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DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

„Association of enoxaparin-mediated anti-Xa activity and the incidence of deep vein thrombosis in intensive care patients“

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Magister der Pharmazie (Mag.pharm.)

Wien, 2020 / Vienna, 2020

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	UA 449
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Diplomstudium Pharmazie
Betreut von / Supervisor:	Assoc. Prof. PD Dr. Eva Schaden

I Abstract

Enoxaparin is a drug in the class of low-molecular-weight heparins and is frequently used in intensive care medicine for the prevention and treatment of venous thromboembolism, particularly deep vein thrombosis and pulmonary embolism. Enoxaparin activates antithrombin III, which then very selectively inhibits coagulation factor Xa. The factor is thus no longer available for the coagulation cascade. This inhibition of the coagulation factor Xa, also known as anti-Xa activity, can be determined and measured by laboratory tests. The manufacturers of enoxaparin recommend monitoring anti-Xa activity in therapeutic doses to prevent accumulation of the drug and the resulting risk of bleeding.

There is great disagreement about the usefulness of measuring anti-Xa activity in prophylactic doses of enoxaparin to monitor sufficient thromboprophylaxis This issue is to be addressed and examined in detail in this diploma thesis.

Retrospectively, patient records of a half year of 6 intensive care units of the General Hospital of the City of Vienna are examined and evaluated. After filtering for enoxaparin administration and presence of anti-Xa activity measurements, a total of 220 patients can be included in this evaluation. The patient files of these patients are examined for documented thromboses, especially deep vein thrombosis. After the research, the measured Anti-Xa activity values are set in relation to the occurrence of thrombotic events.

II Zusammenfassung

Enoxaparin ist ein Arzneistoff der Substanzklasse der niedermolekularen Heparine und wird in der Intensivmedizin sehr oft zur Prophylaxe und Therapie von venösen Thromboembolien, d.h. von tiefen Venenthrombosen und Pulmonalembolien, häufig eingesetzt. Durch Enoxaparin wird Antithrombin III aktiviert, welches daraufhin sehr selektiv den Gerinnungsfaktor Xa hemmt. Der Faktor steht somit nicht mehr für die Gerinnungskaskade zur Verfügung. Diese Hemmung des Gerinnungsfaktors Xa, auch Anti-Xa Aktivität genannt, kann mithilfe von Labortests bestimmt und gemessen werden. Die Hersteller von Enoxaparin empfehlen in hohen therapeutischen Dosierungen eine Kontrolle der Anti-Xa Aktivität, um eine Akkumulation des Arzneistoffs und ein daraus resultierendes Blutungsrisiko zu verhindern.

Über die Messung der Anti-Xa Aktivität in prophylaktischer Dosierung von Enoxaparin um eine ausreichende Thromboseprophylaxe zu überwachen herrscht große Uneinigkeit bezüglich der Sinnhaftigkeit dieses Vorgehens. Diese Fragestellung soll in der vorliegenden Diplomarbeit aufgearbeitet und genau beleuchtet werden.

Retrospektiv werden Patientenakten eines halben Jahres von 6 Intensivstationen des Allgemeinen Krankenhauses der Stadt Wien untersucht und ausgewertet. Nachdem nach Enoxaparingabe und Vorhandensein von anti-Xa Aktivitätsmessungen gefiltert wird, können insgesamt 220 Patientinnen und Patienten in diese Auswertung eingeschlossen werden. Die Patientenakten dieser Patientinnen und Patienten werden nach dokumentierten Thrombosen, speziell nach tiefen Venenthrombosen, untersucht. Nach der Recherche werden die gemessenen Anti-Xa Aktivitätswerte in Relation zum Auftreten von thrombotischen Ereignissen gesetzt.

III Acknowledgements

At this point I would like to thank some people who made this diploma thesis possible.

First of all, I want to thank Assoc. Prof. PD Dr. Eva Schaden, who gave me the great opportunity to write my diploma thesis at the Medical University of Vienna. We had many interesting discussions about the topic and with her expertise she supported me from the beginning. Thank you for also being so enthusiastic about this interdisciplinary cooperation!

I would also like to thank Dr. med.-univ. Christoph Dibiasi for the excellent on-site support. Due to his technical talent he did a lot of preliminary work for this diploma thesis. Despite his own workload, he always took the time to plan and discuss all the single steps with me, explained many procedures in intensive care and made me even more enthusiastic about the topic.

I also want to thank the incredibly warm-hearted team of the intensive care unit 13C1 of the General Hospital of the City of Vienna, where I spent a lot of time. Here I could see many things in practice that I only knew from theory until then.

From the University of Vienna, I would like to thank ao. Univ.-Prof. Mag. Dr. Walter Jäger, who supported me in many organizational fields.

Also, not to be left unmentioned is my family, which has always actively supported me. Thank you for always believing in me and motivating me to continue my studies even in difficult situations. Without you I would never have reached the point of my diploma thesis. I am very grateful to you!

Last but not least I would like to thank all the friends who have accompanied me over the whole time and whom I can always rely on. Thank you for all the hours we spent together, whether it was studying, having fun, having a good time, working in the rescue service or in the student council.

Thank you all!

IV Contents

V Abbreviations

ADP	Adenosine diphosphate
ANOVA	Analysis of variance
aPTT	Activated partial thromboplastin time
AT-III	Antithrombin III
ATP	Adenosine triphosphate
COX-1	Cyclooxygenase-1
CrCl	Creatinine clearance
CT	Computed tomography
CTEPH	Chronic thromboembolic pulmonary hypertension
DNA	Deoxyribonucleic acid
DVT	Deep vein thrombosis
HIT	Heparin induced thrombocytopenia
i.v.	Intravenous administration
ICU	Intensive care unit
INR	International normalized ratio
ISI	International sensitivity index
LMWH	Low molecular weight heparin
MRI	Magnetic resonance imaging
p.o.	Peroral administration
PAI-1	Plasminogen activator inhibitor 1
PDGF	Platelet derived growth factor
PDMS	Patient data management system
PE	Pulmonal embolism
PF4	Platelet factor 4
PTS	Post thrombotic syndrome
RBC	Red blood cell concentrates
s.c.	Subcutaneous administration
t-PA	Tissue-type plasminogen activator
TF	Tissue factor
TPA	Tissue plasminogen activator
TXA_2	Thromboxane A_2
u-PA	Urokinase-type plasminogen activator
UFH	Unfractionated heparin
VKA	Vitamin K antagonist
VTE	Venous thromboembolism
vWF	Von Willebrand factor
WHO	World Health Organization

VI List of Figures

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VII List of Tables

1 Introduction

1.1 Human Blood

"Blood is thicker than water" – German proverb

In fact, blood and water have very similar densities (0.994 and 0.998 g/ml, 23°C).[1] There are many myths and legends surrounding blood, no other body fluid is as important in literature as human blood. Although blood has aroused the interest of people for thousands of years, it is still in the main focus of research today.

The interest of mankind in blood is probably related to its essential connection with life. It is crucial that the blood circulates continuously through the body, otherwise our lives will quickly come to an end. All organs are in constant contact with each other through the blood, be it through oxygen, nutrients, metabolic products and messenger substances. The blood itself is called a liquid body organ. Besides its function as a transport and communication system, it is also important because of its defense function against pathogens. Another important component, one that this thesis will deal with in detail, is the coagulation system. It protects the body from bleeding out in the event of injury and initiates repair of injured tissue.[2]

Blood makes up 6-8% of the fat-free body mass of an adult. The composition of the blood can be divided into blood plasma and cellular components. The cells present in the blood include erythrocytes, leukocytes and platelets. Erythrocytes, also called red blood cells, take over the oxygen transport already mentioned. The entirety of their mass is also called hematocrit. Leukocytes, white blood cells, are crucial for the defense against pathogens. Platelets, also known as thrombocytes, are essential for primary hemostasis, as described in chapter 1.2.1.[2,3]

The aqueous part of the blood is called plasma. 10% of plasma is dissolved substances, two thirds proteins and one third low molecular substances. Among the proteins are coagulation factors such as prothrombin (factor II) and fibrinogen (factor I). Antithrombin, as the antagonist of thrombin activated from prothrombin, is also present in the blood and mediates a balance between coagulation and anticoagulation. Plasminogen, the precursor of plasmin, is important for dissolving clots (i.e. fibrinolysis).[2,4]

1.2 Hemostasis

Blood coagulation represents a highly sophisticated defense mechanism to avoid exsanguination following body injury. The main features of our current understanding were first presented in 1964 as the Waterfall/Cascade model.[5] Essentially, coagulation can be divided into two main parts, the primary hemostasis and the secondary hemostasis. The interplay of both forms a clot and stops the bleeding.[6]

Five components are highly relevant for hemostasis: Endothelial cells of blood vessels, platelets, coagulation factors, coagulation inhibitors and fibrinolysis. Hemostasis forms an interaction of all components.[4]

1.2.1 Primary Hemostasis

Primary hemostasis starts with injury to vascular endothelial cells. When circulating platelets come in contact with subendothelial collagen, it triggers an accumulation of platelets at the site. Subendothelial tissue factor activates the extrinsic pathway of secondary hemostasis (see 1.2.2).[7,8]

Thrombocytes, or platelets, are anucleate, discoid cells with a length diameter of 1.5 to 4 µm. In healthy humans there are 160,000 up to 300,000 platelets per microliter of blood. They are split off megakaryocytes in the bone marrow and have a lifetime of approximately 7-10 days.[4,9]

Primary hemostasis consists of four steps: Platelet activation, adhesion to endothelium, secretion and aggregation. Activated platelets recruit other platelets and activate coagulation factors as part of secondary coagulation (see 1.2.2). Glycoprotein receptors, to which e.g. von Willebrand factor, fibrinogen and other platelets can bind, are also attached to the membrane.[4]

Table 1: Membrane proteins of platelets

The most important membrane proteins of platelets, their role under physiological conditions and clinical significance.[4]

Membrane protein	Role	Clinical significance
GP1a	Binds collagen Activated intracellular pathways leading to thromboxane A2 (TXA_2) generation	Aspirin suppresses TXA_2 synthesis by inhibiting COX
GP1b	Binds von Willebrand factor	Defective in Bernard Soulier disease – results in bleeding disorders
GPVI	Binds collagen	GP VI Absence results in severe bleeding diathesis
GPIIb/IIIa	Binds fibrinogen and von Willebrand factor Binding site for other platelets in aggregation	Defective in Glanzmann's thrombasthenia – results in bleeding disorders
Membrane phospholipid	Activates coagulation factors	Activates factor X → Xa and factor II → IIa in combination with factor VIIIa and IXa, respectively factor Xa
P2Y12	Activated by ADP, leads to generation of TXA_2 and aggregation	P2Y12 Inhibited by clopidogrel and ticagrelor

1.2.1.1 Platelet activation

The activation of platelets can be triggered e.g. by subendothelial collagen, circulating ADP or serotonin. As shown in 1.2.1, collagen binds to the membrane receptors GP1a and GPVI, which activates cyclooxygenase-1 and thereby produces thromboxane A2. TXA_2 leads to vasoconstriction, recruitment of other platelets via TXA_2 receptors and platelet aggregation.[4,8,10]

1.2.1.2 Platelet aggregation

Platelet cross-linking is mediated via GPIIb/IIIa receptors. After platelet activation, GPIIb/GPIIIa receptors change their conformation, whereupon they can bind to fibrinogen. Platelets can therefore interact with the fibrin network generated in secondary hemostasis and form an important structural component of the blood clot.[4,10]

1.2.1.3 Platelet secretion

Thrombocytes contain two types of storage granules. The α-granules contain p-selectin, fibronectin, fibrinogen, factor V & VIII, platelet factor 4 (PF4), tumor growth factor-α and platelet derived growth factor. The dense granules contain adenosine di- and triphosphate, Ca^{2+}, histamine, serotonin and epinephrine. In total, these granules contains more than 300 different substances.[4,10]

Procoagulants, e.g. ADP and TXA_2, are released by platelets in a secondary wave after initial activation and create a positive feedback mechanism. The substances contained in the granules such as serotonin, fibrinogen, fibronectin and PDGF are also released.[4,10]

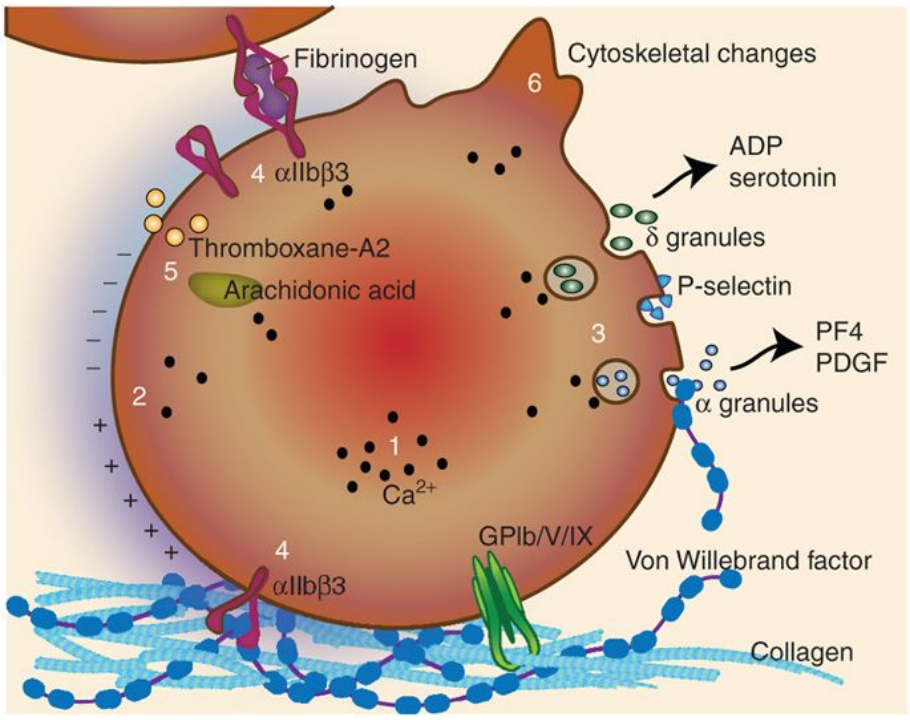

Figure 1: Activated platelet and schematic process of primary hemostasis

Subendothelial collagen, which has normally no contact to blood and the contained cells, is exposed after blood vessel injury. GPIb/V/IX receptor complex binds to von Willebrand factor, which is bound to collagen. The platelet adheres and gets activated. When activated, the intracellular Ca^{2+} level increases (1), which in turn results in exposure of phosphatidylserine and a negative charged surface (2). α- and dense granules release procoagulants, as ADP, serotonin, PF4 and PDGF (3). Because of these procoagulants, GPIIb/IIIa receptors change their conformation and are highly affine to Fibrinogen (4). COX-1 produces TXA_2 from arachidonic acid (5), through activation, the platelet changes its shape (6).[11]

1.2.2 Secondary Hemostasis – Plasmatic coagulation

Secondary hemostasis is traditionally represented by the cascade model. In this model, three pathways can be distinguished: intrinsic and extrinsic pathway, both leading to the common pathway. The clotting factors in these pathways are mostly serine proteases, which are activated one after the other. Finally, prothrombin is activated to thrombin, which in turn converts soluble fibrinogen to insoluble fibrin. The solid fibrin forms the clot. This process takes place in parallel with primary hemostasis, thus forming a thrombus of activated platelets and fibrin.[5,4]

A paradigm shift in hemostasis lead to the currently used cell-based model, which emphasizes that plasmatic coagulation takes place on cell surfaces. Here 3 states can be distinguished: initiation, amplification and propagation.[5,4]

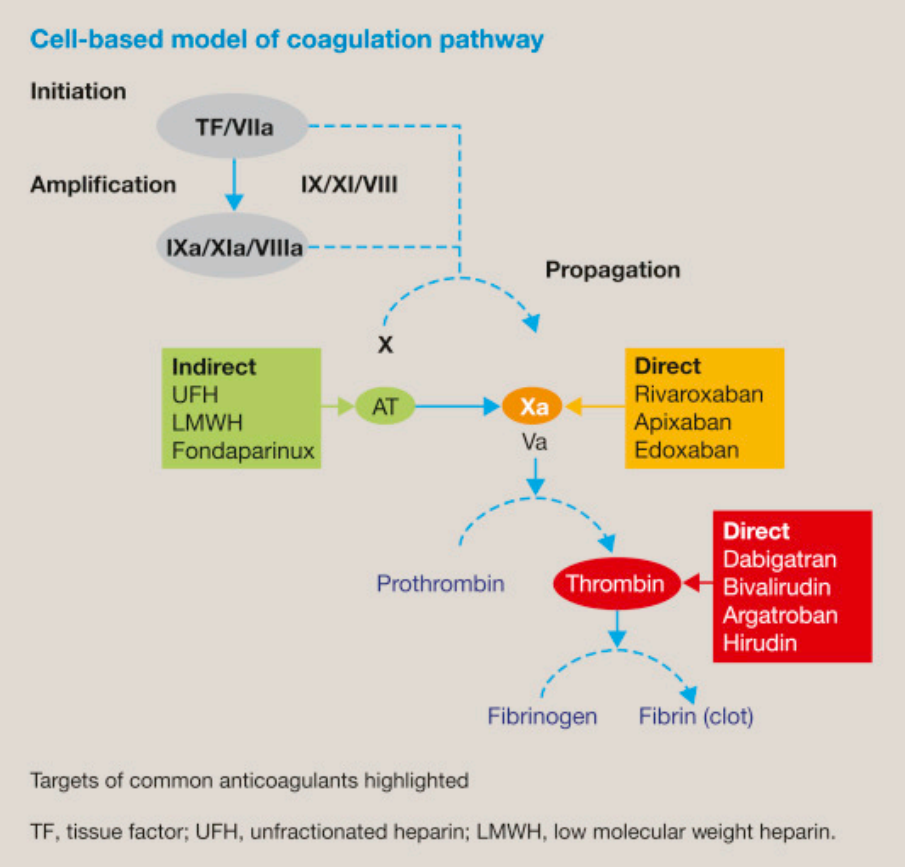

Figure 2: Cell-based model of coagulation

Coagulation is divided to three phases: initiation, amplification and propagation. Tissue factor is released by an initial stimulus, after which the signal is amplified. Finally, insoluble fibrin is formed from soluble fibrinogen, which forms a stable clot.[4]

1.2.2.1 Initiation Phase

In the initiation phase, tissue factor is released after vascular injury, which activates coagulation factor VII to factor VIIa. The complex of factor VIIa and TF in turn activates factor IX and X. Factor X is quickly deactivated by antithrombin and tissue factor pathway inhibitor. A small amount of thrombin is generated.

