hemoglobin, hematocrit, platelet count and white blood cell count of the blood. In the case of cardiac surgery patients, the samples were taken after weaning from CPB, and after the heparin was neutralized with protamine. Also one tube each of citrate and hirudin type was taken before the start of the surgery, to allow for comparison of the coagulation capacity of the blood before and after surgery. The blood samples from the patients on platelet inhibitors were taken two hours after the morning dosage of the platelet inhibitors were given.

3.3 Study Group Characteristics

In **Table 3.1**, study group characteristics can be viewed. The healthy volunteers, cardiac surgery patients and the patients treated with platelet inhibitors had an age of 42 ± 8.8, 66 ± 9.0 and 63 ± 9.2 (mean ± standard deviation) years respectively, and a body mass index (BMI) of 24 ± 3.2, 30 ± 4.0 and 26 ± 4.1 $kg*m^{-2}$. The respective platelet counts were 230 ± 50, 210 ± 49 and 240 ± 59 $*10^9*L^{-1}$, for the healthy volunteers, cardiac surgery and platelet inhibited patients. Hemoglobin levels were 150 ± 9.4, 110 ± 9.9 and 130 ± 15 $g*L^{-1}$, hematocrit 0.44 ± 0.029, 0.31 ± 0.028 and 0.39 ± 0.049 $L*L^{-1}$, and the white blood cell counts were 6.0 ± 0.92,

[12] A state that can occur if a person is Rh-negative and receives transfusion of Rh-positive red blood cells, or if an Rh-negative woman carries an Rh-positive fetus, which can cause complications in future transfusions

16 ± 3.5 and 7.8 ± 2.0 $*10^9*L^{-1}$, for the healthy volunteers, operated patients and patients on platelet inhibitors, respectively.

Table 3.1 Study group characteristics (mean ± standard deviation)

Variable	Healthy volunteers	Cardiac surgery patients	Patients on platelet inhibitors
Age (years)	42 ± 8.8	66 ± 9.0	63 ± 9.2
BMI ($kg*m^{-2}$)	24 ± 3.2	30 ± 4.0	26 ± 4.1
Hb ($g*L^{-1}$)	150 ± 9.4	110 ± 9.9	130 ± 15
HCT ($L*L^{-1}$)	0.44 ± 0.029	0.31 ± 0.028	0.39 ± 0.049
PLT (10^9*L^{-1})	230 ± 50	210 ± 49	240 ± 59
WBC (10^9*L^{-1})	6.0 ± 0.92	16 ± 3.5	7.8 ± 2.0

BMI; body mass index, Hb; hemoglobin, HCT; hematocrit, PLT; platelet count, WBC; white blood cell count.

3.4 Sample Preparation

In **Table 3.2**, the contents of the sample preparations for ROTEM® and Multiplate® analysis are displayed. Sample A is a control sample, with blood and phosphate buffered saline (PBS, 10 mM, pH 7.4), B_1-B_3 are samples with increasing concentrations of fibrinogen (Haemocomplettan®, CSL Behring GmbH, Marburg, Germany), C_1-C_3 are samples with increasing amounts of platelet concentrate, and D_1-D_3 are samples with increasing concentrations of fibrinogen and platelets combined. The added amounts of fibrinogen and platelets were chosen so that the total concentrations in the samples would reflect normal concentrations *in vivo* before and after transfusions. For the cardiac surgery patients, samples A were analyzed with ROTEM® and Multiplate® before surgery, for comparison of the pre- and postoperative blood clot formation and platelet aggregation (Appendix B).

Table 3.2 Sample contents for ROTEM® and Multiplate® analysis

	ROTEM® sample content (µl)				Multiplate® sample content (µl)			
Sample ID	Fibrinogen*	Platelets**	PBS	Whole blood	Fibrinogen*	Platelets**	PBS	Whole blood
A	-	-	227	760	-	-	300	1000
B_1	38	-	189	760	50	-	250	1000
B_2	76	-	151	760	100	-	200	1000
B_3	113	-	114	760	150	-	150	1000
C_1	-	38	189	760	-	50	250	1000
C_2	-	76	151	760	-	100	200	1000
C_3	-	114	113	760	-	150	150	1000
D_1	38	38	151	760	50	50	200	1000
D_2	76	76	75	760	100	100	100	1000
D_3	113	114	-	760	150	150	-	1000

* A solution made of Haemocomplettan® and PBS with the fibrinogen concentration 13 $g*l^{-1}$

**Platelet concentrate with the platelet concentration 1 $200*10^9$ l^{-1}. If the true platelet concentration was higher, PBS was added to reach the wanted concentration.

The total fibrinogen concentrations of the samples with varying endogenous fibrinogen concentrations are visualized in **Table 3.3**. The normal range of endogenous fibrinogen concentration is 2-4 g/l [37]. The concentrations are calculated for the samples for ROTEM® analysis, but the numbers are approximately the same for those intended for Multiplate® analysis.

Table 3.3 Calculated fibrinogen concentration after supplementation. All concentrations are in $g*L^{-1}$.

Endogenous fibrinogen concentration	Sample A	Sample B_1 and D_1	Sample B_2 and D_2	Sample B_3 and D_3
2.00	1.54	2.04	2.54	3.04
2.50	1.92	2.42	2.92	3.42
3.00	2.31	2.81	3.31	3.81
3.50	2.69	3.19	3.69	4.19
4.00	3.08	3.58	4.08	4.58

In **Table 3.4**, the total platelet concentrations of the samples with varying endogenous platelet concentrations are given. $150\text{-}400*10^9$ platelets per liter is the normal endogenous range [38]. Again, the concentrations are calculated for the samples intended for ROTEM® analysis, and are approximately the same as those for Multiplate® analysis.

Table 3.4 Calculated platelet concentration after supplementation. All concentrations are in 10^9*L^{-1}.

Endogenous platelet concentration	Sample A	Sample C_1 and D_1	Sample C_2 and D_2	Sample C_3 and D_3
100	76.9	123	169	215
150	115	162	208	254
200	154	200	246	292
250	192	238	285	331
300	231	277	323	369
350	269	315	362	408
400	308	354	400	446

3.5 Sample Analysis

All ten samples that were intended for ROTEM® analysis were analyzed with both Fibtem and Extem reagent, and the time of measurement was set to 30 minutes. The ten Multiplate® samples were all analyzed with the reagents ADPtest HS, ASPItest and TRAPtest. For both instruments, the samples were always analyzed in the same order, from A-D_3, four at the time and five at the time for ROTEM® and Multiplate®, respectively. If a test had to be repeated, it was placed first in line for the next round of analysis.

3.6 Data Analysis

The data were processed with the software programs Microsoft Excel 2010 and SPSS Statistics 19, to evolve mean values, standard deviations and to make correlation analyses. Correlation analyses were made using Spearman's correlation coefficient.

Statistical analyses were made to examine whether a dose trend existed for any treatment (fibrinogen and/or platelet concentrate). It was also made to examine if there were differences in dose trends between study populations within treatments or between treatments within study groups. The data were fitted to a linear model according to the principle "absolute value of measurement minus baseline". Each comparison was made pairwise and it was taken into account that there were repeated measurements (three doses for each individual). These statistical analyses were carried out using PROC MIXED, SAS® v9.2 (SAS Institute, Cary, NC).

For all statistical analyses, a P value less than 5% was considered statically significant.

4. Results

The results are divided into how they are presented; in absolute values (**Table 4.2**) and in percental change (**Table 4.3**) when fibrinogen and/or platelets were added. For all parameters tested in ROTEM® and Multiplate®, baselines, and three increasing doses of fibrinogen and/or platelets, were evolved. For simplification, only baseline and the highest of the three doses of fibrinogen and/or platelets are presented in the text. The low and median doses are presented in the tables though.

4.1 Results in Absolute Values

The results for the parameters Extem Clotting time and Maximum clot firmness, Fibtem Clotting time and Maximum clot firmness, ADP high sensitivity Area under the curve, ASPI Area under the curve, and TRAP Area under the curve are listed in absolute values in **Table 4.2**, with mean ± standard deviation. Below, the results for the baseline and the highest of the three doses will be presented separately, according to the parameter measured. But first a comparison of the study group characteristics and the baseline values will be presented.

4.1.1 Study Group Characteristics and Baseline Values

For comparison of the groups healthy volunteers, cardiac surgery patients and patients treated with platelet inhibitors, their ages, blood characteristics and baseline values evolved from ROTEM® and Multiplate® analysis are listed in **Table 4.1**. The mean age was approximately 20 years higher in the patient groups, compared to the healthy volunteers, and the BMI was

Table 4.1 Study group characteristics and baseline values (mean ± standard deviation) for the ROTEM® and Multiplate® analysis.

Variable	Healthy volunteers	Cardiac surgery patients	Patients on platelet inhibitors
Age (years)	42 ± 8.8	66 ± 9.0	63 ± 9.2
BMI ($kg*m^{-2}$)	24 ± 3.2	30 ± 4.0	26 ± 4.1
Hb ($g*L^{-1}$)	150 ± 9.4	110 ± 9.9	130 ± 15
HCT ($L*L^{-1}$)	0.44 ± 0.029	0.31 ± 0.028	0.39 ± 0.049
PLT (10^9*L^{-1})	230 ± 50	210 ± 49	240 ± 59
WBC (10^9*L^{-1})	6.0 ± 0.92	16 ± 3.5	7.8 ± 2.0
Extem CT (s)	71± 8.7	88 ± 9.7	57 ± 4.2
Extem MCF (mm)	55 ± 3.8	58 ± 6.2	63 ± 5.5
Fibtem CT (s)	70 ± 7.4	87 ± 10	54 ± 5.0
Fibtem MCF (mm)	9.8 ± 2.5	11 ± 2.5	18 ± 6.0
ADP HS AUC (AU*min)	47 ± 8.7	34 ± 26	12 ± 8.1
ASPI AUC (AU*min)	53 ± 20	16 ± 11	6.6 ± 5.4
TRAP AUC (AU*min)	82 ± 11	72 ± 29	62 ± 24

largest in the operated patients. Hemoglobin (Hb) levels and hematocrit (HCT) were highest in the healthy volunteers and lowest in the surgical patients. The platelet count (PLT) was highest among platelet inhibited patients and lowest in cardiac surgery patients, and white blood cell count (WBC) was highest in the operated patients and lowest among healthy subjects. In terms of TEM, the clot formation was the fastest and firmest in blood from platelet inhibited patients, while they had the lowest AUC in all three Multiplate® tests. In cardiac patients, induction of clotting took the longest, but the clot was firmer than in the healthy vol-

unteers. Platelet aggregation was a bit suppressed in the operated patients compared to the healthy, especially the COX dependent platelet aggregation stimulated by arachidonic acid.

4.1.2 Extem Clotting Time

For the healthy volunteers, the baseline value for the Extem CT parameter was 71 ± 8.7 (mean ± standard deviation) seconds. When the highest dose of fibrinogen, platelets and both fibrinogen and platelets was added, Extem CT decreased to 59 ± 8.5, 57 ± 7.2 and 52 ± 4.4 seconds, respectively (**Figure 4.1**). The clot formation was thus initiated fastest when fibrinogen and platelets were combined, and platelets decreased the clotting time slightly more than fibrinogen did. In the blood from cardiac surgery patients, Extem CT started at a baseline of 88 ± 9.7 seconds, which was a longer clotting time compared to the healthy volunteers. Fibrinogen, platelets and both combined decreased the CT to 75 ± 9.0, 70 ± 6.6 and 66 ± 6.0 seconds, respectively (**Figure 4.1**). The baseline Extem CT for the patients on platelet inhibitors was 57 ± 4.2 seconds, and was therefore the group which had the fastest clot initiation time. After adding of the blood products, the clotting time decreased to 51 ± 6.4, 51 ± 5.6 and 44 ± 3.9 seconds (fibrinogen, platelets and both) (**Figure 4.1**) . The three groups showed similar tendencies, where the CT decreased most when platelets and fibrinogen were combined, and the fibrinogen decreased it the least, or just as much as the platelets, as in the blood from patients treated with platelet inhibitors.

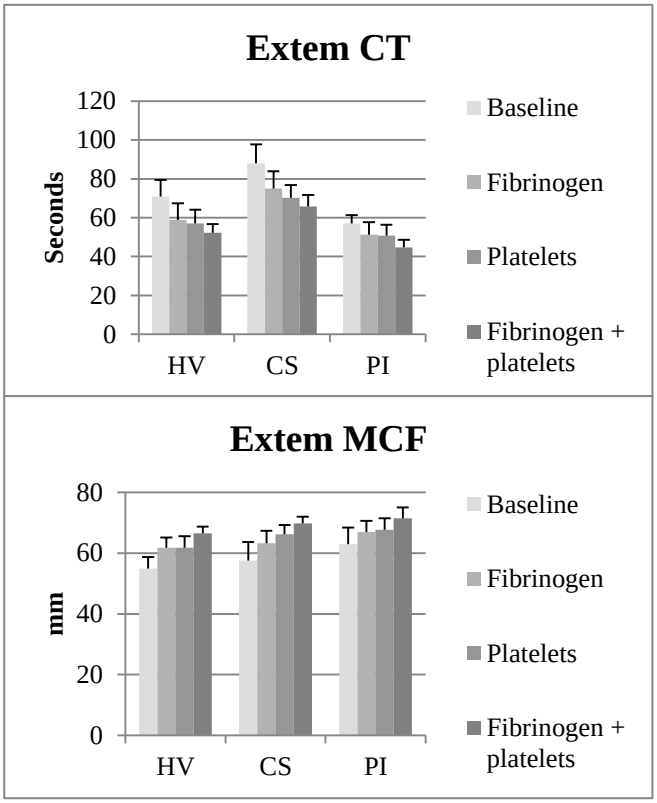

Figure 4.1 Results from Extem CT and MFC. From the left; healthy volunteers (HV), cardiac surgery patients (CS) and patients treated with platelet inhibitors (PI). Here, only the highest dose of each treatment (fibrinogen and/or platelets) is presented.

4.1.3 Extem Maximum Clot Firmness

The Extem MCF baselines for the healthy volunteers, cardiac surgery patients and patients treated with platelet inhibitors were 55 ± 3.8, 58 ± 6.2 and 63 ± 5.5 mm, respectively (**Figure 4.1**). Consequently, the highest MCF was found in the group of patients on platelet inhibitors, and the lowest in the healthy volunteers. When the highest doses of fibrinogen, platelets and both fibrinogen and platelets were added to the blood from healthy volunteers, MCF increased to 62 ± 3.3, 62 ± 3.7 and 67 ± 2.2 mm, respectively. For the cardiac surgery patients, the numbers were 63 ± 4.2, 66 ± 3.1 and 70 ± 2.2 mm, and for the patients on platelet inhibitors, 67 ± 3.7, 68 ± 3.8 and 72 ± 3.5 mm (**Figure 4.1**). The mean MCF was increased in all cases, but the largest effect was caused by the combination of fibrinogen and platelets.

Table 4.2 Results in absolute values (mean ±standard deviation) for healthy volunteers, cardiac surgery patients and patients on platelet inhibitors. The units for the ROTEM® parameters Extem and Fibtem CT are in seconds, and Extem and Fibtem MCF are in mm. The Multiplate® variables ADP HS, ASPI and TRAP AUC are in AU*min.

