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ACADEMIC DISSERTATION
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ACADEMIC DISSERTATION
Tampere University, Faculty of Medicine and Health Technology
Tampere University Hospital, Department of Intensive Care Medicine
Finland

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PunaMusta Oy – Yliopistopaino
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For my family

ABSTRACT

Critical illness increases blood coagulation and therefore patients in the intensive care unit (ICU) are prone to venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE). It is recommended to use thromboprophylaxis, most commonly with low-molecular-weight heparins (LMWHs), as a part of critical care. Nonetheless, despite pharmacological thromboprophylaxis, the incidence of DVT is still 5–15%. The guidelines for thromboprophylaxis are based on studies conducted in medical and surgical patients whereas much less data are available from ICU patients. However, standard thromboprophylaxis in critically ill patients may lead to ineffective anticoagulation.

The main objective of this thesis was to study how to make thromboprophylaxis safer and more effective in critically ill patients. In addition, the association of critical illness with blood coagulation was investigated. The coagulation status was mainly evaluated in patients with aneurysmal subarachnoid hemorrhage (aSAH) who are considered high-risk patients for VTE. At the same time, the risk of bleeding persists, which makes the delivery of anticoagulation particularly challenging.

This thesis consists of four studies. Study I was a systematic review of the literature, examining 18 original publications where LMWH thromboprophylaxis had been monitored with an anti-Xa level measurement in critically ill patients. The anti-Xa levels in critically ill patients were lower in comparison with ward patients. However, no association was seen with clinical adverse events, e.g., bleeding or VTE.

In Study II and III, the route of anticoagulation administration was investigated in a randomized controlled study. Forty ICU patients were randomized to receive 40 mg of enoxaparin thromboprophylaxis either as a continuous intravenous infusion (CII) over 24 hours or as a subcutaneous bolus (SCB) every 24 hours for 72 hours. In Study II, the maximum concentration of anti-Xa was higher with SCB dosing of enoxaparin at 0–24 and 0–72 hours than attained with CII. However, at the final trough level at 72 hours, the anti-Xa level was higher with CII.

In Study III, additional blood clotting parameters were analyzed; these included prothrombin fragment 1+2 (F1+2), antithrombin, fibrinogen, and D-dimer. Moreover, the formation of thrombin was assessed with a calibrated automated thrombogram from a subset of patients. The F1+2 levels were lower when CII

thromboprophylaxis was used than with SCB. These results may indicate a more pronounced anticoagulation with CII thromboprophylaxis.

In Study IV, coagulation changes after aSAH were examined by rotational thromboelastometry (ROTEM). This study was a prospective observational study conducted in 17 aSAH patients and 16 elective neurosurgical patients as controls. The main result was that after aSAH, the blood coagulation increased, and this could be measured by ROTEM. The increment in blood coagulation seemed to be further associated with a worse neurological outcome.

In conclusion, the reported anti-Xa levels with LMWH thromboprophylaxis were not related to clinical events in ICU patients. When LMWH thromboprophylaxis was given as an SCB, the maximum anti-Xa concentrations were higher than with CII. However, the CII led to a higher final trough level of anti-Xa and to more pronounced factor Xa inhibition. These effects of CII suggest that it results in more continuous anticoagulation in ICU patients than can be attained with SCB. Blood coagulation increased after aSAH and associated with worse neurological outcomes.

TIIVISTELMÄ

Useimmat tehohoitoon johtaneet sairaustilat lisäävät veren hyytymistaipumusta ja altistavat potilaat laskimotukoksille ja keuhkoveritulpille. Tämän vuoksi tehohoitopotilailla suositellaan käytettäväksi laskimotukoksia ehkäisevänä lääkityksenä pienimolekyylisiä hepariineja (LMWH). Käytetystä estolääkityksestä huolimatta laskimotukoksia ilmaantuu 5–15 %:lle potilaista tehohoidon aikana. Tämän hetkiset hoitosuositukset pohjautuvat lähinnä vuodeosastopotilailla tehtyihin tutkimuksiin ja näyttöä LMWH-valmisteiden käytöstä tehopotilailla on vähän. Alustavissa tutkimuksissa on saatu viitteitä, että käytettäessä LMWH-valmisteita muilla potilasryhmillä sopiviksi havaituilla annoksilla, saattaa niiden veritulppia ehkäisevä vaikutus jäädä tehohoitopotilailla odotettua heikommaksi. Väitöskirjatutkimuksen tavoitteena on selvittää, miten laskimotukoksia ehkäisevä hoito voitaisiin toteuttaa nykyisiä hoitomuotoja tehokkaammin ja turvallisemmin. Lisäksi tutkitaan miten kriittinen sairaus vaikuttaa veren hyytymiseen ja miten tätä muutosta voitaisiin monitoroida parhaiten. Tätä tarkastellaan erityisesti äkilliseen lukinkalvonalaisen verenvuotoon (subraknoidaalivuoto, SA-vuoto) sairastuneilla tehohoitopotilailla, joiden laskimotukoksia ehkäisevän hoidon toteuttaminen on haastavaa samanaikaisesti ilmenevän kohonneen verenvuotoriskin vuoksi.

Väitöskirja koostuu neljästä osatyöstä, joista ensimmäisessä tutkittiin systemaattisen kirjallisuuskatsauksen avulla, osoittaako veren hyytymistekijä X:n aktiivisuuden estovaikutus LMWH-estolääkityksen tehoa. Tutkimuksessa todettiin, että anti-Xa pitoisuudet olivat tehopotilailla suositeltuja matalammat, mutta nämä eivät olleet yhteydessä lisääntyneeseen tukostaipumukseen.

Toinen ja kolmas osatyö perustuivat satunnaistettuun ja kontrolloituun tutkimukseen tehohoitopotilailla, jossa selvitettiin, voidaanko laskimotukoksia ehkäisevä hoito LMWH-valmiste enoksapariinilla toteuttaa tehokkaammin jatkuvalla suonensisäisellä tiputuksella kuin tavanomaisella ihonalaisella annostelulla. Aiempaa tutkimustietoa jatkuvan suonensisäisen enoksapariinihoidon soveltuvuudesta tehohoitopotilaiden laskimotukosten ehkäisyyn ei ole. Tutkimukseen osallistui yhteensä 40 tehohoitopotilasta. Tutkimuksessa todettiin, että ensimmäisen vuorokauden aikana veren anti-Xa:n huippupitoisuus oli suurempi ihonalaista annostelua saaneilla. Suonensisäistä annostelua saaneilla pitoisuudet pysyivät

kuitenkin tasaisempina tutkimuksen ajan ja 72 tunnin kohdalla suuremmalla osalla oli havaittavissa veren hyytymisen estovaikutusta vertailuryhmään nähden.

Kolmatta osatyötä varten potilailta kerättiin pakasteverinäytteitä, joista määritettiin tarkempia veren hyytymistä kuvaavia verikokeita, kuten protrombiinifragmentti (F1+2), D-dimeeri ja trombiinin muodostuminen. Annettaessa enoksapariinia jatkuvana suonensisäisenä tiputuksena veren hyytymistaipumus väheni merkitsevästi ihonalaiseen annosteluun verrattuna.

Neljännessä osatyössä tarkasteltiin prospektiivisella seurantatutkimuksella SA-vuodon aiheuttamia muutoksia veren hyytymiseen kokoverestä tehtävillä trombolelastometria-analyyseillä (ROTEM). SA-vuodon jälkeistä hyytymistaipumusta ei ole aiemmin tutkittu ROTEM:n avulla. Tutkimukseen osallistui 17 SA-vuotoon sairastunutta tehohoitopotilasta ja vertailuryhmän muodostivat 16 elektiivistä aivoleikkauspotilasta. Päälöydöksenä todettiin, että SA-vuoto lisää veren hyytymistaipumusta, joka on havaittavissa ROTEM-mittauksella. Lisääntynyt hyytymistaipumus oli myös yhteydessä myöhäisen aivoiskemian ilmaantuvuuteen ja sen seurauksena huonompaan neurologiseen selviytymiseen.

Väitöskirjatutkimuksen ensimmäinen osatyö osoitti, ettei tavanomainen laskimotukoksia ehkäisevä hoito saavuta riittävää estovaikutusta tehohoitopotilailla. Osatöiden kaksi ja kolme perusteella näyttäisi siltä, että laskimotukosten estohoitoon jatkuvalla suonensisäisellä annostelulla liittyy tehohoitopotilailla tasaisempi lääkevaikutus. Tällöin myös veren hyytymisen aktivoituminen vähenee tehokkaammin ihonalaiseen annosteluun verrattuna. Osatyön neljä perusteella voidaan todeta ROTEM-analyysin soveltuvan lisääntyneen hyytymistaipumuksen tunnistamiseen. Kokonaisuutena tutkimustulosten avulla voidaan parantaa tehohoitopotilaan hyytymistaipumuksen tunnistamista ja estolääkitykseen liittyviä hoitokäytänteitä.

CONTENTS

ABBREVIATIONS

Anti-Xa	Anti-factor Xa
ACCP	American College of Chest Physicians
APACHE	Acute Physiology and Chronic Health Evaluation
APC	Activated protein C
APTT	Activated partial thromboplastin time
aSAH	Aneurysmal subarachnoid haemorrhage
AT	Antithrombin
AUC	Area under the time-concentration curve
BID	Bis in die (twice a day)
CII	Continuous intravenous infusion
CFT	Clot formation time
CT	Clotting time
COPD	Chronic obstructive pulmonary disease
CRT	Catheter-related thrombosis
CVC	Central venous catheter
DCI	Delayed cerebral ischemia
DVT	Deep vein thrombosis
DIC	Disseminated intravascular coagulation
EBI	Early brain injury
E.t.	Et alii (and others)
ETP	Endogenous thrombin potential (TGA-CAT)
F 1+2	Prothrombin fragment 1+2
FX	Coagulation Factor X
FXa	Activated coagulation Factor X
GAG	Glycosaminoglycan
GCS	Graduated compression stockings
GOSe	Extended Glasgow Outcome Wcore
GRADE	Grading of Recommendations Assessment, Fevelopment and Evaluation
HcII	Heparin co-factor II

HIT	Heparin-induced thrombocytopenia
IPC	Intermittent pneumatic compression
ICU	Intensive care unit
I.e.	Id est (that is)
IQR	Interquartile range
ITT	Intention-to-treat
LMWH	Low-molecular-weight heparin
MCF	Maximal clot firmness
MOOSE	Meta-analysis Of Observational Studies in Epidemiology
OD	Omne in die (once a day)
PF4	Platelet factor 4
PE	Pulmonary embolism
Peak	Peak amount of generated thrombin (TGA-CAT)
PP	Per-protocol
PPP	Platelet-poor plasma
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCT	Randomized controlled trial
ROTEM	Rotational thromboelastometry
SCB	Subcutaneous bolus
SD	Standard deviation
TEG	Thromboelastography
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TG	Thrombin generation
TGA-CAT	Thrombin generation assay by calibrated automated thrombogram
TM	Thrombomodulin
tt-Peak	Time-to-Peak
UFH	Unfractionated heparin
VTE	Venous thromboembolism

ORIGINAL PUBLICATIONS

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II Vahtera, A., Valkonen, M., Huhtala, H., Pettilä, V., & Kuitunen, A. (2017). Plasma anti-FXa concentration after continuous intravenous infusion and subcutaneous dosing of enoxaparin for thromboprophylaxis in critically ill patients. A randomized clinical trial. Thromb Res. 2017 Oct; 158:71-75.

III Vahtera, A., Szanto, T., Lassila, R., Valkonen, M., Sivula, M., Huhtala, H., Pettilä, V., & Kuitunen, A. (2019). Continuous intravenous infusion is superior to subcutaneous administration of enoxaparin in controlling thrombin formation in critically ill patients. Data from the ENOKSI thromboprophylaxis RCT. Submitted.

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1 INTRODUCTION

Critically ill patients have a high risk of venous thromboembolism (VTE), i.e., deep vein thrombosis (DVT) and pulmonary embolism (PE). Without thromboprophylaxis, the incidence of DVT is around 30% (Cade, 1982; Fraisse et al., 2000; Hirsch et al., 1995). VTE increases morbidity (Cook et al., 2005a; Cook et al., 2005b), and in-hospital mortality is relatively high (Bahloul et al., 2010; Patel et al., 2005).

Pharmacological thromboprophylaxis either with low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH) is recommended for all patients in the intensive care units (ICUs) to prevent VTE unless contraindicated (Gould et al., 2012; Kahn et al., 2012; Lassila et al., 2016). However, with the recommended prophylaxis, the risk of DVT is not completely eliminated but only decreased to 5–15% (Cook et al., 2011; Fraisse et al., 2000).

There are multifactorial reasons for the persistent risk for VTE in critically ill patients (Cook et al., 2005b). First, thromboprophylaxis might be omitted because of the surgical procedure or the concomitant bleeding risk (Lauzier et al., 2014). Second, the bioavailability of subcutaneously administered thromboprophylaxis might be impaired (Dörffler-Melly et al., 2002; Haas et al., 2005; Priglinger et al., 2003). Third, it has been claimed that the current thromboprophylaxis regimens might be inadequate (Robinson et al., 2013) to overcome the hypercoagulable state caused by critical illness (Sivula et al., 2009).

Monitoring anticoagulation achieved by LMWH thromboprophylaxis is not generally advised (Garcia et al., 2012). In some clinical situations, e.g., renal failure or obese patients, it is suggested that plasma anti-Xa activity (anti-Xa) should be assayed. However, the ability of anti-Xa to predict the bleeding or thrombotic risk is limited (Bara et al., 1992). This could partly be explained by the principle of anti-Xa measurement method. The anti-Xa level reflects only the inhibitory effect of activated coagulation factor X, and for example the direct inhibition of thrombin is ignored. Thus, there could be discrepancy between anti-Xa levels and clinical events (Walenga et al., 1991). However, there is a lack of evidence to support monitoring LMWH anticoagulation with anti-Xa in critically ill patients.

Aneurysmal subarachnoid hemorrhage (aSAH) patients are one of the most challenging populations for implementing thromboprophylaxis. The risk of VTE is high, but at the same time, the bleeding risk persists with potentially life-threatening consequences (Fujii et al., 1996; Ray et al., 2009). After the initial bleeding, it is known that a hypercoagulation state is present almost immediately (Ettinger, 1970). It is not known, however, if these changes can be detected by rotational thromboelastometry (ROTEM).

The primary purpose of the present study was to assess the pharmacokinetics of one of the most common LMWHs, enoxaparin, after continuous intravenous infusion (CII) in comparison with standard subcutaneous thromboprophylaxis dosing in critically ill patients. The measurement of the anti-Xa level, as well as other coagulation parameters, were used. Second, the current literature of the anti-Xa levels after LMWH thromboprophylaxis were systematically reviewed. Third, the coagulation changes after aSAH were prospectively evaluated by ROTEM.

2 REVIEW OF THE LITERATURE

2.1 Overview of the blood coagulation

Under physiological conditions, blood coagulation is activated when hemostasis is needed. This process, however, can be extreme or happen at the wrong location, resulting in either venous or arterial thrombosis. These blood clots can further travel through the bloodstream as an embolus.

The process of blood coagulation was previously described either as a waterfall (Hoffman et al., 2001) or as an enzymatic cascade (Macfarlane, 1964). These models were protein-centric, and they further were refined as the understanding of the biochemistry of the coagulation factors improved. Typically, the coagulation process has been divided into three pathways. First, the extrinsic pathway, where coagulation is initiated by tissue factor (TF) that is located outside of the bloodstream, second, the intrinsic pathway where all components of coagulation are in the blood and third, the final common pathway where these two pathways interact (Figure 1).

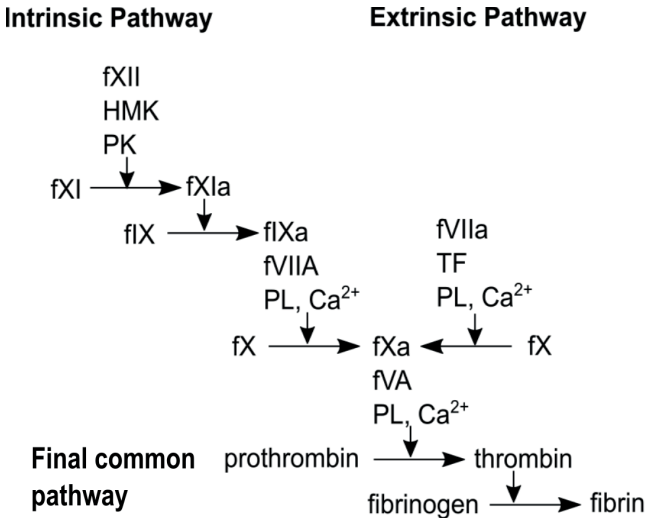

Figure 1. Coagulation cascade, the classical interpretation. Abbreviations: fXII, coagulation factor XII; HMK, high-molecular-weight kininogen; PK, pre-kallikrein; PL, phospholipid; TF, tissue factor. Modified from (Hoffman et al., 2001).