This thrombin in turn activates platelets and factor VIII and V, which are responsible for an increase in thrombin generation. In addition, factor XI is activated by the initial thrombin, which leads to amplification.[5,4,7]

1.2.2.2 Amplification Phase

The signal from the tissue factor-releasing cells is therefore transmitted to the surface of the platelets. As already described in 1.2, the platelets interact with exposed collagen via von Willebrand factor. As a result, the platelets are partially activated and adhere to the injury site. Complete activation is achieved by thrombin from the initiation phase. The factors V, VIII and XI are activated by thrombin as well. It also binds to protease-activated receptor 4 on the surface of the platelets, which induces a release of ADP, thromboxane A_2 and serotonin. These transmitters in turn activate other platelets, which then release factor V from their α-granules. Factor V is subsequently activated from thrombin or factor Xa.[5,4]

1.2.2.3 Propagation Phase

Factor VIIIa binds to factor IXa, which was formed in the initiation phase. They form a highly active complex on the platelet surface. This complex activates factor X very strongly. Factor Xa, with its cofactor VIIa and Ca^{2+} ions, produces thrombin from prothrombin in a highly effective manner.[5,4]

1.2.2.4 Fibrinogen conversion

Fibrinogen is a symmetrical dimer which consists of 3 pairs of 3 entwined polypeptide chains, held together by disulfide bonds. These chains extend from the central core in opposite direction forming the peripheral D domains. In the center the E domain consists. Thrombin cleaves short segments of the NH_2-termini of two chains in the E domain. This creates a surface structure that binds into notches in the D domains and polymerization to protofibril begins. Factor XIIIa, which acts as a transglutaminase and is activated by thrombin, enhances the binding of fibrin molecules by cross-linking the D domains.[12]

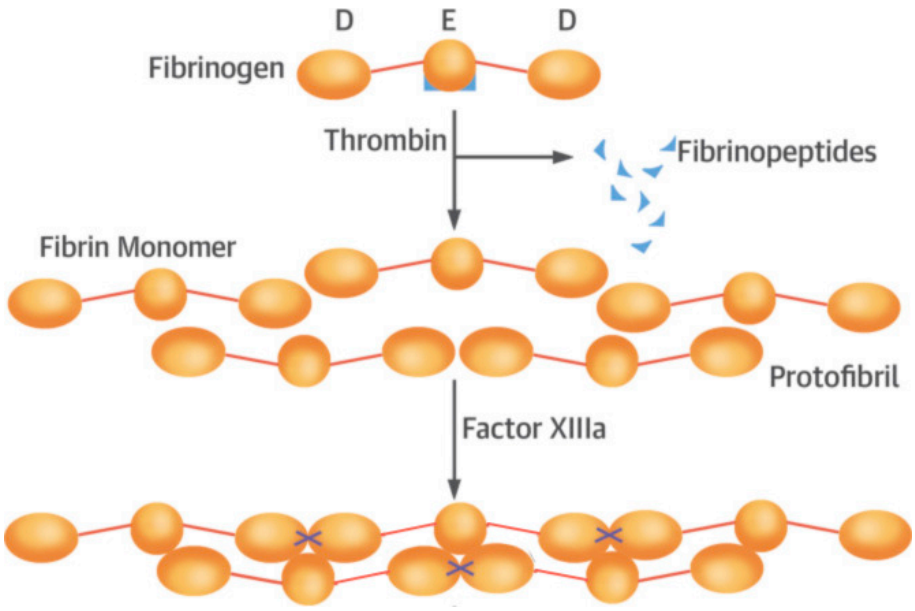

Figure 3: Fibrinogen conversion

Thrombin cuts off fibrinopeptides at the E domains of fibrinogen. These can then bind to the D domains of other fibrinogen monomers and polymerization begins. Factor XIIIa cross-links the D domains. Modified from [12]

1.2.2.5 Coagulation inhibitors

Coagulation should only occur at the site of injury. If the clot would spread further, it would manifest in thromboembolic events. Therefore, there are naturally occurring coagulation inhibitors, which maintain balance in the coagulation system. The main representatives are tissue factor pathway inhibitor, heparin co-factor II, antithrombin, protein C and protein S.[5,4]

In the initiation phase, TFPI intervenes by deactivating factor Xa, VIIa and tissue factor. It is synthesized in the endothelium and stored in platelets. TFPI also occurs in small amounts in the blood. Heparin is also found in the blood. When heparin binds to antithrombin, its affinity to factor Xa is increased thousand times or more and inactivation of factor Xa is very likely.[5,4]

The two serine proteases protein C and protein S inhibit factor Va and factor VIIIa. Protein C and Protein S are activated by thrombomodulin, which in turn is activated by thrombin. Protein S serves Protein C as a cofactor and increases its effectiveness.[5,4]

1.2.3 Fibrinolysis

As persistent blood clots would disrupt physiological blood flow, clot break-up is necessary after blood vessel injury is repaired. This is facilitated by the main fibrinolytic enzyme plasmin, which cleaves the connection between D and E domains of fibrin fibers. A (DD)E intermediate product is formed in which the two D fragments are non-covalently bound to the E fragment. Subsequently, the E fragment is split off, leaving the covalently bound D-dimer. The concentration of D-dimer in the blood is an important parameter for identifying coagulation processes and the resulting fibrinolysis in the body (see 1.3.3.2).[12]

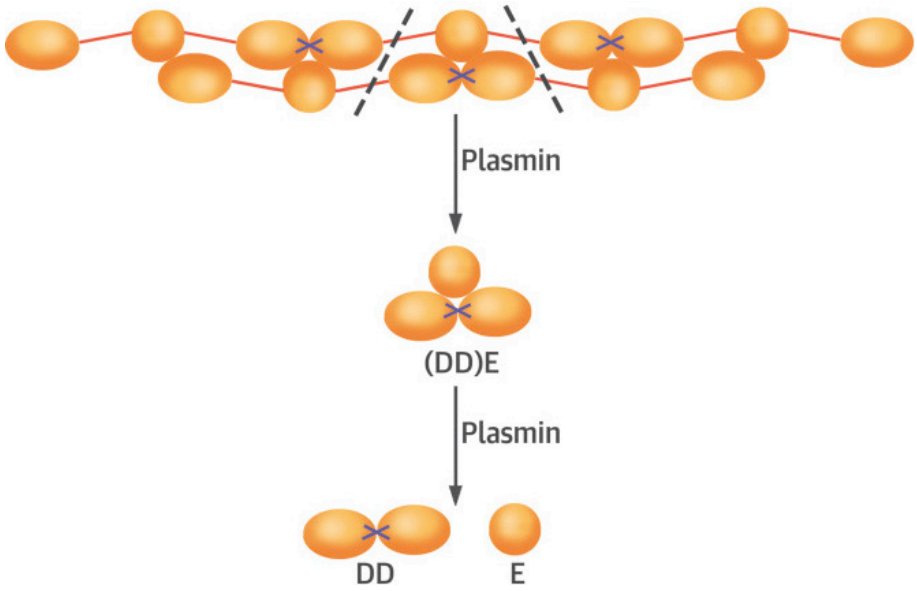

Figure 4: Degradation of fibrin

Plasmin degrades fibrin strands to (DD)E fragments, from which the noncovalent bound E fragments dissociate. What remains are the D-dimers, which can be measured in the blood as a marker for coagulation processes. Modified from [12]

Tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) convert plasminogen to plasmin. In addition to procoagulant substances, t-PA is also present in the endothelial cells and is secreted when injured. Thrombin and vasoactive substances can also induce t-PA. Fibrin forms the cofactor of t-PA and ensures the activation of plasminogen is localized at the thrombus site.

The fibrinolytic system has feedback mechanisms as well. Plasminogen activator inhibitor 1 (PAI-1) targets t-PA and u-PA and inactivates them.[5,4,13]

1.3 Deep vein thrombosis

Deep vein thrombosis (DVT) is one of the most widespread causes of mortality and morbidity in this world.[14,15] It represents, like pulmonary embolism (PE), a subset of venous thromboembolism (VTE) which affects every 1 - 1.6 per 1000 people annually. DVT is ultimately responsible for two thirds of these cases. The last third will be covered by PE, a potentially life-threatening complication of DVT.[14,16]

Clinically, DVT is characterized by an edematous, red and painful extremity. Pruritus, paresthesia and cramps can also occur.[17,18] Untreated, DVT leads to PE in 50% of those affected, which leads in 10% of individuals to death within one hour of the onset of the first symptoms.[17]

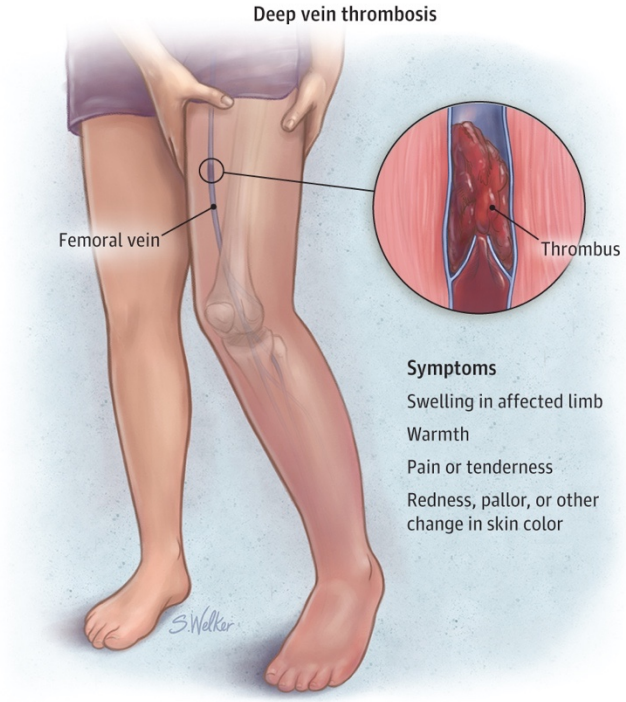

Figure 5: Signs and symptoms of deep vein thrombosis

Deep vein thrombosis shows typically as swollen, warm, red and painful extremity. The thrombus forms in a deep vein and prevents the outflow of blood.[19]

Long term morbidity of DVT is caused to a large extent by development of post-thrombotic syndrome (PTS).[14] Within two years after DVT this concerns 43 - 50% of patients. The symptoms of PTS include leg swelling, leg pain, and venous ulcers in severe cases.[14,17] Post-thrombotic syndrome results from elevated blood pressure in the venous system, which is caused by the congestion of blood due to the disturbed outflow

caused by DVT, as well as venous insufficiency. PTS leads to a significant reduction in quality of life, comparable to chronic diseases such as obstructive lung disease, congestive heart failure and diabetes mellitus.[20]

Usually, the lower limb will be affected by DVT. These occur in different sites with varying frequency. The largest amount concerns the distal veins with 40%, followed by the femoral and common femoral veins with 20% each. Popliteal veins are affected up to 16% and iliac veins up to 4%.[16] If thrombi form in the popliteal vein, the symptoms usually appear in the calf area. Further proximal, in the femoral vein, thrombosis usually presents with fulminant symptoms, which affects the whole leg. This is accompanied by a stronger swelling, more pain and more redness.[17]

The upper limbs are only affected less than 10 percent in general. Very rare are thrombi in the vena cava, they are related to malignancy, vascular abnormalities and compression.[16]

1.3.1 Pathogenesis

Traditionally, the pathogenesis of VTE is illustrated by Virchow's Triad: venous stasis, vascular injury and hypercoagulability.[14,15,21,22]

Rudolf Virchow was a German pathologist and researched the causes of thromboses and pulmonary embolisms. In 1856, he was the first to describe a blood clot as a thread-like network. He recognized that blood cells were embedded in this network. He also found that a thrombus does not form *de novo* in pulmonary arteries, but is formed in the periphery and then washed in.[21]

The majority of DVTs develops around the valves in the venous system. This is proved by many autopsy studies.[14,22] Usually, the valves are responsible to ensure a continuous blood flow through the veins. But they are potential locations for venous stasis as well.[14] In the sinuses next to the valves the blood flow decelerates, which leads to stasis – one of Virchow's conditions of thromboembolism.[14,15] It has been shown that contrast media requires on average up to 27 minutes to leave the valves.[22] Venous stasis represents thereby the most consequential factor but it is not able to lead to thrombus formation by itself.[14]

In addition to stasis, hypoxia occurs in the affected area. When the blood flow decreases, the oxygen supply decreases as well. This has the effect of reducing the function of antithrombotic proteins like thrombomodulin or endothelial protein C receptor,

which are expressed on venous valves. Furthermore, procoagulants are expressed in hypoxia.[14,22] One of them is P-selectin, an adhesion protein that binds neutrophils and monocytes containing tissue factor to the endothelium. Although it is not yet clear whether tissue factor is released by immunologic cells in extravascular tissue or by the endothelium, it is generally agreed that tissue factor plays a central role in thrombus formation.[14,15,22] All in all the venous stasis and the effects of hypoxia may result in a hypercoagulative microenvironment around the venous valves.[14,22]

Blood vessel damage seems able to induce clot formation as well: If the endothelium is injured, it uncovers tissue factor from the subendothelial membrane, which thereupon induces thrombosis. However, there are pathology studies that have not identified vessel damage in the area of venous thromboses.[15,22] An exception are thrombi after surgeries.[22] The main role of the endothelium seems to be more related to the recruitment or activation of immunological cells and platelets as well as to the reduction of the expression of anticoagulant proteins.[14,15]

The clinical conditions leading to DVT are basically related to the Virchow's Triad: prolonged immobility, surgery or trauma, malignancy, pregnancy, varicose veins, congestive heart failure, advancing age, obesity and a medical history of DVT.[14] Furthermore, it is now known that high levels of coagulation factors or defects of natural anticoagulative factors can significantly increase the risk of thrombosis. These genetic defects are associated with other risk factors as described in 1.3.2.[22]

Venous thrombi are usually quite similar in structure. On the inside there is a white thrombus, which is rich in platelets. The core is surrounded by a scaffold, which consists of fibrin complexed with extracellular DNA and histones. These characteristics of the outer layer are subsequently important for thrombolysis by tissue plasminogen activator (TPA).[14]

1.3.2 Risk factors

Although a large part is already known about VTE today, one third to a half of all incidents have no clearly explainable cause. These events are summarized as idiopathic.[18]

Due to a lack of exercise and the resulting blood stasis, there are many risk factors for VTE. These include travelling for more than 4 hours, fractures and consequent immobilization, paralysis and bedriddenness. Congestion of blood flow caused by varicose

veins also leads to VTE.[23] All in all, there are a lot of risk factors, some are hereditary, some are acquired, and some can be modified.[15,18]

1.3.2.1 Age

One of the strongest unmodifiable risk factors is the age of the individual.[14,15,23] VTE is very rare below an age of 45 years. After that, the incidence rises very strongly. For instance, at 85 years of age the expected risk for VTE is ten times higher than for 45-year-olds.[15] Although age is a risk factor in its own, it is difficult to establish a direct link with VTE. In most cases, age leads to the occurrence of various accompanying risk factors. These include obesity, prolonged periods of immobilization and increased occurrence of chronic diseases, cancer or other comorbidities. In addition, with higher age the balance between naturally occurring procoagulants and anticoagulants, such as protein C, in the body shifts.[14]