Healthy Volunteers

Variable	Baseline	Fibrinogen			Platelets			Fibrinogen and platelets		
		Low dose	Median dose	High dose	Low dose	Median dose	High dose	Low dose	Median dose	High dose
Extem CT	71 ± 8.7	65 ± 7.7	61 ± 8.5	59 ± 8.5	63 ± 5.9	60 ± 6.8	57 ± 7.2	58 ± 7.6	54 ± 6.9	52 ± 4.4
Extem MCF	55 ± 3.8	57 ± 3.5	59 ± 2.8	62 ± 3.3	56 ± 3.8	58 ± 3.4	62 ± 3.7	59 ± 2.2	65 ± 2.7	67 ± 2.2
Fibtem CT	70 ± 7.4	61 ± 7.9	55 ± 8.9	52 ± 9.0	61 ± 8.0	57 ± 11	58 ± 6.0	54 ± 8.1	51 ± 6.9	48 ± 5.6
Fibtem MCF	9.8 ± 2.5	13 ± 2.4	18 ± 3.1	21 ± 3.7	10 ± 2.7	11 ± 3.2	12 ± 3.1	13 ± 2.3	19 ± 2.4	22 ± 2.8
ADPHS AUC	47 ± 8.7	42 ± 8.7	38 ± 7.3	36 ± 8.5	43 ± 12	45 ± 11	45 ± 14	42 ± 10	37 ± 7.4	40 ± 6.3
ASPI AUC	53 ± 20	49 ± 18	46 ± 17.5	42 ± 16	64 ± 19	70 ± 19	74 ± 22	63 ± 14	62 ± 18	64 ± 20
TRAP AUC	82 ± 11	72 ± 12	72 ± 8.9	68 ± 13	86 ±10	90 ± 12	91 ± 9.9	84 ± 7.9	83 ± 7.4	80 ± 11

Cardiac Surgery Patients

Variable	Baseline	Fibrinogen			Platelets			Fibrinogen and platelets		
		Low dose	Median dose	High dose	Low dose	Median dose	High dose	Low dose	Median dose	High dose
Extem CT	88 ± 9.7	81 ± 7.8	78 ± 8.1	75 ± 9.0	74 ± 7.8	72 ± 7.3	70 ± 6.6	75 ± 7.9	70 ± 6.5	66 ± 6.0
Extem MCF	58 ± 6.2	59 ± 5.2	61 ± 4.6	63 ± 4.2	61 ± 4.6	63 ± 3.8	66 ± 3.1	62 ± 4.4	67 ± 2.8	70 ± 2.2
Fibtem CT	87 ± 10	74 ± 10	73 ± 7.7	67 ± 7.4	73 ± 8.0	67 ± 5.5	71 ± 6.0	70 ± 8.1	66 ± 7.0	61 ± 4.8
Fibtem MCF	11 ± 2.5	15 ± 2.8	20 ± 2.3	24 ± 2.6	13 ± 2.3	13 ± 2.6	14 ± 2.5	16 ± 2.0	22 ± 2.1	24 ± 3.6
ADPHS AUC	34 ± 26	31 ± 27	31 ± 23	28 ± 23	39 ± 26	51 ± 24	50 ± 25	35 ± 22	36 ± 24	42 ± 23
ASPI AUC	16 ± 11	16 ± 13	13 ± 6.3	14 ± 6.4	31 ± 22	58 ± 31	79 ± 29	27 ± 23	44 ± 27	67 ± 24
TRAP AUC	72 ± 29	66 ± 23	64 ± 32	57 ± 29	94 ± 25	120 ± 32	120 ± 17	82 ± 22	91 ± 18	100 ± 18

Patients on Platelet Inhibitors

Variable	Baseline	Fibrinogen			Platelets			Fibrinogen and platelets		
		Low dose	Median dose	High dose	Low dose	Median dose	High dose	Low dose	Median dose	High dose
Extem CT	57 ± 4.2	53 ± 4.8	53 ± 6.4	51 ± 6.4	52 ± 4.8	51 ± 3.1	51 ± 5.6	52 ± 6.7	47 ± 5.5	44 ± 3.9
Extem MCF	63 ± 5.5	64 ± 4.5	66 ± 4.0	67 ± 3.7	64 ± 5.2	66 ± 5.4	68 ± 3.8	65 ± 3.8	70 ± 3.9	72 ± 3.5
Fibtem CT	54 ± 5.0	49 ± 5.1	50 ± 4.8	46 ± 5.3	48 ± 3.9	47 ± 3.1	49 ± 5.0	47 ± 4.8	46 ± 4.4	42 ± 4.6
Fibtem MCF	18 ± 6.0	21 ± 5.1	26 ± 5.0	30 ± 4.5	19 ± 6.3	19 ± 6.1	19 ± 5.1	21 ± 4.5	27 ± 5.3	30 ± 5.1
ADPHS AUC	12 ± 8.1	12 ± 7.3	14 ± 10	12 ± 9.3	14 ± 5.9	14 ± 5.7	20 ± 9.0	12 ± 7.2	14 ± 8.2	18 ± 10
ASPI AUC	6.6 ± 5.4	6.9 ± 4.4	7.6 ± 5.4	6.4 ± 4.5	40 ± 20	58 ± 19	76 ± 19	37 ± 20	53 ± 19	65 ± 21
TRAP AUC	62 ± 24	55 ± 24	54 ± 26	49 ± 23	74 ± 23	83 ± 18	90 ± 15	64 ± 26	74 ± 22	76 ± 19

4.1.4 Fibtem Clotting Time

The baseline and the highest doses of fibrinogen, platelets and both fibrinogen and platelets were for the Fibtem CT of the healthy volunteers 70 ± 7.4, 52 ± 9.0, 58 ± 6.0 and 48 ± 5.6 seconds, respectively (**Figure 4.2**). For the cardiac surgery patients, those numbers were 87 ± 10, 67 ± 7.4, 71 ± 6.0 and 61 ± 4.8 seconds, while the patients on platelet inhibitors had the values 54 ± 5.0, 46 ± 5.3, 49 ± 5.0 and 42 ± 4.6 seconds (**Figure 4.2**). As with the variable Extem CT, the patients treated with platelet inhibitors had the shortest clotting time, and the cardiac surgery patients had the longest, regarding the baseline values. In all groups, all three types of additives decreased the CT, the combination giving the largest effect, and platelets the smallest.

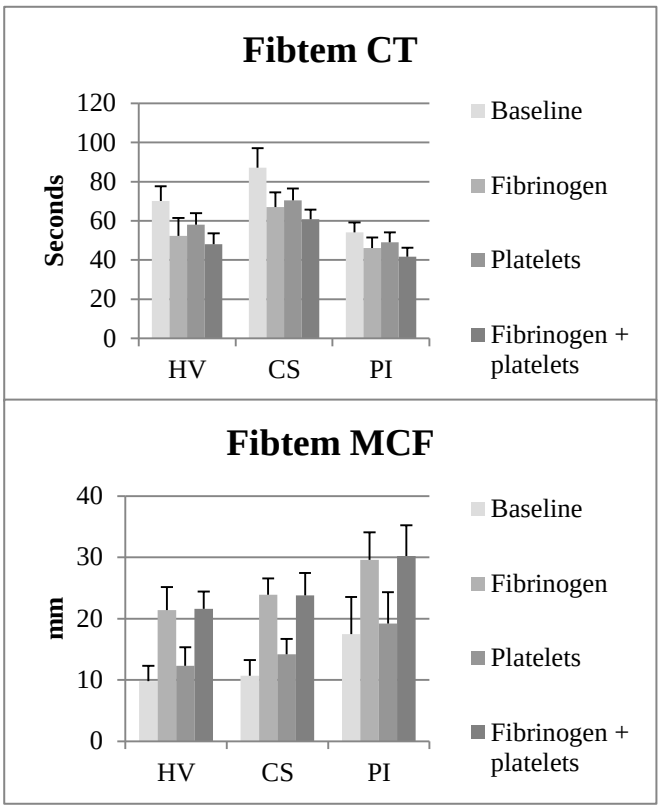

Figure 4.2 Results from Fibtem CT and MFC. From the left; healthy volunteers (HV), cardiac surgery patients (CS) and patients treated with platelet inhibitors (PI). Only the highest dose of each treatment (fibrinogen and/or platelets) is presented here.

4.1.5 Fibtem Maximum Clot Firmness

For the healthy volunteers, cardiac surgery patients and patients treated with platelet inhibitors, the respective baselines for Fibtem MCF were 9.8 ± 2.5, 11 ± 2.5 and 18 ± 6.0 mm (**Figure 4.2**). As with all the above described parameters, clot formation was improved also in terms of Fibtem MCF for the patient group with platelet inhibitors, compared to the other study groups. When fibrinogen, platelets and a combination of both products were added to blood from healthy volunteer, MCF increased to 21 ± 3.7, 12 ± 3.1 and 22 ± 2.8 mm, respectively. For the cardiac surgery patients, MCF increased to 24 ± 2.6, 14 ± 2.5 and 24 ± 3.6 mm, and for patients on platelet inhibitors 30 ± 4.5, 19 ± 5.1 and 30 ± 5.1 mm (**Figure 4.2**). All additives increased the MCF, the combination and fibrinogen only to a similar amount, and platelets to a smaller amount.

4.1.6 ADP High Sensitivity Area under the Curve

In the blood from healthy volunteers, the ADP HS AUC decreased form the baseline 47 ± 8.7 AU*min to 36 ± 8.5, 45 ± 14 and 40 ± 6.3 when fibrinogen, platelets and both fibrinogen and platelets were added (**Figure 4.3**). In cardiac surgery patient, the baseline were 34 ± 26 AU*min, an increase in fibrinogen resulted in a decrease of the AUC to 28 ± 23, platelets increased it to 50 ± 25 and the combination to 42 ± 23 AU*min. Baseline and the blood with added fibrinogen, platelets and the combination for patients on platelet inhibitors gave the AUC values 12 ± 8.1, 12 ± 9.3, 20 ± 9.0 and 18 ± 10, respectively (**Figure 4.3**), so fibrinogen

did not have any effect, while platelets and the combination gave an increase, with the largest increase due to the platelets only. For this parameter, baseline values for healthy volunteers were the highest, and the lowest were represented by the patients treated with platelet inhibitors.

4.1.7 ASPI Area under the Curve

The baseline ASPI AUC values for the healthy, operated and platelet inhibited individuals were 53 ± 20, 16 ± 11 and 6.6 ± 5.4 AU*min, respectively (**Figure 4.3**), so the patients evolved much lower results than the healthy volunteers. The highest doses of fibrinogen, platelets and both combined gave 42 ± 16, 74 ± 22 and 64 ± 20 AU*min in the blood from healthy volunteers, whereas 14 ± 6.4, 79 ± 29 and 67 ± 24 AU*min evolved from the cardiac surgery patients. The same results from platelet inhibited patients were 6.4 ± 4.5, 76 ± 19 and 65 ± 21 AU*min (**Figure 4.3**). Platelets and the combination increased the AUC in all study groups, platelets only increased it most. Fibrinogen resulted in a decrease in AUC compared to the baseline among healthy and operated individuals, whereas it stayed unchanged in the patients on platelet inhibitors.

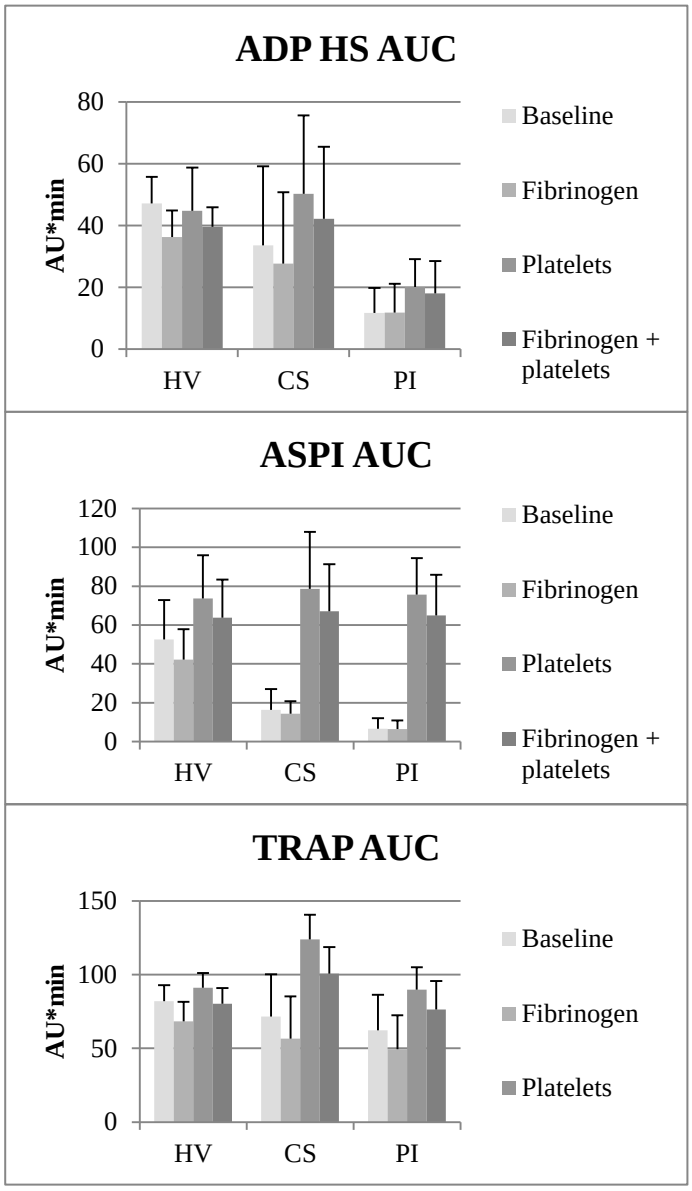

Figure 4.3 Results from AUC ADP HS, ASPI and TRAP. From the left; healthy volunteers (HV), cardiac surgery patients (CS) and patients treated with platelet inhibitors (PI). Here, only the highest dose of each treatment (fibrinogen and/or platelets) is presented. Here, only the highest dose of each treatment (fibrinogen and/or platelets) is presented.

4.1.8 TRAP Area under the Curve

The TRAP AUC increased for all study groups compared to the baseline when platelets was added, fibrinogen caused a decrease, and the combination of platelets and fibrinogen did no change in AUC in the blood from healthy volunteers, but in the two other groups there was an increase (**Figure 4.3**). The baseline value and those for fibrinogen, platelets and the combination were for the healthy individuals 82 ± 11, 68 ± 13, 91 ± 9.9 and 80 ± 11 AU*min, respectively. For the operated patients they were 72 ± 29,

57 ± 29, 120 ± 17, and 100 ± 18 AU*min, and the results evolved from the platelet inhibited were 62 ± 24, 49 ± 23, 90 ± 15 and 76 ± 19 AU*min.

4.2 Change in Percent

The results, in percentage change compared to the baseline, for the variables Extem Clotting time and Maximum clot firmness, Fibtem Clotting time and Maximum clot firmness, ADP high sensitivity Area under the curve, ASPI Area under the curve, and TRAP Area under the curve are listed in **Table 4.3**, with mean ± standard deviation. The results for the baseline and the highest of the three doses will be presented separately in the following text, according to the parameter measured.