In the 1990s, this classification was challenged since it did not correlate with *in vivo* observations (Roberts et al., 1992). For example, why does the extrinsic activation of factor X (fX) not compensate for the lack of fVIII or fIX in hemophiliacs? Furthermore, the extrinsic and intrinsic pathways overlap in several situations. For example, the fVIIa/TF-complex can also active fIX (Osterud et al., 1977), thrombin can directly activate fXI (Gailani et al., 1993) and activated platelets can provide a surface for the activation of fXI (Oliver et al., 1999). Moreover, fXII, high-molecular-weight kininogen, and pre-kallikrein might not be needed for the activation of coagulation (Baglia et al., 1998). Instead, the primary coagulation activator seems to be TF (Hoffman et al., 1996).

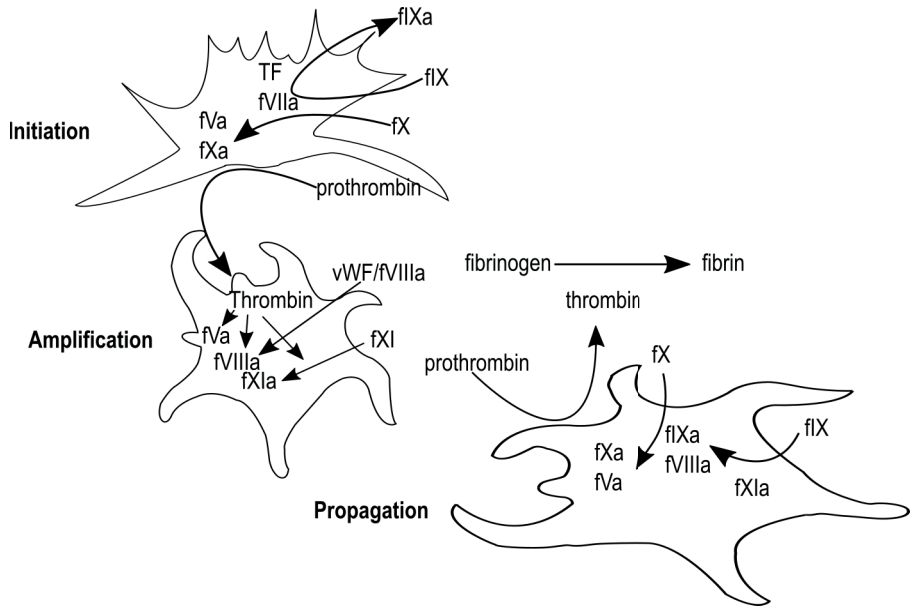

Figure 2. Coagulation cascade, the cell-based interpretation. Abbreviations: TF, tissue factor; vWF, von Willebrand factor. Modified from (Hoffman et al., 2001) .

At the moment the most prevalent model for coagulation is a cell-based system (Hoffman et al., 2001). In contrast to the previous models, it emphasizes the role of the cellular environment in the clotting. In this model, the coagulation does not occur linearly but in the three parallel phases on different cell surfaces, complicating the binary classification into extrinsic and intrinsic pathways. Here, the blood coagulation is divided into three phases. The first phase is called initiation, where the release of TF starts the coagulation process. Under normal conditions, TF is located

on most types of extravascular cells, and it does not make any contacts with plasma coagulation factors (Banner et al., 1996). During inflammatory states, however, TF can be detected in blood by monocytes and endothelial cells. Typically, hemostasis is activated when vascular injury allows coagulation factors in plasma to contact with extravascular TF-bearing cells. Here fVII binds to TF, and the formed complex activates both coagulation, namely fX and fIX, and anticoagulation proteins, e.g. proteins C and S (Wildgoose et al., 1989). Activated fX (fXa) activates fV, and together, they can produce a small amount of thrombin from plasma prothrombin (Monkovic et al., 1990). However, the majority of the FXa-fV complex is readily inhibited by tissue factor pathway inhibitor (TFPI) terminating the thrombin formation.

The second phase involves amplification which takes place on the surface of the platelets. Subsequently, the small amount of thrombin formed during the initiation phase activates the platelets, fV, fVIII, and fXI (Díaz-Ricart et al., 2000; Monroe et al., 1996). This results in the formation of activated platelets with activated cofactors, fVa, and fVIIIa bound to their surfaces (Hung et al., 1992).

The third phase is a propagation on the activated platelets. It begins when the fVIIIa/fIXa complex (called "tenase") activates fX on the platelet surface. FXa is now able to form a complex with its cofactor V, which is bound to the platelet surface. Together they form a complex (called "prothrombinase") that eventually catalyzes the enzymatic reaction where prothrombin is converted into thrombin (Oliver et al., 1999). The propagation phase results in the large-scale formation on thrombin. The amount of formed thrombin is proportional to the clot strength. During this process, prothrombin fragments 1+2 (F1+2) are formed (Figure 3), and the level of F1+2 is an indirect sign of thrombin formation (Schutgens et al., 2004).

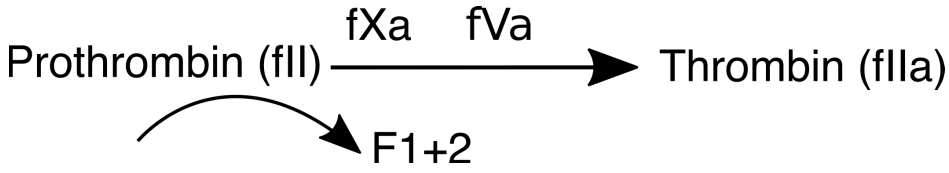

Figure 3. Formation of prothrombin fragments. Abbreviations: fXa, activated coagulation factor X; F1+2, prothrombin fragment 1+2.

Thrombin cleaves fibrinogen, originally called fI, into insoluble fibrin monomer (Riedel et al., 2011). These monomers form first oligomers that aggregate forming protofibrils. The protofibrils interact with each other, building longer fibrin chains

and eventually polymerized fibrin (Weisel et al., 2013). The polymerized fibrin completes the blood clot formation together with platelet adhesion and aggregation.

2.1.1 Regulation of the blood coagulation

As important as it is to have coagulation initiated, it is as crucial to prevent unnecessary clotting. The vascular endothelium is an essential element in the inhibition of blood clotting (Bombeli et al., 1997). It releases anticoagulants such as protein S and TFPI and acts as a source of surface-bound proteoglycans, such as thrombomodulin (TM) and heparan sulfate proteoglycans. The main action is to inactivate thrombin, either inhibiting its function by antithrombin (AT) or binding to TM (Cadroy et al., 1997). When the hemostasis is achieved, the formation of blood clot is further controlled by fibrinolysis (Marder et al., 2013). During fibrinolysis the fibrinolytic system is activated, and fibrin is converted into soluble fibrin degradation products, such as D-dimer (Figure 4). The process is activated by proteases, mainly tissue-type and urinary-type plasminogen activators, that convert plasminogen into active plasmin, which further dissolves fibrine. The main inhibitors of the fibrinolytic system are plasminogen activator inhibitor 1, α_2-antiplasmin and thrombin-activatable fibrinolysis inhibitor.

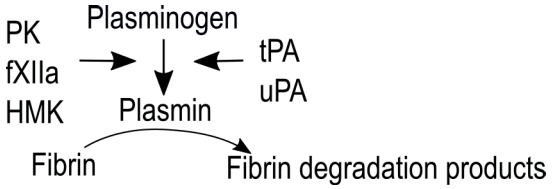

Figure 4. Degradation of fibrin by plasmin. Abbreviations: fXIIa, activated coagulation factor XII, HMK, high-molecular-weight kininogen; PK, pre-kallikrein; tPA, tissue-type plasminogen activator; uPA, urinary-type plasminogen activator.

2.1.1.1 Thrombomodulin-activated Protein C

TM is a specific receptor for thrombin located on intact endothelial cells. When thrombin is bound to TM, its specificity changes and it no longer cleaves fibrinogen or activates platelets (Ye et al., 1991). Instead, it becomes a more efficient protein C activator. Activated protein C (APC) forms a complex with protein S. This complex inactivates fVa and fVIIIa (Fulcher et al., 1984; Walker et al., 1979). Thus, when

thrombin acts via APC it is anti-thrombotic when it is localized on healthy, intact endothelial cells, preventing unnecessary clot formation. In inflammatory states, the expression of TM decreases and this can lead to unnecessary coagulation (Moore et al., 1987).

2.1.1.2 Antithrombin

AT is a powerful endogenous anticoagulant protein in plasma (Murano et al., 1980). It downregulates blood clotting by inhibiting thrombin and other coagulation factors, namely fXa and fIXa, (Biggs et al., 1970; Olds et al., 1994). The native conformation of AT molecule has only a limited inhibitory activity in coagulation, but it is greatly accelerated by heparan sulfate proteoglycans under physiological conditions (Damus et al., 1973). Heparan sulfate proteoglycans are located on the vascular endothelium and in the underlying matrix, and when they interact with AT, conformational changes are induced (Marcum et al., 1984). The pharmaceutical principle of the heparins is their ability to induce a similar conformational change in the AT molecule (Beeler et al., 1979; Lin et al., 2001).

Inherited deficiencies in AT increase the risk for venous thrombosis (Egeberg, 1965). The prevalence is around 0.02 to 0.05%, and disease severity depends on the genetic properties of the deficiency (Patnaik et al., 2008; Tait et al., 1994). An acquired AT deficiency is more common, a phenomenon occurring in association with severe sepsis, trauma, and extracorporeal circulation (White et al., 2001). In general, these conditions lead to consumption coagulopathy, resulting in a decline in AT levels (Harper et al., 1996). AT levels below 60% of normal have been associated with VTE, but supplementation with AT concentrate is controversial or may be even detrimental (Warren et al., 2001).

2.1.1.3 Heparin co-factor

Heparin cofactor II (HcII) is another type of serpine produced in liver (Tollefsen et al., 1981). HcII inhibits thrombin but no other coagulation factors. Both inherited and acquired HcII deficiencies have been described (Tollefsen, 2002). However, the association of low HcII levels with VTE is unclear and no routine testing is advised (Olson, 2002).

2.1.1.4 Tissue factor pathway inhibitor

TFPI can be found mainly from the microvascular endothelium. In plasma, TFPI is bound to lipoproteins; it inhibits fXa directly and produces feedback inhibition of the fVIIa-TF complex (Broze et al., 1988). The anticoagulant properties of TFPI can be further enhanced with heparins (Ho et al., 1997).

2.2 Natural history of venous thromboembolism

"Phenomena due to the irritation of the vessel and its surroundings;
Phenomena due to blood coagulation;
Phenomena due to the interruption of the blood-stream."
(Virchow, 1856)

Virchow's Triad– endothelial damage, hypercoagulability, and venous stasis is the classic foundation of the pathophysiology of VTE. It is a vicious circle that activates the coagulation cascade and leads further to thrombus formation and propagation.

Venous thromboembolism presents either as a DVT or a PE. DVTs can be further subdivided by the location in which they occur i.e. proximal or distal (Kearon, 2003). In lower extremities the divider is the knee and in the upper extremities the elbow. In addition, classifications into upper and lower extremity DVTs and symptomatic versus asymptomatic DVTs have been used.

The natural history of venous thrombosis typically originates from the calf veins, where it might embolize into proximal veins and further to the pulmonary arteries causing a PE (Kakkar et al., 1969). The PE, which has been referred to as "sudden breathless sleep", is the most feared and potentially fatal complication of VTE (Bell et al., 1982). In addition, the natural progression from DVT, PEs can arise from various sources, e.g. thrombi in the right atrium, amniotic fluid, bone marrow, or tumors (BellSimon et al., 1982). Furthermore, there is a rare entity called in situ pulmonary artery thrombosis that usually results from blood stasis in the pulmonary vasculature (Cha et al., 2015).

Without adequate treatment, 28% of symptomatic distal DVT will recur within 90 days' follow-up, 17% with a proximal extension, and 13% with PE (Lagerstedt et al., 1985). Similar results have been observed in postoperative patients in whom nearly 30% experienced a distal DVT in the early postoperative period (Kakkar et

al., 1969). Without treatment, the thrombosis persisted in 20% of patients, from which nearly every fourth had extended into proximal DVT after only 72 hours.

In medical and surgical patients, the presence of DVT is known to associate with coincidental PE in 40 to 50% of cases. Thus, they are often considered as one entity (Kakkar et al., 1969; Moser et al., 1994; Nielsen et al., 1994). Especially with proximal DVT, there is a severe risk for subsequent PE (Moser et al., 1981a). Majority of these PEs are asymptomatic (Moser et al., 1994; Nielsen et al., 1994). In mechanically ventilated ICU patients who required a chest CT scan, the prevalence of PE was 19% with a concurrent DVT rate of 33% (Minet et al., 2012). Though the lower extremity DVTs are strongly associated with PE, it is not precisely known how many of the proximal DVTs embolize from the lower extremity veins into the pulmonary arteries to form a PE.

2.3 Prevalence and incidence of venous thromboembolism in critically ill patients

ICU patients have a higher risk of DVT than other hospitalized patients (Attia et al., 2001; Cade, 1982). The used definition of prevalent DVT in critically ill patients has varied from 6 hours to two days after the ICU admission (Cook et al., 2005b; Cook et al., 2011; Schönhofer et al., 1998). When an ultrasonography screening protocol has been used, the DVT prevalence within 48 hours of ICU admission has been around 3% in the medical-surgical ICU population (Cook et al., 2005b; Cook et al., 2011). In specific ICU patient groups, the prevalence of DVT has been somewhat higher; 8% in surgical patients (Harris et al., 1997) and 11% in patients with acute exacerbation of chronic obstructive pulmonary disease (COPD) requiring ICU admission (Schönhofer et al., 1998).

The incidence of DVT has been about 30% (9–32%), when no thromboprophylaxis has been used (Cade, 1982; Fraisse et al., 2000; Hirsch et al., 1995; Moser et al., 1981b). The study designs are presented in more detailed in Table 1.

Table 1. The incidence of DVT without thromboprophylaxis in critically ill patients. Abbreviations: COPD, chronic obstructive pulmonary disease; DVT, deep venous thrombosis; ICU, intensive care unit; MICU, medical intensive care unit; NS, not specified; PE, pulmonary embolism; RCT, randomized controlled trial; US, ultrasonography.

Author	ICU type	Study design	Screening method	Timing of screening	No of patients	DVT (%)	PE (%)
Moser et al. (1982)	Respiratory	Prospective cohort	Lower extremity 125I-labeled fibrinogen scanning	Twice weekly	34	9	6
Cade et al. (1982)	General	RCT	Lower extremity 125I-labeled fibrinogen scanning	8 d (4–10 d)	119	29	NS
Hirsch et al. (1995)	MICU	Prospective cohort	Lower and upper extremity Doppler US	Within 48 hours and twice weekly	NS	32	1
Fraisse et al. (2000)	COPD	RCT	Lower extremitiy Doppler US and venography	At inclusion and weekly	85	28	None
Joynt et al. (2009)	MICU	Prospective cohort	Lower extremity compression and Doppler US	Within 24 hours and twice weekly	80	19	None

In historical studies, the incidence of DVT has been up to 40% despite pharmacological prophylaxis (Hirsch et al., 1995). In more recent trials, the incidence of proximal DVT during the ICU stay is lower, 5–10%, in the medical-surgical ICU population when pharmacological prophylaxis has been used (Cook et al., 2005a; Cook et al., 2011). In selective ICU patients, DVT rates have varied from 16% in a COPD exacerbation (Fraisse et al., 2000), to 21% in a cohort over-represented with CRRT patients, (Beitland et al., 2019) to 23% in patients with prolonged mechanical ventilation (Ibrahim et al., 2002). In patients with either severe sepsis or septic shock, the incidence of VTE has been the highest – up to 37%, despite of guideline-recommended thromboprophylaxis (Kaplan et al., 2015). Moreover, in severe septic patients, early asymptomatic VTE is common; nevertheless, prophylaxis is used or not (Shorr et al., 2009). The incidence of DVT varies depending on which screening method is used. If the upper extremity DVTs have also been screened, the incidental rate of DVT has been higher (Beitland et al., 2019; Hirsch et al., 1995; Kaplan et al., 2015). The designs of studies where thromboprophylaxis has been used are presented in Table 2.