1.3.2.2 Hormones

An increase of risk in women taking oral contraceptives is described. Compared to non-users ethinylestradiol and progestin, two substances used as combined hormonal contraceptive, quadruple the risk for VTE.[24] Although the risk is increased, just 7 in 10,000 women suffer thromboses during intake of oral contraceptive therapy.[24] This is still lower than in the case of pregnancy, where 20 out of 10,000 women are affected.[23] To prevent peripartum hemorrhage, pregnancy leads to an hypercoagulative state.[25] In comparison to pregnancy, VTE occurs 5 times more frequently in postpartum period. Hormone replacement therapy, used in postmenopausal women, is also an additional risk factor that increases the possibility for VTE.[23]

1.3.2.3 Obesity and metabolic syndrome

In contrast to the age, obesity is considered one of the strongest modifiable risk factors for VTE. Above a BMI of 30 kg/m^2, the risk of suffering a VTE increases 2 to 3 times compared to normal BMI. As obesity is on the rise in population, the risk of thrombosis is expected to continue to increase.[15] High body weight can lead to a mechanical impairment of the venous valve system. This mainly affects the valves in the lower extremities. The resulting stasis leads to a risk of thrombosis.[26]

Metabolic syndrome is defined by the presence of obesity, glucose intolerance, hypertension and hyperlipidemia and leads to a procoagulant state with elevated serum levels of fibrinogen. The metabolic syndrome is also accompanied by endothelial dysfunction, which leads to increased serum concentrations of TF, vWF and PAI-1, whereas the anticoagulant protein C level is decreased. In addition, adipocytes release proinflammatory messenger substances.[26]

1.3.2.4 Care patients

Hospitalized patients and nursing home residents are also at risk to suffer VTE. In these cases, an increased risk of up to 100 times higher compared to general population can be expected. In total, hospitalized patients and nursing home residents account for almost 60% of the VTE incidents.[15,23] Surgical patients make up a large proportion of those affected. In these patients, long periods of immobilization lead to blood stasis, which in turn leads to thrombotic events. Patients with major orthopedic surgeries are at a high risk here, as this group has particularly long periods of immobilization.[15] In the literature up to 1% incidence is reported despite pharmacological prophylaxis.[27] Neurosurgery, thoracic, abdominal and pelvic surgery are also associated with a high risk of VTE.[15,23]

Besides surgical patients, however, cancer patients are also frequently affected, especially those with metastases.[15] This is caused on the one hand by the disease itself, on the other hand also by its treatment, because immunosuppressive therapy and cytotoxic chemotherapy increase the risk of thrombosis.[23]

Another trigger for VTE are central venous catheters. Compared to subclavian access, femoral venous catheters lead to the highest incidence. Besides these catheters, transvenous pacemakers also lead to VTE.[23]

1.3.2.5 Critically ill patients

Critically ill patients in intensive care units have almost twice as much risk of developing VTE as patients in normal wards.[15] As noted in chapter 1.3.2, certain measures performed in intensive care units, such as prolonged immobilization, administration of blood products and central venous catheterization, increase the risk of VTE.[15,28] Studies estimate the risk of developing VTE to be between 5 and 37%. This large range is due to the heterogeneity of patients, as there is no standard intensive care patient.[15]

Furthermore, DVT in critically ill patients often does not manifest itself in the classic symptoms and is therefore overlooked.[15,28]

For the critically ill, the occurrence of DVT means an extension of the duration of mechanical ventilation of 4.85 days and a 7.28-day longer stay in the ICU. The hospital mortality of patients does not increase significantly with DVT (RR 1.31; 95% CI, 0.99 - 1.74; p=0.06), but an upward trend can be observed.[29]

1.3.2.6 Hereditary diseases

Hereditary diseases can also increase the incidence of DVT.[18,30] Factor V Leiden is the most common, occurring in about 3-7% of Europeans. Here, a missense mutation of the factor V polymorphism occurs. As a consequence factor Va is 10 times slower inactivated by activated protein C (APC resistance) and increases thrombin generation. Compared to general population affected persons with heterozygous defects have a 3 to 5 times higher risk to suffer VTE, whereas homozygous defects lead to 10 times heightened risk. Nevertheless, the overall risk of developing VTE is relatively low. Only 5% of patients develop VTE by the age of 65.[30]

Prothrombin gene mutations affect 1-2% of the European population. Prothrombin levels are elevated in this disease. As a result, the risk of VTE is 2 to 4 times higher.

Less frequently, Protein C deficiency (0.3%) and Protein S deficiency (0.1%) occur. Analogous to factor V Leiden, the inactivation of coagulation factor Va is inhibited and thrombin generation is increased.[30]

Antithrombin deficiency, which occurs in 0.02% of the population, can be divided into two classes: Type I and type II. Type I has a quantitative defect, the molecule is produced less. A qualitative defect is present in type II, where the molecule functions abnormally.[30]

Table 2: Overview of risk factors for the occurrence of venous thromboembolism

The risk factors for the occurrence of venous thromboembolism may have clinical and environmental causes or be hereditary.[18]

Clinical and environmental risk factors	Hypercoagulability	Older age
		Active cancer
		Antiphospholipid syndrome
		Estrogen therapy
		Pregnancy or puerperium
		Personal or family history of VTE
		Obesity
		Autoimmune and chronic inflammatory diseases
		Heparin-induced thrombocytopenia
	Vascular damage	Surgery
		Trauma or fracture
		Central venous catheter or pacemaker
	Venous stasis or immobilization	Hospitalization for acute medical illness
		Nursing-home residence
		Long-haul travel for more than 4 h
		Paresis or paralysis
Heritable risk factors		Factor V Leiden
		Prothrombin 20210G→A mutation
		Antithrombin deficiency
		Protein C deficiency
		Protein S deficiency
		Non-0 blood group

1.3.3 Diagnosis

DVTs show, as already noted in 1.3, through their cardinal symptoms of pain, swelling and redness.[14,17] Nevertheless, an exact diagnosis often requires additional laboratory and imaging tests.[17]

1.3.3.1 Wells score

The best known and most common score for risk estimation of DVT is the Wells score.[31] Patients with a score of 3 or more are considered as high risk patients, 74.6% of which develop VTE. Patients with a score of 2 or 1 are considered moderate risk (16.6% risk of VTE), zero or less means low risk (3.0% risk of VTE).

Table 3: Wells Score 1997

For risk assessment of VTE. Values greater than or equal to 3 indicate a high risk.[31]

Clinical feature	Score
Active cancer (treatment ongoing or within previous 6 months or palliative)	1
Paralysis, paresis, or recent plaster immobilization of the lower extremities	1
Recently bedridden for more than 3 days or major surgery, within 4 weeks	1
Localized tenderness along the distribution of the deep venous system	1
Entire leg swollen	1
Calf swelling by more than 3 cm when compared with the asymptomatic leg (measured 10 cm below tibial tuberosity)	1
Pitting edema (greater in the symptomatic leg)	1
Collateral superficial veins (non-varicose)	1
Alternative diagnosis as likely or greater than that of deep-vein thrombosis	-2

In 2003 the Wells score was modified, since then +1 point is also awarded for a previous diagnosed DVT.[14,16]

However, this modified score is highly specific to exclude DVT. If a patient's medical history shows a low value in the risk calculation using the Wells score, then patients can be assumed not to have DVT. In combination with a negative D-dimer test the negative predictive value is 97,7%, an additional duplex sonography will not be needed.[17] It must be noted that this high predictive power only affects patients with a low pretest probability. In high risk patients the predictive power is weakened.[16]

1.3.3.2 D-dimer

Whenever clots are degraded by the fibrinolytic system (see 1.2.3), D-dimer, a soluble fragment of fibrin, remains.[12,14,16] D-dimer is therefore a valuable marker for detecting coagulation processes and resulting fibrinolysis in the body.[12] It can be measured in the blood or in the plasma and the sensitivity for diagnosis of DVT is estimated above 75%.[14,16]

However, the specificity of D-dimer to detect DVT is low and is reported in the literature to be between 26% and 83%. It may be elevated in post-surgical patients, or due to trauma, inflammation, infection, malignancy, liver disease or pregnancy.[14] Therefore, the measurement of D-dimer is only useful in low risk patients (see Wells score in 1.3.3.1) for exclusion of thrombosis. In high risk patients, a deflection is already to be expected on the basis of the underlying disorders and is therefore not useful. In this case diagnostic imaging is indicated.[14,16]

1.3.3.3 Imaging methods

The current standard of care is two-point duplex sonography, which is non-invasive and save, cost-effective, highly specific and sensitive.[14,16] In addition, it can be performed directly at the bedside.[32] This examination should be performed in all patients with a high risk for VTE (see 1.3.3.1 Wells score) and in patients with a low risk but positive D-dimer test.[17] In addition to the presence of DVT, the size, the resulting stenosis and the chronicity can be determined very accurately.[14]

During the examination, the vein of interest is visualized in ultrasound, after which it is carefully compressed by the transducer. Under physiological circumstances, it should be compressible, and the vein should collapse. If the vein cannot be compressed, this is a strong indication for a DVT. The vein is followed proximally, then the test is performed again. In this way DVTs can be localized very precisely.[14,32]

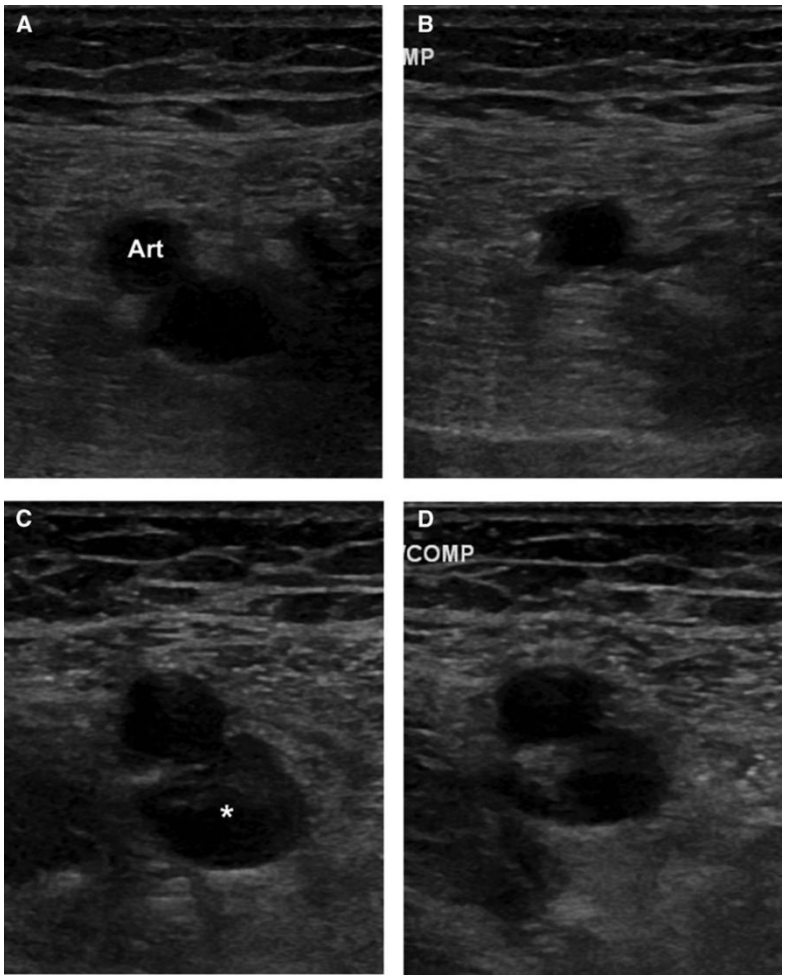

Figure 6: Ultrasound images of normal femoral vein vs. acute DVT

Normal femoral artery and vein physiologically (A) and compressed (B). In (B) the vein collapses because of the examination's applied pressure. Acute DVT uncompressed (C) and non-compressible (D). In (C) an expansion of the vein is visible because of the higher pressure in the venous system. In (D) the vein is deformed to an oval shape, but it is not compressible.[33]

Venography is another method to detect DVTs. There are three different methods of this: Conventional contrast venography, computed tomography (CT) venography and magnetic resonance (MR) venography.[14,17]

The gold standard for the diagnosis of DVT in lower limbs is the conventional contrast venography. However, this is accompanied by a number of disadvantages, including patient discomfort, inability to perform in the case of contrast agent allergy or renal insufficiency, local availability restrictions and possible inadequate visualization. If contrast venography is nevertheless requested, a dorsal vein is cannulated at the foot and contrast agent is infused. At the same time, a tourniquet is attached to the proximal

end of the leg. Now x-rays are taken to visualize the desired vein. If the vein does not fill at a certain point in different images, DVT can be assumed.[14]

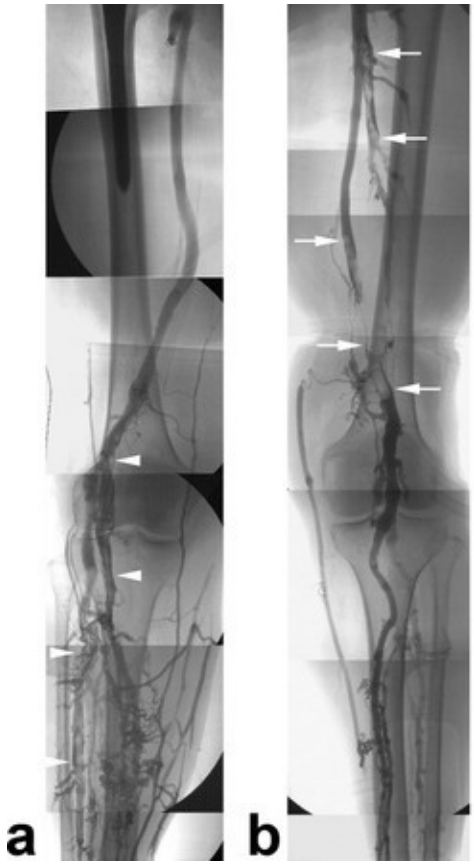

Figure 7: Contrast venography

Bilateral DVT detected in a 76-year-old woman 14 days after hip surgery (right). (a) and (b) are conventional contrast venography. (a) shows the right leg, which was operated. A thrombus is present in the calf and extends till the popliteal vein. (b) shows the left, not operated leg. Here a fulminant thrombosis in the femoral vein is visible. Modified from [34]

Another approach is the CT Venography. Here, contrast medium is infused as well. The opacity of the venous system is timed with the imaging, so DVTs can be detected very sensitively and specifically. CT venography is a particularly useful procedure for suspected PE in connection with DVT. Similar to conventional venography, CT venography is not always feasible, for example in cases of allergy or renal insufficiency. In addition, exposure to ionizing radiation should be considered.[14]

MRI venography offers many of the same advantages. In addition, ionizing radiation can be avoided. Compared to CT venography, the sensitivity and specificity of MRI venography to detect DVTs is very similar.[14]

Magnetic resonance direct thrombus imaging (MR-DTI) can be used to determine the age of the thrombus. Fresh thrombi have a higher level of methemoglobin, which has paramagnetic properties. This allows direct imaging of the thrombus and differentiation between acute thrombotic events and persisting or evolving clots.[35]

If for some reason no contrast agent can be applied, several pulse beats can be recorded in sequence by MRI. In this non-contrast MR venography (NC-MRV), the deep venous system is also visualizable and filling defects can be detected.[14,35]

The disadvantages of MRI venography are due to the MRI technology itself. This is the most expensive of all mentioned procedures, and the corresponding equipment is often not available. Sometimes patients do not tolerate an MRI examination. Nevertheless, MRI venography is becoming more and more important, especially as a substitute when ultrasound examination is not feasible.[14]

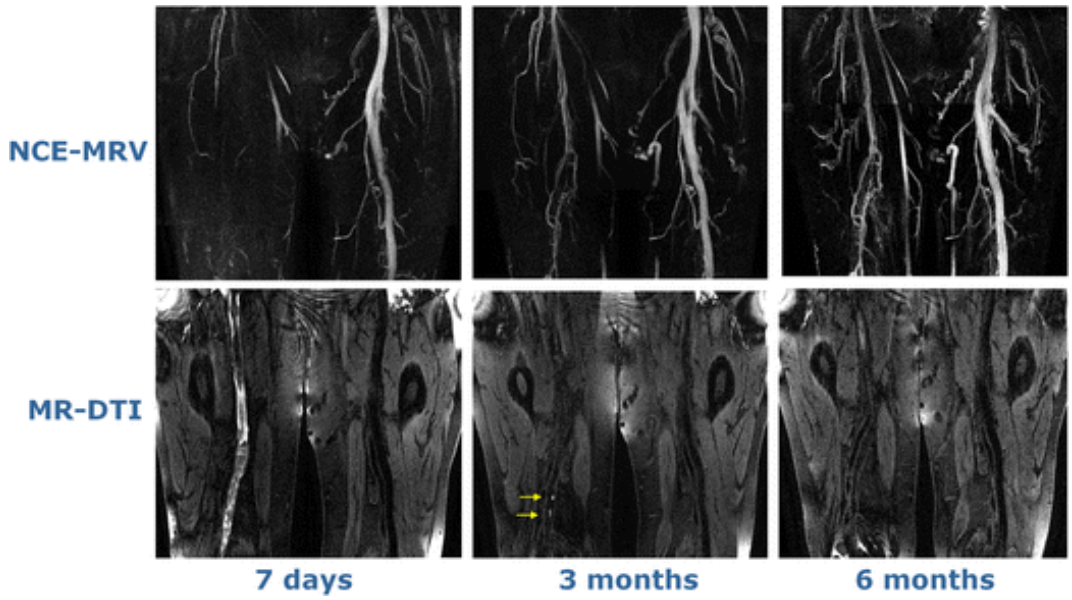

Figure 8: NC-MRV and MR-DTI

In non-contrast magnetic resonance venography (NC-MRV), the occlusion of the femoral vein is visible in all three examinations. In magnetic resonance direct thrombus imaging (MR-DTI), the fresh thrombus is clearly visible in the first examination, only small parts in the second and no more at all in the third.[35]

1.3.4 Complications

The most acute complication of DVT is PE.[15] Here, pulmonary arterial blockage occurs due to a clot that dislodged from the original DVT site.