4.2.1 Extem Clotting Time

The percentage change for the highest doses of fibrinogen, platelets and both fibrinogen and platelets, compared to the baseline, were all negative, meaning that the CT was shortened. The respective changes were for the healthy volunteers a decrease in 17 ± 7.4, 19 ± 5.9 and 26 ± 5.8 %, and for the cardiac surgery patients a decrease in 14 ± 9.1, 20 ± 8.3 and 25 ± 8.2 % (**Figure 4.4**). The according numbers for the patients on platelet inhibitors were a decrease in 10 ± 6.8, 11 ± 8.6 and 22 ± 2.3 %. The patients treated with platelet inhibitors were the only study group were the combination of the two blood product resulted in a doubled effect compared to when only one was given. The effect was larger for the combination in the other groups, but not to the extent of the patients on platelet inhibitors. In the blood from operated patients, the effect of platelets was larger than that caused by fibrinogen in the text below (**Figure 4.4**).

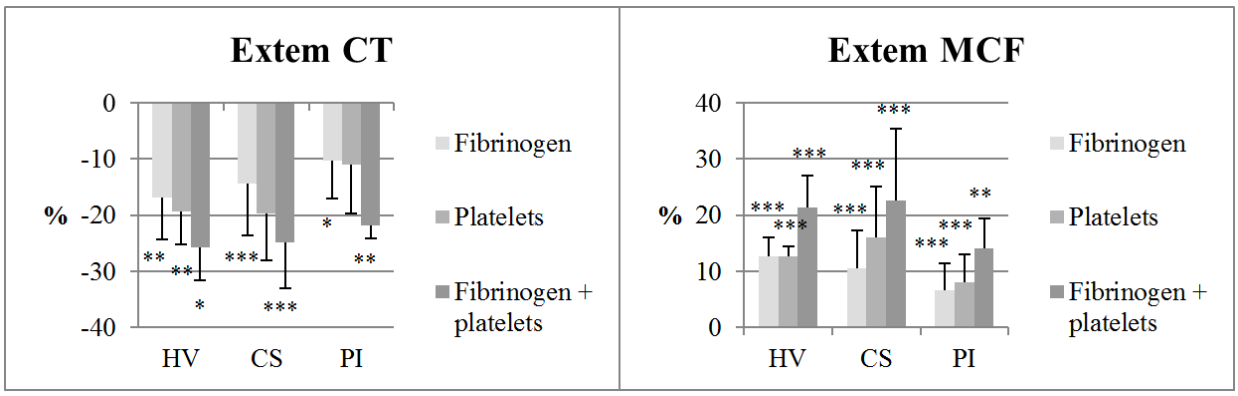

Figure 4.4 Results of percentage change compared to baseline for Extem CT and MFC. From the left; healthy volunteers (HV), cardiac surgery patients (CS) and patients treated with platelet inhibitors (PI). The stars denote for statistical significant dose trends (absolute values for each dose; low, median and high, minus baseline). P values below 0.05, 0.01 and 0.001 is presented with *, ** and ***, respectively. The P values in numbers are to be found in **Table 4.4**.

Table 4.3 Results in percentage change (mean ±standard deviation), compared to the baseline, for healthy volunteers, operated and platelet inhibited patients. The ROTEM® variables Extem and Fibtem CT are in seconds, and Extem and Fibtem MCF are in mm. The units for the Multiplate® parameters ADP HS, ASPI and TRAP AUC are in AU*min.

Healthy Volunteers

Variable	Fibrinogen			Platelets			Fibrinogen and platelets		
	Low dose	Median dose	High dose	Low dose	Median dose	High dose	Low dose	Median dose	High dose
Extem CT	-8.0 ± 5.7	-14 ± 9.4	-17 ± 7.4	-11 ± 6.1	-15 ± 7.0	-19 ± 5.9	-18 ± 4.8	-23 ± 4.6	-26 ± 5.8
Extem MCF	4.4 ± 1.9	8.4 ± 3.8	13 ± 3.4	2.8 ± 1.9	6.5 ± 2.2	13 ± 1.8	7.9 ± 3.8	18 ± 4.0	21 ± 5.6
Fibtem CT	-14 ± 5.6	-21 ± 7.2	-26 ± 7.8	-13 ± 9.5	-18 ± 12	-17 ± 8.5	-24 ± 7.0	-28 ± 3.7	-31 ± 4.3
Fibtem MCF	31 ± 11	86 ± 22	120 ± 27	6.6 ± 13	8.1 ± 20	27 ± 18	38 ± 20	96 ± 26	130 ± 30
ADP HS AUC	-9.7 ± 11	-17 ± 17	-23 ± 13	-9.0 ± 25	-3.4 ± 16	-6.3 ± 20	-10 ± 16	-21 ± 15	-15 ± 7.9
ASPI AUC	-4.6 ± 11	-9.3 ± 17	-17 ± 15	54 ± 140	70 ± 160	83 ± 190	65 ± 180	59 ± 180	46 ± 110
TRAP AUC	-12 ± 9.7	-12 ± 8.4	-17 ± 12	5.0 ± 11	9.7 ± 12	12 ± 13	3.5 ± 8.6	1.9 ± 8.6	-1.5 ± 12

Cardiac Surgery Patients

Variable	Fibrinogen			Platelets			Fibrinogen and platelets		
	Low dose	Median dose	High dose	Low dose	Median dose	High dose	Low dose	Median dose	High dose
Extem CT	-7.2 ± 6.2	-12 ± 6.3	-14 ± 9.1	-16 ± 4.8	-18 ± 6.1	-20 ± 8.3	-14 ± 9.2	-20 ± 7.9	-25 ± 8.2
Extem MCF	3.2 ± 3.4	7.1 ± 5.6	11 ± 6.7	6.3 ± 4.8	10 ± 6.8	16 ± 9.1	9.0 ± 5.4	18 ± 10	23 ± 13
Fibtem CT	-16 ± 6.7	-16 ± 7.8	-23 ± 6.7	-17 ± 5.0	-23 ± 6.2	-18 ± 10	-19 ± 11	-24 ± 11	-30 ± 8.6
Fibtem MCF	42 ± 16	98 ± 45	130 ± 54	20 ± 14	23 ± 17	36 ± 20	54 ± 33	110 ± 55	140 ± 80
ADP HS AUC	-8.0 ± 15	0.59 ± 38	-14 ± 19	28 ± 50	90 ± 97	81 ± 88	19 ± 37	19 ± 60	54 ± 77
ASPI AUC	-15 ± 31	-11 ± 39	-4.4 ± 39	95 ± 92	320 ± 230	530 ± 450	64 ± 80	210 ± 170	450 ± 410
TRAP AUC	-5.8 ± 7.9	-9.1 ± 29	-20 ± 26	39 ± 29	73 ± 47	92 ± 63	21 ± 24	36 ± 32	54 ± 48

Patients on Platelet Inhibitors

Variable	Fibrinogen			Platelets			Fibrinogen and platelets		
	Low dose	Median dose	High dose	Low dose	Median dose	High dose	Low dose	Median dose	High dose
Extem CT	-8.0 ± 3.5	-7.3 ± 6.6	-10 ± 6.8	-9.1 ± 7.3	-10 ± 6.6	-11 ± 8.6	-9.7 ± 8.2	-17 ± 5.3	-22 ± 2.3
Extem MCF	1.8 ± 2.8	4.7 ± 3.7	6.7 ± 4.7	2.2 ± 3.1	4.7 ± 2.8	8.0 ± 5.1	3.1 ± 4.3	11 ± 4.2	14 ± 5.3
Fibtem CT	-9.4 ± 4.7	-8.3 ± 6.9	-15 ± 6.4	-10 ± 4.9	-13 ± 7.4	-8.7 ± 11	-13 ± 9.1	-15 ± 9.4	-23 ± 5.8
Fibtem MCF	22 ± 15	56 ± 26	80 ± 37	10 ± 7.4	7.3 ± 7.6	14 ± 19	24 ± 19	64 ± 28	83 ± 36
ADP HS AUC	14 ± 57	3.3 ± 50	5.5 ± 64	54 ± 71	78 ± 120	120 ± 96	19 ± 42	54 ± 68	94 ± 94
ASPI AUC	82 ± 180	47 ± 100	15 ± 83	1100 ± 1500	1800 ± 2000	2400 ± 2500	1000 ± 1400	1800 ± 2100	2100 ± 2300
TRAP AUC	-15 ± 16	-18 ± 22	-23 ± 15	27 ± 26	66 ± 110	89 ± 150	3.4 ± 16	39 ± 51	56 ± 120

4.2.2 Extem Maximum Clot Firmness

For the healthy individuals, the percentage change of the Extem MCF parameter increased with 13 ± 3.4, 13 ± 1.8 and 21 ± 5.6 %, when fibrinogen, platelets and both product were given (**Figure 4.4**). The changes for the operated patients were an increase of 11 ± 6.7, 16 ± 9.1 and 23 ± 13 %, and for the platelet inhibited, 6.7 ± 4.7, 8.0 ± 5.1 and 14 ± 5.3 %. An almost doubled effect was reached for the combined additive compared to when only one was given, only in blood from patients on platelet inhibitors. As with Extem CT, the effect of platelets was larger than that of fibrinogen in the cardiac surgery patients (**Figure 4.4**).

4.2.3 Fibtem Clotting Time

In all three study groups, CT was decreased when fibrinogen, platelets and both blood product were added. The decrease were for the healthy volunteer 26 ± 7.8, 17 ± 8.5 and 31 ± 4.3 %, for the operated patients 23 ± 6.7, 18 ± 10 and 30 ± 8.6 %, while for the patients on platelet inhibitors it were 15 ± 6.4, 8.7 ± 11 and 23 ± 5.8 % (**Figure 4.5**). Once again, the combination gave a doubled effect in the platelet inhibited patients, and the effect from fibrinogen was larger than that from platelets in all groups.

4.2.4 Fibtem Maximum Clot Firmness

Fibrinogen, platelets and a combination of both added to blood from healthy volunteers gave an increase, compared to baseline, in Fibtem MCF of 120 ± 27, 27 ± 18 and 130 ± 30 %, respectively (**Figure 4.5**). The gain for the patients groups were 130 ± 54, 36 ± 20 and 140 ± 80 % for the operated, and 80 ± 37, 14 ± 19 and 83 ± 36 % for the platelet inhibited. The effect caused by fibrinogen was much larger than that by platelets in every study group, and the combination was not an addition of the individual effects from fibrinogen and platelets in any group (**Figure 4.5**).

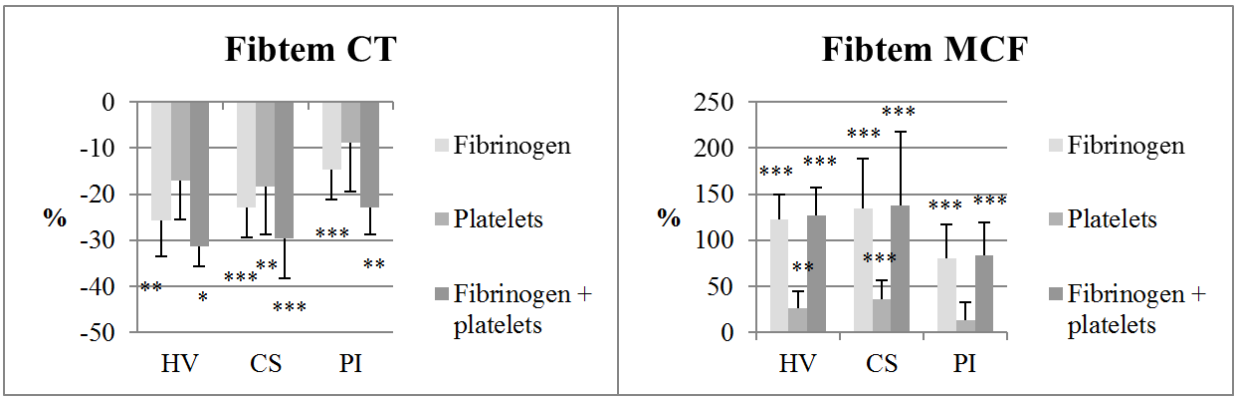

Figure 4.5 Results of percentage change compared to baseline for Fibtem CT and MFC. From the left; healthy volunteers (HV), cardiac surgery patients (CS) and patients treated with platelet inhibitors (PI). The stars denote for statistical significant dose trends (absolute values for each dose minus baseline). P values below 0.05, 0.01 and 0.001 is presented with *, ** and ***, respectively. The P values in numbers can to be viewed in **Table 4.4**.

4.2.5 ADP High Sensitivity Area under the Curve

When fibrinogen was added to the blood from healthy and operated individuals, AUC decreased with 23±13 and 14±19 %, respectively, while in blood from platelet inhibited individuals, it roughly remained unchanged with an increase of only 5.5 ± 64 % (**Figure 4.6**). Platelets decreased AUC with 6.3 ± 20 in healthy and increased it with 81 ± 88 and 120 ± 96 % in operated versus platelet inhibited individuals. The combination of both fibrinogen and platelets resulted in a decrease of 15 ± 7.9 % in healthy volunteers, and gain of 54 ± 77 and 94 ± 94 % in cardiac surgery and platelet inhibited patients (**Figure 4.6**).

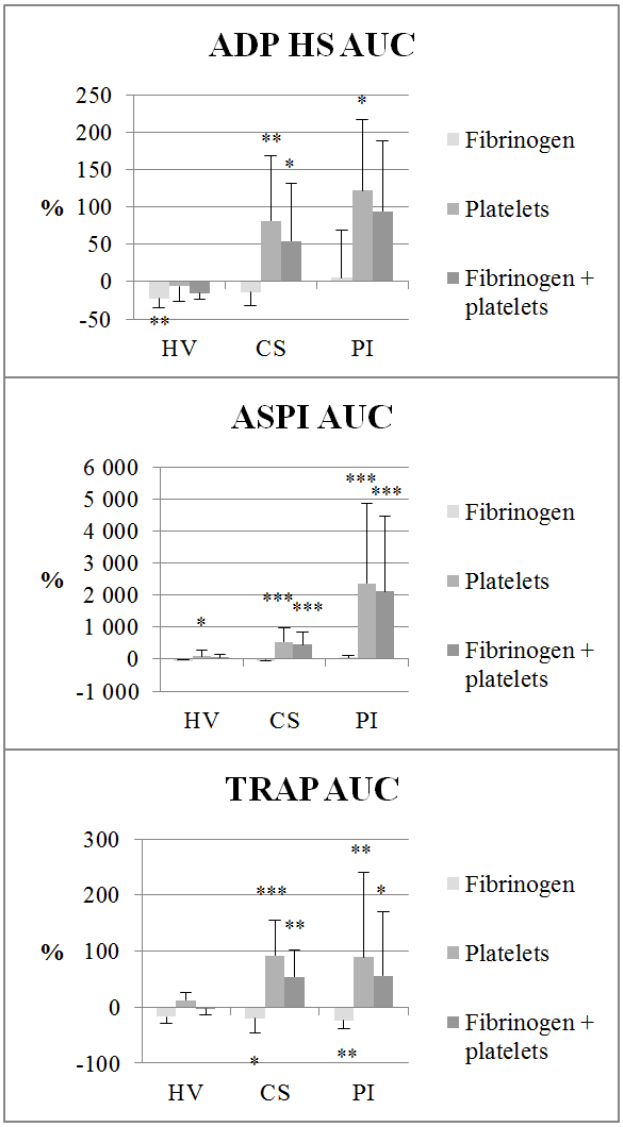

Figure 4.6 Results of percentage change compared to baseline for ADP HS, ASPI and TRAP AUC. From the left; healthy volunteers (HV), cardiac surgery patients (CS) and patients treated with platelet inhibitors (PI). The stars denote for statistical significant dose trends (absolute values for each dose minus baseline). P values below 0.05, 0.01 and 0.001 is presented with *, ** and ***, respectively. The P values in numbers are to be found in **Table 4.4**.