Table 2. The incidence of DVT with thromboprophylaxis in critically ill patients. Abbreviations: COPD, chronic obstructive pulmonary disease; DVT, deep venous thrombosis, GCS, graduated compression stockings; ICU, intensive care unit; IPC, intermittent pneumatic compression; LMWH, low-molecular-weight heparin; MICU, medical intensive care unit; NS, not specified; RCT, randomized controlled trial; PE, pulmonary embolism; UFH, unfractionated heparin; US; ultrasonography.

Author	ICU type	Study Design	Screening method	Timing of screening	Phramacological prophylaxis	Mechanical prophylaxis	No of patients	DVT (%)	PE (%)
Cade et al. (1982)	General	RCT	Lower extremity 125I-labeled fibrinogen scanning	8 d (4–10 Day)	UFH 5000 IU x 2 or placebo	NS	119	13 vs. 29	ns
Hirsch et al. (1995)	MICU	Prospective cohort	Lower and upper extremity Doppler US	Within 48 hours and twice weekly	43% UHF	18% IPC	100	33	1
Marik et al. (1997)	Mixed	Prospective cohort	Lower extremitiy Duplex US	4–7th ICU Day	UFH	If pharmocological contraindicated (26/102)	102	12	4
Fraisse et al. (2000)	COPD	RCT	Lower extremitiy Doppler US and venography	At inclusion and weekly	Nadroparin 3800–5700 IU vs. placebo	NS	223/167	16 vs. 28	None
Cook et al. (2005)	Mixed	Prospective cohort	Lower extremity compression US	Within 48 hours and twice weekly	UFH (81.7%), LMWH (4%), UFH intravenously (6.8%)	If pharmocological contraindicated	261		
Cook et al. (2005)	Mixed	RCT, pilot	Lower extremity compression US	Within 48 hours and twice weekly	UFH vs. dalteparin 5000 IU	NS	129	9	1.6
Cook et al. (2011)	Mixed	RCT	Lower extremity compression US	Within 48 hours and twice weekly	UFH vs. dalteparin 5000 IU	GCS or IPC	3746	5.1 vs. 5.8	1.3 vs. 2.3
Kaplan et al. (2015)	Septic	Prospective cohort	Lower and upper extremity Doppler US	Prior ICU discharge and on request	43% UFH, 34% LMWH, 3% warfarin	20% GCS	113	37	4
Beitland et al. (2019)	Mixed	Prospective cohort	Lower and upper extremity Doppler US	Within 48 hours and twice weekly	Dalteparin 5000 IU	63 % GCS	70	21	6

The incidence of PE in critically ill patients is less clear and no studies could be identified where the frequency has been studied with screening protocols. In general, in hospitalized patients, the estimated prevalence of PE is around 1% (Stein et al., 1995). In critically ill patients, the PE rate varies between 0 and 6% when no thromboprophylaxis has been used (Fraisse et al., 2000; Moser et al., 1981b; Shorr et al., 2009). In a mixed ICU population, the symptomatic PE rate has been reported as 1% with LMWH and 2 to 4% with UFH prophylaxis (Cook et al., 2011; Marik et al., 1997).

Screening for DVT in critically ill patients is not currently advised (Kahn et al., 2012). However, only a minority of the proximal DVTs are clinically suspected (Cook et al., 2005a). Furthermore, the definition of symptomatic DVT is not useful in critically ill patients (Crowther et al., 2005). In the past, the gold standard of DVT diagnosis was the venous angiogram. This has been replaced with a compression and Doppler ultrasonography in medical-surgical patients. The ultrasonography is a non-invasive procedure, but it has not been compared with venography in ICU patients (Crowther et al., 2005).

Furthermore, the sensitivity of ultrasonography in asymptomatic patients has been questioned previously (Marik et al., 1997). It has been speculated that screening for significant occult DVT should be conducted before initiating a mechanical

thromboprophylaxis, especially with a compression device. The rationale behind this approach is to minimize the risk of embolization from DVT. Though this reasoning seems logical, there is no published evidence to support this practice. However, the screening has been proposed before initiation of a mechanical prophylaxis, if the patient has one of the following risk factors: age over 65 years, Acute Physiology and Chronic Health Evaluation (APACHE) II over 12 points or a recent emergence procedure (Harris et al., 1997).

2.4 Risk factors for venous thromboembolism in critically ill patients

The pathophysiology of VTE is complex, and thus the risk of developing a VTE is multifactorial. Commonly, all known risk factors affect at least one of the three components of the Virchow triad.

Thrombotic risk factors are typically divided either into inherited or acquired factors. In the ICUs, the risk factors may be further classified as prior to the current critical illness or directly related to the reason requiring ICU care or the patient's treatment in that unit. The main risk factors before initiating ICU care include age, obesity (Stein et al., 2005), malignancy (Heit et al., 2002), smoking, inherited thrombophilia, and family/personal history of VTE (Cook et al., 2005b; Lim et al., 2015). In critically ill patients, an increasing BMI seems to be associated with VTE risk (Lee et al., 2017; Lim et al., 2015).

The ICU-acquired risk factors include immobilization, central venous catheters (CVCs), and platelet transfusions (Cook et al., 2005a). Furthermore, the duration of mechanical ventilation (Fraisse et al., 2000; Kaplan et al., 2015) as well as the duration of CVC (Ibrahim et al., 2002), seem to be associated with VTE.

Hypercoagulation with accumulation of tissue factor plays a crucial role in the pathophysiology of VTE (Day et al., 2005). Though patients in the ICU are known to be hypercoagulable (Sivula et al., 2009), the association with hypercoagulable state and VTE in critically ill patients has not been proven (Van Haren et al., 2014).

The risk factors for PE are typically similar to those for DVT, as the most common risk factors for PE are male sex, obesity, history of cancer, patient's history of DVT, coma, thrombocytosis (Lim et al., 2015; Minet et al., 2012). In addition, the vasopressor use has been described to increase the risk of PE (Lim et al., 2015). In trauma ICU patients, the main predictive factors for PE are age over 40 years,

Simplified Acute Physiology Score (SAPS) over 25, and hypoxemia (Bahloul et al., 2011).

2.4.1 Catheter-related thrombosis

The introduction of polyurethane catheters diminished the risk of intravascular catheter-related thrombosis (CRT) (Borow et al., 1985). Nevertheless, asymptomatic CRTs are still relatively common in ICU patients (Durbec et al., 1997; Merrer et al., 2001; Timsit et al., 1998). The risk of catheter-related thrombosis varies according to the insertion site, and it seems to highest among femoral catheters (Merrer et al., 2001; Trottier et al., 1995). When upper extremity CVCs were evaluated for CRT, the subclavian route was preferable over an internal jugular vein (Hrdy et al., 2017; Timsit et al., 1998). As well as the insertion site, increasing age, and the absence of therapeutic heparinization seem to be independent risk factors for CRT (Timsit et al., 1998). The results on whether the duration of CVC increases the risk of VTE are conflicting. Some investigators detected no association with CRTs and the length of cannulation (Joynt et al., 2000; Trottier et al., 1995) while others found a positive association (Beitland et al., 2019; Ibrahim et al., 2002). These differences may be at least partly explained by different screening protocols and methods.

The clinical relevance of CRTs is uncertain. It appears to vary according to the size of thrombus and the level of venous occlusion; a smaller thrombus might even dissolve spontaneously (Trottier et al., 1995). However, in one study, CRTs increased the risk of catheter-related sepsis (Timsit et al., 1998).

2.4.2 Clinical outcomes after venous thromboembolism

In patients with either severe sepsis or septic shock, a clinically significant VTE increased the length of ICU stay but did not affect mortality (Kaplan et al., 2015). Similar results have been reported in patients requiring prolonged mechanical ventilation where VTE lengthened both the ICU and hospital stay (Ibrahim et al., 2002).

In a meta-analysis published in 2015, also an incidental DVT prolonged the ICU and hospital stay, as well as the duration of mechanical ventilation (Malato et al., 2015). Hospital or ICU mortality did not reach statistical significance.

Depending on the size and the location of PE, it can cause acute respiratory symptoms, even cardiovascular collapse. In medical patients, PE is known to

increase morbidity and mortality (Heit et al., 1999; Pengo et al., 2004). In ICU patients, the consequences are less evident. The in-hospital mortality among ICU-acquired PE patients has been reported to be over 50% despite recommended thromboprophylaxis and therapeutic anticoagulation after PE diagnosis (Bahloul et al., 2010). On the other hand, if treated early with anticoagulation, the PE does not seem to increase the ICU or hospital stay, nor does it elevate mortality (Minet et al., 2012).

2.5 Overview of indirect parenteral anticoagulants

2.5.1 Heparin

Heparin was initially discovered over 100 years ago from a dog's liver (Greek for liver "Heparin") (McLean, 1959). It belongs to the class of linear acidic polysaccharides, known as glycosaminoglycans (GAG). Heparin consists of a mixture of GAG chains made from disaccharide subunits. These subunits are highly sulfated and therefore, negatively charged. Natural heparin is produced by the mast cells and can be identified in almost all mammalians. The commercial UFHs are usually isolated either from porcine intestinal mucosa or bovine lung.

2.5.1.1 Structure and effects on coagulation

The mean molecular weight of heparin is 15 000 daltons (Da), but the molecular weight of individual polysaccharide chain ranges from 3000 to 30 000 Da (Andersson et al., 1976). The main anticoagulant activity of heparin is mediated through the heparin-AT interaction (Andersson et al., 1976). AT is a weak anticoagulant as such, but with increasing heparin concentrations, the anticoagulant efficacy increases exponentially from 300 to 2000-fold (Beeler et al., 1979). AT forms a covalent bond with the coagulation enzymes, and thereby the procoagulant activity is irreversibly inhibited. Heparin dissociates and inhibits the activities of other procoagulant enzymes.

The formed heparin-AT complex inhibits several coagulation factors, including fIIa (thrombin), fXa, fIXa, fXIa, and fXIIa (Hirsh et al., 2001); with the most sensitive to inhibition being against thrombin and fXa. The heparin molecule needs to have a specific pentasaccharide unit to allow it to bind AT and an additional

saccharide unit with a minimum of 13 chains for binding to the coagulation fIIa (Figure 5A) (Lindahl et al., 1984; Rosenberg et al., 1979). The inhibition of fXa only requires binding to AT at the unique pentasaccharide unit of heparin molecule (Figure 5B) (Casu et al., 1981). Thus, short heparin chains can only inhibit fXa.

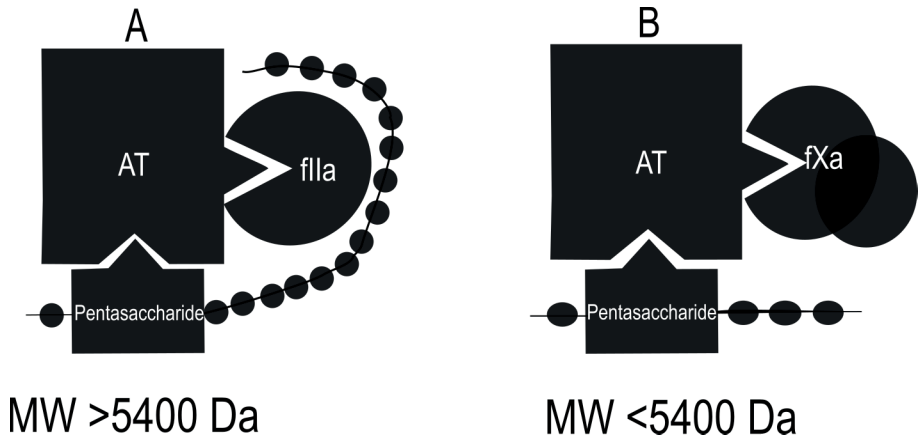

Figure 5. The interaction of heparin chain, AT, fXa, and thrombin. Abbreviations: AT, antithrombin; fIIa, activated coagulation factor II, Da, dalton; MW, molecular weight.

Even heparin chains without the pentasaccharide unit and the capability to interact with AT, can catalyze thrombin inhibition by HcII at high concentrations (TollefsenBlank et al., 1981). The chain length needs to be at least 24 saccharides long. Thus, LMWHs are less effective in the activation of HcII.

In addition to AT mediated actions, heparin possesses numerous other effects, e.g. it releases TFPI, inhibits platelet function (Fernandez et al., 1986), has anti-inflammatory properties (Young, 2008) and enhances the antithrombotic and fibrinolytic function of the vascular endothelium (Blajchman et al., 1989).

In whole blood, the anticoagulation effect of heparin is inhibited by at least two mechanisms. First, platelet factor 4 (PF4) released from activated platelets during coagulation is a potent heparin but not an LMWH inhibitor (Lane et al., 1984). This difference can be explained by different length of polysaccharide chains. Second, when fXa is bound into the prothrombinase complex, it is resistant to heparin inactivation but not to LMWHs (Teitel et al., 1983).

2.5.1.2 Pharmacokinetics

UFH needs to be administered parenterally since its oral absorption is very limited (Arbit et al., 2006). It is usually given either as a CII or as a subcutaneous bolus (SCB). The bioavailability of heparin is 50% when given subcutaneously, and a higher initial dose or an additional IV bolus is often required (Hull et al., 1986). Once it gains access to the bloodstream, heparin binds not only to AT but also to numerous other plasma proteins, endothelial cells, macrophages, and von Willebrand factor (Bârzu et al., 1985). Thus, the pharmacokinetics of heparin is complex, and its anticoagulant effect is unpredictable (Hirsh et al., 2001).

Heparin is cleared by two mechanisms (Bjornsson et al., 1982). First, there is a rapid saturable phase that is due to binding to receptors on endothelial cells (Glimelius et al., 1978) and macrophages (Friedman et al., 1974). Bound heparin is subsequently internalized and depolymerized. Second, there is a slower non-saturable phase when the clearance is mainly renal (de Swart et al., 1982). Because of this dual mechanism of clearance, heparin accumulation in renal insufficiency is less evident.

2.5.1.3 Monitoring anticoagulation

The dose of heparin administered is associated with both its efficacy (Turpie et al., 1989) and safety (Epilog, 1997). While the anticoagulant response displays a wide individual variation, monitoring the effect of heparin is usually advised, especially with therapeutic doses (Garcia et al., 2012). The anticoagulation is generally controlled using the activated partial thromboplastin time, aPTT, a measure of the lag time of thrombin formation in the intrinsic system (Béguin et al., 1988). The suggested aPTT ratio for therapeutic anticoagulation has been between 1.5- and 2.5-times the control value which corresponds to the anti-Xa activity of 0.3–0.7 IU/ml (Basu et al., 1972). However, these targets have not been confirmed in randomized controlled trials (RCTs).

2.5.1.4 Heparin resistance

Heparin resistance is a clinical situation where a patient requires unusually high doses to achieve the targeted aPTT (Hull et al., 1986). This phenomenon has several potential explanations, e.g. AT deficiency, increased heparin clearance (Whitfield et

al., 1983), elevations of heparin-binding proteins (Lijnen et al., 1983) and high levels of factor VIII and/or fibrinogen (Edson et al., 1967) and PF4 (Levine et al., 1984).

In the ICU, unusually large UFH doses are sometimes needed to reach the targeted aPTT. In some patients, this could be acquired (or congenital) contact factor deficiency. In these patients, monitoring of anti-Xa might be useful (Levine et al., 1994).

2.5.1.5 Adverse events

The most common adverse event related to UFH is hemorrhage. The mechanism includes the anticoagulant effect of heparin but also the interaction with platelets and endothelial cells (Blajchman et al., 1989; Fernandez et al., 1986). The risk is proportional to the UFH dose (Morabia, 1986) and with therapeutic dose the risk of major bleeding is around 4% (Costantino et al., 2012). In critically ill patients the major bleeding rate with UFH thromboprophylaxis is around 6% and any bleeding rate 13% (Cook et al., 2011). The anticoagulant effect can be reliably neutralized with protamine sulfate. The neutralization can be verified with a decrease in the aPTT level. Because of the mechanism of action, fresh frozen plasma infusion is ineffective for anticoagulant reversal.

The main non-hemorrhagic adverse events are heparin-induced thrombocytopenia (HIT) (Greinacher et al., 1994) and after long-term use osteoporosis (Shaughnessy et al., 1995). Uncommon reactions include necrotizing skin reactions, hypersensitization, alopecia, hyperkalemia, and transient transaminase elevations.

HIT is a potentially life-threatening disorder where the reaction of heparin with immunoglobulin G type antibodies leads to the activation of platelets (Greinacher, 2015; Warkentin, 2011). The pathogenesis of typical HIT is complex. In brief, some of the heparin molecules form a complex with platelet PF4, a small cytokine that is released from activated platelets (Greinacher et al., 1994). This PF4-heparin complex acts as an antigen for IgG antibody production, resulting in the formation on PF4 /heparin-IgG immune complexes on platelet surfaces. This further activates platelets and promotes PF 4 release. As a consequence, blood coagulation becomes activated in a vicious circle manner. As well as the typical HIT, delayed onset HIT and spontaneous HIT have been described (Warkentin et al., 2001; Warkentin et al., 2014). Their pathophysiology is most likely autoimmune (Warkentin et al., 2016).