Symptoms are shortness of breath, chest pain, sweating and palpitations. Since the symptoms are very non-specific, PEs are often detected very late, which leads to an increase in mortality.[36]

A common chronic complication of DVT is the post thrombotic syndrome. It is characterized by pain, swelling, edema, leg heaviness, skin discoloration and venous ulceration. Venous ulcers are usually found around the ankle, usually recognizable by discoloration of the skin, edema and exudation. In the severe progression PTS can even lead to the loss of a limb. Additionally, PTS significantly increases the probability of recurrence of VTE.[15,16]

Chronic thromboembolic pulmonary hypertension (CTEPH) is also a possible complication, but rarer. CTEPH is manifested by progressive dyspnea, which can occur after 1-2 years after a VTE. This results in a massive reduction in the quality of life, which can only be remedied by surgery.[15]

1.3.5 Outcome and prognosis

PEs cause a large part of cardiac arrests and are thus responsible for 4 - 20% of deaths in hospitals, as shown by autopsy studies. Up to one third of patients affected die within the first 3 months after presentation.[15]

Patients who have survived a VTE have an increased risk of developing it again. Within the first decade after the initial occurrence of a VTE, the cumulative probability that such an event will occur again is 30% with a similar mortality rate. The risk factors for recurrence are the same as those mentioned in chapter 1.3.2 for an initial occurrence of VTE.[15]

1.3.6 Antithrombotic therapy

1.3.6.1 Treatment of acute deep vein thrombosis

Untreated DVT can lead to symptomatic PE in 50% of all cases, with very high mortality: 10 % of patients with PE die within one hour of symptom onset. It follows that proper treatment of DVT is absolutely necessary to avoid occurrence of PE as well as propagation of the thrombus and recurrence of DVT.[17]

Guidelines recommend three phases of anticoagulation: initial phase, long-term phase and extended phase. The initial phase concerns the first week after diagnosis, here the decision has to be made between using direct-acting oral anticoagulants (DOACs) or vitamin K antagonists (VKAs). If the second option is chosen, additional concomitant parenteral anticoagulation is required to bridge at least five days until vitamin K antagonists act effective. The DOACs dabigatran and edoxaban require 5 - 10 days of bridging, whereas rivaroxaban and apixaban do not require such a procedure. Low molecular weight heparins are very well suited for this purpose; they are more strongly recommended by guidelines and therapeutic trials than unfractionated heparin, because of showing lower mortality and greater effectiveness.[14,16,37]

The optimal duration of anticoagulation therapy cannot be determined in general. It depends on the cause of DVT, the risk of bleeding and thrombophilia status. Experts recommend at least three months of therapy for DVT or PE caused by surgery or transient, non-surgical risk factors. If the risk factor persists or is rated as high, lifelong anticoagulative therapy is recommended. Equally, indefinite therapy is also recommended if DVT or PE recurs. Nevertheless, the increased risk of bleeding should never be ignored.[16,37]

1.3.6.2 Prophylaxis of deep vein thrombosis

Basic measures like early mobilization and gymnastic exercises should be applied routinely to every patient. These methods have a positive effect on the locomotor system, cardiovascular system and respiration. In addition, adequate hydration of the patient must be ensured.[38]

For patients at low risk of VTE, basic measures should be applied regularly and can be supplemented by physical measures. Physical measures include medical thrombosis prophylaxis stockings and intermittent pneumatic compression. When then bleeding risk is acceptable patients with medium or high risk of VTE should be treated

with drug based VTE prophylaxis In addition, basic measures should be implemented; physical measures can be applied as well. Approved drugs for medicinal VTE prophylaxis are heparins, danaparoid (parenterally administered heparinoid used in heparin-induced thrombocytopenia), factor Xa inhibitors, thrombin inhibitors and vitamin-K-antagonists (coumarins).[38] In patients deemed at high risk for VTE (see 1.3.2), prophylactic administration of anticoagulants should definitely be considered. Prophylactic anticoagulation has proven to be effective for patient safety and reduction of mortality and also from an financial point of view.[39]

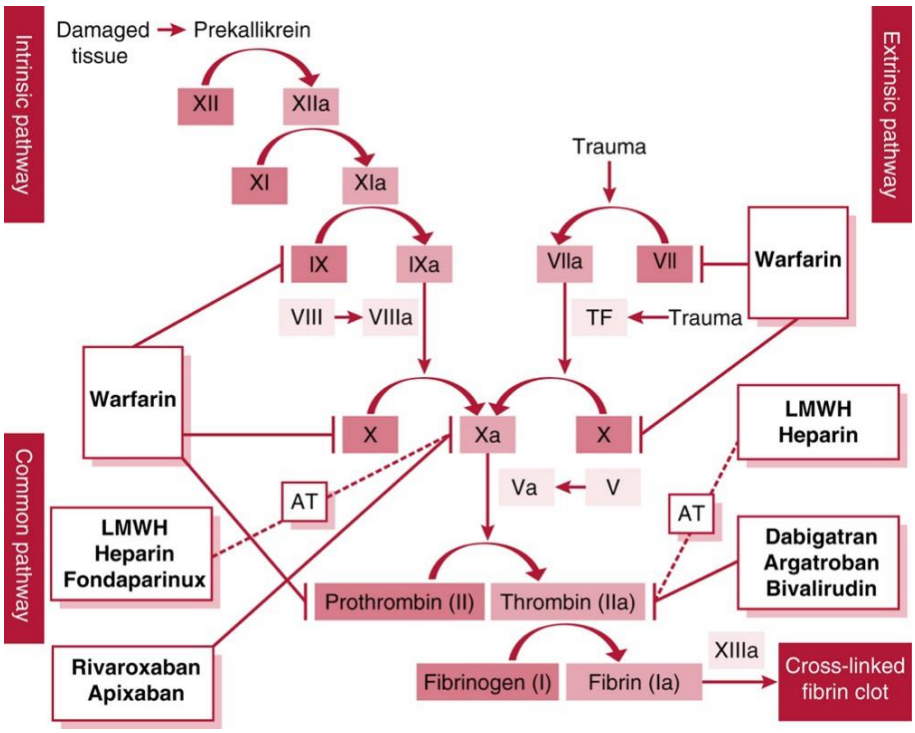

Figure 9: Different intervention points of antithrombotic therapy

Today various anticoagulative drugs are established. Each substance class intervenes in blood coagulation at a different site.[40]

1.3.7 Pharmacological approaches

1.3.7.1 Heparin

Heparin is an endogenous polysaccharide produced by mast cells and basophilic granulocytes.[9] McLean and Howell discovered heparin in 1916 and in 1935 the first clinical trials were undertaken to research it as an anticoagulant drug.[41]

The polyanionic molecule has a molecular weight of 6,000 - 30,000 Da and contains several sulphate and carboxyl residues, which makes the molecule one of the strongest occurring acids in the human body. Structurally typical for heparin is the alternating occurrence of uronic acids and glucosamine, both of them are partially sulphated. The number and position of the sulfate residues differ from molecule to molecule.[9,42,43]

Parenterally administered heparin inhibits the coagulation cascade in several places at once. First and foremost, heparin functions as cofactor for antithrombin III (AT-III) and enhances the inhibition of thrombin and other serine proteases by factor 1000. A specific pentasaccharide structure is of particular importance binding to AT-III. When this structure binds to AT-III, it changes its conformation and forms a complex with thrombin, which is no longer available for coagulation. A sufficient chain length of more than 18 monomers is crucial for the formation of this ternary complex. In addition, the coagulation factors IXa and Xa are inhibited.[9]

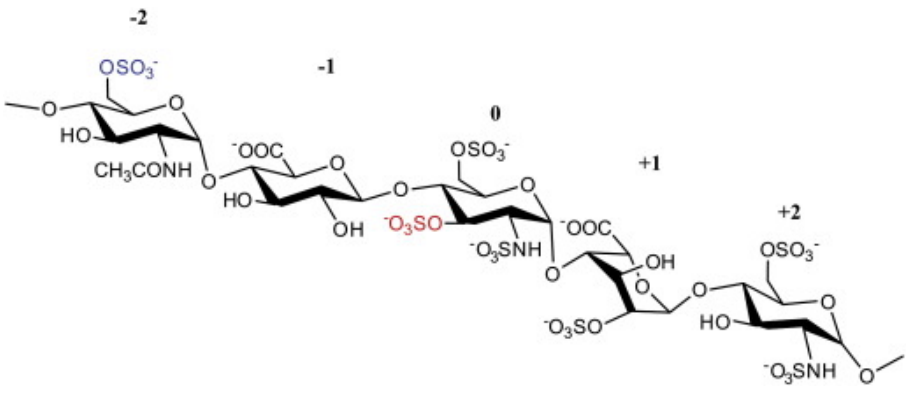

Figure 10: Antithrombin-binding pentasaccharide unit of heparin

This pentasaccharide structure is essential for the binding of heparin to AT-III. The red marked 3-O-sulphate residue at position 0 and the blue marked 6-O-sulphate residue at position -2 are indispensable for binding to antithrombin.[44]

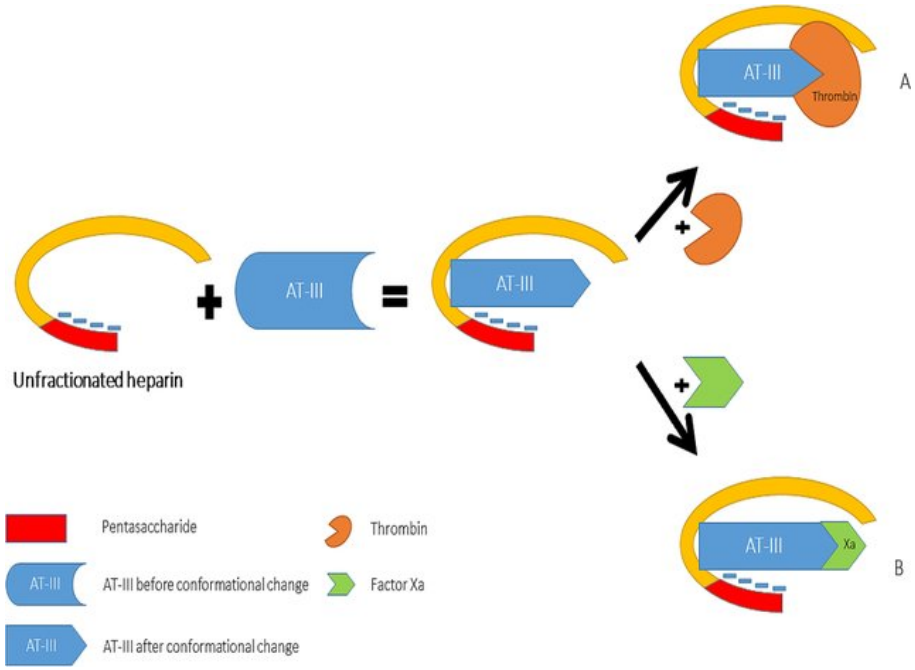

Figure 11: Mechanism of action of heparin

Unfractionated heparin binds to Antithrombin III, which undergoes a change of conformation. Activated AT-III then inhibits thrombin (factor IIa) and factor IXa and Xa.[45]

Shorter heparins also bind to AT-III, but the thrombin-binding properties are lost. Nevertheless, the complex inhibits the coagulation factors IXa and Xa. This fact explains why low molecular weight heparins and Fondaparinux inhibit more selective factor Xa. All heparins and derivatives are suitable for the therapeutic and prophylactic treatment of thromboembolic events. They differ in their pharmacokinetic properties and their specificity to coagulation factors.[9]

Side effects of heparins are the occasional occurrence of skin and mucous membrane bleeding, allergic reactions, hair loss and osteoporosis during long-term therapy. Another side effect is heparin-induced thrombocytopenia (HIT). HIT type I occurs early after the start of therapy. It is characterized by a decrease in platelet count but normalizes quickly and usually remains without complications. Much more dangerous is HIT type II, which occurs 5 - 10 days after the start of therapy. HIT II results in IgG-mediated platelet activation. The antigen is the complex of platelet factor 4 and heparin. Paradoxically, this leads to severe thromboembolic complications. The incidence is 3% for therapy with UFH and 0.3% for LMWH, so if the platelet count drops, HIT diagnostics should be performed.[9,42,43]

1.3.7.1.1 Unfractionated heparin

Heparin occurs naturally in the body and is stored in the granules of mast cells mainly in the liver, lungs and intestinal mucosa. Other mammals also produce heparin in their bodies. Unfractionated heparin (UFH) is obtained from porcine intestinal mucosa or bovine lungs. Composition and sulphation vary, therefore the dosages are given in International Units and not in milligrams. Because of its length, UFH is broken down more quickly than short-chain heparins. The short half-life of about 1 hour can also be used to advantage. For example, anticoagulative therapy can be stopped very quickly or UFH can also be used for bridging to maintain anticoagulation until immediately before surgery. Nevertheless, UFH carries up to ten times the risk of heparin-induced thrombocytopenia than low molecular weight heparins.[14] UFH is usually used in hospital because close monitoring is necessary.[9,14]

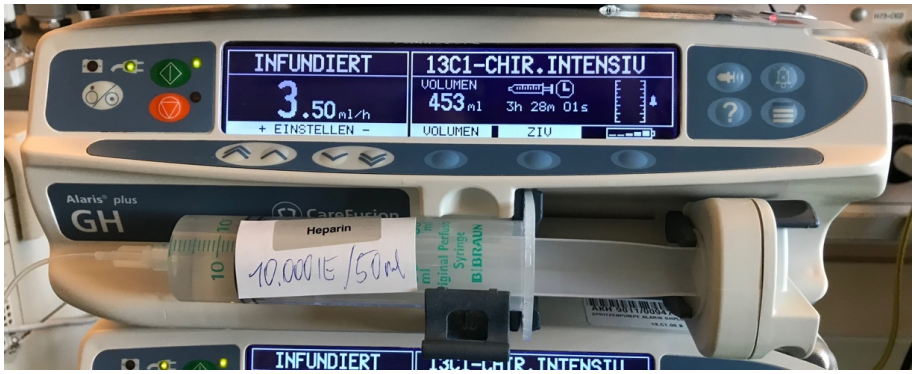

Figure 12: Continuous UFH infusion

The syringe driver continuously emits UFH. Due to the short half-life of UFH it is very controllable and often used in intensive care units.