4.2.6 ASPI Area under the Curve

The highest doses of fibrinogen, platelets, and the combination of both resulted in a change, compared to the baseline, of -17 ± 15, 83 ± 190 and 46 ± 110 % in the healthy volunteers (**Figure 4.6**). In the cardiac surgery patients, the numbers were -4.4 ± 39, 530 ± 450 and 450 ± 410 % and in the patients on platelet inhibitors 15 ± 83, 2400 ± 2500 and 2100 ± 2300 %. So fibrinogen was either slightly decreasing or leaving the AUC unchanged. Platelets increased it, especially among the patient groups, so did the combination (**Figure 4.6**).

4.2.7 TRAP Area under the Curve

In all study groups, fibrinogen resulted in a decrease in AUC, platelets increased it, and platelets and fibrinogen combined increased the AUC in blood from the patient groups and slightly decreased it in the healthy subjects (**Figure 4.6**).. Given in numbers, the change was -17 ± 12, 12 ± 13 and -1.5 ± 12 % for the healthy individuals, -20 ± 26, 92 ± 63, and 54 ± 48 % for the cardiac surgery patients, and -23 ± 15, 89 ± 150 and 56 ± 120 % for the patients on platelet inhibitors.

4.3 Results From Statistical Analysis

Three models have been fitted to each of the measurements; Extem CT and MCF, Fibtem CT and MCF, ADP HS AUC, ASPI AUC and TRAP AUC. One of these models examined if there was any incidence of dosage trend within a study group and for each treatment (three increasing doses of fibrinogen and/or platelets) separately. A second one examined differences in dose trends between treatments within groups. The third model examined differences between dose trends between groups within treatments.

In **Table 4.4**, results from the analyses made to determine whether a treatment had a significant dose trend, can be viewed. The only treatments of the ROTEM® variables that were non-significant were Extem CT for platelets given to the cardiac surgery patients and patients on platelet inhibitors, Fibtem CT for platelets given to healthy volunteers and Fibtem CT and MCF for platelets given to patients treated with platelet inhibitors. For the Multiplate® variables, there were more nonsignificant dosage trends, the most of them found among healthy individuals. A significant P value does not tell whether the trend is positive or negative for increasing doses of the treatment, but combined with the results for percentage changes compared to baseline, that conclusion can be made. For example, when fibrinogen was added to blood from healthy individuals, ADP HS AUC decreased as the dosage increased, while the P value for this dosage trend is 0.006. Thus, that dose trend was negative for increasing doses of fibrinogen.

Table 4.4 P values for dosage trends within a study group and a treatment were calculated for the variables Extem CT and MCF, Fibtem CT and MCF and ADP HS, ASPI and TRAP AUC and for the treatments fibrinogen (F) or platelets (P) or both (F+P).

Group	Treatment	ExCT	ExMCF	FibCT	FibMCF	ADP HS AUC	ASPI AUC	TRAP AUC
Healthy Volunteers	F	0.002**	<.001***	0.001**	<.001***	0.006**	0.104	0.313
	P	0.004**	<.001***	0.577	0.007**	0.639	0.014*	0.129
	F + P	0.025*	<.001***	0.034*	<.001***	0.239	0.934	0.267
Cardiac surgery patients	F	<.001***	<.001***	<.001***	<.001***	0.227	0.137	0.021*
	P	0.158	<.001***	0.007**	<.001***	0.002**	<.001***	<.001***
	F + P	<.001***	<.001***	<.001***	<.001***	0.016*	0.001***	0.004**
Patients on platelet inhibitors	F	0.048*	<.001***	<.001***	<.001***	0.443	0.172	0.009**
	P	0.595	0.002**	0.318	0.108	0.010*	<.001***	0.007**
	F + P	0.003**	<.001***	0.002**	<.001***	0.079	<.001***	0.015*

* Significant at the level 0.05
** Significant at the level 0.01
** Significant at the level 0.001

As can be seen in **Table 4.5**, the most of the treatments had significant different dose trends within the study groups. Some differences in treatments that did not fall out as significant, however, were those in Extem CT in healthy subjects and patients treated with platelet inhibitors and ADP HS and TRAP AUC in healthy volunteers.

Table 4.5 P values for difference in dosage trends between treatments within study groups. The analyses were made for the variables Extem CT and MCF, Fibtem CT and MCF and ADP HS, ASPI and TRAP AUC and for the treatments fibrinogen (F) and/or platelets (FP/P).

Group	Treatments	ExCT	ExMCF	FibCT	FibMCF	ADP HS AUC	ASPI AUC	TRAP AUC
Healthy Volunteers	F,P	0.751	0.277	0.093	<.001***	0.140	<.001***	0.135
	F,FP	0.976	<.001***	0.570	0.689	0.192	0.327	0.781
	P, FP	0.797	<.001***	0.015*	<.001***	0.057	0.020*	0.088
Cardiac surgery patients	F,P	0.384	0.0030**	0.001**	<.001***	0.006**	<.001***	<.001***
	F,FP	0.384	<.001***	0.278	0.210	0.001**	<.001***	<.001***
	P, FP	0.025*	0.0013**	0.024*	<.001***	0.089	0.020*	0.155
Patients on platelet inhibitors	F,P	0.494	0.8085	<.001***	<.001***	0.018*	<.001***	<.001***
	F,FP	0.082	0.006**	0.320	0.106	0.024*	<.001***	0.0020**
	P, FP	0.053	0.003**	0.020*	<.001***	0.737	0.005**	0.262

* Significant at the level 0.05
** Significant at the level 0.01
** Significant at the level 0.001

Whether the dosage trends differ between study populations within treatment type, can be seen in **Table 4.6**. For the ROTEM® parameters, the dose trend of fibrinogen varied the most between the study groups. For Multiplate® parameters, though, the dosage trend differed more when platelets or the combination of platelets and fibrinogen were given.

Table 4.6 P values for difference in dosage trends between study groups; healthy volunteers (HV), cardiac surgery patients (CS) and patients on platelet inhibitors (PI), within treatment were calculated for the variables Extem CT and MCF, Fibtem CT and MCF and ADP HS, ASPI and TRAP AUC and for the treatments fibrinogen and/or platelets.

Group	Treatments	ExCT	ExMCF	FibCT	FibMCF	ADP HS AUC	ASPI AUC	TRAP AUC
Fibrinogen	HV, CS	0.949	0.343	0.004**	0.908	0.501	0.124	0.317
	HV, PI	0.042*	0.016**	0.013*	0.907	0.011*	0.113	0.700
	CS, PI	0.006**	0.176	<0.001***	1.000	0.367	0.024*	0.583
Platelets	HV, CS	0.620	0.935	0.515	0.717	0.066	<.001***	<.001***
	HV, PI	0.036*	0.195	0.335	0.357	0.214	<.001***	0.086
	CS, PI	0.465	0.263	0.074	0.155	0.020*	0.194	0.053
Fibrinogen and platelets	HV, CS	0.142	0.125	0.246	0.863	0.011*	<.001***	<.001***
	HV, PI	0.902	0.454	0.450	0.523	0.027*	<.001***	0.001**
	CS, PI	0.253	0.453	0.037*	0.450	0.699	0.177	0.325

* Significant at the level 0.05
** Significant at the level 0.01
** Significant at the level 0.001

4.4 Summary of the Results

For all study groups, Extem and Fibtem MCF were increased and Extem and Fibtem CT were decreased, when the fibrinogen level in the blood samples were increased. Though, elevated fibrinogen levels either decreased or did not have any effect on the platelet aggregation. The MCF and CT values were increased and decreased, respectively, when the platelet level was elevated, in blood from all study groups. But the dosage trends of increasing platelet levels were not significant in all ROTEM® parameters. In healthy volunteers, platelets either improved the platelet aggregation, or did not have any significant effect. In the patient groups, platelets increased the platelet aggregation significantly and to a large extent, especially in the patients treated with platelet inhibitors. In healthy volunteers and in cardiac surgery patients, the combination of platelets and fibrinogen significantly increased the effect on clotting time and maximum clot firmness (both Extem and Fibtem), but the effect was not as large as the sum of the individual effects of the blood products. However in the blood from patients on platelet inhibitors, the combination gave a sum of the individual effects. In all study groups, the combination roughly gave the sum of the individual effects on the platelet aggregometry analyses.

5. Discussion

Cardiac surgery patients treated with ASA and platelet inhibited patients on ASA and clopidogrel, had lower baseline AUC values of aggregation induced by ASPI or both ADP HS and ASPI reagents, respectively. This was expected, as these drugs inhibit such platelet aggregation inductions. Even though these patients had lower baseline values, the effect of added fibrinogen to the blood sample was better than that in the blood from healthy volunteers, according to the percentage change. The effect from fibrinogen seemed to be better when the platelet aggregation was suppressed. But the most potentiated effect on the platelet aggregation was observed when platelets were added. The more inhibited platelet aggregation, the larger the percentage change seemed to be, when platelets or the combination was given. When looking at the absolute values, the increased ADP induced AUC among platelet inhibited patients did not however reach close to the baseline of the healthy volunteers. ASPI induced aggregation on the other hand, went higher than the reference baseline in both patient groups when platelets or platelets and fibrinogen was added.

ASA and clopidogrel effectively decreased ADP and arachidonic acid induced platelet aggregation, but the clot formation was faster in patients taking these drugs, compared to both healthy volunteers and operated patients. The question is whether these patients already have a faster and firmer clot formation than healthy subjects, or if it is a response to compensate for the platelet inhibition effect.

Li *et al.* demonstrated that fibrinogen and/or platelet concentrates increased the platelet aggregation in platelet inhibited blood, caused by tirofiban or eptifibatide. So their result from infusion of fibrinogen deviated from the platelet aggregometry results obtained in this thesis. One reason for the deviation could be that their methods distinguished from those used in this work. They used *in vitro* platelet inhibited blood from healthy volunteers and not blood from patients actually treated with the drug. Additionally, they used drugs which inhibit GPIIb/IIIa receptor induced platelet aggregation, while drugs used in this work prevent COX dependent and ADP receptor induced aggregation. They were also vague with some details in the methods, making it difficult to figure out how much fibrinogen or platelets that were actually added to the blood samples. Thereby making a fair comparison of their results and those obtained here is impossible.

The effect of the platelets and fibrinogen combined on the platelet aggregation were lower than that of the platelets alone. This tendency was detected when the protocol was tested for the first times. To try out if this was because the combined samples were the last analyze the order of the protocol was changed. Yet the tendency was the same as before, therefore it was presumed that this outcome was not an issue caused by the time aspect.

When fibrinogen and/or platelets were added to the blood samples, the effects on the clot formation were similar in all study groups, with decreasing CT and increasing MCF as a result. Though, the percentage change of the ROTEM® parameters, when the blood products were added to the blood samples, was least for the patients treated with platelet inhibitors. One reason for this could be that these patients had shorter CT and larger MCF at baseline. There may be a limit for how much CT and MCF can be improved, and if the clot formation

already is fast and firm, the effect of added platelets and/or fibrinogen could be small. Yet, the combination of the blood products enhanced the effect to a much wider extent in platelet inhibited patients, compared to when fibrinogen or platelets only where given to the samples. In blood from these patients, the combination gave approximately the sum of the single product, which was not the case with the other study groups.

Even if fibrinogen did not increase the platelet aggregation significantly in any of the study groups, the total effect on the hemostasis should be an improvement, because of the improvement on the clot formation.

Stored blood products have a reduced functionality, compared to the blood components inside the vasculature.[14] The percentage change was lower in both platelet aggregation and clot formation when the blood already contained healthy blood components. One potential reason for this, except from a possible saturation of the hemostasis, could be due to that the added blood product may be of poorer quality than the endogenous. But in patients with inhibited or damaged blood products caused by drugs and cardiac surgery, the added product could be of better quality than the endogenous.

Although the effect of platelets is supposed to be eliminated by cytochalasin D in Fibtem, platelets here showed to increase MCF and decrease CT in all study groups, but the effect of fibrinogen was as presumed larger. The effect of the platelets could be due to remaining fibrinogen content in the platelet concentrate, or due to some surviving platelets despite the present toxin.

To examine whether the clot formation and platelet aggregation results was correlated to the individuals characteristics, for example age, BMI, platelet count, for how long a patient has taken a platelet inhibiting drug or smoking habits, Spearman's correlation coefficients were calculated. These calculations were made within each study group, but showed only a few weak significant correlations. Therefore it is presumed that the individuals within each group can be regarded as uniform enough, and that the results from the different groups are comparable.

6. Conclusions

The effect of infused fibrinogen and/or platelets on the platelet aggregation varied between the study groups. The more inhibited platelet aggregation at baseline, the better the response of added blood products seemed to be. However, the clot formation response to fibrinogen and platelet transfusion was comparable in all three study populations. But also in terms of clot formation, the effect of infused blood product seemed to be smaller when the clot formation already was fast and firm at baseline. These findings may have an impact on future transfusion protocols.

7. Future Work

As mentioned in the introduction, there are blood products more than platelets and fibrinogen that may affect the clot formation and platelet aggregation. One such is coagulation factor XIII, which in its active form act by crosslinking fibrin monomers into a stable mesh. Another one is factor VII, which together with TF initiates the extrinsic pathway of clot formation [9]. Examination of how these coagulation factors affect clot formation and platelet aggregation could therefore provide knowledge which may affect future transfusion protocols.

In the discussion it was mentioned that the patients treated with platelet inhibitors had faster and firmer clot formation than the reference group, which could be either a response effect to compensate for the platelet inhibition, or that these patients were in this state even before medication. A way of studying this would be to examine the blood from patients who will start a treatment with platelet inhibitors, with the TEM method, before the first dosage and when the drug has an effect. Then a potential increase in MCF and decrease in CT would be detected.