In all types of HIT, activated platelets shed highly thrombogenic microparticles that provide a surface for the coagulation cascade to promote the generation of

thrombin (WarkentinKelton et al., 2001). The activated platelets themselves are removed from the circulation, stimulating thrombocytopenia. As a consequence, both venous and arterial thromboses rather than bleeding are triggered. The clinical assessment of HIT is conducted with a pretest probability scoring system, the 4 T's: Thrombocytopenia (from 0 to 2 scores), Timing of thrombocytopenia or thrombosis (from 0 to 2 scores), Thrombosis (from 0 to 2 scores), and no oTher explanation for thrombocytopenia (from 0 to 2 scores) (Lo et al., 2006). A total score is 8 where scores 1–3, 4–5, and 6–8 are considered to correspond to a low, intermediate, and high probability of HIT, respectively. The diagnosis is confirmed from the blood tests (Watson et al., 2012).

HIT usually develops 5–7 days after the initiation of heparin, and it is more common after UFH than after LMWH, especially in surgical patients (Greinacher, 2015). In the ICU, the rate of HIT is 0.3–0.5%, whereas the overall frequency of thrombocytopenia is nearly 100-fold (Cook et al., 2011).

2.5.2 Low-Molecular-Weight Heparins

The development of LMWHs was stimulated by two key findings. First, it was observed that when the heparin molecule was fractionated, it started to progressively lose its ability to prolong aPTT while maintaining the capability to inhibit factor Xa (Andersson et al., 1976). Second, LMWHs seemed to cause less bleeding than heparin in experimental models (Cade et al., 1984).

2.5.2.1 Structure and effects on coagulation

All currently available LMWHs are depolymerized from UFH either chemically (with nitrous acid or alkaline hydrolysis) or enzymatically (with heparinase) (Table 3) (Hirsh et al., 2001). During the depolymerization process, the saccharide chains of UFH are cleaved, resulting in more standardized molecules, with molecular weights from 3000 to 6000 Da. The mean molecular weight of LMWHs is around 5000 Da corresponding to 15 saccharide units (Lane et al., 1984). Though the molecular weight of LMWH is more uniform than in UFH, differences in manufacturing methods lead to differences in LMWH's physical structure, biological, and pharmacological properties (Table 3). Therefore, their interchangeability has been questioned (Baglin et al., 2006). However, in vivo, the anticoagulant activities of different LMWHs seem to be more homogenous.

Table 3. The characteristics of UFH and LMWHs. Abbreviations: Da, dalton; MW, molecular weight; T_{max}, time to peak plasma anti-Xa level, T ½, half-time; UFH, unfractionated heparin (Marder et al., 2013).

Product	MW (Da)	Method of depolymerization	Anti-Xa (IU/mg)	Anti-fIIa (IU/mg)	Ratio	T_{max}	$T_{1/2}$ (h)
UFH	15 000	None			1	minutes	0.5–1.5
Enoxaparin	4034	Alkaline	105	27	3.9	3-5 h	4.5–7
Dalteparin	5663	Nitrous acid	130	58	2.2	3–5 h	3.5–4
Nadroparin	4279	Nitrous acid	95	27	3.5	3–5 h	3.5
Tinzaparin	4500	Heparinase	83	45	1.8	3–4 h	3

The anticoagulant effect of LMWHs, like UFHs, is mainly mediated through AT (Figure 5) (Casu et al., 1981). Because of the depolymerization, the number of saccharide units is lower than in UHF (Andersson et al., 1976). Thus, the capability of LMWHs to catalyze thrombin inhibition is reduced (Figure 5A), and the majority of LMWH molecules can only inactivate fXa (Figure 5B) (Lane et al., 1984). Therefore, the anti-Xa activity of all LMWHs is superior to their anti-IIa activity. To a lesser extent, LMWHs also have AT-independent anticoagulation properties by HcII. However, the clinical relevance is less clear.

2.5.2.2 Pharmacokinetics

The LMWHs pharmacokinetics and the anticoagulant response are more predictable than UFH (Bratt et al., 1986; Frydman et al., 1988; Handeland et al., 1990). The bioavailability after subcutaneous injection is 90% (Hirsh et al., 1992). The elimination half-life of LMWHs is dose-independent, varying between 3 to 7 h. The peak level is 3 to 5 h after SCB. All LMWHs are predominately cleared through the kidneys, which means that their biological half-life is prolonged in patients with renal failure (Palm et al., 1987).

The pharmacokinetic properties of LMWHs and UFH are shown in more detail in Table 3. The anti-Xa-anti-IIa ratio of LMWHs varies from 1.8 (tinzaparin) to 3.9 (enoxaparin) (Baglin et al., 2006). There is no clinical evidence that these ratios reflect their in vivo anticoagulation activities since they are based on assays performed in vitro using platelet-poor plasma (Garcia et al., 2012).

2.5.2.3 Monitoring anticoagulation

LMWHs are typically administered in fixed doses for thromboprophylaxis and weight-adjusted doses for therapeutic situations (Garcia et al., 2012). In general, routine monitoring of LMWH anticoagulation is not advised. In specific conditions, e.g. in morbid obesity and renal insufficiency, monitoring might be beneficial (Garcia et al., 2012; Samama, 1995). In cases of chronic renal insufficiency, anti-Xa levels have a positive correlation with the creatinine clearance (Goudable et al., 1991).

The prolongation of aPTT is only seen significantly with therapeutic doses of LMWH, although the degree varies with different reagents. The recommended test for LMWH monitoring is the chromogenic anti-Xa assay where results are expressed in IU of anti-Xa, accordingly to a LMWH reference preparation for assay calibration. The suggested targets for therapeutic anticoagulation administered BID of enoxaparin are 0.6–1.0 IU/ml assayed at 4 h after SCB representing the peak level (Bara et al., 1985).

There are no anti-Xa targets with fixed doses of LMWH thromboprophylaxis (Garcia et al., 2012), however, in research a peak target of 0.2–0.4 IU/ml is often used (Levine et al., 1989). In general, the anti-Xa levels after LMWH thromboprophylaxis have been lower in critically ill than in ward patients. The exact reason is not known, but a reduced bioavailability of subcutaneously administered LMWH because of a concomitantly administered vasoconstrictor infusion has been suggested (Dörffler-Melly et al., 2002).

2.5.2.4 Adverse events

The affinity of LMWHs for plasma proteins and cells is reduced in comparison with UFH. This leads to more desirable safety profile. The most common side effect of LMWHs is also bleeding, but the risk is lower compared to UFH. In critically ill patients, no difference was seen in major bleeding rates when thromboprophylaxis doses of UFH was compared with dalteparin (Cook et al., 2011). However, since all LMWHs are cleared by the kidneys, in patients with renal failure, there is a risk of accumulation and thus an increased risk of bleeding. This was shown in a meta-analysis by Lim et al., where creatine clearance < 30 ml/min increased the bleeding risk by more than 2-fold after a therapeutic dose of LMWH, but not after a prophylactic dose. The reversal of anticoagulant effects of LMWH is yet to proven. Protamine neutralizes the anticoagulant effect only party, since it mainly neutralizes the anti-IIa activity.

Other possible side effects of LMWHs are similar to UFH, including HIT, transient increase in transaminase levels, osteoporosis, skin reactions, and hyperkalemia (Shaughnessy et al., 1995). Their incidence is lower since LMWHs interact with plasma proteins to a lesser extent. e.g. the incidence of HIT is threefold lower with LMWHs than with heparin (Hirsh et al., 1992).

2.6 Current guidelines for pharmacological thromboprophylaxis in critically ill patients

The Finnish "Deep venous thrombosis and pulmonary embolism: The Current Care Guideline" recommends using thromboprophylaxis in critically ill patients (Lassila et al., 2016). The proposed method of thromboprophylaxis is not specified for critically ill patients, but for high-risk (the overall risk for VTE 4-10%) surgical and medical patients, LMWH is recommended. In high-risk surgical patients, it is recommended to provide supplemental graduated compression stockings (GCS).

The most detailed recommendation for preventions of VTE in critically ill patients can be found in the 9th edition of the American College of Chest Physicians Guidelines (ACCP), especially for non-surgical patients (Kahn et al., 2012) and non-orthopedic surgical patients (Gould et al., 2012). The ACCP guidelines use the GRADE (Grading of Recommendations Assessment, Development, and Evaluation) system for categorizing the quality of evidence (high, moderate, low, and very low) and the strength of recommendations (Guyatt et al., 2008).

2.6.1 Critically ill non-surgical patients

The current ACCP guideline for non-surgical critically ill patients recommends using pharmacological thromboprophylaxis, either LMWH or UFH, in favor of no prophylaxis (GRADE 2C, weak recommendation, low-quality evidence) (Kahn et al., 2012). This recommendation is based on 5 RCTs, one of UFH vs. placebo (Cade, 1982), one of LMWH vs. placebo (Fraisse et al., 2000), and three of UFH vs. LMWH (Cook et al., 2011; De et al., 2010; Shorr et al., 2009). Despite the classification one of the included RCTs was done in surgical patients (De et al., 2010).

2.6.1.1 Unfractionated heparin vs. placebo

Cade et al. studied 119 critically ill patients randomized to receive either UFH 5000 BID or placebo (Cade, 1982). The incidence of lower extremity DVT was evaluated daily by ^{125}I-labeled fibrinogen scanning. The total risk of DVT was reduced when UFH was compared with placebo (13% vs. 29%, respectively; RR, 0.46; 95% CI, 0.22–0.99, $p<0.05$). The rates of proximal DVT, PE, bleeding, or mortality were not reported.

2.6.1.2 Low-molecular-weight heparin vs. placebo

In the study by Fraisse et al., nadroparin was compared to placebo in 223 in COPD patients requiring mechanical ventilation (Fraisse et al., 2000). The nadroparin dose was adjusted for body weight, 3800, or 5700 IU SC OD. The incidence of lower extremity DVT was assessed by venography at planned study completion, early permanent discontinuation or during the study to confirm Doppler ultrasonography diagnosis of DVT. A slight reduction in all DVT was found with nadroparin, 16 vs. 28%, respectively (RR 0.55; 95% CI, 0.30–1.00, $p <0.05$). No difference was detected in proximal DVT, major bleeding or mortality rates.

2.6.1.3 Unfractionated heparin vs. low-molecular-weight heparin

Two of the studies investigated UFH in comparison with enoxaparin (De et al., 2010; ShorrWilliams et al., 2009) and one with dalteparin (Cook et al., 2011). All studies were done using subcutaneous administration of heparin and LMWHs.

The RCT of De et al. examined 154 surgical ICU patients undergoing major elective surgery who received either UFH 5000 IU BID or enoxaparin 40 mg OD (De et al., 2010). The major surgery was defined as any surgical procedure that was assumed to require at least 6 days hospitalization of which at least 24 h in the ICU. The DVT rate was assed once between 5th and 7th days by Doppler ultrasonography. No difference was observed in either all DVT, 2.7% (2/75 patients) in the UFH group vs. 1.2% (1/81 patients) in the enoxaparin group, $p=0.51$ or the major bleeding rate, 2.7% (2/75 patients) vs. 1.2% (1/81 patients), $p=0.48$, respectively. It was reported that UFH increased both minor bleeding 21.3% (16/75 patients) vs. 8.6% (7/81 patients), $p=0.02$ and overall bleeding, 24% (18/75 patients) vs. 9.9%

(8/81 patients), p=0.01, respectively. The PE rate or the location of DVTs were not reported.

Shorr et al. randomized severe sepsis or septic shock patients to receive either UFH 5000 IU BID, enoxaparin 40 mg OD or placebo. In addition, all patients received concomitant drotrecogin alfa for 6 days (Levi et al., 2007; et al., 2009). Drotrecogin alfa is a recombinant form of APC and has both antithrombotic and anti-inflammatory properties. The study included 1935 patients: 498 in UFH, 478 in enoxaparin, and 959 in the placebo arm. DVTs were screened with bilateral lower extremity compression ultrasonography between day 4 and day 6. Ultrasonography was performed earlier if symptomatic DVT was suspected. No difference was seen in 28-day mortality, 29.3% (145/495 patients) in the UFH group vs. 27.3% (130/477 patients) in the enoxaparin group vs. 31.9% (305/955 patients) in the placebo group, p= 0.20 (Levi et al., 2007). Neither UFH nor LMWH showed any beneficial or detrimental effects within the study days 0–28 when compared to placebo on symptomatic DVT : 6.3% (26/498 patients) vs. 5.9% (23/478 patients) vs. 7.0% (67/959 patients), PE: 0.4% (2/498 patients) vs. 0.4% (4/478 patients) vs. 0.8% (8/959 patients) or any VTE: 5.6% (28/498 patients), 5.9% (28/478 patients), 7.0% (67/959 patients), respectively (Shorr et al., 2009). The use of heparin did not increase the risk of any bleeding: 12.4% (121/976 patients) vs. 10.9% (105/959 patients), p=0.06 or serious bleeding: 3.9% (38/976 patients) vs. 5.2% (50/959 patients), respectively, p=0.16. Interestingly, a withdrawal of anticoagulation seemed to be harmful since both the 28-day mortality, 35.6% (p=0.03), and the rate of VTE seemed to be highest (8.1%) in patients who had received any prior heparin before randomization and then allocated into the placebo arm (Levi et al., 2007; Shorr et al., 2009) . One possible explanation could be the so-called "rebound hypercoagulation" that has been described in acute coronary syndromes (Granger et al., 1995). However, these results should be interpreted with caution since drotrecogin alfa is no longer available due to safety and efficacy issues (Ranieri et al., 2012).

In a large multicenter trial, 3764 medical-surgical ICU patients were randomized to receive either UFH 5000 IU BID or dalteparin 5000 IU OD during the ICU stay (Cook et al., 2011). A lower extremity compression ultrasonography was performed within 2 days after ICU admission and then twice weekly. No difference in proximal DVT: 5.1% in the UFH group vs. 5.8% in the dalteparin group (96/1873 patients vs. 109/1873 patients, HR 0.92; 95% CI 0.68–1.23, p=0.57) , all DVT: 7.4 vs. 8.6% (138/1873 patients vs. 161/1873 patients HR 0.93; 95% CI 0.72–1.19, p=0.54), major bleeding: 5.5 vs. 5.6% (103/1873 patients vs. 105/1873 patients, HR 1.00; 95% CI 0.75–1.34, p=0.98), respectively. Both ICU mortality: 15.2 vs. 16.2%

(284/1873 patients vs. 304/1873 patients, HR 0.97; 95% CI 0.82–1.15, p=0.71) and hospital mortality: 22.1 vs. 24.5% (414/1873 patients vs. 459/1873 patients, HR 0.92; 95% CI 0.80–1.05, p=0.21), respectively, remained indifferent. Dalteparin reduced the incidence of any PE: 2.3 vs. 1.3% (43/1873 patients vs. 24/1873 patients, RR 0.51; 95% CI 0.30–0.88, p<0.01), respectively.

Overall, when UFH or LMWH has been compared with placebo, the risks of symptomatic DVT, symptomatic PE, major bleeding, and mortality have remained the same. A reduction in asymptomatic DVT rates has been shown both with UFH and LMWH (Cade, 1982; Fraisse et al., 2000). LMWHs seem to have a marginally more favorable profile over UFH: less bleeding (De et al., 2010) and more reduction in PE rate (Cook et al., 2011). However, ACCP guidelines decided to consider them as equivalent since the beneficial treatment effect of PE was only attributable to a small difference in events (Cook et al., 2011). Moreover, the treatment effect was biased because of screening of DVT and further treating asymptomatic DVT with therapeutic anticoagulation (Kahn et al., 2012).

2.6.2 Critically ill surgical patients

Surgical patients in the ICU are typically considered as high-risk patients for VTE (around 6%) (Gould et al., 2012). Moreover, major surgery seems to be one of the main risk factors for VTE (Barsoum et al., 2010). Surgical procedures with an especially high VTE incidence of 2 to 3% include lower extremity vascular surgery, invasive neurosurgery and total or partial hip arthroplasty major orthopedic surgery, and abdominal and urologic cancer surgery (White et al., 2003). In addition to the risk of VTE, the risk of bleeding needs to be evaluated when planning thromboprophylaxis, especially in the early postoperative period. However, the risk of bleeding can only be evaluated indirectly, since in most RCTs, those patients with increased risk for bleeding are excluded. Typically risk factors for major bleeding complications are active bleeding, previous major bleeding, severe renal or hepatic failure, thrombocytopenia, acute stroke, uncontrolled hypertension, and concomitant use of anticoagulants (Gould et al., 2012).