1.3.7.1.2 Low molecular weight heparins

Low molecular weight heparins are obtained by degrading naturally occurring, unfractionated heparin.[9,41,46] In the course of time, several processes have been developed to produce LMWH. Originally, physical separation processes were used, but these are not suitable for an industrial scale. More suitable are chemical processes to depolymerize heparin. But during chemical degradation, the polysaccharides are randomly broken down, which in turn produces a very heterogeneous product. Better product quality and better environmental compatibility can be achieved by enzymatic degradation. The heparinases I, II and III cleave UFH at precisely defined sites, thus ensuring

a homogenous product. Nowadays there are also mixed forms of production, such as the physicochemical or photochemical method. However, these are not as selective and often show desulfation.[29,43]

Due to the different manufacturing processes many preparations with different molecular weights are on the market today. Among the most important are Enoxaparin (4,500 Da), Dalteparin (6,100 Da), Certoparin (5,400 Da), Nadroparin (4,300 Da), Reviparin (4,400 Da) and Tinzaparin (6,500 Da).[9] Today there are already some biosimilars available.[46]

Figure 13: Enoxaparin biosimilars in different dosages

Enoxaparin is usually sold in ready-to-use syringes. The subcutaneous application can be performed by the patient himself or by health care professionals.[47]

With 4-6 hours after subcutaneous administration, the half-lives of LMWHs are significantly longer than those of UFH. Due to the higher product homogeneity and the more specific mechanism of action, LMWH effects are more predictable than with UFH and do not require further drug monitoring in most instances.[9]

1.3.7.1.3 Fondaparinux

Fondaparinux consists only of the antithrombin-binding pentasaccharide of heparin and is produced synthetically. It has a similar mechanism of action as LMWH and selectively inhibits factor Xa and does not affect thrombin. Because of its selectivity, it was postulated that it would not bind with platelet factor 4. Nevertheless, antibodies against PF4 could be detected in some cases, but HIT II never occurred. Due to the very much shortened chain length, it is degraded more slowly. Half-lives of about 15 hours or more can be achieved when administered subcutaneously. A single administration per day is possible.[9,14]

1.3.7.2 Vitamin K antagonists

Vitamin K antagonists (VKA), are among the oldest representatives of anticoagulants.[9] They are established, effective and relatively cheap.[16] Unlike other anticoagulants, VKA have extremely long half-lives (warfarin: 40 hours, phenprocoumon: 150 hours). In addition, warfarin is metabolized via CYP2C9, which makes it difficult to use with other CYP-interacting substances. Phenprocoumon, on the other hand, is metabolized via CYP3A4.[9] Experience in usage by physician and patient is required and close monitoring is necessary.[37] Traditionally, phenprocoumon is more commonly used in Europe whereas in the rest of the world, warfarin is commonly prescribed.[48]

VKA are derived from the naturally occurring coumarins. Cows were observed to have an increased tendency to bleed after eating spoiled clover. This sweet clover disease brought this substance class into the focus of research.[48]

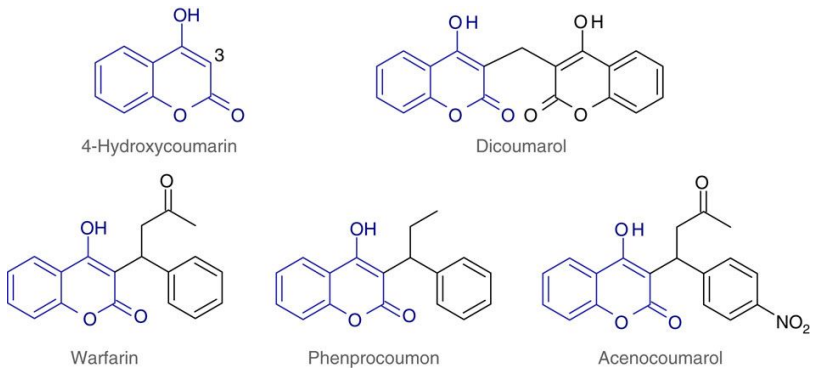

Figure 14: Coumarins

Derived from the naturally occurring 4-hydroxycoumarin, the drugs phenprocoumon, acenocoumarol and warfarin, which are widely used today, were developed. The first drug in this class was dicoumarol.[48]

VKAs prevent the vitamin K-mediated γ-carboxylation of glutamic acid in precursors of the coagulation factors IX, X, VII and II as well as anticoagulants protein C and S. Since it takes a few days before the supply of coagulation factors is depleted, whereas protein C and S levels fall more quickly, the coagulant balance shifts to a prothrombotic state directly after initiation of VKA therapy. This is why additional anticoagulation, e.g. with LMWH, is warranted until International normalized ratio (INR), the coagulation test for monitoring VKAs, has reached the therapeutic target.[9,49]

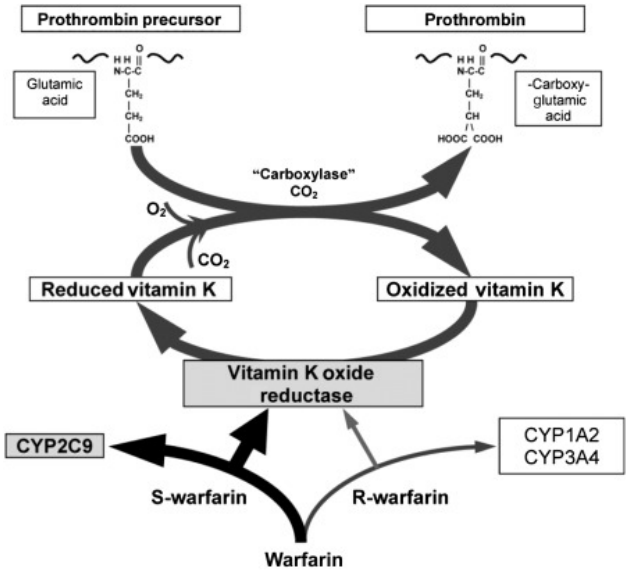

Figure 15: Schematic mechanism of action of vitamin K antagonists

Vitamin K antagonists as warfarin inhibit vitamin K epoxide reductase, which in turn is not able to reduce vitamin K epoxide. Reduced vitamin K hydroquinone is indispensable for synthesis of various coagulation factors, as prothrombin in this figure.[49]

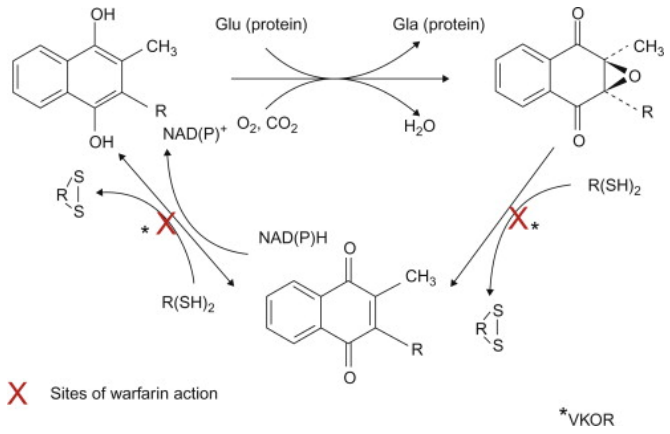

Figure 16: Chemical mechanism of action of vitamin K antagonists

Vitamin K antagonists (as warfarin) inhibit the reduction of vitamin K epoxide by vitamin K epoxide reductase (VKOR). The epoxy form can no longer be converted to hydroquinone, which is important for the carboxylation of glutamic acid in precursors of coagulation factors.[49]

1.3.7.3 Direct acting oral anticoagulants

In recent years, direct acting oral anticoagulants (DOACs) have increasingly established themselves as a low-complication alternative to vitamin K antagonists. They do not require close monitoring, bridging procedures are not needed due to their short half-life and there are fewer medication interactions.[16,9] Due to comparable effectiveness with less side effects, DOACs are currently preferred by guidelines to vitamin K antagonists.[16,18]

The name comes from the direct interaction with coagulation factors, for example dabigatran inhibits thrombin (factor IIa) and rivaroxaban, apixaban, edoxaban inhibit factor Xa.[16]

Patients treated with edoxaban or dabigatran require additional anticoagulation with LMWH at least for 5 days before starting therapy, whereas this is not needed with rivaroxaban and apixaban.[16,18] DOACs are contraindicated in patients with severe renal and/or liver insufficiency. They are not recommended in case of high bleeding risk, thrombocytopenia and malignancy.[16] Special attention should be paid to possible complications when taking p-glycoprotein inhibitors (e.g. verapamil, clarithromycin) and CYP3A4 inhibitors or inducers (e.g. antimycotics, antiepileptics, protease inhibitors) at the same time.[18]

DOACs have often been criticized for lack of antidotes in case of major bleeding. Meanwhile, idarucizumab, an antibody that can antagonize dabigatran, is licensed and available on market.[18] Since May 2018 andexanet alfa, a recombinant but inactive form of the human coagulation factor Xa is approved by the US Food and Drug Administration for antagonization of rivaroxaban and apixaban in case of major bleeding.[50]

1.3.7.4 Thrombolysis

Thrombolysis is an option that can be considered for severe PE in unstable patients. Due to the high risk of fatal bleeding, this method should only be used if normal anticoagulation therapy does not achieve a sufficient effect.[37]

As shown in chapter 1.2.3, plasmin can degrade insoluble fibrin polymers. Plasminogen, the precursor of plasmin, can be activated by plasminogen activators (t-PA, u-PA). This results in an over thousand-fold increased affinity to fibrin, which is then broken down. Today, recombinantly produced plasminogen activators exist: alteplase, reteplase and tenecteplase. They differ in their half-life and affinity to fibrin.[51]

If systemic thrombolysis is unable to resolve the thrombus, catheter-directed thrombolysis may still be considered. Alternatively, surgical removal of the thrombus can be attempted.[37]

Table 4: Dosage recommendations for VTE prophylaxis

Guidelines currently recommend the following dosages for the high-risk prophylaxis of VTE. Depending on the medication selected, the scheme below must be followed.[38]

	Drug	Dose	Scheme	Route
Unfractionated heparins	Heparin Ca^{2+}/Na^{+}	5000 IE	3x/d	s.c.
	Heparin Ca^{2+}/Na^{+}	7500 IE	2x/d	s.c.
Low molecular weight heparin	Enoxaparin	40mg	1x/d	s.c.
Penta-saccharid	Fondaparinux	2,5mg	1x/d	s.c.
Direct acting oral anticoagulants (DOACs)	Rivaroxaban	10mg	1x/d	p.o.
	Apixaban	2.5mg	2x/d	p.o.
	Dabigatran-etexilat	220mg	1x/d	p.o.
Vitamin-K-antagonists	Phenprocoumon	adjusted dose	INR 2.0 – 3.0	p.o.
	Warfarin	adjusted dose	INR 2.0 – 3.0	p.o.

Table 5: Dosage recommendations for the therapy of acute DVT or PE

Guidelines currently recommend the following dosages for the therapy of DVT and PE. Depending on the medication selected, the scheme below must be followed.[52]

	Drug	Initial dose	Maintenance dose	Scheme	Route
Low molecular weight heparins (LMWH)	Enoxaparin	1,0 mg/kg	1,0 mg/kg	2x/d	s.c.
	Dalteparin	100 *or* 200 IU/kg	100 *or* 200 IU/kg	2x *or* 1x/d	s.c.
	Certoparin	8000 IU	8000 IU	2x/d	s.c.
	Nadroparin	0,1 ml/10kg	0,1 ml/10kg	1x or 2x/d	s.c.
	Tinzaparin	175 IU/kg	175 IU/kg	1x/d	s.c.
	Reviparin	3436 IU	3436 IU	2x/d *(45-60 kg)*	s.c.
		10307 IU	10307 IU	1x/d *(> 60 kg)*	s.c.
Pentasaccharid	Fondaparinux	7,5 mg	7,5 mg	1x/d	s.c.
		5 mg *(< 50 kg)*	5 mg *(< 50 kg)*	1x/d	s.c.
		10 mg *(> 100 kg)*	10 mg *(> 100 kg)*	1x/d	s.c.
Unfract. heparins	Heparin Ca^{2+}	5000 IU	15 – 20 IU/kg/h	Bolus + Infusion	i.v.
	Heparin Na^{+}	5000 IU	15 – 20 IU/kg/h	Bolus + Infusion	i.v.
Direct acting oral antico-agulants (DOACs)	Rivaroxaban	2x 15 mg (3 weeks)	20 mg	2/d or 1/d	p.o.
	Apixaban	2x 10 mg	5 mg (6m: 2,5 mg)	2x/d	p.o.
	Edoxaban	LMWH, UFH, FDX	60 mg	1x/d	p.o.
	Dabigatran-etexilat	LMWH, UFH, FDX	150 mg	2x/d	p.o.
Vit.-K-an-tagonists	Phenprocoumon	6 mg (1. - 2.d)	1,5 mg – 4,5 mg	INR 2-3	p.o.
	Warfarin	2,5 – 5 mg (1.+2.d)	2,5 – 10,0 mg	INR 2-3	p.o.

1.4 Coagulation assays

1.4.1 Prothrombin time, International normalized ratio

For determination of prothrombin time citrated blood plasma is mixed with Ca^{2+} and thromboplastin, a reagent which contains recombinant or extracted tissue factor, which initiates coagulation via the extrinsic coagulation pathway. The resulting clotting time is measured with mechanical or optical methods and should be between 10 and 14 seconds, however normal ranges vary between different laboratories.[53,54]

Available reagents vary in the amount and origin of tissue factor, which can lead to different prothrombin times. With the international normalized ratio (INR) a comparable value is created, which is independent of the test system used. The World Health Organization established the international sensitivity index (ISI), which indicates the responsiveness of the used reagent and is included in the calculation of the INR.[53,54]

$$INR = \left(\frac{Prothrombintime_{sample}}{Prothrombintime_{normal}}\right)^{ISI}$$

1.4.2 Activated partial thromboplastin time

The activated partial thromboplastin time (aPTT) is also determined from citrated plasma. A surface activator and a diluted phospholipid are added to the sample. As no tissue factor is present in thromboplastin reagent, it is called partial thromboplastin. The sample is incubated, then Ca^{2+} is added. The aPTT corresponds to the time until the formation of a clot. Usually the aPTT values are between 22 and 40 seconds. Since coagulation is not triggered by tissue factor, this assay is used to investigate the intrinsic coagulation.[53,54]

1.4.3 Anti-Xa activity

As shown in 1.3.7.1.2, Enoxaparin and other low molecular weight heparins mainly inhibit factor Xa, which leads in turn to prolonged clotting times.[55] Anti-Xa activity is therefore the assay of choice for the monitoring of LMWH. This can be done by a variety of assays.[55,56]

In general, factor Xa substrates bound to chromophores are used in these assays. Factor Xa cleaves these substrates, thereby releasing the chromophore, which can be measured by a spectrophotometer. The color development is directly proportional to the factor Xa present in the assay. A certain amount of factor Xa is added to the sample. If LMWH is present in the sample, it inhibits via antithrombin the factor Xa and thus the color development of the assay. A calibration curve can be used to calculate the existing LMWH concentration from the result of the assay.[53]

Since LMWHs generally have very good subcutaneous bioavailability, they are considered to be particularly safe. Therefore, monitoring during VTE prophylaxis is usually not necessary for clinically stable patients without concomitant diseases.[55–57] Nevertheless, it should be mentioned that the measurement of anti-Xa activity can increase the efficiency and safety of anticoagulation therapy. In the context of a therapeutic dosage of LMWHs, measurement of anti-Xa activity at the beginning of therapy makes sense to prevent inefficient or excessive anticoagulation.[55]

Maximum plasma levels of LMWH can be measured 1 to 5 hours after subcutaneous application. Therefore, a sample for a peak level should be taken after approximately 4 hours. Peak levels have a stronger correlation with efficiency and safety than trough levels.[55,57]

2 Background

As shown in 1.3.2.4, DVT is a very common occurrence in intensive care patients. Therefore, this patient collective is considered to be at high risk. As a consequence, many of these patients receive pharmacological VTE prophylaxis, often with LMWHs. LMWHs, such as enoxaparin, intervene in the coagulation cascade via antithrombin III-mediated inhibition of factor Xa and thus prevent the formation of clots.

Chapter 1.4.3 demonstrates that the resulting inhibition of factor Xa can be measured by the anti-Xa activity assay. In therapeutic doses, i.e. in the presence of VTE, the anti-Xa activity is used to detect possible accumulation of enoxaparin, which is associated with a high risk of bleeding.

However, as shown in this thesis, anti-Xa activity is also frequently determined in patients receiving a prophylactic dosage of enoxaparin. Logically, these values should be much lower than those measured at the therapeutic dose. In the literature there is some disagreement about the usefulness of anti-Xa activity measurement in patients who receive enoxaparin as prophylaxis of thrombosis.

The main aim of this thesis is to investigate the relationship between VTE and anti-Xa activity in intensive care patients receiving prophylactic enoxaparin therapy. It shall be examined whether patients with VTE have a significantly changed anti-Xa activity compared to patients who were not diagnosed with VTE. If these patients have reduced anti-Xa activity, because factor Xa for some reason is less inhibited, this reduction could be associated with the occurrence of thrombosis. Subsequently, it could be postulated that a certain level of anti-Xa activity should be targeted. If, on the other hand, no significant difference in anti-Xa activity can be observed, this would be further evidence that the anti-Xa activity value should not be used as guidance for VTE prophylaxis with enoxaparin. From a scientific point of view, it is of high interest whether this correlation exists. In financial terms, a large number of unnecessary measurements could be avoided if clear statements are published.

As one of the secondary objectives, correlation of the enoxaparin dose and anti-Xa activity is examined. The relation to renal function will also be investigated. In contrast to the occurrence of VTE, the patient collective will also be screened for bleeding events and a possible connection to enhanced anti-Xa activity.