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References

1. Coakley M, et al., *Assessment of thrombin generation measured before and after cardiopulmonary bypass surgery and its association with postoperative bleeding.* J Thromb Haemost, 2011. **9**(2): p. 282-292.
2. Moulton MJ, et al., *Reexploration for bleeding is a risk factor for adverse outcomes after cardiac operations.* J Thorac Cardiovasc Surg, 1996. **111**(5): p. 1037-1046.
3. Karthik S, et al., *Reexploration for bleeding after coronary artery bypass surgery: risk factors, outcomes, and the effect of time delay.* Ann Thorac Surg, 2004. **78**(2): p. 527-534.
4. Luddington RJ, *Thrombelastography/thromboelastometry.* Clin Lab Haem, 2005. **27**(2): p. 81-90.
5. Romlin BS, et al., *Intraoperative Thromboelastometry Is Associated with Reduced Transfusion Prevalence in Pediatric Cardiac Surgery.* Anesthesia and Analgesia, 2011. **112**(1): p. 30-36.
6. Solomon C, et al., *Platelet concentrates transfusion in cardiac surgery in relation to preoperative point-of-care assessment of platelet adhesion and aggregation.* Platelets, 2010. **21**(3): p. 221-228.
7. Velik-Salchner C, et al., *The effect of fibrinogen concentrate on thrombocytopenia.* J Thromb Haemost, 2007. **5**: p. 1019-1025.
8. Reece M, et al., *Near-patient platelet function testing in patients undergoing coronary artery surgery: a pilot study.* Anaesthesia, 2011. **66**(2): p. 97-103.
9. Temenoff JS and Mikos AG, *Biomaterials: The Intersection of Biology and Materials Science*. 2008, Upper Saddle River, New Jersey: Prentice Hall.
10. Karlsson M, et al., *Plasma fibrinogen level, bleeding, and transfusion after on-pump coronary artery bypass grafting surgery: a prospective observational study.* TRANSFUSION, 2008. **48**(10): p. 2152-2158.
11. Fries D, et al., *The Effect of Fibrinogen Substitution on Reversal of Dilutional Coagulopathy: An In Vitro Model.* Anesth Analg, 2006. **102**: p. 347-351.
12. Li YF, Spencer FA, and B. RC, *Comparative efficacy of fibrinogen and platelet supplementation on the in vitro reversibility of competitive glycoprotein IIb/IIIa (αIIb/β3) receptor-directed platelet inhibition.* American Heart Journal, 2001. **142**(2): p. 204-210.
13. (SSTH), S.S.f.T.o.H., *Hemostas vid allvarlig blödning*. 2010, SSTH, Koagulationscentrum, Sahlgrenska University Hospital, Karolinska University Hospital.: Malmö. p. 6-14.
14. Spiess BD, *Transfusion of Blood Products Affects Outcome in Cardiac Surgery.* Semin Cardiothorac Vasc Anesth, 2004. **8**(4): p. 267-281.
15. Tóth O, et al., *Multiple electrode aggregometry: A new device to measure platelet aggregation in whole blood.* Thromb Haemost, 2006. **96**(6): p. 781-788.
16. Alberts B, et al., *MOLECULAR BIOLOGY OF THE CELL*. Fourth edition ed. 2002, New York: Garland Science. 1463.
17. Scheraga HA, *The thrombin-fibrinogen interaction.* Biopysical Cemistry, 2004. **112**: p. 117-130.
18. Dirkmann D, et al., *Hypothermia and Acidosis Synergistically Impair Coagulation in Human Whole Blood.* Anesthesia and Analgesia, 2008. **106**(6): p. 1627-1632.
19. Depta JP and Bhatt DL, *Aspirin and Platelet Adenosine Diphosphate Receptor Antagonists in Acute Coronary Syndromes and Percutaneous Coronary Intervention: Role in Therapy and Strategies to Overcome Resistance.* Am J Cardiovasc Drugs, 2008. **8**(2): p. 91-112.

20. Caplain H, *Enhanced Antiplatelet Effects of Clopidogrel plus Acetylsalicylic Acid Compared with Acetylsalicylic Acid Alone or Combined with Extended-Release Dipyridamole in Healthy Volunteers.* Cerebrovasc Dis, 2005. **19**: p. 214-219.
21. Hollopeter G, et al., *Identification of the platelet ADP receptor targeted by antithrombotic drugs.* Nature, 2001. **409**(6817): p. 202-207.
22. *Brilique*. 2011 [cited 2011 September 2nd]; http://www.fass.se/LIF/produktfakta/artikel_produkt.jsp?NplID=20100114000225&DocTypeID=6].
23. *Efient*. 2011 [cited 2011 July 5th]; http://www.fass.se/LIF/produktfakta/artikel_produkt.jsp?NplID=20080321000013&DocTypeID=3].
24. *INTEGRILIN*. 2010 [cited 2011 April 21st]; http://www.fass.se/LIF/produktfakta/artikel_produkt.jsp?NplID=19990701000028&DocTypeID=3].
25. *Aggrastat*. 2011 [cited 2011 September 6th]; http://www.fass.se/LIF/produktfakta/artikel_produkt.jsp?NplID=19990730000037&DocTypeID=3].
26. Bechmann LP, et al., *Low-molecular-weight heparin in patients with advanced cirrhosis.* Liver International, 2011. **31**(1): p. 75-82.
27. *Waran*. 2005 [cited 2011 April 21st]; http://www.fass.se/LIF/produktfakta/artikel_produkt.jsp?NplID=19640101000028&DocTypeID=3&UserTypeID=0].
28. *WHEN MINUTES COUNT to stop the bleeding*, in *www.rotem.de*. 2009, Pentapharm GmbH: München.
29. Lang T and v.D. M, *Possibilities and limitations of thromboelastometry/thromboelastography.* Hämostaseologie, 2006. **26**(Suppl 1): p. 21-29.
30. Haas T, et al., *The In Vitro Effects of Fibrinogen Concentrate, Factor XIII and Fresh Frozen Plasma on Impaired Clot Formation After 60% Dilution.* Anaesthesia & Analgesia, 2008. **106**(5): p. 1360-1365.
31. Jarvis GE, *Platelet Aggregation in Whole Blood; Impedance and Particle Counting Methods*, in *Platelets and Megakaryocytes*, JM Gibbins and M. Mahaut-Smith, Editors. 2004, Humana Press Inc.: Totowa, New Jersey. p. 77-78.
32. Paniccia R, et al., *Comparison of methods for monitoring residual platelet reactivity after clopidogrel by point-of-care tests on whole blood in high-risk patients.* Thromb Haemost, 2010. **104**(2): p. 287-292.
33. Cusack NJ and H. SM., *Differential inhibition by adenosine or by prostaglandin E1 of human platelet aggregation induced by adenosine 5'-O-(1-thiodiphosphate) and adenosine 5'-O-(2-thiodiphosphate).* British Journal of Pharmacology, 1982. **75**(2): p. 257-259.
34. *multiplate*. 2008 [cited 2011 September 5th]; multiplate.net].
35. Gertler R, et al., *Are the Point-of-Care Diagnostics MULTIPLATE and ROTEM Valid in the Setting of High Concentrations of Heparin and Its Reversal with Protamine?* Komplettera.
36. Petrie A and Sabin C, *Medical Statistics at a Glance*. Third ed. 2009, London: Wiley-Blackwell. 180.
37. Arif S, Khan AS, and K. AR, *Changes in fibrinogen level in liver cirrhosis.* J Ayub Med Coll Abbottabad, 2002. **14**(2): p. 19-21.
38. Herlitz LC, et al., *Light chain proximal tubulopathy.* Kidney International, 2009. **76**: p. 792-797.

Appendix A

Here the calculations for how to determine the fibrinogen and platelet concentrations of the different samples for the *ex vivo* study are demonstrated. These calculations are for the samples intended for Multiplate® analysis, but the calculations for samples intended for ROTEM® analysis give approximately the same concentrations.

Fibrinogen concentration calculation:

$$\frac{x \cdot 1.0 \cdot 10^{-3} + 13 \cdot y \cdot 10^{-6}}{1.0 \cdot 10^{-3} + 300 \cdot 10^{-6}} = total\ \ concentration\ in\ sample\ [g * L^{-1}]$$

where x denotes for the endogenous fibrinogen concentration (~2-4 $g*L^{-1}$), and y for the amount of added fibrinogen (0-300 µL).

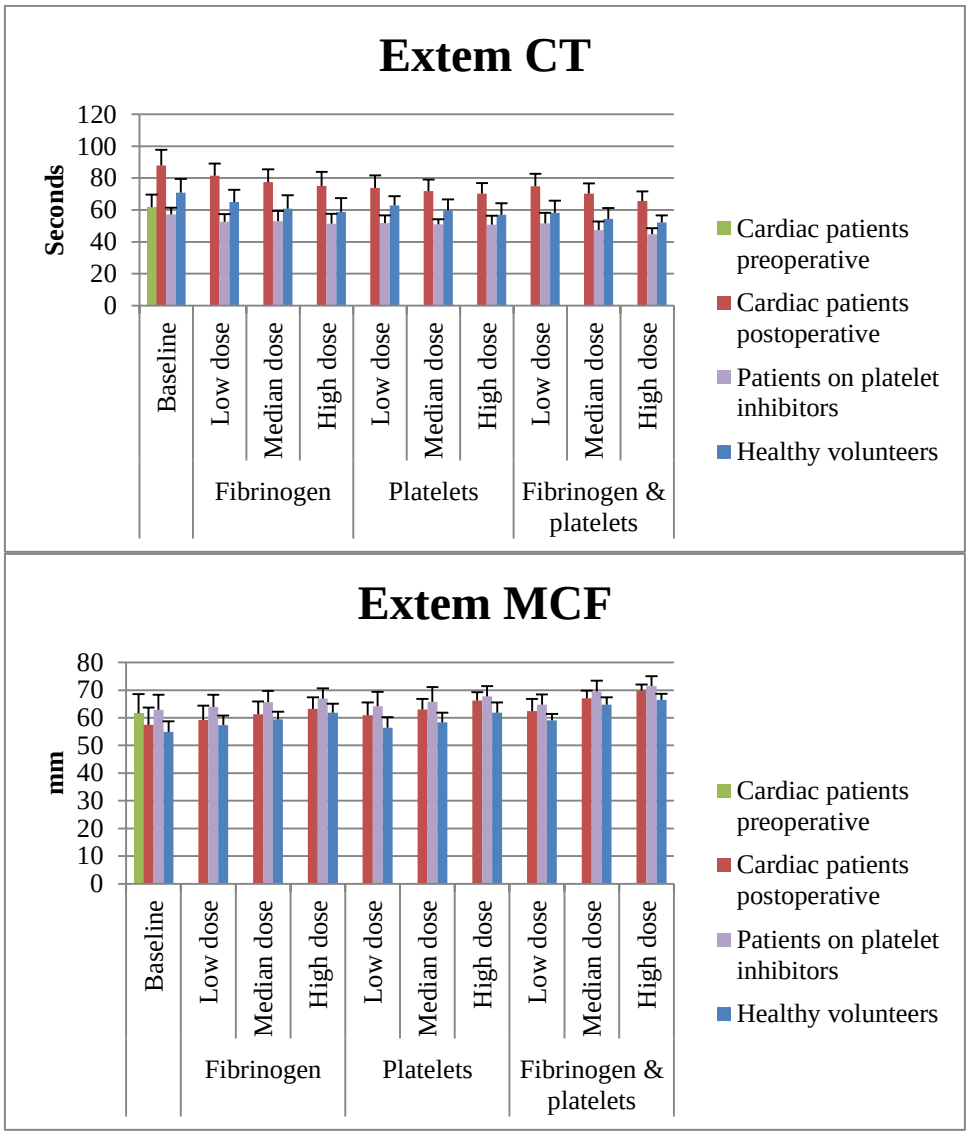
Platelet concentration calculation:

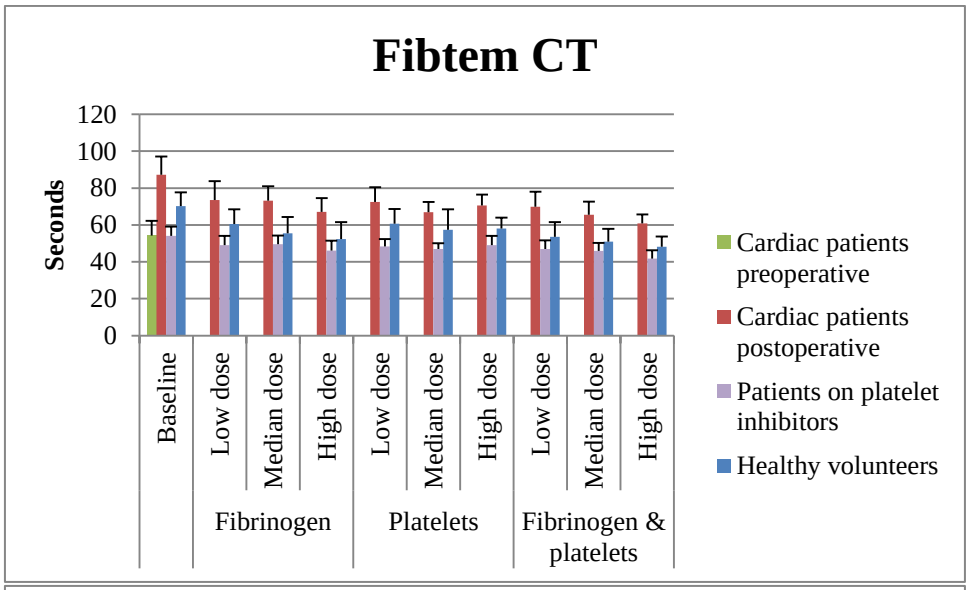
$$\frac{x \cdot 1.0 \cdot 10^{-3} + 1200 \cdot 10^{9} \cdot y \cdot 10^{-6}}{1.0 \cdot 10^{-3} + 300 \cdot 10^{-6}} = total\ \ concentration\ in\ sample\ [10^{9} * L^{-1}]$$

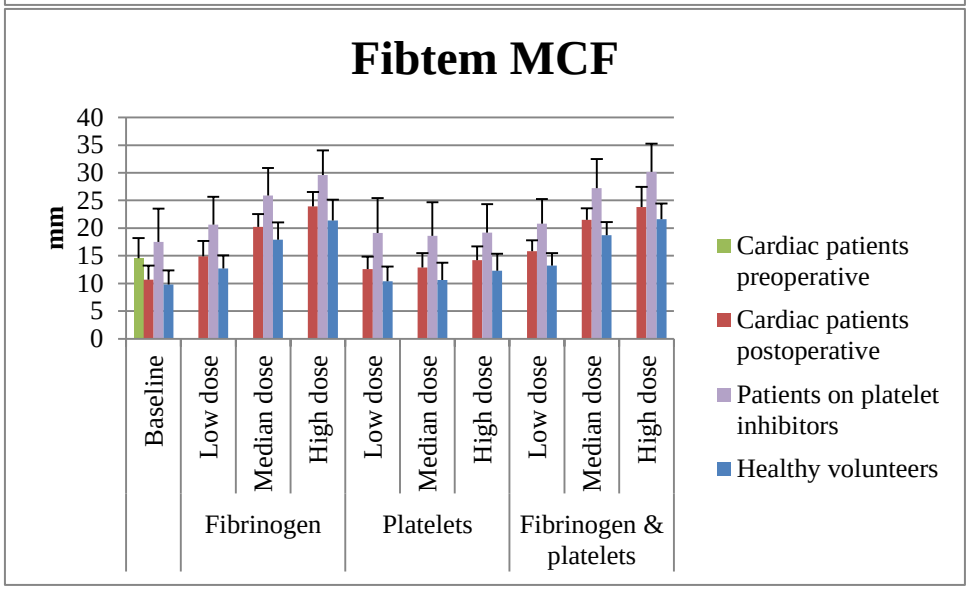
where x is the endogenous platelet concentration (150-400$*10^{9}*L^{-1}$), and y the amount of platelet concentrate added (0-300 µL).