In thoracic, general and abdominal surgery patients at high risk for VTE, but not at high risk for bleeding: pharmacological thromboprophylaxis with UFH or LMWH (GRADE 1B) is preferable over no prophylaxis (Gould et al., 2012). This is based on the meta-analysis performed by Collins et al. where UFH reduced the risk of fatal PE from 0.9% (55/6777 patients) to 0.3% (19/7307 patients), p<0.001 (Collins et

al., 1988). In LMWHs, the risk reduction was also halved i.e. from 12 per 1000 patients to 6 (95% CI 3–13) per 1000 patients in comparison with placebo (Mismetti et al., 2001). It has been suggested that mechanical prophylaxis with elastic stockings or intermittent pneumatic compression (IPC) should be supplemented to pharmacological prophylaxis (GRADE 2C) (Kakkar et al., 2010), (Roderick et al., 2005). If the bleeding risk is considered major, only mechanical prophylaxis is advised as long as the risk of bleeding persists (GRADE 2 C) (Eppsteiner et al., 2010).

The postoperative risk of VTE seems to be lower in cardiac surgery patients than in other ICU patients, approximately 0.5 to 1.0% (White et al., 2003). The risk of major postoperative bleeding is 4.7%. Thus, the risk of VTE is considered moderate, but the risk for major bleeding is substantial. In uncomplicated cardiac surgery patients, only IPC is preferred to no prophylaxis (GRADE 2C) or pharmacologic prophylaxis (GRADE 2C). If hospitalization is prolonged, it is suggested to combine pharmacological prophylaxis with mechanical prophylaxis (GRADE 2C).

2.6.2.1 Critically ill trauma patients

In major trauma patients, the baseline risk for VTE is approximately 3–5%, but it increases up to 8–10% among patients with traumatic brain injury or spinal cord injury (Gould et al., 2012). Furthermore, an incidence of proximal DVT as high as 18% (63/349 patients, from whom only 3 symptomatic) has been reported with venography screening when no prophylaxis has been used (Geerts et al., 1994). There are limited RCT data supporting interventions involving thromboprophylaxis in trauma patients, especially those in ICU. Therefore, the ACCP's recommendation for thromboprophylaxis in trauma patients is based on estimates of the relative risk in other populations (Gould et al., 2012). For major trauma patients at high risk for VTE but an average risk for bleeding, it is recommended to have a combination of pharmacological (either LMWH or UFH) and mechanical prophylaxis (GRADE 2C). If pharmacological prophylaxis is contraindicated, mechanical prophylaxis, preferably with IPC, is suggested (GRADE 2 C). When the risk of bleeding recedes, then it is possible to initiate pharmacological prophylaxis (GRADE 2C).

2.7 Current guidelines for mechanical thromboprophylaxis in critically ill patients

Mechanical thromboprophylaxis includes GCS and IPC (Limpus et al., 2006). Both approaches have a similar mechanism of action as they generate an external pressure that reduces the diameter of the venous lumen, and accelerates venous blood flow (Stanton et al., 1949) further reducing venous stasis (Agu et al., 1999). The external pressure provided by GCS is static, while IPC generates a more dynamic approach mimicking the physiological effects of the venous valve system and sustaining pulsatile blood flow in the deep veins (MacLellan et al., 2007).

The mechanical thromboprophylaxis offers two potential benefits. First, it may be useful when pharmacological prophylaxis is contraindicated. Second, while reducing the venous stasis –one of the three elements of Virchow's Triad– it might confer synergistic benefits with pharmacological prophylaxis. The side effects include patient discomfort, skin injury, and reduced mobility (CLOTS, 2009).

The ACCP guideline for non-surgical ICU patients recommends using mechanical thromboprophylaxis, either GCS or IPC, for as long as the risk of bleeding persists. Subsequently, mechanical thromboprophylaxis should be replaced by pharmacological thromboprophylaxis (GRADE 2C). In major trauma patients with a high risk for VTE, it is suggested to supplement mechanical thromboprophylaxis, preferable IPC, with pharmacological prophylaxis (GRADE 2C) (Gould et al., 2012). IPC should be used at least 18 h a day. If GCS are used efforts should be made to achieve proper with and ankle pressure of 18 to 22 mmHg.

When the current ACCP guidelines were released, the RCT data on mechanical thromboprophylaxis in critically ill were limited. Thus, the recommendation is based on results originating from surgical ward patients where combining IPC with pharmacological prophylaxis was found to be beneficial (Kakkos et al., 2016).

2.7.1 Intermittent pneumatic compression vs. low-molecular-weight heparin

Four RCTs studying mechanical thromboprophylaxis in ICU patients have been published. The thromboprophylaxis study of Ginzburg et al. examined 442 moderate to severe trauma patients who were randomized either to an IPC group or to an LMWH group (enoxaparin 30 mg BID) (Ginzburg et al., 2003). Doppler ultrasonography of lower extremities was performed within 24 hours of ICU admission and weekly thereafter. The rate of all DVT did not differ, 2.7% (6/224

patients) in IPC group vs. 0.5% (1/218 patients) in enoxaparin group (p=0.122). No difference was seen in the incidence of PE (1/224 patients vs. 1/218 patients), minor bleeding (4/224 patients vs. 9/218 patients, p=0.237) or major bleeding rates (4/224 patients vs. 4/218 patients), respectively. Mortality was not reported.

Kurtoglu et al. studied 120 severe head or spinal trauma patients who received either IPC thromboprophylaxis or LMWH (enoxaparin 40 mg) (Kurtoglu et al., 2004). The DVTs were screened by lower extremity Doppler ultrasonography on admission to the ICU, weekly and one week after discharge. The DVT rate did not differ, 6.6% (4/60 patients) in IPC group vs. 5% (3/60 patients) in enoxaparin. No difference was seen in PE (2/60 patients vs. 4/60 patients), mortality (7/60 patients vs. 8/60 patients) or exacerbation of intracranial hematoma rates (1/60 patients vs. 1/60 patients), respectively.

In the study of Serin et al., 259 mechanically ventilated ICU patients received either both IPC and GCS or LMWH thromboprophylaxis (enoxaparin 4000 IU) (Serin et al., 2010). No difference in asymptomatic DVT rate was seen: 1% (1/94 patients) in IPC+GCS group vs. 2% (3/152 patients) in enoxaparin group.

In the largest RCT studying mechanical thromboprophylaxis, 2003 medical, surgical or trauma patients were investigated (Arabi et al., 2019). They were randomized within 48 hours after ICU admission to receive either IPC with pharmacological prophylaxis (UFH or LMWH) or only pharmacological prophylaxis. IPC was used a median of 22 hours (interquartile range, IQR, 21–23 hours) daily for a median of 7 days (IQR 4–13 days). There was no difference in the incidence of proximal DVT 3.9% (37/957 patients) vs. 4.2% (41/985 patients) (RR 0.93, 95% CI 0.60–1.44, p=0.74). Adjunctive IPC did not result in beneficial or detrimental effects on PE: 0.8% (8/991 patients) vs. 1.0% (10/1012 patients) (RR 0.82; 95% CI 0.32–2.06), nor any DVT: 9.6% (95/991 patients) vs. 8.4% (85/1012 patients), (RR 1.14; 95% CI 0.86–1.51), skin injury: 2.5% (25/991 patients) vs. 2.2% (22/1012 patients), (RR 1.16; 95% CI 0.66–2.04), or mortality at 28 days: 14.6% (145/990 patients) vs. 16.5% (167/1011 patients), (RR 0.89; 95% CI 0-72–1.09).

The ACCP guideline gives already a GRADE 2C recommendation of pharmacological thromboprophylaxis over mechanical thromboprophylaxis in critically ill patients (Kahn et al., 2012). Based on the results of Arabi et al., the grading of the recommendation is most likely to be upgraded (Arabi et al., 2019). No subgroup analysis has yet been published. In major trauma patients, however, the recommendation to combine mechanical prophylaxis to pharmacological prophylaxis might also be revised (Gould et al., 2012).

In a large observational study including 271 ICUs and nearly 300 000 hospital discharge events, the used VTE prophylaxis was evaluated (Lilly et al., 2014). Interestingly, only the use of pharmacological prophylaxis was associated with reduced in-hospital mortality OR 0.84 (95% CI 0.82–0.86, p<0.0001). Mechanical thromboprophylaxis was associated with a higher mortality risk, OR 1.11 (1.09–1.14, p<0.001) (Lilly et al., 2014). Furthermore, the risk persisted even when mechanical thromboprophylaxis was combined with anticoagulation although the reason for this is unknown.

2.8 Aneurysmal subarachnoid hemorrhage patients

Despite a decrease in the annual incidence of aSAH (Korja et al., 2016) the 1-year mortality after aSAH is still 50% (Korja et al., 2013). In the acute phase of aSAH, the blood coagulation and fibrinolytic systems are activated, resulting in increased coagulation (Ettinger, 1970). This provokes cerebral microthrombosis, a phenomenon that has been suggested to be a part of some clinical entities, such as early brain injury (EBI) and delayed cerebral ischemia (DCI) (Macdonald, 2014). An overview of the proposed pathophysiology is shown in Figure 6. EBI and DCI are both significant risk factors for poor neurological outcomes and increased mortality (Ahn et al., 2018; Broderick et al., 1994; Frontera et al., 2009). Moreover, because of the increased coagulation, the aSAH patients are considered as high-risk patients for VTE.

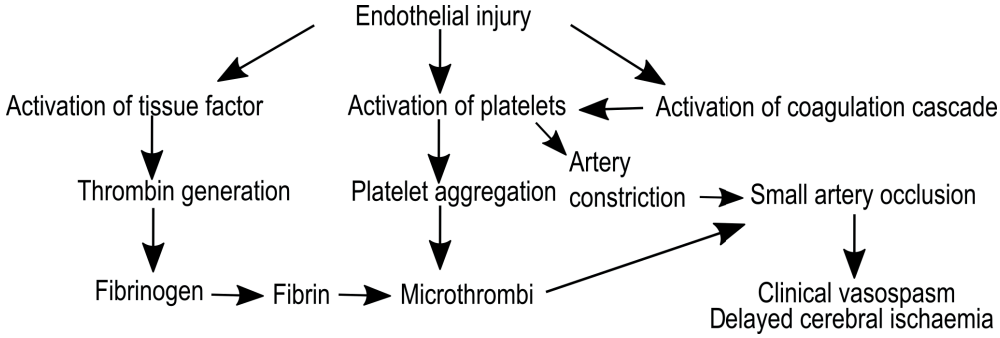

Figure 6. Pathophysiology of delayed cerebral ischemia

2.8.1 Overview of the coagulation changes after aneurysmal subarachnoid hemorrhage patients

Managing thromboprophylaxis in aSAH patients is challenging. In the early stage of the disease course, the risk of re-bleeding is around 17% (31/179 patients) surpassing the risk of thrombosis (Fujii et al., 1996). After the aneurysm has been secured, the risk of bleeding varies depending on whether a craniotomy has been performed, whether there is an intracerebral hemorrhage, an external ventricular drain, a need for antithrombotic medications due to endovascular stenting or a need for further neurosurgical procedures. Nevertheless, the concomitant risk of VTE exists.

In retrospective cohorts, the overall incidence of DVT after aSAH has ranged from 3.5% up to 18% (Kshettry et al., 2014; Ray et al., 2009), and the incidence seems to be higher than in other neurosurgical populations (Kim et al., 2009). When ultrasonography screening has been applied, the incidence of overall DVT is somewhat higher, around 9.7 to 24% (Ray et al., 2009; Serrone et al., 2013). On average, DVT is detected later if no screening has been conducted (Kim et al., 2009; Serrone et al., 2013). The symptomatic PE rate is 1.2 – 2% (Kshettry et al., 2014; Ray et al., 2009; Serrone et al., 2013).

The known independent risk factors for DVT after aSAH are tobacco smoking, black race and long hospital stay (Serrone et al., 2013). Furthermore, the severity of aSAH correlates with the risk of DVT (Ray et al., 2009). VTE has been associated with in-hospital complications (e.g. pulmonary/cardiac and infectious complications) and the length of hospital stay (Kshettry et al., 2014).

2.8.2 Pharmacological thromboprophylaxis in aneurysmal subarachnoid hemorrhage patients

There are very limited RCT data on pharmacological thromboprophylaxis in aSAH patients. There is weak evidence that after surgical occlusion of the aneurysm, enoxaparin thromboprophylaxis might increase intracranial bleeding (4/85 patients) in comparison with placebo (0/85 patients) (Siironen et al., 2003). However, none of the four patients required any additional treatment. The incidence of symptomatic DVT remained unchanged: 3/85 patients in the placebo group vs. 1/85 patients in the enoxaparin group. In a meta-analysis conducted in a mixed neurosurgical population, the efficacy of LMWH and IPC was considered to be equal in DVT reduction, LMWH vs. GCS RR 0.60; 95% CI 0.44–0.81 and IPC vs. placebo RR 0.41: 95% CI 0.21–0.78 (Collen et al., 2008). No statistical difference was seen in the

numbers of intracerebral hemorrhages, LMWH vs. mechanical prophylaxis RR 1.97; 95% CI 0.64–6.09. The safety and efficacy profiles of LMWH and UHF were similar.

2.8.3 Mechanical thromboprophylaxis in aneurysmal subarachnoid hemorrhage patients

No RCT trials have been done investigating the use of mechanical thromboprophylaxis in the aSAH population, but the closest surrogates might be immobilized stroke patients. In CLOTS-1, 2518 stroke patients were investigated in a multicenter RCT and randomized 1:1 to either regular care plus thigh-length GCS or routine care without GCS (CLOTS, 2009). No benefit was observed from GCS treatment within 30 days of randomization: the incidence of all DVT was 10.0% vs. 10.5%. More skin breaks, ulcers, and blisters occurred in the study group: 5 vs. 1%, OR 4.18 (95% CI 2.40–7.27). Mainly based on these results, the most recent Cochrane review does not recommend the routine use of GCS in stroke patients (Naccarato et al., 2010).

In CLOTS-3, 2876 non-ambulatory stroke patients were randomized 1:1 to receive IPC care or no IPC (CLOTS, 2013). After randomization clinicians were allowed to start both prophylactic and therapeutic dose of UFH/LMWH. The incidence of DVT decreased significantly with IPC: 8.5 vs. 12.1%, RR 3.6% (95% CI 1.4–5.8). There was no difference in the incidences of either symptomatic DVT or PE. Skin breaks were more common in the IPC group (3% vs. 1.5% p=0.002). Pharmacological prophylaxis was used only in 17% of patients in both groups.

2.8.4 Current guidelines for thromboprophylaxis in aneurysmal subarachnoid hemorrhage patients

Both the Neurocritical Care Society and the European Stroke Organization guidelines advise starting mechanical thromboprophylaxis prior to occlusion of the aneurysm (Diringer et al., 2011; Steiner et al., 2013). After the aneurysm is secured, additional pharmacological thromboprophylaxis is recommended. This could be started 12–24 h after surgical occlusion and immediately after coiling (Steiner et al., 2013). While the use of antifibrinolytic therapy prevents rebleeding (Hillman et al., 2002; Starke et al., 2008), it increases the risk of DVT (Starke et al., 2008). Thus, it is considered to be contraindicated in patients with a high risk of thromboembolic complications (Diringer et al., 2011). However, an early (<48 h after the onset of

aSAH symptoms, short (<72 hours) course of antifibrinolytic treatment with tranexamic acid might be beneficial.

3 AIMS OF THE STUDY

The main aim of this thesis was to study how to make thromboprophylaxis safer and more effective in critically ill patients. Furthermore, the association of critical illness with blood coagulation was studied.

The more detailed objectives were:

1. To systematically review the literature of anti-Xa levels in critically ill patients receiving LMWH thromboprophylaxis and to evaluate the association of anti-Xa levels with clinically meaningful patient-centered endpoints (e.g., VTE or bleeding) (I)

2. To compare the pharmacokinetics of enoxaparin using plasma anti-Xa levels when thromboprophylaxis was given either as a CII or a SCB in critically ill patients (II)

3. To study coagulation parameters when enoxaparin thromboprophylaxis was given either as a CII or a SCB in critically ill patients (III)

4. To analyze the coagulation after aSAH using ROTEM measurements in comparison with patients undergoing clipping of a non-ruptured aneurysm (IV)

4 MATERIALS AND METHODS

4.1 Study I

4.1.1 Study design

Study I was a systematic review of the literature designed to assess anti-Xa levels in critically ill patients receiving LMWH thromboprophylaxis. The study design was published in PROSPERO (www.crd.york.ac.uk/PROSPERO, number CRD42015025744) before data extraction. The Cochrane Collaboration (Higgins et al., 2011), PRISMA Statement (Moher et al., 2009), and Meta-analysis Of Observational Studies in Epidemiology (MOOSE) reporting (Stroup et al., 2000) recommendations were used.