3 Material and Methods

3.1 Submission to the Ethics Committee

This retrospective chart review study was approved by the ethical review committee of the Medical University of Vienna (EK No. 1936/2019). Informed consent was deemed not necessary.

3.2 Patient recruitment

All patients over 18 years, who were admitted to an intensive care ward of the Department of Anesthesia, Intensive Care Medicine and Pain Medicine, Medical University of Vienna, for at least one day between 01/2018-05/2018, received subcutaneous enoxaparin and had at least one measurement of anti-Xa activity were included in this study.

Patients were excluded when DVT or PE were present at ICU admission. If they received direct-acting oral anticoagulants and unfractionated heparin or if they received extracorporeal membrane oxygenation therapy, the anti-Xa activity values of these days were not further considered. Values of days without these procedures were included in this study.

3.3 Data retrieval

Patient data was exported from the electronic patient data management system (PDMS; IntelliSpace Critical Care and Anesthesia, Philips Austria GmbH, Vienna, Austria).

For the demographic analysis, body weight, height, age, gender, length of stay and primary diagnosis were collected as baseline parameters. Based on this data, body mass index and diagnosis category could be assigned. Prescription and administration of medication was also searched for enoxaparin, the total amount administered per day was recorded.

The measured anti-Xa activity was matched to the last administered enoxaparin dose and classified into peak, trough12 and trough24 according to the time of measurement after the last enoxaparin administration. Anti-Xa activity was also matched to last

measured creatine clearance within 5 days. The amount of red blood cell (RBC) concentrates was also included to the analysis to detect bleeding events.
All this data was extracted from the PDMS, stored in Microsoft Excel sheets and used for further analysis.

3.4 Chart review

To determine the presence or absence of VTE, patient histories, physician and nursing notes and transfer reports of the included patients were reviewed. In addition, all ultrasound, CT and MRI reports, which were performed during the stay intensive care were screened for the presence of VTE. Documented transthoracic or transesophageal echocardiography was also screened for detection of cardiac thrombosis. The information found was collected in a Microsoft Excel sheet for the further analysis.
Presence or absence of the following parameters was noted: diagnosed DVT, diagnosed PE, diagnosed venous thrombosis (other than DVT), diagnosed embolism (other than PE) and suspected thrombosis/embolism in case of reasonable suspicion but no further diagnostic confirmation. Already existing thrombosis on admission were recorded separately.

3.5 Classification of values

3.5.1 Classification of enoxaparin dosage

As already stated in chapter 1.3.6, there are two different dose ranges in which anticoagulative therapy can be applied: therapeutic and prophylactic dosing.[58] For each patient day, the total dose of enoxaparin administered was calculated. If an amount of 40 or less milligrams of enoxaparin per day was given, the day of stay was assigned to the category of prophylactic regime, else the day of stay was assigned to non-prophylactic regime. For patients with a body weight over 90 kg or a BMI over 30 kg/m^2, doses of up to 80 mg per day were counted as part of the prophylactic regime.
The total days in the two regimes were counted and compared. Finally, the whole stay was evaluated, divided into prophylactic (85% or more days in prophylactic regime), non-prophylactic (85% or more days in non-prophylactic regime) and mixed (neither of the two regimes to 85%) categories.

3.5.2 Classification of peak and trough anti-Xa activity

As described in 1.4.3, enoxaparin pharmacokinetics lead to different anti-Xa activity depending on the timing of blood sampling after enoxaparin administration. Here, peak anti-Xa activity was defined as blood sampling between 3 – 5 hours after last enoxaparin administration, whereas trough values were defined as trough12 and trough24 anti-Xa activity. Trough12 values enclosure the measurements between 11 – 13 hours after enoxaparin administration, trough24 values stand for measurements at the same time or 23 – 24 hours after enoxaparin administration. In contrast to peak and trough12, the time interval for trough24 anti-Xa activity is obviously shorter. In most cases, enoxaparin is administered every 24 hours. That means, if measurements after more than 24 hours are taken into account, the next enoxaparin dose would already be detected. This would bias the mean value. The likelihood that enoxaparin could be exposed for one day was considered very low and the values obtained should not significantly affect the results.

3.6 Blood products

Besides the incidence of thrombosis, bleeding, one of the most serious adverse effects of enoxaparin, should also be investigated in this thesis. Similar to the detection of thrombosis, there is no exactly defined endpoint for bleeding in the PDMS. The available data must therefore be interpreted and analyzed accordingly. For this diploma thesis, the number of red blood cell (RBC) concentrates administered was chosen as endpoint for clinically significant bleeding. However, it should not be overlooked that in intensive care medicine RBC concentrates are often administered to compensate for anemia due to reasons besides bleeding. To account for different lengths of ICU stays, the number of RBC concentrates is divided by the length of stay in days.

3.7 Data analysis and plotting

3.7.1 KNIME

Data processing and analysis was performed with the Konstanz Information Miner (KNIME) Analytics Platform.

KNIME is a free Java-based workflow program based on Eclipse open source platform. In contrast to Eclipse, KNIME has a graphical interface in which so-called nodes can be connected to each other. Each node stands for one task, when a task is finished, the next one starts. The workflow platform is widely usable due to a large number of plug-ins, some of them also for creating graphics.[59,60]

KNIME was originally developed for data mining in 2004, but quickly gained a foothold in the field of cheminformatics. Today it is widely used in biotech, pharmaceutical research and many other areas.[60]

The table resulting from the data retrieval and the chart review (chapters 3.3 and 3.4) is imported into a KNIME workflow. The classifications mentioned in 3.5 are then carried out. The inclusion and exclusion criteria are also implemented. As shown in Figure 17, calculations are performed using the gained data.

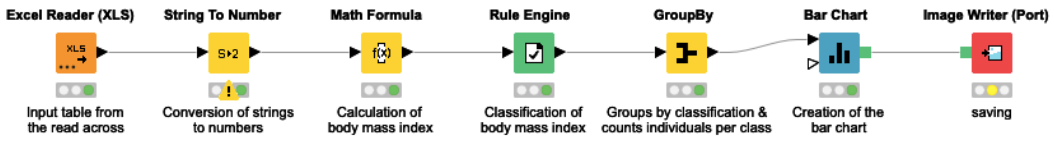

Figure 17: KNIME workflow for calculation of body mass indices

The calculation of the body mass indices of the patients was automated with KNIME. Each node stands for one task. When all nodes are executed, the resulting bar chart is saved as an image.

3.7.2 R Studio

The graphics of this diploma thesis were created with RStudio. RStudio is an integrated development environment that is freely available, highly functional and used in several academic disciplines. In contrast to the nodes in KNIME, scripts are generated directly in an editor mode.[61] For plotting, the package ggplot2 was used. The underlying data was taken from the data retrieval and the chart review (see 3.3 and 3.4).

3.8 Statistics

The main research goal of this diploma thesis is to find a possible distinction of the anti-Xa activity in the patient groups with and without thrombosis in prophylactic enoxaparin regime. Anti-Xa activity can be measured at 3 different levels (see 3.5.2), which were considered separately. In order to determine a general interaction of the anti-Xa activity at the different levels and occurrence of thrombosis, a two-factorial analysis of variance (ANOVA) was performed.

For more detailed differentiation within peak, trough12 and trough24 levels a t-test was established to evaluate significant differences in anti-Xa activity in patients with and without thrombosis. Since the patients were only divided into one of the two groups and never occur in both groups, a t-test with independent samples was performed. For t-tests it is also relevant whether the variances are equal or unequal. Therefore, a Levene test was carried out. Differences of anti-Xa activity between the patient groups with and without thrombosis were shown graphically using boxplots.

In order to evaluate secondary targets, scatterplots with implemented correlation lines were created. This allowed an initial assessment of positive or negative correlation. After creating the plots, the Pearson correlation coefficient (r) was calculated. Coefficients of 1 mean complete correlation, whereas coefficients of 0 mean no correlation at all. Coefficients of -1 stand for negative correlations. The statistical significance was described by a p-value, which stands for the probability that the correlation can occur randomly by an uncorrelated system. P-values below 0.05 were considered as significant. In contrary to the main research goal, general relationships are to be examined in the secondary objectives. Therefore, the anti-Xa activity values of all enoxaparin regimes (i.e. prophylactic, non-prophylactic and mixed) are taken into account.

4 Results

4.1 Results of the data collection

Initial screening revealed 286 adult intensive care patients, who were admitted between 01.01.2018 to 31.05.2018 and received enoxaparin as anticoagulation therapy. 80 patients have to be removed because no according to the inclusion criteria usable anti-Xa activity was determined during the stay. 12 individuals are excluded due to thrombosis and PE already existing at admission, 3 of them were already excluded due to not usable anti-Xa activity values. In total, 197 patients remained for further analysis.

4.2 Demographic data analysis

The 197 included patients are examined according to demographic criteria. The duration and the reasons for the stay in intensive care and the number of deceased patients is also evaluated. Detailed information can be found in Table 6 and Figure 18 to Figure 21.

Table 6: Patient demographics

Demographic data of the patient collective.

	Mean	**Std. dev.**		**Absolute**	**Relative**
Age (years)	61.5	17.1	Male	115	58.4%
Weight (kg)	79.2	27.3	Surgical	142	72.1%
Height (cm)	170.2	9.6	Non-surgical	48	24.4%
BMI (kg/m^2)	27.4	9.9	Trauma	7	3.5%
Days in ICU	12.8	16.6	Died in ICU	11	5.6%

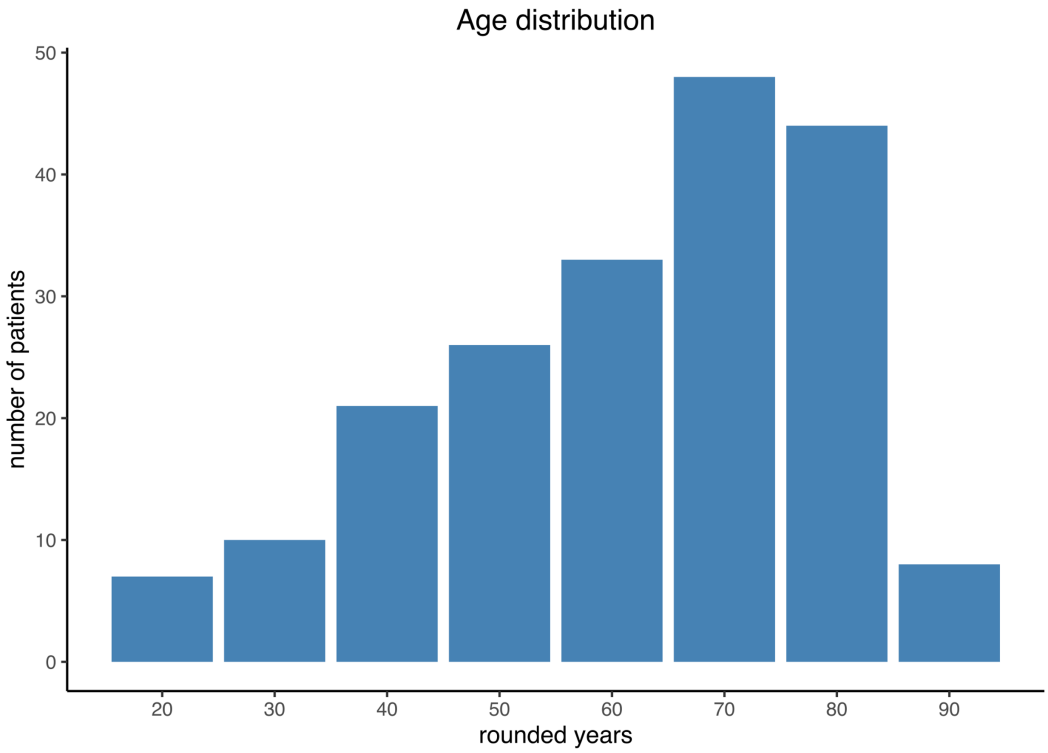

Figure 18: Age distribution

The age of the included patients clustered to 10-year steps.

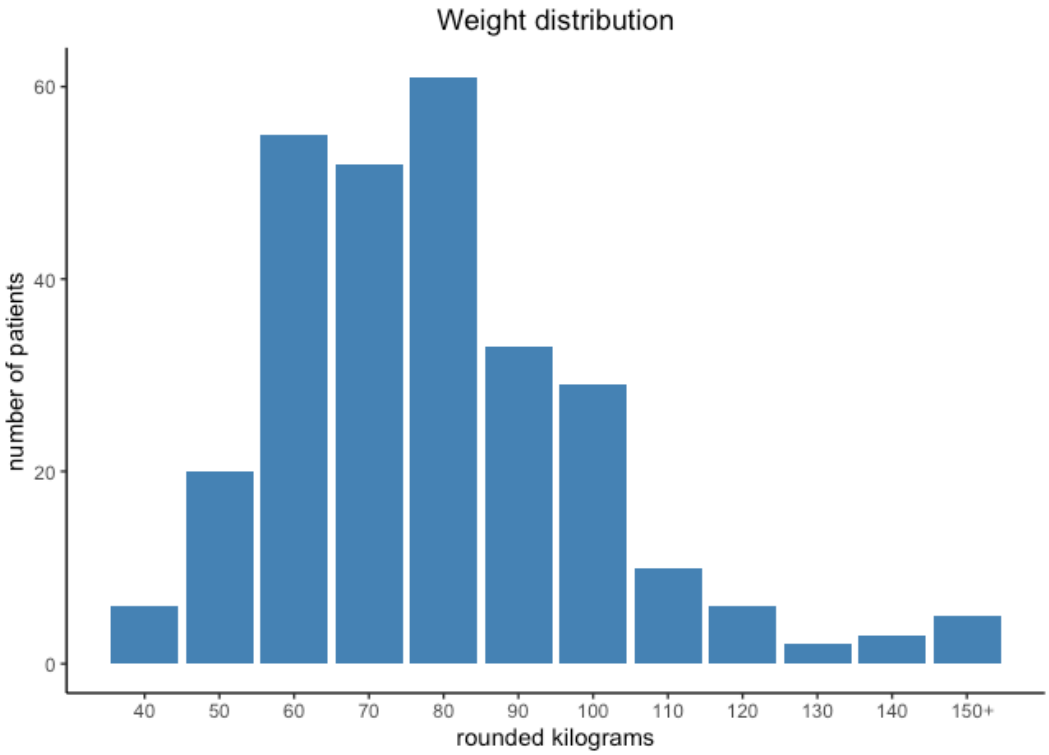

Figure 19: Weight distribution

The body weight of the patients rounded to steps of 10 kilograms. In 4 cases the weight was not sufficiently documented, therefore the patients are not included to this graphic. Patients with 150 or more kilograms of body weight are combined as 150+.

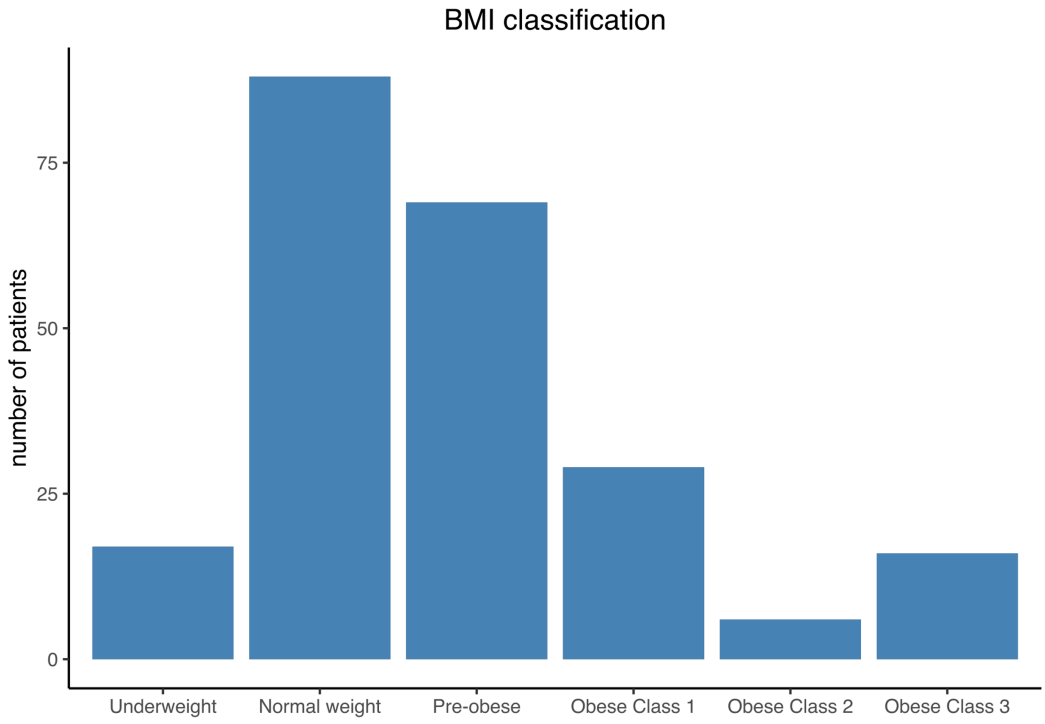

Figure 20: BMI classification

BMIs (kg/m^2) are classified by current WHO standards.[62] Normal BMI is between 18.50 and 24.99. Below, patients are considered underweight. Pre-obese means BMI up to 29.99, obese class 1 up to 34.99, class 2 up to 39.99 and class 3 everything above 40. In 61 cases the BMI was not calculable.