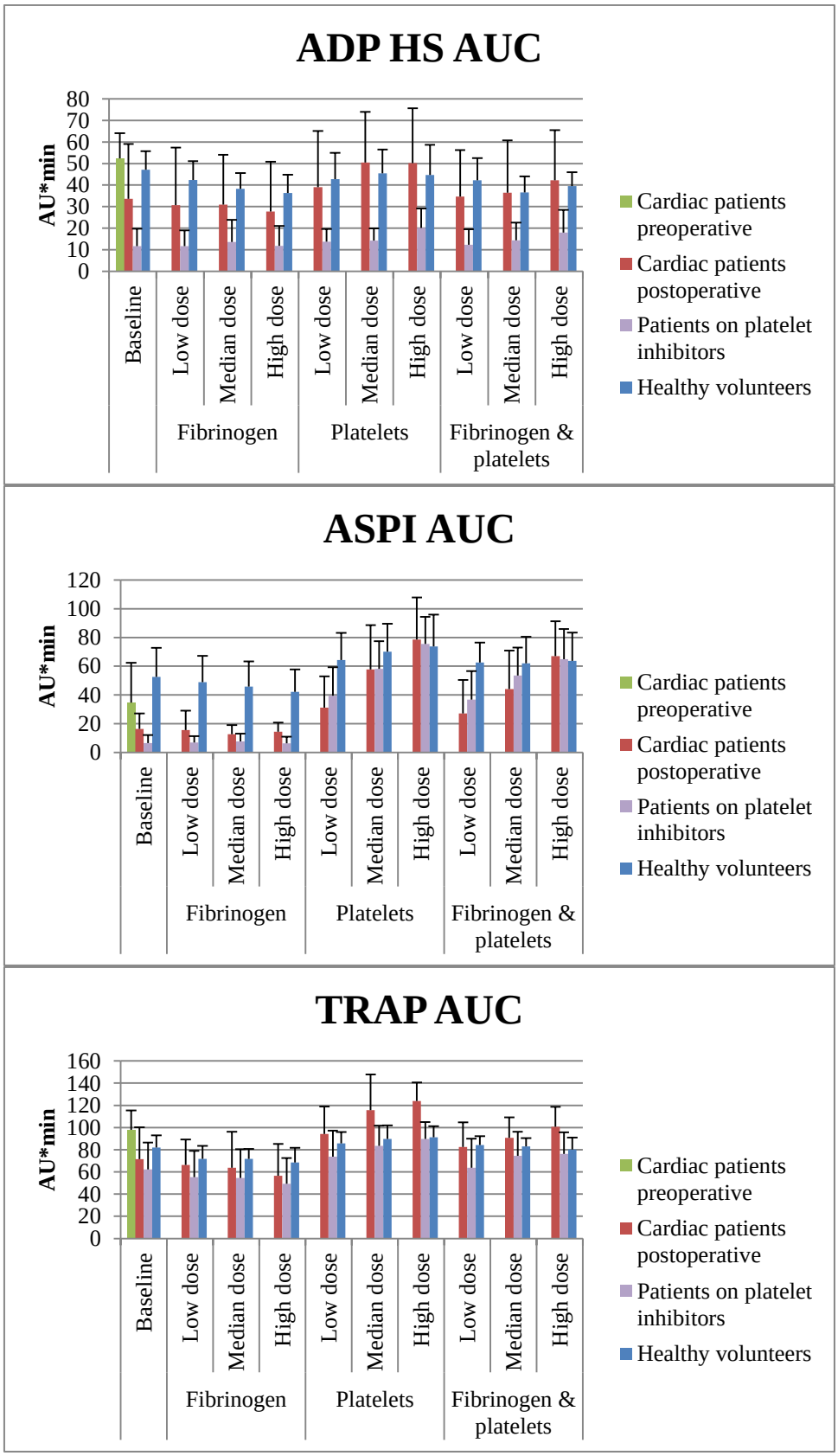
Appendix B

In the report, results were visualized in figures for only the highest of the three doses of fibrinogen, platelets and the combination of both fibrinogen and platelets. Here, figures with results, in absolute values, for all three doses and baseline values are to be seen. At first, the results for the ROTEM variables Extem clotting time (CT) and maximum clot firmness (MCF) are shown, then Fibtem CT and MCF, and finally ADP HS, ASPI and TRAP AUC. All results are visualized in mean + standard deviation.




Fibtem CT
Seconds
120
100
80
60
40
20
0
Baseline
Low dose
Median dose
High dose
Low dose
Median dose
High dose
Low dose
Median dose
High dose
Fibrinogen
Platelets
Fibrinogen & platelets
Cardiac patients preoperative
Cardiac patients postoperative
Patients on platelet inhibitors
Healthy volunteers


Fibtem MCF
mm
40
35
30
25
20
15
10
5
0
Baseline
Low dose
Median dose
High dose
Low dose
Median dose
High dose
Low dose
Median dose
High dose
Fibrinogen
Platelets
Fibrinogen & platelets
Cardiac patients preoperative
Cardiac patients postoperative
Patients on platelet inhibitors
Healthy volunteers


ADP HS AUC
AU*min
80
70
60
50
40
30
20
10
0
Baseline
Low dose
Median dose
High dose
Low dose
Median dose
High dose
Low dose
Median dose
High dose
Fibrinogen
Platelets
Fibrinogen & platelets
Cardiac patients preoperative
Cardiac patients postoperative
Patients on platelet inhibitors
Healthy volunteers
ASPI AUC
AU*min
120
100
80
60
40
20
0
Baseline
Low dose
Median dose
High dose
Low dose
Median dose
High dose
Low dose
Median dose
High dose
Fibrinogen
Platelets
Fibrinogen & platelets
Cardiac patients preoperative
Cardiac patients postoperative
Patients on platelet inhibitors
Healthy volunteers
TRAP AUC
AU*min
160
140
120
100
80
60
40
20
0
Baseline
Low dose
Median dose
High dose
Low dose
Median dose
High dose
Low dose
Median dose
High dose
Fibrinogen
Platelets
Fibrinogen & platelets
Cardiac patients preoperative
Cardiac patients postoperative
Patients on platelet inhibitors
Healthy volunteers

Appendix C

Below results from correlation analysis, calculated with the Spearman's correlation coefficient, are displayed. Significant correlations are denoted with yellow marks. The significant correlations are quite randomly distributed, and many of their scatter plots (not shown here) do not form a linear curve and often have outliners, so the varying parameters within the study groups do not seem to have any great effect on the results.

Table C1 Correlations for the healthy volunteers, between the variables age, BMI, smoker habits and variables for the ROTEM® and Multiplate® results, in absolute values. Yellow marks denote for significant correlations.

			Age	BMI	PLT	Smoker (no=0, ex=1, occasional=2, yes=3,)	ex-ct-bl	ex-ct-f3	ex-ct-t3	ex-ct-ft3	ex-mcf-bl	ex-mcf-f3	ex-mcf-t3	ex-mcf-ft3	fib-ct-bl	fib-ct-f3	fib-ct-t3	fib-ct-ft3	fib-mcf-bl	fib-mcf-f3	fib-mcf-t3	fib-mcf-ft3	adp-auc-bl	adp-auc-f3	adp-auc-t3	adp-auc-ft3	aspi-auc-bl	aspi-auc-f3	aspi-auc-t3	aspi-auc-ft3	trap-auc-bl	trap-auc-f3	trap-auc-t3	trap-auc-ft3
Spearman's rho	Age	Correlation Coefficient	1,000	,644	-,273	,455	-,118	-,170	-,382	-,170	,390	,154	,271	-,025	-,182	,031	-,340	-,098	,358	,615	,499	,585	,103	,067	,006	-,079	,444	,195	,547	,139	,539	,529	,590	,394
		Sig. (2-tailed)	.	,044	,446	,187	,745	,638	,276	,638	,265	,670	,449	,946	,614	,933	,336	,788	,310	,058	,142	,075	,776	,854	,987	,829	,199	,590	,102	,701	,108	,116	,073	,260
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	BMI	Correlation Coefficient	,644	1,000	-,292	-,007	,199	-,220	,122	,058	-,266	-,505	-,278	-,303	-,140	-,049	,140	-,135	-,245	,106	-,182	,110	-,299	-,320	-,280	-,584	,226	,134	,293	-,116	-,158	,165	,530	,146
		Sig. (2-tailed)	,044	.	,413	,984	,581	,542	,738	,874	,457	,137	,437	,394	,699	,893	,699	,710	,496	,771	,615	,762	,402	,367	,434	,077	,531	,712	,412	,751	,663	,649	,115	,688
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	PLT	Correlation Coefficient	-,273	-,292	1,000	-,037	,056	,219	-,188	,426	,439	,401	,597	,426	,079	-,043	-,255	,477	,241	,106	,320	,030	,304	,657	,164	,345	-,188	-,006	,225	,042	,030	,073	,219	-,055
		Sig. (2-tailed)	,446	,413	.	,919	,878	,544	,603	,220	,204	,250	,068	,220	,828	,906	,476	,163	,503	,772	,367	,933	,393	,039	,651	,328	,602	,987	,532	,907	,934	,841	,544	,881
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Smoker (no=0, ex=1, occasional=2, yes=3,)	Correlation Coefficient	,455	-,007	-,037	1,000	-,283	-,015	-,022	-,172	,398	,205	,363	,319	,097	,159	-,142	,053	,509	,573	,477	,510	,292	-,022	,142	,276	-,307	-,127	-,172	-,410	,306	-,112	-,120	-,186
		Sig. (2-tailed)	,187	,984	,919	.	,428	,967	,951	,635	,255	,570	,302	,369	,789	,662	,695	,885	,133	,083	,163	,132	,413	,951	,696	,440	,389	,726	,635	,239	,390	,758	,742	,606
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Table C2 Correlations for the healthy volunteers, between the variables age, BMI, smoker habits and variables for the ROTEM® and Multiplate® results, in percentage changes. Yellow marks denote for significant correlations.

			Age	BMI	PLT	Smoker (no=0, ex=1, occasional=2, yes=3,)	dexctf3%	dexctt3%	dexctft3%	dexmcff3 %	dexmcft3 %	dexmcfft3 %	dfibctf3%	dfibctt3%	dfibctft3%	dfibmcff3 %	dfibmcft3 %	dfibmcfft3 %	dadpaucf3 %	dadpauct3 %	dadpaucft 3%	daspiaucf 3%	daspiauct 3%	daspiaucft 3%	dtrapaucf3 %	dtrapauct3 %	dtrapaucft 3%
Spearman's rho	Age	Correlation Coefficient	1,000	,644	-,273	,455	,055	-,523	,006	-,590	-,365	-,539	-,006	-,006	,067	,365	,285	-,006	-,042	-,224	-,709	,152	,261	-,042	,176	-,067	-,164
		Sig. (2-tailed)	.	,044	,446	,187	,881	,121	,987	,073	,300	,108	,987	,987	,855	,300	,425	,987	,907	,533	,022	,676	,467	,907	,627	,855	,651
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	BMI	Correlation Coefficient	,644	1,000	-,292	-,007	-,535	-,216	-,237	-,561	,235	,091	,024	,401	-,067	,576	,000	,492	-,195	-,225	-,693	,049	,267	-,316	,231	,644	,116
		Sig. (2-tailed)	,044	.	,413	,984	,111	,548	,510	,092	,514	,802	,947	,250	,854	,081	1,000	,148	,590	,532	,026	,894	,455	,374	,521	,044	,751
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	PLT	Correlation Coefficient	-,273	-,292	1,000	-,037	-,006	-,201	,345	-,389	-,006	-,370	-,248	-,285	,442	-,316	-,236	-,564	,685	-,006	,164	,382	,358	,527	,091	-,042	,067
		Sig. (2-tailed)	,446	,413	.	,919	,987	,578	,328	,266	,987	,293	,489	,425	,200	,374	,511	,090	,029	,987	,651	,276	,310	,117	,803	,907	,855
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Smoker (no=0, ex=1, occasional=2, yes=3,)	Correlation Coefficient	,455	-,007	-,037	1,000	,544	,127	,097	-,247	-,022	-,306	,082	-,321	-,127	-,097	,082	-,365	-,231	-,186	-,142	,544	-,022	,157	-,291	-,365	-,410
		Sig. (2-tailed)	,187	,984	,919	.	,104	,726	,790	,492	,951	,390	,822	,366	,727	,789	,822	,299	,521	,606	,696	,104	,951	,666	,415	,299	,239
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Table C3 Correlations for the cardiac surgery patients, between variables such as age, BMI, type of operation, amount of bleeding, type of diagnosis, medications, smoker habits and the ROTEM® and Multiplate® results, in absolute values. Yellow marks denote for significant correlations.