In data gathering, the following databases were searched: MEDLINE, SCOPUS, Cochrane Library, and ClinicalTrials.com in collaboration with a university librarian. The following keywords were combined using Boolean Operators: Venous Thrombosis/thromboprophylaxis/ Venous Thromboembolism AND Critical Care/Intensive Care Unit/Critical Illness AND LMWH. No restriction on language, date, or type of publication was applied. The data search was completed in May 2015.

Studies were selected using predefined inclusion criteria. The inclusion criteria were prospective in design, performed in adult (age >18 years) critical care patients, included more than ten patients, used any LMWH thromboprophylaxis, and included at least one anti-Xa measurement at a known time point after a specified LMWH administration. Two reviewers independently evaluated all identified titles and abstracts. Inter-rater agreement was calculated, and any disagreements were resolved through discussions.

The studies retrieved for more detailed evaluation were also independently evaluated by the same two investigators. The following data were extracted from selected studies: study design, patient population, anti-Xa results, and clinical outcomes (i.e., DVT, symptomatic DVT, PE, symptomatic PE, and major or minor bleeding. The Downs and Black checklist was used for assessing the quality of the selected studies (Downs et al., 1998).

4.2 Studies II and III

4.2.1 Study design (II and III)

Studies II and III were based of a randomized, single-blind, controlled trial conducted in 40 critically ill patients who were recruited from ICUs of Tampere and Helsinki University hospitals between March 2014 and July 2016.

Inclusion criteria were: ICU patients age between 18 and 80 years with an indication for pharmacological thromboprophylaxis; a BMI between 18–30 kg/m^2 and expected ICU stay over 72 hours. The following exclusion criteria were applied: indications for anticoagulant therapy other than thromboprophylaxis; intracranial hemorrhage or central neurosurgical operation within 3 months of ICU admission; diagnosis of disseminated intravascular coagulation according to International Society on Thrombosis and Haemostasis criteria (Taylor et al., 2001); known HIT; known hypersensitivity to enoxaparin or heparin; blood platelet count < 20 × 10^9/l, prothrombin time (PT) < 20% or International Normalized Ratio (INR) > 1.7; major hemorrhage within the last week unless definitively treated; glomerular filtration rate <50 ml/min/1.73 m^2 (Cockcroft et al., 1976) or chronic dialysis; known HIV, hepatitis B or hepatitis C infection; pregnancy; and known liver disease. If a patient had received LMWH thromboprophylaxis within 24–72 hours before inclusion, randomization was done if the anti-Xa level was <0.1 IU/ml.

Block randomization was performed using sequentially numbered and sealed envelopes that were stratified according to use of vasopressor. The randomization was conducted independently in each study site.

4.2.2 Study intervention and clinical management (II and III)

After obtaining consent, patients were randomized to receive 40 mg enoxaparin (Klexane, Sanofi, Espoo, Finland) either as a CII or a SCB for 72 hours. The CII was prepared by a pharmacist or an ICU nurse. The daily dose of 40 mg of enoxaparin sodium was diluted in 100 ml 0.9% sodium chloride solution that was divided into two 50 ml syringes and infused intravenously 4.2 ml/h via either a central or peripheral venous catheter using an automated pump. The study period lasted 72 hours, during which any discontinuations were recorded and if the break was over 2 hours, then the patient was excluded. The SCB of enoxaparin was given

once daily from a prefilled single-dose syringe containing 40 mg of enoxaparin sodium. Mechanical thromboprophylaxis was undertaken according to the regular clinical practice of each study site. The dosing of enoxaparin was not allowed to change during the study period. After the study period of 72 hours, the thromboprophylaxis was continued according to the regular ICU practice.

4.2.3 Blood sampling (II and III)

The blood samples for immediate anti-Xa analysis were taken at 0, 3, 6, 9, 12, 15, 18, 24, 27, 48, 51, and 72 hours after the beginning of the study drug. Additional blood samples were obtained at 1.5 and 4.5 hours from patients in the CII group. Complete blood count, platelet and leukocyte counts, serum C-reactive protein concentrations were checked daily and INR or PT when clinically indicated. All study blood samples were taken from a heparin-naïve arterial line.

In study III, additional blood samples were collected at 0 (presenting the baseline), 51 (presenting the final peak level for the SCB group), and 72 (representing the final trough level for the SCB group) hours. These were collected into sodium citrate anticoagulant (3.2%, 109 nM) tubes. Samples for AT, F1+2, fibrinogen, and D-dimer were centrifuged 2500 g for 15 min.

Further additional samples were collected from patients treated in Helsinki University Hospital. These blood samples were first centrifuged at 2000 g for 10 min and then at 10 000 g for 10 min to obtain platelet-poor plasma (PPP) for thrombogram analysis.

All plasma was divided into 0.5ml plastic tubes and stored at -80 C in a freezer at each study cite until analysis.

4.2.4 Outcome measures

4.2.4.1 Study II

The primary outcome measure was the maximum plasma anti-Xa concentration within the 24 hours after the beginning of the study drug ($C_{max0–24h}$). The secondary outcomes were the maximum anti-Xa concentration within 72 hours ($C_{max0–72\,h}$), area under the time-concentration curve (AUC) at 24 ($AUC_{0–24\,h}$), and 72 ($AUC_{0–72\,h}$) hours. The influence of vasoconstrictor infusion (yes/no) as well as total

vasoconstrictor dose on anti-Xa $C_{max0–24h}$ and $AUC_{0–24\ h}$ were analyzed in order to evaluate the effect on the bioavailability of the SCB of enoxaparin.

The following clinical adverse events were registered: major bleeding (requiring >2 units transfusion of red blood cells, intracranial bleeding or bleeding requiring therapeutic intervention or resulting in death), minor bleeding (any other bleeding), symptomatic DVT confirmed by compression ultrasound, symptomatic PE confirmed by CT pulmonary angiography and HIT (Cuker et al., 2012). Asymptomatic VTE events were not screened.

At baseline, basic patient characteristics, comorbidities and APACHE II score were collated. The daily Sequential Organ Failure Assessment score was calculated during the study period of 72 hours. The length of ICU stay, and all-cause mortality at day 90 after ICU admission were further registered.

4.2.4.2 Study III

The primary outcome measure was F 1+2 levels at 51 and 72 hours after the beginning of the study drug. Further markers of coagulation (anti-Xa, AT, D-dimer, and fibrinogen) were assessed. From these, the DIC scores were calculated (Taylor et al., 2001). Additionally, thrombin generation was analyzed with a calibrated automated thrombogram (TGA-CAT) from patients treated in Helsinki University Hospital.

4.3 Study IV

4.3.1 Study design

Study IV (ClinicalTrials.gov identifier, NCT02540005) was a prospective, observational study conducted in Tampere University Hospital between October 2015 and June 2016. During the study period of 72 hours, the coagulation status in aSAH patients was evaluated and compared with preoperative values in patients undergoing clipping of non-ruptured aneurysms.

All consecutive patients who were admitted to the ICU because of aSAH were considered eligible. Inclusion criteria for the study group were age over 18 years, aSAH confirmed by brain computed tomography (CT) and confirmed aneurysmatic origin either with CT angiography (CTA) or digital subtraction angiography (DSA),

the onset of symptoms less than 12 hours, and an expected ICU stay over 72 hours. Inclusion criteria for the control group were aged over 18 years, elective craniotomy due to a non-ruptured intracranial aneurysm, and expected hospital stay of over 72 hours. The exclusion criteria for both groups were pregnancy, any long-term anticoagulant medication, except for aspirin <150 mg/day and known active cancer.

4.3.2 Blood sampling

The overall coagulation was evaluated using ROTEM. Additional blood samples for ROTEM analysis were collected at 12, 24, 48, and 72 hours since the onset of aSAH symptoms. From the control group, ROTEM samples were taken preoperatively (i.e., baseline), and these also served as a local reference range for ROTEM. From the aSAH patients, complete blood count, platelet and leukocyte counts, C-reactive protein, and international normalized ratio concentrations were measured daily during the study period of 72 hours.

All study blood samples were taken from a heparin-naïve arterial line and analyzed immediately.

4.3.3 Clinical management and outcome measures

The primary outcome measure was EXTEM-MCF by ROTEM in the aSAH patients compared with baseline values from the control group. Secondary outcomes included other measured ROTEM parameters: EXTEM-CT, EXTEM-CFT, FIBTEM-MCF, INTEM-MCF, INTEM-CT, and INTEM-CFT. To further assess the contribution of platelets to the strength of the blood clot, the difference between EXTEM-MCF and FIBTEM-MCF was calculated. In addition, the association ROTEM parameters with the incidence of EBI and DCI was analyzed.

During the ICU stay, aSAH patients were treated according to the neurointensive care guidelines (Diringer et al., 2011; Steiner et al., 2013). After the aneurysm was secured, all patients received pharmacological thromboprophylaxis, 4500 IU tinzaparin OD. If the aneurysm was secured by coiling, then the thromboprophylaxis was started at the same evening and if by clipping on the following evening. No additional mechanical thromboprophylaxis was used. The control patients received standard perioperative treatment.

A bilateral compression ultrasonography of the lower extremity veins was done in both study and control patients once within days 3–5 (from the onset of aSAH

symptoms or from the operation day). If a DVT was detected, it was treated accordingly. If PE was suspected, a CT pulmonary angiography was done.

The clinical severity of aSAH at admission was evaluated retrospectively to detect EBI. For this Hunt-Hess score was applied, scores 4–5 were classified as severe, and scores 1–3 as mild (Ahn et al., 2018; Hunt et al., 1968). A Fisher grading scale (grades 1–4) was used to evaluate the severity of bleeding from the first head CT (Fisher et al., 1980). The incidence of symptomatic vasospasm is associated with the Fisher scale and being highest in grade 3 (Frontera et al., 2006).

DCI was evaluated retrospectively using the ICU database (Centricity Critical Care Clinisoft; GE Healthcare, Barrington, Illinois, USA) at 24 hours to 14 days from the onset of aSAH. Criteria for DCI presented by Vergouwen et al. was used (Vergouwen et al., 2010). Accordingly, DCI was defined either clinically or radiologically. The clinical criteria included either a neurological deterioration for over 1 hour or a new neurological symptom, both of which could not be explained by other features. The radiological criterion was a new ischemic lesion on neuroimaging data that was not related to the primary aSAH insult or neurosurgery.

Outcome data were evaluated using the Glasgow outcome scale score on day 90 (Wilson et al., 1998).

4.4 Laboratory methods

4.4.1 Chromogenic Anti-Xa measurement (II)

Anti-Xa activity was measured in fresh blood samples using a validated chromogenic assay without adding AT. The method used in Tampere University Hospital was STA-Liquid anti-Xa, Diagnostica Stago, and in Helsinki University Hospital HemosIL Liquid Anti-Xa on an ACL TOP 750 Analyzer, Instrumentation Company Laboratory/Werfen.

The chromogenic anti-Xa measurement uses an fXa substrate that has a chromophore attached. When it interacts with fXa, the chromophore is cleaved (Figure 7) (Walenga et al., 2004). The released chromophore induces a color change that can be measured with a spectrophotometer. The magnitude is directly proportional to the amount of fXa in the plasma. During the anti-Xa measurement, a known amount of fXa is added to the analyzed plasma. If there is heparin or LMWH present, they will inhibit fXa by AT. Thus, less fXa is available to cleave the

substrate and render the color change. The amount of heparin can be further calculated using a standard curve with the known heparin concentration (Bates et al., 2005). The results are presented as IU of anti-fXa.

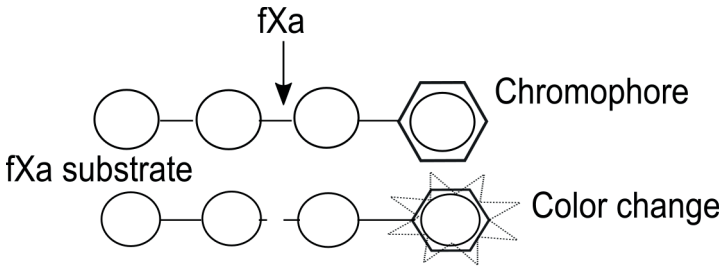

Figure 7. The principle of chromogenic anti-Xa measurement. Abbreviations: fXa, activated coagulation factor X.

4.4.2 Other markers of coagulation (III)

AT levels in plasma samples were analyzed by chromogenic assays using the HemosiL (HemosIL Liquid Antithrombin and HemosIL Liquid Anti-Xa reagents on an ACL TOP 750 Analyzer) with reagents from Instrumentation Laboratory, F1+2 by an enzyme immunoassay (Enzygnost® F1+2, monoclonal, Siemens Healthcare Diagnostics, Espoo, Finland), fibrinogen with HemosIL Fibrinogen activity (IL QFA Thrombin, Clauss method) on an ACL Top Analyzer and D-dimer with an immunoturbidimetric assay (Quantia D-Dimer, Abbot Diagnostics, Espoo, Finland) on an Architect –analyzer. All analyses were performed in the core laboratory of Helsinki University Hospital.

4.4.3 Thrombogram (III)

Thrombin generation was measured by CAT using Thrombinosope software (Thrombinoscope, Stago, Asnières-sur-Seine, France) (Dargaud et al., 2012; Helin et al., 2015; Hemker et al., 2002). Measurements were done in 96-microtiter plates, where 80 µL of PPP was supplemented with 20 µL of either the inner method calibrator reagent or TF–containing reagent. Reagents comprised 1–5 pM TF and 4 µM phospholipids in PPP (PPP Reagent Low). TGA-CAT was initiated by 20 µL of FluCa reagent mixture (Thrombinoscope, Stago, Asnières-sur-Seine, France). Analysis was conducted for a minimum of 60 min. The following TGA-CAT

parameters were registered: lag time, endogenous thrombin potential (ETP), peak amount of generated thrombin (Peak), and time-to-Peak (tt-Peak) (Figure 8). Additionally, PPP from 11 healthy controls served as a reference range.

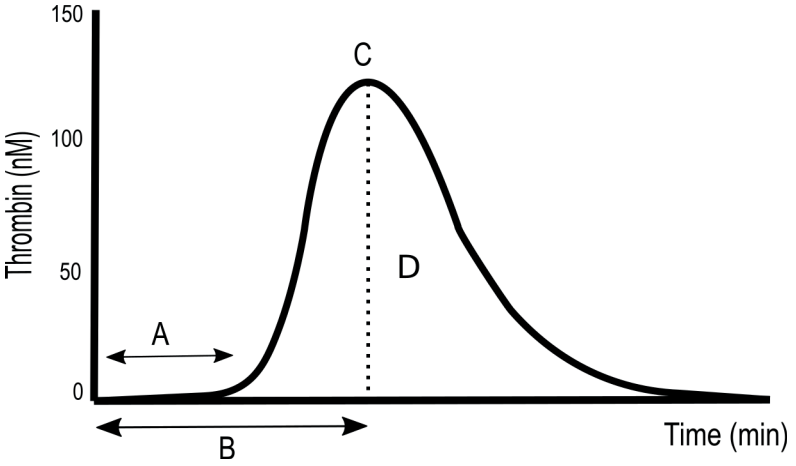

Figure 8. Thrombogram. Abbreviations: A, Lag time; B, Time-to-Peak (tt-Peak); C, Peak amount of generated thrombin; D, Endogenous thrombin potential (ETP).

4.4.4 Rotational thromboelastometry (IV)

ROTEM analysis was conducted using a ROTEM delta analysis system (TEM Innovations GmbH, Munich, Germany). The analysis was performed in the central laboratory of Tampere University Hospital immediately after the blood sample was drawn.

Each analysis included EXTEM, INTEM and FIBTEM analysis from which the following parameters were measured: clotting time (CT), clot formation time (CFT), and maximum clot firmness (MCF) (Figure 9). In brief, CT represents the interval to initiation of clot formation, CFT is the duration of 20 mm wide clot formation after CT, and it represents increasing polymerization of fibrin and beginning interaction between fibrinogen and platelets and MCF represents the overall maximum clot strength. The increased MCF is a sign of hypercoagulation (Akay et al., 2009). Every analysis was done using single-use reagents, and a more detailed description is shown in Table 4.