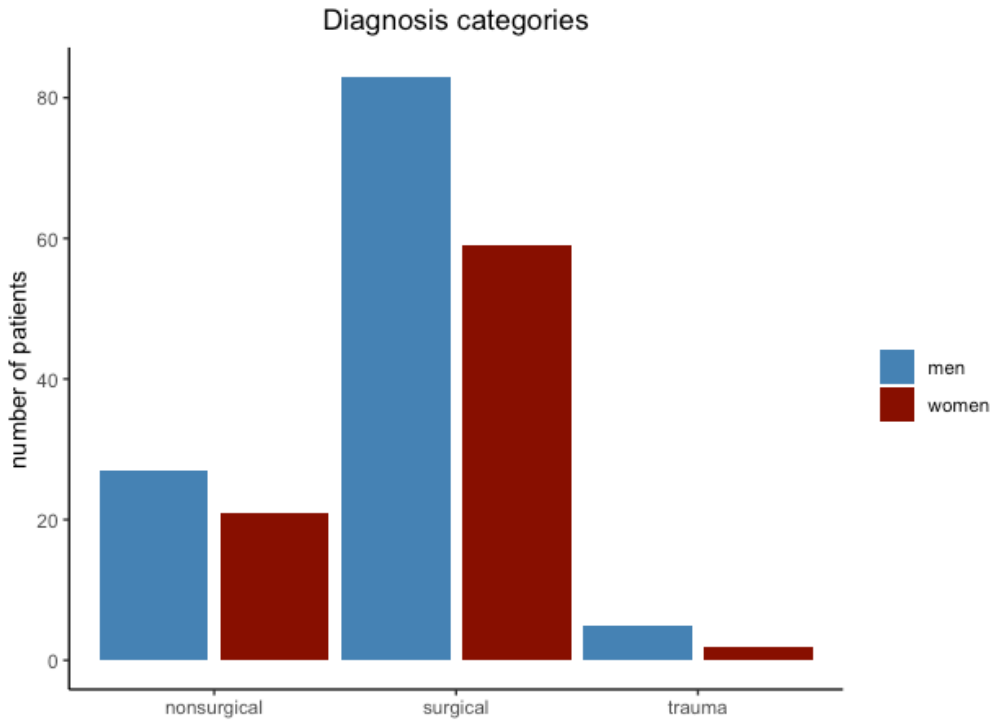

Figure 21: Diagnosis categories

Causes of stay in intensive care divided into non-surgical, surgical and trauma. Men and women are applied separately.

4.3 Thrombotic events in the patient collective

As described in chapter 3.4, patient records of the included individuals were searched for the presence of VTE. During the screening, patients with DVT, PE and other thromboses were differentiated. Other thromboses mainly consist of upper extremity venous thrombosis. The numbers of documented thrombotic events can be found in Table 7.

Table 7: Occurrence of thrombotic events in the patient collective

The manual evaluation of the patient data management system yields the following numbers of cases. The numbers are given in absolute terms and relative to the patient collective. All patients, whether they were in prophylactic or non-prophylactic regime, were included in this evaluation. Some patients meet more than one criterion. Therefore, the number of total thrombotic events is lower than the sum of single thrombotic events.

	Absolute occurrence	**Relative occurrence**
Deep vein thrombosis	2	1.02%
Pulmonal embolism	5	2.54%
Other thromboses	5	2.54%
Total thromboembolic events	9	4.57%

4.4 Analysis of Enoxaparin administration

In total, 5305 enoxaparin injections were administered in the included intensive care units between 01.01.2018 and 31.05.2018. This corresponds to a sum of 226,800 mg pure enoxaparin. The patients included in this study received a total of 2024 injections of enoxaparin.

As discussed in 3.5.1, the overall dose regimes of enoxaparin are divided into prophylactic, non-prophylactic and mixed. The number of patients according to the regimes is shown in Figure 22.

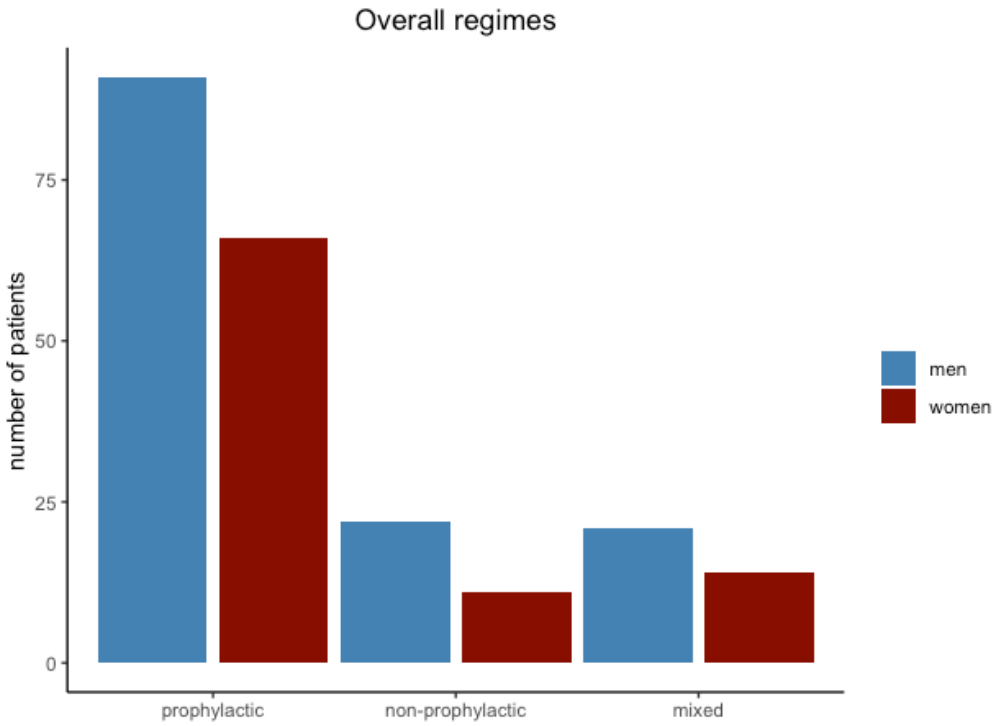

Figure 22: Dose regimes

The different dose regimes of enoxaparin are divided into prophylactic (85% of days ≤40 mg enoxaparin; 85% of days ≤80 mg for patients with a body weight above 90 kg or a BMI above 30 kg/m^2), non-prophylactic (85% of days not in prophylactic regime) and mixed regimes (neither prophylactic or non-prophylactic). Men and women are applied separately.

4.5 Analysis of anti-Xa activity

4.5.1 Descriptive overview

Since the anti-Xa activity plays a central role in this thesis, it will be examined in detail. First, the measurement rate of the anti-Xa activity is determined. In Figure 23 it can be seen that the measurements are not stochastically distributed, but rather in fixed time periods. The largest number of measurements is taken 4 to 5 hours after enoxaparin administration. A second and a third increase in the measurement rate are evident around 12 and 24 hours after the last enoxaparin administration. As described in chapter 3.5.2, the measurements 3 to 5 hours after administration meet the requirements for determining the peak value. 12 and 24 hours after administration, the trough level can be effectively monitored.

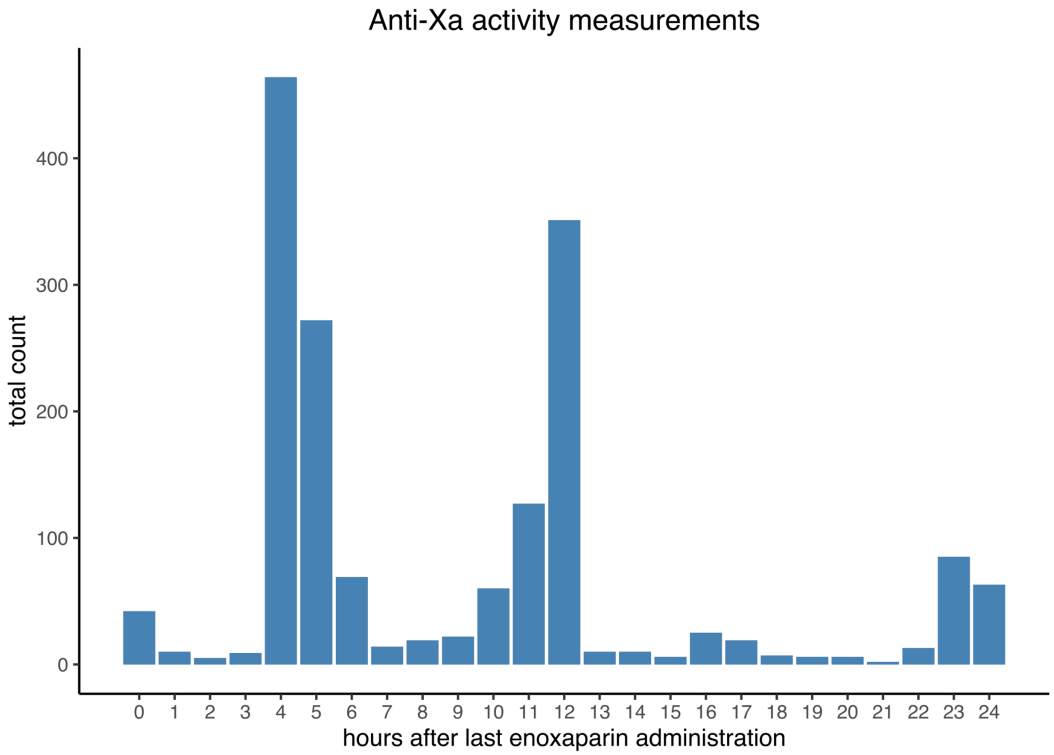

Figure 23: Measurements of anti-Xa activity after last enoxaparin administration

Anti-Xa activity measurement rates after last Enoxaparin administration. Most measurements are performed during the peak period. Trough values are mainly examined around 12 and 24 hours after last administration.

In a second step the anti-Xa activity values are collected and assigned to different patient groups. The mean values of the anti-Xa activity, their standard deviation and the numbers of measurements are displayed in Table 8.

Table 8: Anti-Xa activities in different patient groups

Mean anti-Xa activity assigned to peak/trough levels, therapy regime of the day and administered enoxaparin dose. The number of available anti-Xa activity values is given, as well as the total amount of values in each class.

Level	Regime	Enoxaparin (mg)	Mean anti-Xa activity (IU/ml)	Std. dev.	Values	Sum
peak	prophylactic	20	0,30	0,19	51	252
		40	0,32	0,22	181	
		60	0,32	0,14	8	
		80	0,47	0,11	12	
	non-prophylactic	20	0,30	0,15	23	462
		40	0,39	0,21	320	
		60	0,47	0,22	75	
		80	0,64	0,29	40	
		100	0,40	0,18	3	
		120	0,68	0,00	1	
trough12	prophylactic	20	0,13	0,12	37	420
		40	0,11	0,12	381	
		60	0,16	0,00	1	
		80	0,00	0,00	1	
	non-prophylactic	20	0,35	0,18	5	49
		40	0,30	0,21	29	
		60	0,35	0,17	11	
		80	0,23	0,12	4	
trough24	prophylactic	20	0,05	0,09	42	176
		40	0,04	0,10	132	
		80	0,07	0,10	2	
	non-prophylactic	40	0,14	0,03	3	6
		60	0,08	0,11	2	
		80	0,28	0,00	1	

4.5.2 Anti-Xa activity in patients with and without VTE

The main objective of this diploma thesis is to determine, whether anti-Xa activity differs between patients with vs. without documented VTE. For this objective, only patients receiving prophylactic enoxaparin will be analysed. Anti-Xa values obtained after initiation of ECMO therapy or after the onset of a thromboembolic event were disregarded. Table 9 and Figure 24 give an overview of the included anti-Xa activity values of the respective patient groups.

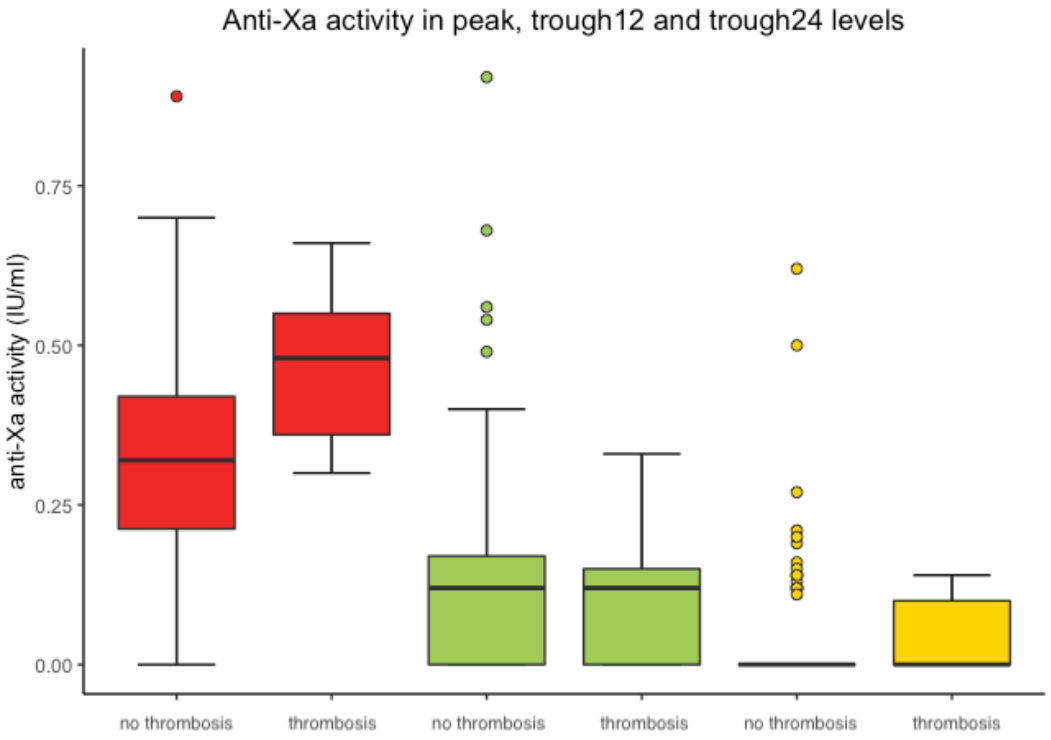

Figure 24: Anti-Xa activity in patients with vs. without thrombosis

In this box plot different patient groups who received prophylactic enoxaparin doses are compared regarding their anti-Xa activity. The anti-Xa activity levels are divided into peak, trough12 and trough24 based on the time elapsed since the last enoxaparin administration. In addition, values of patients with and without thrombosis (pos and neg) are distinguished.

Table 9: Mean anti-Xa activity of patients with vs. without thrombosis

The mean anti-Xa activity values of patients with and without thrombosis are compared regarding the levels.

	No thrombosis		Thrombosis	
	Mean anti-Xa activity (IU/ml)	**Std. dev.**	**Mean anti-Xa activity (IU/ml)**	**Std. dev.**
Peak	0.318	0.165	0.478	0.120
Trough12	0.113	0.117	0.101	0.084
Trough24	0.028	0.086	0.036	0.055

To determine a general interaction of the data, a two-factorial ANOVA is performed. To get a first impression of an interaction, the data is displayed graphically. Then the calculation is performed.