			Age	Operation (ACB=1, AVR=2)	BMI	PLT	EF%	Higgins	ASA (no=0, 75mg=1, 160mg=2)	Clopidogrel (no=0, 75mg Plavix=1)	LMH (no=0, Arixtra2.5 mg=1)	Angina (no=0, stable=1, unstable=2)	Heart attack (no=0, yes=1)	Smoker (no=0, ex=1, occasional=2, yes=3,)	ECC time (min)	Clamp time (min)	Peroperative bleeding (mL)	Postoperative bleeding <12h (mL)	ex-ct-bl	ex-ct-fi3	ex-ct-fi3	ex-ct-fi3	ex-mcf-bl	ex-mcf-fi3	ex-mcf-fi3	ex-mcf-fi3	fib-ct-bl	fib-ct-fi3	fib-ct-fi3	fib-ct-fi3	fib-mcf-bl	fib-mcf-fi3	fib-mcf-fi3	fib-mcf-fi3	adp-auc-bl	adp-auc-fi3	adp-auc-fi3	adp-auc-fi3	aspi-auc-bl	aspi-auc-fi3	aspi-auc-fi3	aspi-auc-fi3	trap-auc-bl	trap-auc-fi3	trap-auc-fi3	trap-auc-fi3
Spearman's rho	Age	Correlation Coefficient	1,000	,058	-,170	-,561	,101	,756	,262	-,642	,392	,407	,000	-,139	-,565	-,640	,578	,018	-,295	,213	-,396	-,580	,216	,000	,273	,231	-,372	-,136	-,511	-,381	,554	,281	,149	,669	-,024	,006	,061	,107	-,243	-,156	-,024	-,328	-,046	-,182	-,620	-,517
		Sig. (2-tailed)		,873	,638	,092	,782	,011	,465	,046	,262	,244	1,000	,701	,089	,046	,080	,960	,407	,555	,257	,079	,548	1,000	,445	,522	,290	,708	,131	,278	,096	,432	,682	,034	,947	,987	,868	,769	,498	,667	,947	,354	,900	,614	,056	,126
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Operation (ACB=1, AVR=2)	Correlation Coefficient	,058	1,000	,174	-,407	,480	-,060	-,667	-,272	-,272	-,577	-,408	,133	,406	,407	,182	-,406	,059	,522	,407	,295	-,180	,295	-,186	,178	-,058	,530	,292	,414	,236	,354	-,177	,177	,058	,116	,290	,058	,058	-,058	,175	,174	,175	,406	,406	-,058
		Sig. (2-tailed)	,873	.	,631	,243	,160	,870	,035	,447	,447	,081	,242	,715	,244	,243	,615	,244	,872	,122	,243	,409	,620	,409	,606	,622	,873	,115	,413	,235	,511	,316	,624	,624	,873	,749	,416	,873	,873	,873	,629	,631	,629	,244	,244	,873
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	BMI	Correlation Coefficient	-,170	,174	1,000	,237	,132	,012	-,174	,142	,142	,040	,071	,180	,491	,523	-,298	-,115	-,252	-,321	,231	-,068	,488	,566	,428	,255	-,122	-,154	-,122	,241	,099	,012	,241	-,228	,370	,292	-,285	-,304	,309	,348	-,182	-,030	,109	,224	,345	,176
		Sig. (2-tailed)	,638	,631	.	,510	,717	,973	,631	,695	,695	,912	,845	,618	,150	,121	,403	,751	,483	,365	,521	,853	,153	,088	,217	,478	,738	,671	,737	,503	,786	,973	,503	,526	,293	,413	,425	,393	,385	,325	,614	,934	,763	,533	,328	,627
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	PLT	Correlation Coefficient	-,561	-,407	,237	1,000	-,063	-,697	,000	,285	-,463	,057	,107	,045	-,195	-,021	-,737	-,219	-,074	-,334	,186	,244	,417	,478	,400	,293	,369	-,025	,251	,403	-,307	-,105	,403	-,220	,596	,518	,158	,366	,608	,560	,503	,565	,573	,316	,559	,699
		Sig. (2-tailed)	,092	,243	,510	.	,863	,025	1,000	,425	,177	,875	,769	,901	,590	,953	,015	,544	,839	,345	,607	,497	,230	,162	,252	,411	,294	,946	,485	,249	,389	,773	,249	,542	,069	,125	,663	,298	,062	,093	,138	,089	,083	,374	,093	,024
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	EF%	Correlation Coefficient	,101	,480	,132	-,063	1,000	-,332	-,450	-,368	-,074	-,139	,074	,373	-,006	-,173	-,279	-,816	,356	,194	,387	,293	,547	,637	,564	,656	,060	,064	,259	,422	,722	,892	,623	,514	,483	,472	,276	,233	,602	,619	,261	,263	,434	,433	,452	,314
		Sig. (2-tailed)	,782	,160	,717	,863	.	,348	,191	,296	,840	,702	,840	,288	,986	,633	,436	,004	,313	,590	,269	,411	,102	,048	,089	,039	,870	,861	,470	,225	,018	,001	,054	,128	,157	,168	,440	,517	,065	,057	,466	,462	,210	,211	,190	,377
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Higgins	Correlation Coefficient	,756	-,060	,012	-,697	-,332	1,000	,358	-,219	,694	,389	,183	-,046	-,118	-,178	,795	,424	-,391	,019	-,563	-,604	-,125	-,377	-,110	-,236	-,553	-,310	-,640	-,562	,244	-,108	-,206	,168	-,368	-,319	-,168	-,228	-,536	-,436	-,478	-,592	-,450	-,443	-,760	-,679
		Sig. (2-tailed)	,011	,870	,973	,025	,348	.	,310	,543	,026	,266	,613	,899	,745	,622	,006	,222	,264	,959	,090	,064	,730	,283	,762	,511	,097	,383	,046	,091	,496	,767	,567	,642	,296	,369	,642	,526	,110	,208	,162	,071	,192	,200	,011	,031
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	ASA (no=0, 75mg=1, 160mg=2)	Correlation Coefficient	,262	-,667	-,174	,000	-,450	,358	1,000	,408	,408	,625	,102	-,548	-,348	-,349	,045	,261	-,088	-,174	-,698	-,619	,045	-,442	,000	-,268	,349	-,221	-,613	-,621	-,133	-,221	,133	-,089	-,348	-,393	-,522	-,349	-,435	-,263	-,437	-,522	-,437	-,174	-,522	-,261
		Sig. (2-tailed)	,465	,035	,631	1,000	,191	,310	.	,242	,242	,053	,779	,101	,324	,323	,901	,466	,809	,631	,025	,057	,902	,201	1,000	,455	,323	,540	,060	,056	,714	,540	,714	,808	,324	,261	,122	,323	,209	,463	,207	,122	,207	,631	,122	,466
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Clopidogrel (no=0, 75mg Plavix=1)	Correlation Coefficient	-,642	-,272	,142	,285	-,368	-,219	,408	1,000	,167	,079	,250	-,041	,426	,499	-,186	,071	,144	-,213	-,285	,036	-,257	-,325	-,342	-,437	,428	,000	-,036	-,036	-,434	-,253	-,036	-,652	-,213	-,214	-,284	-,321	-,142	-,072	-,356	-,071	-,249	,213	,284	,284
		Sig. (2-tailed)	,046	,447	,695	,425	,296	,543	,242	.	,645	,829	,486	,911	,219	,142	,607	,845	,692	,554	,425	,921	,474	,360	,333	,206	,218	1,000	,922	,921	,210	,481	,921	,041	,554	,553	,426	,366	,695	,844	,312	,845	,487	,554	,426	,426
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	LMH (no=0, Arixtra2.5 mg=1)	Correlation Coefficient	,392	-,272	,142	-,463	-,074	,694	,408	,167	1,000	,432	,667	,325	,000	-,143	,557	,213	,144	-,071	-,392	-,361	,147	-,325	,114	-,291	-,356	-,361	-,322	-,253	,507	,144	,145	,036	-,213	-,143	-,284	-,285	-,284	-,143	-,570	-,355	-,321	-,142	-,426	-,142
		Sig. (2-tailed)	,262	,447	,695	,177	,840	,026	,242	,645	.	,212	,035	,359	1,000	,694	,094	,554	,692	,845	,262	,306	,686	,360	,754	,414	,312	,306	,365	,480	,135	,691	,690	,921	,554	,694	,426	,425	,426	,693	,085	,314	,366	,695	,219	,695
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Angina (no=0, stable=1, unstable=2)	Correlation Coefficient	,407	-,577	,040	,057	-,139	,389	,625	,079	,432	1,000	,471	-,004	-,389	-,450	,137	,147	-,414	-,663	-,722	-,851	,301	,037	,441	,350	-,286	-,704	-,661	-,611	,177	,082	,450	,440	,255	,208	-,114	-,077	-,107	,260	-,050	-,322	-,050	-,087	-,328	-,074
		Sig. (2-tailed)	,244	,081	,912	,875	,702	,266	,053	,829	,212	.	,169	,992	,267	,192	,707	,684	,235	,037	,018	,002	,399	,918	,202	,321	,424	,023	,038	,061	,624	,823	,191	,203	,478	,564	,754	,832	,768	,469	,890	,365	,890	,811	,354	,840
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Heart attack (no=0, yes=1)	Correlation Coefficient	,000	-,408	,071	,107	,074	,183	,102	,250	,667	,471	1,000	,773	-,142	-,214	,260	-,071	,072	-,355	-,321	-,072	,293	,036	,342	,073	-,428	-,541	-,036	,072	,434	,325	,471	,109	,355	,428	,284	,285	,284	,429	,000	,213	,249	,071	,071	,426
		Sig. (2-tailed)	1,000	,242	,845	,769	,840	,613	,779	,486	,035	,169	.	,009	,695	,553	,468	,845	,843	,314	,366	,843	,411	,921	,333	,841	,218	,106	,922	,842	,210	,360	,170	,765	,314	,218	,426	,425	,426	,216	1,000	,554	,487	,845	,845	,219
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Smoker (no=0, ex=1, occasional=2, yes=3,)	Correlation Coefficient	-,139	,133	,180	,045	,373	-,046	-,548	-,041	,325	-,004	,773	1,000	,118	,056	,225	-,257	,119	-,153	,157	,317	,211	,313	,278	,235	-,577	-,289	,345	,459	,484	,451	,318	,166	,513	,609	,576	,452	,499	,516	,264	,499	,483	,208	,402	,513
		Sig. (2-tailed)	,701	,715	,618	,901	,288	,899	,101	,911	,359	,992	,009	.	,746	,879	,532	,474	,743	,674	,666	,372	,558	,378	,436	,514	,080	,418	,328	,182	,156	,191	,371	,647	,129	,062	,082	,190	,142	,127	,460	,142	,157	,564	,249	,129
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	ECC time (min)	Correlation Coefficient	-,565	,406	,491	-,195	-,006	-,118	-,348	,426	,000	-,389	-,142	,118	1,000	,948	-,108	,127	,080	-,164	,286	,252	-,356	-,092	-,409	-,280	-,049	,012	,116	,049	-,315	-,215	-,395	-,568	-,297	-,322	-,333	-,596	-,139	-,140	-,498	-,261	-,432	-,042	,273	-,103
		Sig. (2-tailed)	,089	,244	,150	,590	,986	,745	,324	,219	1,000	,267	,695	,746	.	,000	,767	,726	,827	,651	,424	,482	,312	,800	,241	,434	,894	,973	,750	,892	,376	,550	,258	,087	,405	,364	,347	,069	,701	,699	,143	,467	,213	,907	,446	,777
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Clamp time (min)	Correlation Coefficient	-,640	,407	,523	-,021	-,173	-,178	-,349	,499	-,143	-,450	-,214	,056	,948	1,000	-,133	,152	-,043	-,116	,277	,284	-,401	-,056	-,469	-,318	,024	,142	,153	,152	-,477	-,352	-,455	-,669	-,231	-,250	-,243	-,473	-,116	-,171	-,354	-,116	-,305	,049	,371	-,018
		Sig. (2-tailed)	,046	,243	,121	,953	,633	,622	,323	,142	,694	,192	,553	,879	,000	.	,713	,675	,906	,751	,438	,427	,250	,879	,172	,371	,947	,696	,673	,676	,163	,319	,186	,034	,521	,486	,498	,168	,751	,636	,316	,751	,392	,894	,291	,960
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Peroperative bleeding (mL)	Correlation Coefficient	,578	,182	-,298	-,737	-,279	,795	,045	-,186	,557	,137	,260	,225	-,108	-,133	1,000	,380	-,141	,253	-,394	-,338	-,451	-,489	-,387	-,344	-,597	-,016	-,188	-,261	,255	-,048	-,384	,255	-,329	-,178	,203	,048	-,564	-,519	-,153	-,184	-,219	-,177	-,481	-,405
		Sig. (2-tailed)	,080	,615	,403	,015	,436	,006	,901	,607	,094	,707	,468	,532	,767	,713	.	,279	,697	,480	,260	,340	,191	,152	,270	,330	,068	,965	,603	,466	,477	,895	,273	,477	,353	,623	,574	,896	,090	,124	,674	,611	,543	,624	,159	,245
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Postoperative bleeding <12h (mL)	Correlation Coefficient	,018	-,406	-,115	-,219	-,816	,424	,261	,071	,213	,147	-,071	-,257	,127	,152	,380	1,000	-,153	-,285	-,134	-,351	-,550	-,677	-,506	-,628	-,334	-,172	-,128	-,494	-,512	-,874	-,741	-,296	-,564	-,547	-,455	-,474	-,745	-,695	-,371	-,382	-,590	-,576	-,600	-,430
		Sig. (2-tailed)	,960	,244	,751	,544	,004	,222	,466	,845	,554	,684	,845	,474	,726	,675	,279	.	,672	,425	,713	,320	,099	,032	,136	,052	,345	,634	,724	,147	,130	,001	,014	,406	,090	,102	,187	,166	,013	,026	,291	,276	,073	,082	,067	,214
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Table C4 Correlations for the cardiac surgery patients, between variables such as age, BMI, type of operation, amount of bleeding, type of diagnosis, medications, smoker habits and the ROTEM® and Multiplate® results, in percentage changes. Yellow marks denote for significant correlations.

			Age	BMI	PLT	EF%	Higgins	ASA (no=0, 75mg=1, 160mg=2)	Clopidogrel (no=0, 75mg Plavix=1)	LMH (no=0, Arixtra2.5 mg=1)	Angina (no=0, stable=1, unstable=2)	Heart attack (no=0, yes=1)	Smoker (no=0, ex=1, occasional=2, yes=3,)	ex-ct-bl	ex-ct-f3	ex-ct-t3	ex-ct-ft3	ex-mcf-bl	ex-mcf-f3	ex-mcf-t3	ex-mcf-ft3	fib-ct-bl	fib-ct-f3	fib-ct-t3	fib-ct-ft3	fib-mcf-bl	fib-mcf-f3	fib-mcf-t3	fib-mcf-ft3	adp-auc-bl	adp-auc-f3	adp-auc-t3	adp-auc-ft3	aspi-auc-bl	aspi-auc-f3	aspi-auc-t3	aspi-auc-ft3	trap-auc-bl	trap-auc-f3	trap-auc-t3	trap-auc-ft3
Spearman's rho	Age	Correlation Coefficient	1,000	-,311	-,304	,093	.	,175	.	-,114	,349	-,191	-,290	,130	,318	-,256	-,034	,046	-,081	-,269	,166	,326	,031	,031	,034	,216	,315	,232	,382	-,407	-,550	-,310	-,182	-,162	-,213	-,322	-,231	-,249	-,286	-,134	-,255
		Sig. (2-tailed)	.	,382	,393	,797	.	,629	.	,753	,323	,598	,416	,720	,370	,475	,926	,900	,825	,452	,647	,358	,933	,933	,927	,548	,375	,518	,277	,243	,099	,383	,615	,656	,554	,364	,521	,487	,424	,713	,476
		N	10	10	10	10	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	BMI	Correlation Coefficient	-,311	1,000	,578	,137	.	-,175	.	,495	-,044	-,038	,007	,359	,183	,475	,340	,557	,604	,731	,537	,104	,338	,511	,512	,396	,297	,498	,314	,722	,725	,650	,753	,262	,207	,687	,608	,912	,888	,377	,790
		Sig. (2-tailed)	,382	.	,080	,706	.	,629	.	,145	,905	,917	,986	,308	,612	,165	,336	,095	,065	,016	,110	,776	,339	,131	,130	,257	,405	,143	,377	,018	,018	,042	,012	,464	,565	,028	,062	,000	,001	,283	,007
		N	10	10	10	10	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	PLT	Correlation Coefficient	-,304	,578	1,000	-,503	.	-,174	.	-,190	-,174	,342	,526	,672	,524	,357	,471	,732	,871	,799	,710	,298	,693	,585	,602	,535	,329	,470	,423	,177	,250	,636	,591	-,328	-,158	,527	,600	,515	,564	,261	,685
		Sig. (2-tailed)	,393	,080	.	,138	.	,631	.	,599	,631	,334	,118	,033	,120	,311	,169	,016	,001	,006	,022	,403	,026	,075	,066	,111	,353	,171	,223	,625	,486	,048	,072	,354	,663	,117	,067	,128	,090	,467	,029
		N	10	10	10	10	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	EF%	Correlation Coefficient	,093	,137	-,503	1,000	.	,297	.	,506	,446	-,350	-,789	-,039	-,025	,114	,132	-,125	-,177	-,056	-,050	,287	-,069	,013	,100	-,075	-,100	-,100	-,094	,306	,388	-,155	,000	,623	,523	,354	,217	,205	,081	,466	,217
		Sig. (2-tailed)	,797	,706	,138	.	.	,404	.	,135	,196	,321	,007	,915	,945	,755	,717	,731	,624	,877	,891	,422	,849	,973	,784	,837	,783	,783	,795	,389	,268	,668	1,000	,054	,120	,315	,546	,570	,824	,175	,546
		N	10	10	10	10	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Higgins	Correlation Coefficient	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		Sig. (2-tailed)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ASA (no=0, 75mg=1, 160mg=2)	Correlation Coefficient	,175	-,175	-,174	,297	.	1,000	.	-,218	,667	-,509	-,315	,243	,000	,059	,351	-,234	-,059	-,175	-,117	-,116	-,294	,000	-,058	-,233	-,175	-,350	-,059	-,292	-,234	-,174	-,471	-,175	-,058	,290	,174	-,290	-,290	-,290	-,058
		Sig. (2-tailed)	,629	,629	,631	,404	.	.	.	,545	,035	,133	,375	,499	1,000	,872	,319	,516	,871	,628	,747	,749	,410	1,000	,873	,517	,628	,321	,872	,413	,516	,631	,169	,629	,873	,416	,631	,416	,416	,416	,873
		N	10	10	10	10	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Clopidogrel (no=0, 75mg Plavix=1)	Correlation Coefficient	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		Sig. (2-tailed)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		N	10	10	10	10	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	LMH (no=0, Arixtra2.5 mg=1)	Correlation Coefficient	-,114	,495	-,190	,506	.	-,218	.	1,000	,218	-,524	-,619	-,238	-,229	,501	-,192	-,076	-,193	,000	-,077	,038	,038	,420	,076	-,038	-,076	-,076	-,038	,650	,535	-,038	,116	,800	,800	,038	-,190	,494	,342	,342	,114
		Sig. (2-tailed)	,753	,145	,599	,135	.	,545	.	.	,545	,120	,057	,507	,524	,140	,596	,834	,592	1,000	,833	,917	,916	,226	,834	,917	,834	,834	,916	,042	,111	,917	,750	,005	,005	,917	,599	,147	,334	,334	,754
		N	10	10	10	10	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Angina (no=0, stable=1, unstable=2)	Correlation Coefficient	,349	-,044	-,174	,446	.	,667	.	,218	1,000	-,764	-,472	,364	,306	,354	,351	-,131	,044	-,131	,000	,306	,088	,306	,262	-,131	-,131	-,263	,000	-,088	-,088	-,348	-,398	,175	,349	,174	,000	-,174	-,174	-,087	,087
		Sig. (2-tailed)	,323	,905	,631	,196	.	,035	.	,545	.	,010	,168	,301	,389	,316	,319	,718	,903	,718	1,000	,391	,809	,389	,465	,718	,718	,463	1,000	,810	,810	,324	,255	,629	,323	,631	1,000	,631	,631	,811	,811
		N	10	10	10	10	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Heart attack (no=0, yes=1)	Correlation Coefficient	-,191	-,038	,342	-,350	.	-,509	.	-,524	-,764	1,000	,619	,040	,076	-,501	,038	,459	,310	,459	,345	,076	,269	-,268	,114	,457	,382	,573	,269	-,268	-,115	,190	,386	-,495	-,572	,038	,342	,038	,038	,190	,190
		Sig. (2-tailed)	,598	,917	,334	,321	.	,133	.	,120	,010	.	,057	,913	,834	,140	,916	,182	,384	,182	,329	,834	,452	,455	,753	,184	,276	,083	,452	,455	,752	,599	,271	,145	,084	,917	,334	,917	,917	,599	,599
		N	10	10	10	10	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Smoker (no=0, ex=1, occasional=2, yes=3,)	Correlation Coefficient	-,290	,007	,526	-,789	.	-,315	.	-,619	-,472	,619	1,000	,175	,093	-,237	,176	,328	,389	,364	,199	-,254	,100	-,238	,142	,234	,288	,371	,160	-,281	-,205	,164	,177	-,703	-,713	-,046	,145	-,164	,007	-,421	,092
		Sig. (2-tailed)	,416	,986	,118	,007	.	,375	.	,057	,168	,057	.	,628	,799	,509	,627	,355	,267	,301	,581	,479	,784	,507	,696	,515	,420	,292	,659	,431	,570	,650	,625	,023	,021	,899	,690	,650	,986	,226	,800
		N	10	10	10	10	0	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Table C5 Correlations for the patients treated with platelet inhibitors, between variables such as age, BMI, type of diagnosis, medication, smoker habits and the ROTEM® and Multiplate® results, in absolute values. Yellow marks denote for significant correlations.