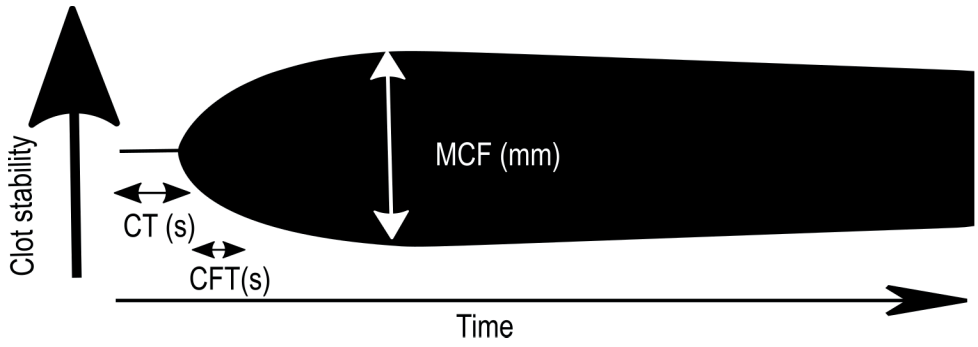

Figure 9. Basic principle of ROTEM. Abbreviations: CT, clotting time; CFT, clot formation time; MCF, maximum clot firmness.

Table 4. Description of different ROTEM assays used. Abbreviations: TF, tissue factor.

Assay	Activation method	Citration	Recalcification	Interpretation
EXTEM	TF	Yes	Yes	Extrinsic pathway (platelets and fibrinogen)
INTEM	Contact	Yes	Yes	Clot formation via the contact phase
FIBTEM	TF	Yes	Yes	Clot formation after platelet inhibition with cytochalasin D

4.5 Statistical methods

In study II, the primary endpoint was maximum anti-Xa concentration (C_{max}) after a SCB of enoxaparin. No data on anti-Xa levels after a CII of enoxaparin were available. Thus, based on previous results after a SCB of enoxaparin, a clinically meaningful increase in the anti-Xa level was considered as 0.1 IU/ml (standard deviation, SD, 0.11 IU/ml). Accordingly, at least 20 patients in both groups were needed to achieve 80% power with a significance level of 5%. A stratification according to use of vasoconstrictor was used to further assess the impact of vasoconstrictor infusion on anti-Xa levels.

In study IV, the primary endpoint was MCF (mm) by EXTEM. The sample size was calculated using previous results from a non-cardiac population (Hincker et al., 2014). Accordingly, at least 16 patients in each group were needed to achieve an 80% power to detect a clinically meaningful increase from 65mm to 70 mm (SD 5 mm) in MCF with a significance level of 5%.

Data are presented in absolute numbers and percentages (%), in medians with IQR or means with SD as appropriate. The threshold for the statistical significance

level was set at a p-value <0.05. No Bonferroni correction for multiple comparisons was done.

The normality of data was assessed with Shapiro-Wilk test. Comparisons between continuous variables were performed using Student's t-test for normally distributed variables and Mann-Whitney U test for non-normally distributed variables. For categorical variables, univariate analysis with Fisher's exact test was performed. Spearman's rank correlation test was used to determine the relationship between two continuous variables. Pair-wise comparisons of non-parametric variables were made using Wilcoxon signed-rank test. Inter-rater agreement was calculated by Cohen's kappa (k)

All statistical analyses were performed using the SPSS statistical software, version 23.0 or 24.0 (IBM Corp., Armonk, New York, USA).

5 ETHICAL ASPECTS (STUDIES II–IV)

All clinical study protocols (II–IV) were independently assessed in the local ethics committee of Pirkanmaa. The study protocols II and III were further approved by the Finnish Medical Association (FIMEA) and each study hospital. All trials were conducted in accordance with the amended Declaration of Helsinki. Written informed consent was obtained from all patients or their next of kin before study enrolment.

5.1 Studies II and III

The pharmacokinetics of LMWHs in the critically ill is poorly understood. Based on two pilot studies, decreased bioavailability after subcutaneous dosing of LMWH has been suspected (Dörffler-Melly et al., 2002; Priglinger et al., 2003). Thus, investigating enoxaparin pharmacokinetics using a CII was warranted.

The dosage and modes of administration of enoxaparin were in agreement with current recommendations and did not significantly differ from the current guidelines for thromboprophylaxis in critically ill patients (Gould et al., 2012; Kahn et al., 2012). An intravenous bolus of enoxaparin is generally used e.g. during percutaneous coronary intervention (Montalescot et al., 2011). However, intravenous loading dose was omitted, since immediately after intravenous bolus anti-Xa levels are known to be high before drug distribution (Aiach et al., 1983). Thus, no additional risks for bleeding or thromboembolic complications were anticipated when enoxaparin thromboprophylaxis was given as a CII. During the study period, a total of 30-34 samples (total 186-210 ml blood) was taken depending on the study group. The amount of blood can be considered insignificant. All blood samples were taken from an arterial line already in place.

5.2 Study IV

All patients received routine care in the ICU and the neurosurgical ward. The sampling did not influence the treatment, and the treating physician was unaware of the results. The total amount of extra blood samples was small (9-14 ml/patient), and the samples were drawn from an arterial line already in place. A compression ultrasound of the lower extremities was conducted once in all patients on days three to five. It is a non-invasive procedure with minimal complication risk. If a DVT was detected, the treating physician was informed, and the treatment was started accordingly.

6 RESULTS

6.1 Study I

The literature search revealed 5206 potentially relevant studies, of which 31 were retrieved for more detailed evaluation. The number of selected studies was 18, including two RCTs and 16 observational studies. A total number of patients was 1644. Enoxaparin had been studied in 12 publications (Costantini et al., 2013; Gouya et al., 2012; Haas et al., 2005; Lim et al., 2013; Malinoski et al., 2010; Mayr et al., 2002; Priglinger et al., 2003; Robinson et al., 2010; Robinson et al., 2013; Rutherford et al., 2005; Vincent et al., 2011; Zenáhlíková et al., 2010), dalteparin in four publications (Douketis et al., 2008; Droege et al., 2014; Rabbat et al., 2005; Rommers et al., 2006), certoparin in one report (Jochberger et al., 2005) and nadroparin in one trial (Dörffler-Melly et al., 2002). The detailed data of selected studies are presented in Table 5 and the target values for adequate anticoagulant levels in Table 6.

The median peak anti-Xa varied from <0.1 (Jochberger et al., 2005) to 0.35 IU/ml (Douketis et al., 2008) and the mean peak anti-Xa from 0.09 to 0.40 IU/ml (Dörffler-Melly et al., 2002; Robinson et al., 2013). Only four studies reported trough anti-Xa levels, and the medians were between undetectable and <0.1 IU/ml (Douketis et al., 2008; Jochberger et al., 2005; Mayr et al., 2002).

The majority, 12 out of 18 studies, reported clinical events, such as VTE and bleeding. These were not associated with anti-Xa levels. In one study done in critically ill trauma and surgical patients, a trough level below 0.1 IU/ml correlated with all DVT incidence (37 vs. 11%, p=0.026) when routine screening bilateral lower and upper extremity Duplex ultrasonography was used within 48 hours of admission and then weekly (Malinoski et al., 2010).

The median overall Downs and Black Quality score was 19 (IQR 15–20) of 27 consisting of the following subscores: 8.5 (IQR 7–10) of 11 for reporting, 1 (IQR 1–2) of 3 for external validity, 5 (IQR 4–5) of 7 for internal validity-bias, and 3 (IQR 2–5) of 7 for internal validity-confounding and power.

Table 5. Selected studies for systematic review (I). Abbreviations: BID, twice a day; CrCl, creatinine clearance; ICU, intensive care unit; LMWH, low-molecular-weight heparin; MICU, medical intensive care unit; NS, not specified; OD, once a day; SICU, surgical intensive care unit; TICU, trauma intensive care unit.

Study	Population	LMWH dose	Peak anti-Xa IU/ml	Trough anti-Xa IU/ml
Certoparin				
Jochberger et al. (2005)	Mixed ICU (n=30+32)	3000 IU OD or bid	<0.1 (<0.1–0.2) vs. <0.1 (<0.1–0.28)	<0.1 (<0.1–0.17) vs. <0.1
Dalteparin				
Douketis et al. (2008)	ICU, CrCl <30 ml/min (n=138/156)	5000 IU OD	d3:0.29 (0.20–0.42), d10:0.35 (0.24–0.43), d17:0.34 (0.27–0.45)	<0.1 (<0.1–<0.1)
Droege et al. (2014)	TICU (n=190/785)	5000 IU OD or bid	NS	NS
Rabbat et al. (2005)	Mixed ICU, CrCl >30 ml/min (n=19)	5000 IU OD	0.3	NS
Rommers et al. (2006)	Mixed ICU (n=7+7)	2500 IU OD	0.15 (±0.05) vs. 0.14 (±0.06)	0.05 (± 0.06) vs. 0.02 (±0.02)
Enoxaparin				
Costantini et al. (2013)	TICU (n=61)	30–60 mg bid	NS	NS
Gouya et al. (2012)	MICU vs. ward (n=15+16)	40 mg OD	0.16 (0–0.22) vs. 0.2 (0.15–0.27)	NS
Haas et al. (2005)	TICU nonedematous (n=11/14) vs. edematous	30 mg bid	0.27 vs. 0.12	NS
Lim et al. (2013)	MICU (n=55)	40 mg OD	0.22 (0.17–0.26)	0 (0–0.03)
Malinoski et al. (2010)	SICU (n=54), through <0.1 IU/ml) vs. through > 0.1	30 mg bid	0.17 (±0.1) vs. 0.27 (±0.1)	NS
Mayr et al. (2002)	Mixed ICU (n=89)	40 mg OD	0.18 (<0.1–0.52)	<0.1 (<0.1–0.43)
Priglinger et al. (2003)	Mixed ICU vs. ward (n=16+13)	40 mg OD	NS	NS
Robinson et al. (2010)	Mixed ICU (n=18+16+20+18)	40, 50, 60 or 70 mg OD	0.13, 0.14, 0.27 vs. 0.29	NS
Robinson et al. (2013)	Mixed ICU (n=20+20+19+19)	40 mg OD, 30 mg bid, 40 mg bid or 1 mg/kg	0.20, 0.08, 0.17 vs. 0.34, d3: 0.13, 0.15,	NS
Rutherford et al. (2005)	TICU (n=17)	40 mg OD	0.19 (±0.09)	0.04 (±0.04)
Vincent et al. (2011)	Mixed ICU (n=36)	30 mg bid	0.24 (0.19)	0.10 (0.09)
Zenáhlíková et al. (2009)	MICU (n=16)	40 mg OD	0.17 (±0.17)	NS
Nadroparin				
Dörffler-Melly et al. (2002)	ICU vs. ward (n=15+15+15)	2850 IU OD	0.09 vs. 0.23vs. 0.28	NS

Table 6. Target levels for thromboprophylaxis (I) and the used references in selected studies. Abbreviations: NS, not specified

Study	Target for anti-Xa IU/ml	Reference
Certoparin		
Jochberger et al. (2005)	Any time 0.1-0.3	Levine et al. (1989)
Dalteparin		
Douketis et al. (2008)	Peak 0.2–0.4	Levine et al. (1989)
Droege et al. (2014)	At 12h >0.1	NS
Rabbat et al. (2005)	Peak 0.2–0.4	Levine et al. (1989)
Rommers et al. (2006)	NS	
Enoxaparin		
Costantini et al. (2013)	Peak 0.2–0.4	Samama et al (1995) Nutescu et al. (2009)
Gouya et al. (2012)	NS	
Haas et al. (2005)	Peak >0.1	Mayr et al. (2002), Levine et al. (1989)
Lim et al. (2013)	Peak 0.1–0.3	Levine et al. (1989)
Malinoski et al. (2010)	Trough >0.1	Levine et al. (1989)
Mayr et al. (2002)	Any time 0.1-0.3	Levine et al. (1989)
Priglinger et al. (2003)	NS	
Robinson et al. (2010)	Any time 0.1–0.3	Mayr et al. (2002), Levine et al. (1989)
Robinson et al. (2013)	Peak 0.1–0.4	Levine et al. (1989)
Rutherford et al. (2005)	Any time 0.1–0.2	NS
Vincent et al. (2011)	Any time >0.1	Levine et al. (1989)
Zenáhlíková et al. (2009)	Peak 0.2–0.4	Rabbat et al. (2005)
Nadroparin		
Dörffler-Melly et al. (2002)	Peak 0.3–0.4	Leyvraz et al. (1991)

6.2 Study II

Forty patients were randomized but one subject had to be excluded because of an infusion pump failure, leaving 39 patients to modified intention-to-treat (ITT) analysis. Furthermore, 7 patients were excluded leaving 32 in the per-protocol (PP) analysis. The study flowchart is illustrated in Figure 9.

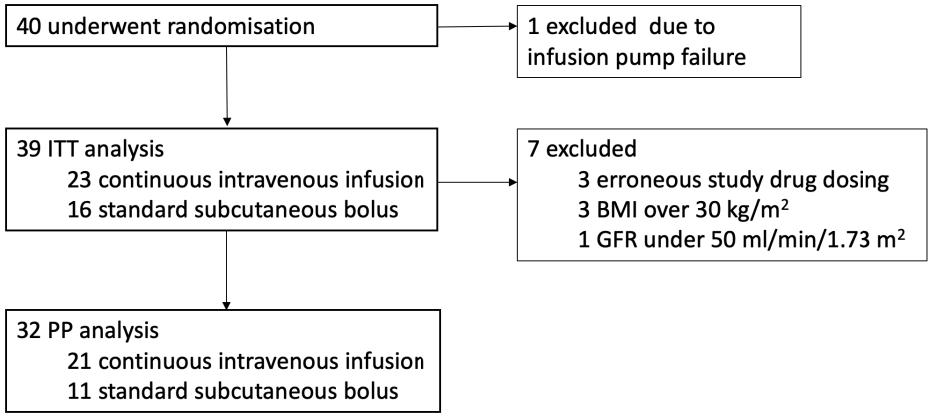

Figure 10. Flowchart of the study enrollment (II). Abbreviations: BMI, body mass index; GFR, glomerular filtration rate; ITT, intention-to-treat; PP; per-protocol analysis.

The baseline characteristics and laboratory values are shown in Table 7. No differences existed between the groups.

Table 7. Baseline and laboratory characteristics (II). Data are presented as n (%) or median (interquartile range). Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; BMI, body mass index; Hct, hematocrit; ICU, intensive care unit; LMWH, low-molecular-weight heparin; WBC, white blood cell.

Patient characteristics	Continuous intravenous infusion n=23	Subcutaneous bolus n=16
Male sex	13 (57)	14 (88)
Age, years	52 (45–59)	56 (42–64)
BMI, kg/m^2	27 (23–30)	27 (24–30)
Active cancer	2 (9)	2 (13)
Diabetes	4 (17)	2 (13)
Smoking	13 (57)	5 (31)
Alcohol abuse	11 (48)	8 (50)
Hypertension	9 (39)	6 (38)
APACHE II	16 (14–20)	18 (11–26)
Creatine Clearance, ml/min	121 (84–152)	122 (94–162)
Norepinephrine	8 (35)	7 (44)
Sepsis	7 (30)	7 (44)
Septic shock	2 (9)	1 (6)
Primary admission diagnosis		
Cardiovascular	1 (4)	0
Respiratory	10 (44)	6 (38)
Gastrointestinal	7 (30)	6 (38)
Neurologic	1 (4)	0
Infection	2 (9)	2 (13)
Trauma	1 (4)	1 (6)
Metabolic	1 (4)	1 (6)
LMWH prior inclusion	11 (48)	5 (31)
Length of ICU stay before LMWH (hh:mm)	18:56 (10:18–26:47)	17:33 (13:55–24:55)
Mechanical ventilation	15 (65)	12 (75)
Platelet count, $10^9/l$	195 (153–319)	142 (122–192)
WBC count, $10^9/l$	13 (8–17)	12 (10–21)
C reactive protein mg/l	236 (140–295)	262 (190–393)
Hct %	0.36 (0.30–0.38)	0.34 (0.29–0.43)
Bilirubin µmol/l	12 (7–20)	13 (9–15)

Within the first 24 hours, the median anti-Xa $C_{max0–24h}$ was lower in the CII in comparison with the SCB group: 0.05 IU/ml (IQR 0.05–0.18 IU/ml) vs. 0.18 IU/ml (IQR 0.12–0.33 IU/ml), respectively ($p<0.05$), Table 8. Within 72 hours the difference was similar: median anti-Xa $C_{max0–72\ h}$ was 0.14 IU/ml (0.05–0.22 IU/ml) in the CII group and 0.23 IU/ml (0.20–0.38 IU/ml) in the SCB group ($p<0.05$). At 72 hours the median anti-Xa was higher in the CII than in the SCB group: 0.12 IU/ml (IQR 0.05–0.17 IU/ml) vs. 0.05 IU/ml (IQR 0.01–0.05), $p<0.05$. The AUC values did not differ. PP analysis did not affect the results. Norepinephrine infusion did not significantly alter plasma anti-Xa levels or AUCs.