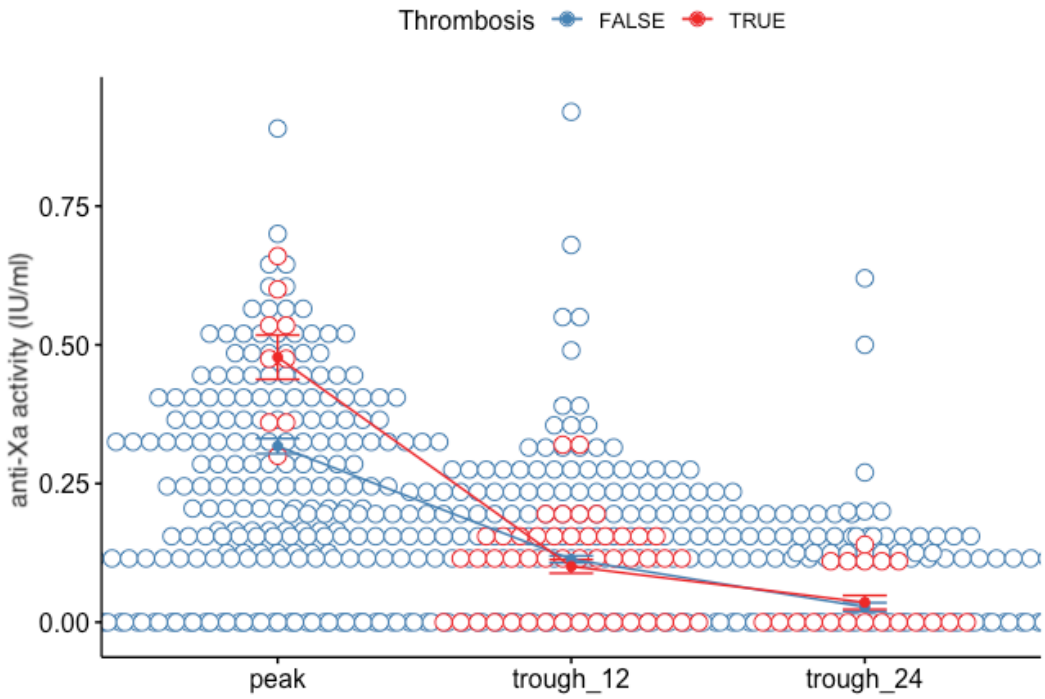

Figure 25: Data visualization for ANOVA

The influence of peak, trough12 and trough24 levels and the occurrence of thrombosis on the anti-Xa activity is shown graphically.

Table 10: Two-factorial ANOVA

A two-factorial ANOVA was performed. Anti-Xa activity levels and occurrence of thromboses are used as factors. The interaction of the two factors is investigated.

	Sum Sq	Df	f value	p value
level	7.501	2	254.868	$< 2.2\ e^{-16}$
anyThrombosis	0.014	1	0.952	0.330
level:anyThrombosis	0.212	1	7.190	0.001

Data analysis using ANOVA shows that the level of anti-Xa activity has a significant influence on the value of anti-Xa activity. The presence of VTE does not show a significant influence on anti-Xa activity. The interaction of the both factors shows a significant influence on the anti-Xa activity. For an accurate analysis within the anti-Xa levels additional t-tests are performed.

Two-sided t-tests are used to compare mean peak, trough12 and trough24 antiXa activities between patients with and without VTE. As patients can either appear in only one group (with or without VTE), independent t-tests are performed.

For this purpose, a variance comparison by the Levene test must first be carried out. The Levene Test assumes in its null hypothesis that the variances are equal. The alternative hypothesis would indicate unequal variances.

Table 11: Levene test

The Levene test was carried out to determine similar variances.

level	t (Levene)	p (Levene)
peak	1.027	0.312
trough12	2.437	0.119
trough24	0.007	0.932

Since all p-values in the Levene test are above 0.05, no evidence for significant unequal variances can be found in any of the groups. Conversely, this means that the variances are in a similar range. Therefore, a t-test is performed on independent samples with equal variances.
The two-sided t-test is used to test for significant differences in anti-Xa activity levels between the two patient groups. The results of the t-test can be seen in Table 12.

Table 12: Independent groups t-test
The two-sided t-test was performed separately for the levels of peak, trough12 and trough24 anti-Xa activity values. The mean anti-Xa activity of the patient groups with and without thrombosis was tested for equality.

level	t	df	p-value (2-tailed)	CI
peak	-2.863	153	0.005	0.95
trough12	0.726	391	0.468	0.95
trough24	-0.404	142	0.687	0.95

The result of the two-sided t-test shows that the mean values of the patient groups with and without thrombosis differ significantly in the peak levels. The peak levels are significantly higher in patients with thrombosis than in patients without thrombosis. In trough12 and trough24 levels no significant difference between the two groups could be observed.

4.5.3 Anti-Xa activity in relation to the enoxaparin dose

The relationship of anti-Xa activity with last administered enoxaparin dose is considered as a secondary objective. As for all secondary objectives, all anti-Xa activity values were considered, i.e. those from prophylactic, non-prophylactic and mixed regimes. In Figure 26, the correlation between enoxaparin dose and anti-Xa activity is investigated graphically.

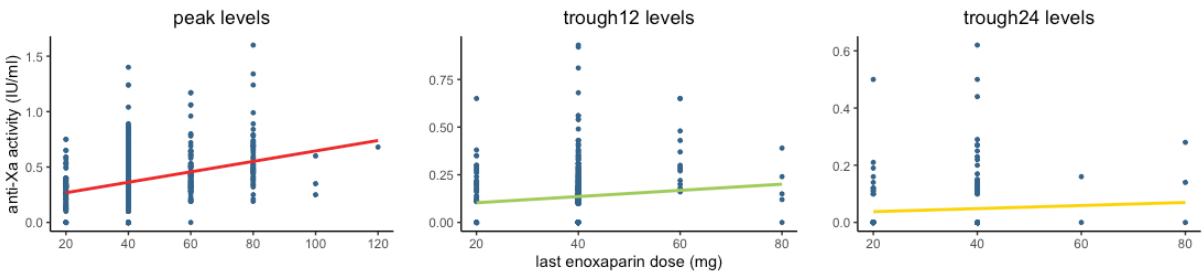

Figure 26: Enoxaparin mediated peak, trough12 and trough24 anti-Xa activity

The measured levels of anti-Xa activity are examined separately for correlation with enoxaparin dose. The correlation is displayed graphically with the straight line.

Table 13: Enoxaparin mediated anti-Xa activity

The correlation of enoxaparin dose and the mediated anti-Xa activity was calculated using the Pearson correlation coefficient. The p-value indicates the probability that an uncorrelated system has the same correlation.

level	*r*	p	df
peak	0.296	6.66 e^{-16}	712
trough12	0.077	0.096	467
trough24	0.063	0.398	180

As shown in Figure 26 and Table 13, peak level anti-Xa activity correlates best with the enoxaparin dose administered. In contrast to an uncorrelated system, a clear significance of the correlation between anti-Xa activity and enoxaparin dose can be shown by a very low p-value. Regarding trough12 and trough24 levels, no significant relationship can be determined.

4.5.4 Anti-Xa activity in relation to renal function

The relationship between anti-Xa activity and renal function should also be investigated. The last available creatinine clearance within 5 days before the measurement of anti-Xa activity is used as endpoint for renal function. For 1281 anti-Xa activity values, last creatinine clearance data is available.

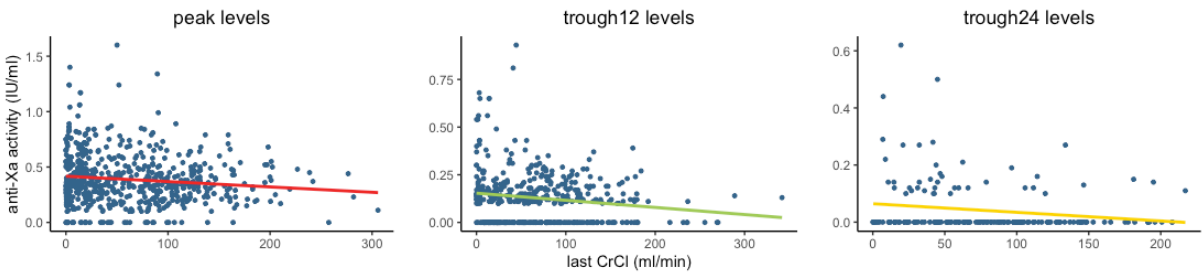

Figure 27: Anti-Xa activity and last creatinine clearance

The measured anti-Xa activity values are set in relation to the last measured creatinine clearance. It should be noted that a high creatinine clearance stands for a good renal function.

Table 14: Correlation between anti-Xa activity and creatinine clearance

The last creatinine clearance and anti-Xa activity are correlated using the Pearson correlation coefficient. All anti-Xa activity levels show a high significance in their correlation to creatinine clearance.

level	*r*	p	df
peak	-0.119	0.002	654
trough12	-0.157	0.001	445
trough24	-0.175	0.020	176

Based on Figure 27 and Table 14, it can be shown that anti-Xa activity is negatively correlated with renal function across peak, trough12 and trough24 levels.

4.5.5 Bleeding complications in relation to anti-Xa activity

To determine the association between mean anti-Xa activity to RBC concentrates administered (adjusted to length of stay), Pearson's correlation coefficients were calculated for mean peak, mean trough12 and mean trough24 levels. No significant correlation was found.

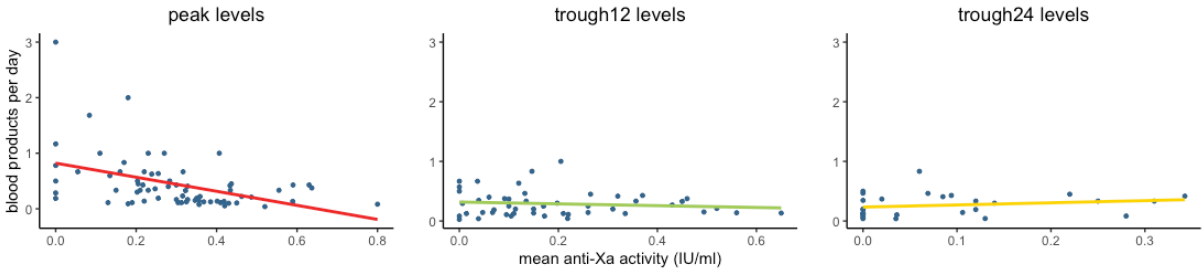

Figure 28: Red cell concentrates in relation to mean peak and trough12 anti-Xa levels

The consumption of red cell concentrates per day is correlated with mean peak, trough12 and trough24 anti-Xa activity values.

Table 15: Red cell concentrates per day related to mean anti-Xa activity

The total number of red cell concentrates is related with the days of stay to units per day. These units per day are related to the mean anti-Xa activity. The Pearson coefficient of correlation is calculated.

level	*r*	p	df
peak	-0.440	1.526	67
trough12	-0.119	0.415	47
trough24	0.200	0.299	27

5 Discussion

As the main research goal, the anti-Xa activity values of the patients with and without DVT should be investigated. Since only two of the patients had a confirmed DVT, the research question was extended to VTE in general. DVTs in two patients represent 1.02% of the patient population and are therefore clearly below the incidence reported in intensive care patients in the literature (5-37%).[15,63]

From a statistical point of view, trough12 and trough24 anti-Xa activity levels do not significantly differ between patients with and without VTE. Only in the peak anti-Xa activity level a significant difference could be observed. However, contrary to expectations, the mean anti-Xa activity in the group with VTE was higher than in the group without VTE. Higher anti-Xa activity is associated with higher levels of enoxaparin, which in turn should reduce the risk of thrombosis. Therefore, no statement can be given whether a specific anti-Xa activity value should be aimed in prophylactic enoxaparin dosage. To avoid bias, anti-Xa activities measured after the first documentation of VTE were not further considered in this thesis.

In the literature different results are presented. Ko et al. showed in trauma patients that the incidence of VTE could be reduced by monitoring anti-Xa activity. In this prospective study subprophylactic anti-Xa activity was counteracted by increased doses of enoxaparin. Using this method, the occurrence of VTE was reduced from 7.6% to 1.1% ($p = 0.046$).[64] Thus, the incidence of VTE should depend on the anti-Xa activity. In this thesis the dependence on anti-Xa activity could not be verified, because, as described above, mean trough anti-Xa activity did not differ significantly between patient groups with and without VTE. Mean peak anti-Xa activity levels showed paradoxically higher values in the patient group with VTE.

In a further study, Malinoski et al. searched prospectively for DVT using duplex screening. It was found that patients with DVT had a lower mean peak anti-Xa activity than patients without DVT (0.17 vs. 0.27 IU/ml). This contradicts the results of this thesis. As guidelines do not recommend active search for DVT many asymptomatic DVTs are possibly overlooked. It should also be noted that only DVTs were considered in the mentioned study and not VTE in general.

In the literature often subprophylactic trough anti-Xa activity (≤ 0.1 IU/ml) despite regular LMWH dosage resulting in an increased risk of VTE are reported.[64–66] . Also in the

patients included in this study, the trough12 and trough24 anti-Xa activity values often fell below this limit, equally in the patient groups with and without VTE.

Regarding the relationship between enoxaparin dosage and anti-Xa activity, a highly significant correlation was found for peak levels, as compared to trough12 and trough24 levels. Based on these results, it can be stated that peak anti-Xa activity levels reflect the last enoxaparin dose given, whereas through levels should better be used to determine accumulation of enoxaparin. However, as no differences regarding rate of thromboembolic complications can be made, the clinical utility of determining peak anti-Xa activity for prophylactic enoxaparin still remains questionable.

Enoxaparin is excreted renally and it is widely known that drug accumulation occurs in renal dysfunction.[64,65,67] This was also confirmed by the results of this diploma thesis. Especially in cases of very low creatinine clearance (≤ 30 ml/min) indicating poor renal function, monitoring of anti-Xa activity is recommended to avoid an accumulation of enoxaparin and increased bleeding risk.[67] From the perspective of this study, this recommendation can be endorsed.

In the last research question the connection between anti-Xa activity and administered RBCs should be investigated. Higher anti-Xa activity values could promote the occurrence of bleeding events. All correlation coefficients are not deemed statistically significant. The sample size would have to be increased to make a clearer statement. However, this missing correlation is supported by Ko et al., where no significant association between enoxaparin-mediated anti-Xa activity and administered RBCs was found as well.[64] Malinoski et al. also detected no analogous connection in UFH-mediated anti-Xa activity levels, neither did Droege et al. with regard to dalteparin.[65,66]

5.1 Limitations

Overall, only two newly occurring DVTs were documented in the entire collective of 197 patients, which were not known at the time of admission. This can have several causes. As stated in 1.3.3.3, current guidelines do not recommend an active search for DVT, only after the appearance of clinical signs or in case of a high-risk scoring result. Therefore, DVTs that are asymptomatic are unlikely to be detected. In addition, the PDMS does not have a separate field or form to document DVTs. DVT must thus be documented manually in the course, which also can lead to a loss of information. In addition, DVT was often suspected based on clinical signs and a CT angiography

was ordered for the following day. As a first step, the enoxaparin dosage was increased. The next day no DVT could be recorded by imaging procedures.
Another indication that undetected DVTs must be present in the collective is that new PE occurred in 5 patients. PE, as already noted in chapter 1.3.4, almost always have their origin in DVT. In contrast to DVT, PE shows up more symptomatic. It should be mentioned that not every DVT necessarily leads to a PE. All in all, that means that an estimated number of unreported cases of DVT can be expected.
In any case, a higher number of included patients would eliminate many uncertainties. The amount of highly invasive measures in intensive care leads to many exclusions from the patient collective. Compared to other wards, the patient collective in intensive care is very heterogeneous and has many concomitant diseases. Therefore, more patients should be enrolled for further investigations.

6 Conclusion

The aim to relate anti-Xa activity with DVT did not succeed due to the small number of cases. Therefore, the study was extended to VTE.

Based on the underlying data, no anti-Xa target level could be defined to prevent VTE in prophylactic enoxaparin dosing. In the trough level range, the anti-Xa activities of the patient groups with and without VTE differentiate not significantly. Only the peak anti-Xa activity levels differ significantly - but these are higher in the group of patients with VTE, which makes it impossible to establish a target level for VTE prophylaxis.

As a secondary objective, the correlation between administered enoxaparin dose and the mediated anti-Xa activity was investigated. Only in peak levels of anti-Xa activity a significant correlation could be shown.

A clear significant correlation between anti-Xa activity and creatine clearance was demonstrated. Therefore, the anti-Xa activity can be monitored in case of poor renal function.

With regard to bleeding risk, no significant correlation between administered RBCs and measured anti-Xa activity could be shown.

7 Future Perspective

This diploma thesis represents the beginning of a larger study of the Medical University of Vienna. In total, the time period will be extended to a period of 4 years and thus, significantly more patients will be included. With this diploma thesis the first empirical values are collected, methods are specified, and the procedure is tested. Due to the higher amount of data, a more precise statement can be made about the question of whether the risk of thrombosis can be estimated with the help of anti-Xa activity. The results obtained are to be published and thus create added value for science and medicine.

Within this diploma thesis, a list of keywords was established in order to achieve possible hits with machine searches. The aim is to increase the speed of the evaluation. Patient records containing these keywords are filtered out and can be checked manually. Documents that do not produce any hits no longer need to be viewed. This saves a lot of time, since this large part of the documents cannot be used for the study.

Currently there is no separate protocol in the patient data management system for the archiving or documentation of thromboses. The documentation depends on the treating physician and must be entered manually in the course. Information can be lost here. If there were a separate section for the archiving of thromboses, this would make work much easier for future studies.

A further study could also prospectively look for DVT in ICU patients. The mentioned number of potentially undetected DVTs could thus be avoided. However, this method is much more complex and requires well-trained personnel. In addition, a significant increase in costs and time requirement can be expected. In contrast, the patient data management system of the General Hospital of Vienna provides available data any time and can be processed automatically.

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