			dextf3%	dextt3%	dextft3%	dexmcf3%	dexmcft3%	dexmcfft3%	dfibctf3%	dfibctt3%	dfibcfft3%	dfibmcf3%	dfibmcft3%	dfibmcfft3%	dadpaucf3%	dadpauct3%	dadpaucft3%	daspiaucf3%	daspiauct3%	daspiaucft3%	dtrapaucf3%	dtrapauct3%	dtrapaucft3%
Spearman's rho	Age	Correlation Coefficient	,596	-,073	-,073	-,601	-,210	-,287	,383	,170	-,006	-,555	-,757	-,326	,231	,365	,280	,708	,229	,103	-,280	-,079	-,219
		Sig. (2-tailed)	,069	,841	,841	,066	,560	,422	,275	,638	,987	,096	,011	,358	,521	,300	,434	,022	,525	,776	,434	,828	,544
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Operation (ACB=1, AVR=2)	Correlation Coefficient	,406	,174	,174	,407	-,299	,175	,406	,174	,407	-,175	-,294	-,175	,290	,290	,174	-,294	,175	,174	,522	,174	,058
		Sig. (2-tailed)	,244	,631	,631	,243	,401	,629	,244	,631	,243	,629	,410	,629	,416	,416	,631	,410	,629	,631	,122	,631	,873
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	BMI	Correlation Coefficient	-,079	,491	,321	-,079	-,625	-,480	,127	,176	,511	-,286	,104	-,274	-,418	-,309	-,661	,006	-,511	-,200	,139	-,091	,212
		Sig. (2-tailed)	,829	,150	,365	,828	,053	,160	,726	,627	,132	,424	,774	,444	,229	,385	,038	,987	,132	,580	,701	,803	,556
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	PLT	Correlation Coefficient	-,426	,195	,073	,018	-,210	-,293	,012	-,213	-,034	,341	,775	,177	-,195	-,766	-,590	-,382	-,183	-,122	-,292	-,687	-,511
		Sig. (2-tailed)	,220	,590	,841	,960	,560	,412	,973	,555	,927	,334	,008	,625	,590	,010	,073	,277	,613	,738	,413	,028	,132
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	EF%	Correlation Coefficient	-,201	-,213	-,251	-,333	-,696	-,485	,251	,132	,035	-,547	-,292	-,680	-,314	-,263	-,376	-,254	-,277	-,351	,000	-,232	-,176
		Sig. (2-tailed)	,578	,554	,484	,346	,025	,156	,484	,717	,924	,101	,413	,031	,377	,462	,284	,479	,439	,320	1,000	,519	,627
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Higgins	Correlation Coefficient	,605	,087	,181	-,319	,132	-,009	,106	,324	,184	-,394	-,726	-,156	,224	,661	,343	,839	,013	,044	-,044	,330	,287
		Sig. (2-tailed)	,064	,811	,617	,369	,717	,979	,771	,361	,610	,260	,018	,666	,533	,038	,332	,002	,973	,905	,905	,351	,422
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	ASA (no=0, 75mg=1, 160mg=2)	Correlation Coefficient	,000	-,522	-,435	-,393	,225	-,044	-,522	-,522	-,698	,131	,088	,175	,174	,087	,174	,529	,218	,261	,000	,087	,087
		Sig. (2-tailed)	1,000	,122	,209	,261	,533	,905	,122	,122	,025	,718	,809	,629	,631	,811	,631	,116	,545	,466	1,000	,811	,811
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Clopidogrel (no=0, 75mg Plavix=1)	Correlation Coefficient	-,284	-,284	-,142	,392	,330	,285	-,640	-,284	-,285	,428	,504	,249	,142	,000	-,071	-,072	-,107	,071	,569	,213	,426
		Sig. (2-tailed)	,426	,426	,695	,262	,352	,425	,046	,426	,425	,218	,138	,487	,695	1,000	,845	,843	,769	,845	,086	,554	,219
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	LMH (no=0, Arixtra2.5 mg=1)	Correlation Coefficient	,000	-,284	-,213	-,428	,000	-,321	-,071	,426	,071	-,642	-,647	-,570	-,142	,355	,071	,647	-,321	-,142	,071	,213	,569
		Sig. (2-tailed)	1,000	,426	,554	,218	1,000	,366	,845	,219	,845	,046	,043	,085	,695	,314	,845	,043	,366	,695	,845	,554	,086
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Angina (no=0, stable=1, unstable=2)	Correlation Coefficient	,034	-,080	-,141	-,481	,024	-,232	-,328	,040	-,299	-,192	-,068	-,040	-,114	-,235	-,322	,787	-,081	-,322	-,181	-,201	-,107
		Sig. (2-tailed)	,927	,825	,698	,160	,947	,519	,354	,912	,401	,596	,852	,912	,754	,514	,365	,007	,825	,365	,617	,578	,768
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Heart attack (no=0, yes=1)	Correlation Coefficient	-,213	-,071	,000	-,285	,000	-,356	,000	,640	,214	-,463	-,288	-,535	-,213	-,071	-,355	,432	-,570	-,569	-,142	-,284	,142
		Sig. (2-tailed)	,554	,845	1,000	,425	1,000	,312	1,000	,046	,553	,177	,420	,111	,554	,845	,314	,213	,085	,086	,695	,426	,695
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Smoker (no=0, ex=1, occasional=2, yes=3,)	Correlation Coefficient	-,139	,250	,264	,017	-,161	-,268	,340	,874	,626	-,501	-,337	-,588	-,250	-,076	-,388	,049	-,602	-,624	-,062	-,277	,083
		Sig. (2-tailed)	,702	,487	,462	,962	,657	,454	,337	,001	,053	,140	,341	,074	,487	,834	,267	,893	,066	,054	,864	,438	,819
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	ECC time (min)	Correlation Coefficient	-,176	,248	,273	,559	,081	,359	-,370	,055	,292	,195	,178	,140	-,248	,152	-,139	-,325	-,316	-,139	,588	,661	,709
		Sig. (2-tailed)	,627	,489	,446	,093	,823	,309	,293	,881	,413	,590	,623	,700	,489	,676	,701	,359	,374	,701	,074	,038	,022
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Clamp time (min)	Correlation Coefficient	-,061	,371	,389	,695	,113	,412	-,267	,012	,366	,348	,332	,287	-,067	,128	-,116	-,369	-,213	,000	,608	,517	,565
		Sig. (2-tailed)	,868	,291	,266	,026	,756	,237	,455	,973	,298	,325	,348	,422	,854	,725	,751	,294	,554	1,000	,062	,126	,089
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Peroperative bleeding (mL)	Correlation Coefficient	,602	,013	,114	,095	,399	,283	,253	,551	,286	-,334	-,744	-,130	,469	,722	,602	,616	,241	,152	,190	,272	,285
		Sig. (2-tailed)	,066	,972	,754	,793	,254	,429	,480	,099	,423	,346	,014	,720	,172	,018	,066	,058	,502	,675	,599	,446	,425
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Postoperative bleding <12h (mL)	Correlation Coefficient	-,018	,224	,115	,255	,688	,432	-,236	,079	,043	,267	,055	,517	-,079	,188	,394	,227	,243	,248	-,067	,442	,442
		Sig. (2-tailed)	,960	,533	,751	,476	,028	,213	,511	,829	,907	,455	,880	,126	,829	,603	,260	,528	,498	,489	,855	,200	,200
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10

Table C6 Correlations for the patients treated with platelet inhibitors, between variables such as age, BMI, type of diagnosis, medication, smoker habits and the ROTEM® and Multiplate® results, in percentage changes. Yellow marks denote for significant correlations.

			dexctf3%	dexctt3%	dexctft3%	dexmcff3%	dexmcft3%	dexmcfft3%	dfibctf3%	dfibctt3%	dfibctft3%	dfibmcff3%	dfibmcft3%	dfibmcfft3%	dadpaucf3%	dadpauct3%	dadpaucft3%	daspiaucf3%	daspiauct3%	daspiaucft3%	dtrapaucf3%	dtrapauct3%	dtrapaucft3%
Spearman's rho	Age	Correlation Coefficient	,340	-,265	-,003	-,103	-,334	,122	-,274	-,426	-,389	-,170	-,322	-,182	-,640	,316	,261	-,215	,140	,036	-,412	,237	,018
		Sig. (2-tailed)	,336	,459	,993	,776	,345	,738	,444	,220	,266	,638	,364	,614	,046	,374	,466	,550	,700	,920	,237	,510	,960
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	BMI	Correlation Coefficient	-,085	-,183	,091	-,310	-,444	-,802	,316	,286	,492	-,353	-,024	-,389	,079	-,426	-,474	-,338	-,170	-,085	,457	-,936	-,620
		Sig. (2-tailed)	,815	,613	,802	,383	,199	,005	,374	,424	,148	,318	,947	,266	,828	,220	,166	,339	,638	,815	,184	,000	,056
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	PLT	Correlation Coefficient	,127	-,201	,006	-,455	-,515	-,624	,552	,345	,406	-,527	,139	-,552	,383	,200	,091	,252	,394	,479	,462	-,673	-,285
		Sig. (2-tailed)	,726	,578	,987	,187	,128	,054	,098	,328	,244	,117	,701	,098	,275	,580	,803	,483	,260	,162	,179	,033	,425
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	EF%	Correlation Coefficient	-,304	-,025	,025	,118	,180	-,043	-,603	-,304	-,317	,118	-,093	,118	-,069	-,541	-,565	-,132	-,652	-,652	-,249	,043	-,130
		Sig. (2-tailed)	,392	,946	,946	,745	,618	,905	,065	,392	,372	,745	,798	,745	,851	,107	,089	,716	,041	,041	,487	,905	,719
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Higgins	Correlation Coefficient	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		Sig. (2-tailed)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		N	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	ASA (no=0, 75mg=1, 160mg=2)	Correlation Coefficient	-,406	-,349	,233	,406	,406	,290	-,406	,058	-,290	,406	,058	,290	,291	,174	-,406	,411	,058	,058	-,407	,174	,290
		Sig. (2-tailed)	,244	,323	,517	,244	,244	,416	,244	,873	,416	,244	,873	,416	,415	,631	,244	,238	,873	,873	,243	,631	,416
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Clopidogrel (no=0, 75mg Plavix=1)	Correlation Coefficient	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		Sig. (2-tailed)	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	LMH (no=0, Arixtra2.5 mg=1)	Correlation Coefficient	,114	,495	-,114	,114	-,038	-,190	,266	,342	,038	,038	-,342	,038	-,381	-,798	-,646	-,269	-,798	-,798	,267	-,342	-,646
		Sig. (2-tailed)	,754	,145	,753	,754	,917	,599	,458	,334	,917	,917	,334	,917	,277	,006	,044	,452	,006	,006	,456	,334	,044
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Angina (no=0, stable=1, unstable=2)	Correlation Coefficient	,000	-,044	,044	,348	,087	,087	-,261	-,087	-,522	,261	,000	,174	,000	-,174	-,435	,264	-,261	-,261	,000	,087	,087
		Sig. (2-tailed)	1,000	,905	,905	,324	,811	,811	,466	,811	,122	,466	1,000	,631	1,000	,631	,209	,460	,466	,466	1,000	,811	,811
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Heart attack (no=0, yes=1)	Correlation Coefficient	-,190	-,305	,000	-,646	-,190	-,342	-,038	-,266	,266	-,570	,114	-,494	,267	,494	,722	-,038	,570	,570	-,191	,038	,266
		Sig. (2-tailed)	,599	,392	1,000	,044	,599	,334	,917	,458	,458	,086	,754	,147	,456	,147	,018	,916	,086	,086	,598	,917	,458
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	Smoker (no=0, ex=1, occasional=2, yes=3,)	Correlation Coefficient	-,053	-,261	,195	-,362	-,191	-,303	,270	-,026	,408	-,276	,257	-,276	,343	,513	,651	-,047	,770	,803	,228	-,059	,454
		Sig. (2-tailed)	,885	,467	,590	,304	,597	,395	,451	,942	,242	,440	,474	,440	,332	,129	,041	,898	,009	,005	,527	,871	,188
		N	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10