No DVTs were diagnosed. Three patients had PE; two in CII and one in SCB groups. Four minor bleedings were seen. The 90-day mortality was 12.8%. None of these adverse events displayed any associations with anti-Xa levels.

Table 8. Pharmacokinetic parameters between study groups (II). Data are presented median (interquartile range). Abbreviations: anti-Xa, anti-factor Xa; AUC, area under the time-concentration curve; C_{max}, maximum concentration; ITT, intention-to-treat; PP, per-protocol ✦ $p<0.05$.

	Pharmacological parameters	Continuous intravenous infusion	Subcutaneous bolus
ITT	Anti-Xa $C_{max0–24\ h}$	0.05 (0.05–0.18)✦	0.18 (0.12–0.33)✦
	Anti-Xa $C_{max0–72\ h}$	0.14 (0.05–0.22)✦	0.23 (0.20–0.38)✦
	AUC $_{(0\text{-}24\ h)}$	1.2 (1.0–2.9)	1.54 (1.2–4.1)
	AUC $_{(0\text{-}72\ h)}$	5.4 (3.6–10.9)	7.7 (5.9–11.7)
PP	Anti-Xa $C_{max0–24\ h}$	0.05 (0.05–0.16)✦	0.19 (0.11–0.34)✦
	Anti-Xa $C_{max0–72\ h}$	0.11 (0.05–0.19)✦	0.22 (0.18–0.34)✦
	AUC $_{(0\text{-}24\ h)}$	1.2 (0.8–2.7)	1.6 (1.3–4.8)
	AUC $_{(0\text{-}72\ h)}$	4.1 (1.8–8.0)	5.7 (3.1–8.0)

6.3 Study III

From 40 initially randomized patients, two patients were excluded (one because of infusion pump failure resulting in an erroneous enoxaparin dose and one because of missing frozen samples), leaving 22 in the CII and 16 in the SCB group. TGA-CAT

was performed in 18 patients from whom 5 pM TF stimulation triggered thrombin generation in 13 (72%) patients: 7 in the CII and 6 in the SCB group (Figure 11). Only an ITT analysis was carried since in study II, the results of ITT and PP analyses did not differ.

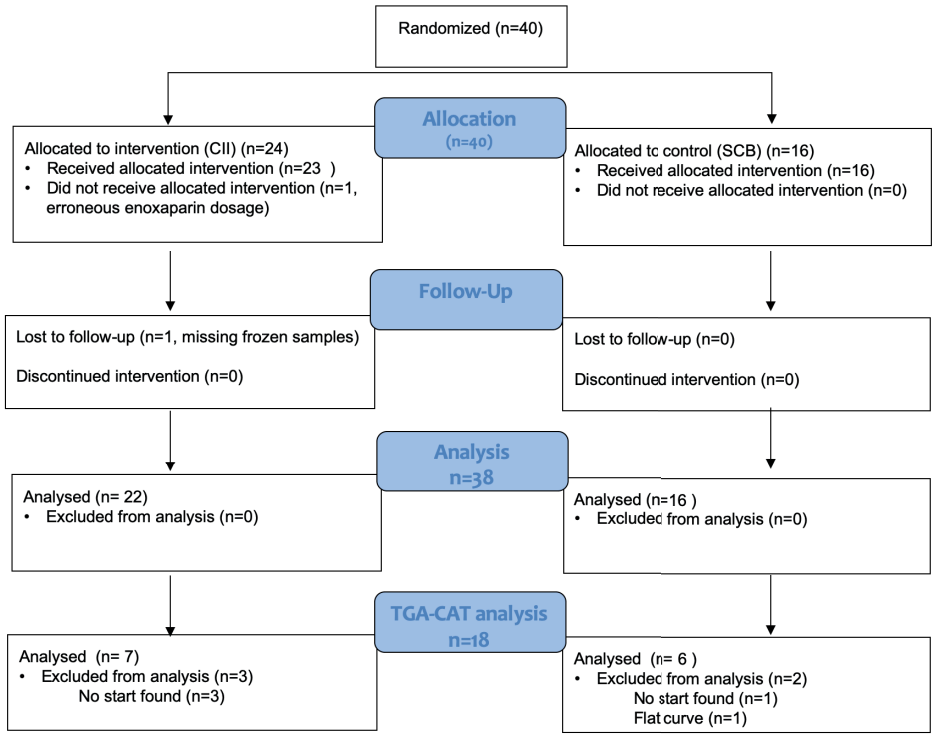

Figure 11. CONSORT flowchart (III). Abbreviations: CII, continuous intravenous infusion; SCB, subcutaneous bolus; TGA-CAT, thrombin generation assay by calibrated automated thrombogram.

At baseline, no difference was seen in coagulation parameters. D-dimer, F 1+2, and fibrinogen were markedly above the upper reference level and AT was below the lower reference level in all patients. At 51 and 72 hours, F1+2 levels were significantly lower in the CII in comparison with the SCB group: 372 pM/l (IQR 237–627 pM/l) and 374 pM/l (IQR 287–531 pM/l) vs. 634 pM/l (IQR 501–846 pM/l) and 657 pM/l (IQR 482–880 pM/l), $p<0.05$ (Figure 12). AT levels nearly normalized in the CII group, but not in the SCB group. The detailed coagulation parameters are shown in Table 9.

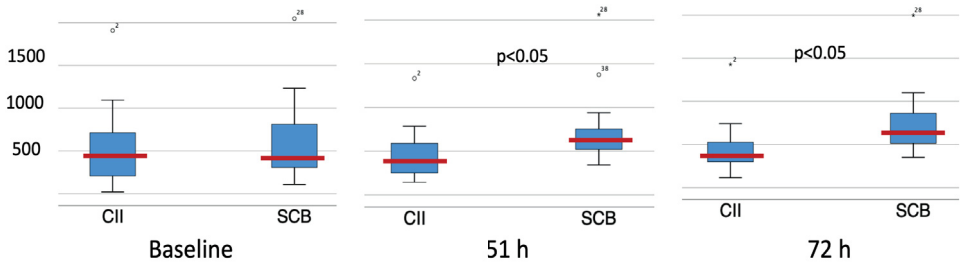

Figure 12. Comparison between F1+2 levels in study groups (III). Abbreviations: CII, continuous intravenous infusion; SCB, subcutaneous bolus.

Table 9. The coagulation variable results (III). Data are presented median (interquartile range). Abbreviations: Anti-Xa, anti-factor Xa; AT, antithrombin; CII, continuous intravenous infusion; DIC, disseminated intravascular coagulation; F1+2, prothrombin fragment 1+2; NA, not applicable; SCB, subcutaneous bolus. ✦ p <0.05 within groups (baseline vs. 51 h or baseline vs. 72 h from the study start. respectively), ❋ p <0.05 between groups (CII vs. SCB)

Variable	Reference range	Study group	0 h n=38		51 h (at peak for SCB)		72 h (at trough for SCB)	
AT (%)	85–125	CII	65	(52–89)	79	(59–94)✦	83	(64–96) ✦
		SCB	70	(63–81)	70	(54–82)	69	(54–110)
Anti-Xa (IU/ml)	NA	CII	0.05	(0–0.05)	0.10	(0.05–0.13)❋✦	0.12	(0.05–0.17)✦
		SCB	0.05	(0–0.05)	0.21	(0.09–0.25)❋✦	0.05	(0.01–0.05)
D-dimer (mg/l)	<0.5	CII	3.1	(1.7–6.1)	5.2	(1.8–8.5)	6.1	(2.2–7.1)
		SCB	5.4	(1.7–7.0)	5.5	(2.5–9.1)	3.1	(2.7–7.3)
DIC-score	NA	CII	3	(2–3)	3	(0–3)	3	(1–3)
		SCB	3	(2–3)	3	(2–3)	2	(2–3)
F 1+2 (pM/l)	69–229	CII	438	(197–731)	372	(237–627)❋	374	(287–531)❋
		SCB	413	(300–831)	634	(501–846)❋	657	(482–880)❋
Fibrinogen (g/l)	2–4	CII	6.9	(4.9–8.8)	7.9	(6.8–9.1)	7.5	(6.8–8.7)
		SCB	7.9	(5.9–9.3)	8.6	(6.6–9.5)	7.6	(5.9–9.2)

At 51 hours, both lag time and time to peak were shorter in the CII group than in the SCB group: 4.3 min (IQR 4.0–6.0 min) vs. 7.5 min (IQR 5.8–14.8 min), respectively, p <0.05 and 7.7 min (IQR 6.3–8.3 min) vs. 14.3 min (IQR 9.5–27.1min), respectively, p <0.05. The peak concentration was lower in the CII than in the SCB group at 72 hours 271 vs. 356 nM, respectively (p <0.05) (Table 10).

Table 10. The thrombogram results (III). Data are presented median (interquartile range). Abbreviations: ETP, endogenous thrombin potential; CII, continuous intravenous infusion; Peak, peak amount of thrombin; SCB, subcutaneous bolus; TGA-CAT, thrombin generation assay by calibrated automated thrombogram. ✤values of healthy adults ✦p <0.05 within groups (baseline vs. 51 h or baseline vs. 72 h from the study start. respectively), ❋p <0.05 between groups (CII vs. SCB)

TGA-CAT parameter	Reference range✤	Study group	0 h		51 h		72 h	
			CII n=7, SCB n=6		CII n=7, SCB n=7		CII n=8, SCB n=5	
Lag time, min	2.6 (2.3–2.7)	CII	3.7	(3.5–4.7)	4.3	(4.0–6.0) ❋	4.8	(4.5–6.5)
		SCB	4.8	(3.5–6.8)	7.5	(5.8–14.8) ✦❋	4.7	(2.9–8.2)
ETP, nM/min	1278 (1123–1467)	CII	1543	(1349–1556)	1448	(1289–1531)	1411	(1219–1471)
		SCB	1495	(1244–1543)	1038	(843–1677)	1545	(1326–2293)
Peak, nM	210 (161–232)	CII	295	(288–302)	303	(225–309)	271	(215–299)
		SCB	256	(238–280)	125	(29–180)	364	(287–430) ❋
Time to peak, min	6.1 (5.5–7.3)	CII	6.2	(6.0–7.0)	7.7	(6.3–8.3) ❋	7.3	(7.2–9.5)
		SCB	7.4	(5.7–9.4)	14.3	(9.5–27.1) ✦❋	6.7	(4.7–10.5)

No correlation was evident with any of the TGA-CAT parameters and anti-Xa levels. In the CII group, the lag time (.882) and tt-Peak (.791) correlated significantly with AT, but inversely with F 1+2 (-.937) and C-reactive protein (-.847) at 51 hours. At the same time, F 1+2 levels and Peak (.929) had a significant correlation in the SCB group.

6.4 Study IV

The study included 17 aSAH and 16 control patients. The demographics are detailed in Table 11. The groups were reasonably balanced; however, the number of smokers was higher in the aSAH group, and the aSAH patients were younger than the control group. Majority of ruptured aneurysms were secured with coiling (Table 12). Severe EBI was diagnosed in four and DCI in 7 of the 17 aSAH patients. However, on day 90, a majority, 60%, of the patients showed functional neurological recovery (Table 12). The 90-day mortality was 5.9%. Two aSAH patients had an asymptomatic DVT, and in both, a PE was confirmed irrespective of ROTEM analysis results.

Table 11. The baseline demographics of included patients (IV). Data are presented as n (%) or median (interquartile range). Abbreviations: aSAH, aneurysmal subarachnoid haemorrhage; ACA, anterior communicating artery; BA, basilar artery; BMI, body mass index; HTA, hypertensio arterialis; ICA, internal carotid artery; MCA, middle cerebral artery; PCA, posterior cerebral artery; PA, pericallosal artery. ✦p<0.05.

Characteristic	aSAH patients (n=17)	Control patients (n=16)
BMI	30 (25–33)✦	28 (25–31)✦
Age	49 (40–60)	62 (56–67)
Sex male	6 (35.3)	7 (43.8)
Smoking	10(58.8)✦	6 (37.5)✦
Alcohol abuse	2 (12.5)	0
HTA	7 (41.2)	11 (68.8)
Diabetes	0	3 (18.8)
Known cancer	1 (5.9)	1 (6.3)
Low-dose aspirin	1 (5.9)	5 (31.3)
Aneurysm location		
ACA	6 (35.3)	
BA	2 (11.8)	
ICA	4 (23.5)	
MCA	4 (23.5)	15 (93.8)
PCA	1 (5.9)	
PA	0	1 (6.3)

Table 12. Neurological outcome of aSAH patients (IV). Abbreviations: GOSe, Glasgow outcome score extended; LMWH, low-molecular-weight heparin; TXA, tranexamic acid.

Characteristics	Mean (SD)	n (%)
Hunt Hess	2.4 (1)	
1		2 (11.8)
2		10 (58.8)
3		1 (5.9)
4		4 (23.5)
Fisher scale	3 (0.9)	
Fisher 1		0
Fisher 2		6 (35.3)
Fisher 3		5 (29.4)
Fisher 4		6 (35.3)
Treatment		
Clipping		4 (23.5)
Coiling		13 (76.5)
TXA		1 (5.9)
LMWH during ICU		15 (88.2)
GOSe score at 90 d	6.7 (1.9)	
Death		1 (5.9)
Vegetative state		0
Lower severe disability		0
Upper severe disability		0
Lower moderate disability		1 (5.9)
Upper moderate disability		3 (17.6)
Lower good recovery		3 (17.6)
Upper good recovery		7 (41.2)

The laboratory results and ROTEM assays are in Table 13. At 72 hours EXTEM-MCF and FIBTEM-MCF were significantly higher in aSAH when compared to the baseline values from the control group: 68.0 mm (IQR 66.0–71.0 mm) vs. 64.5 mm (IQR 59.5–66.8 mm), p=0.024 and 23.9 mm (IQR 19.0–25.0 mm) vs. 15.4 mm (IQR 12.5–17.8 mm), p=0.001, respectively.

Table 13. ROTEM results. Data presented as median (interquartile range) or mean (standard deviation) (IV). Abbreviations: aSAH, aneurysmal subarachnoid hemorrhage; CFT, clot formation time; CRP, C-reactive protein; CT, clotting time; Hb, hemoglobin; MCF, maximum clot firmness. ✦p<0.05.

			From the onset of aSAH (h)			
Parameter	Reference range	Control group	12	24	48	72
EXTEM						
MCF (mm)	50–72	64.5 (59.5–66.8)	64 (62.0–69.5)	68 (63.0–70.0)	66 (65.5–69.0)	68.0 (66.0–71.0)✦
CT (s)	38–79	48 (45–58)	53 (48–57)	54 (45–61)	51 (47–62)	52 (45–61)
CFT (s)	34–159	96.5 (81.3–120.5)	100 (71.5–109.5)	101 (68.5–111.5)	86.0 (69.0–92.0)✦	74.0 (65.0–89.0)✦
INTEM						
MCF (mm)	50–72	65.5 (61.3–68.0)	65.0 (62.5–71.5)	68 (64.0–71.5)	67 (65.5–70.0)	67.5 (65.8–71.3)
CT (s)	100–240	158 (149–170)	142 (129–167)	145 (129–167)	149 (138–167)	149 (143–159)
CFT (s)	30–110	66 (58.5–78.5)	72 (51.5–82.5)	65 (51.0–74.5)	61 (52.5–79.0)	59 (50.5–66.8)
FIBTEM						
MCF (mm)	9–25	15.4 (12.5–17.8)	15 (13.5–21.5)	16 (14.5–22.5)	19 (16.5–23.0)	23.0 (19.0–25.0)✦
Platelet (10^9/l)	150–360	244 (88)		240 (50)	217 (66)	221 (66)
Leucocyte (10^9/l)	3.3–8.2	7.2 (1.6)		13.7 (3.9)	13.5 (3.9)	12.3 (3.5)
Hb(g/l)	134–167	145 (13)		132 (15.2)✦	124 (12.5)✦	127 (12.6)✦
CRP (mg/l)	< 10	23.7 (13.0–36.8)		8.3 (3.7–13.2)	19.5 (9.2–45.5)	34 (6.2–88.5)

At 72 hours in patients who further developed DCI, FIBTEM-MCF was significantly higher in comparison to aSAH patients without DCI: 25.0 mm (IQR 66.8–69.8) vs. 19.0 mm (IQR 16.5–22.5 mm), p=0.012 (Figure 13). The results were comparable in patients with EBI.

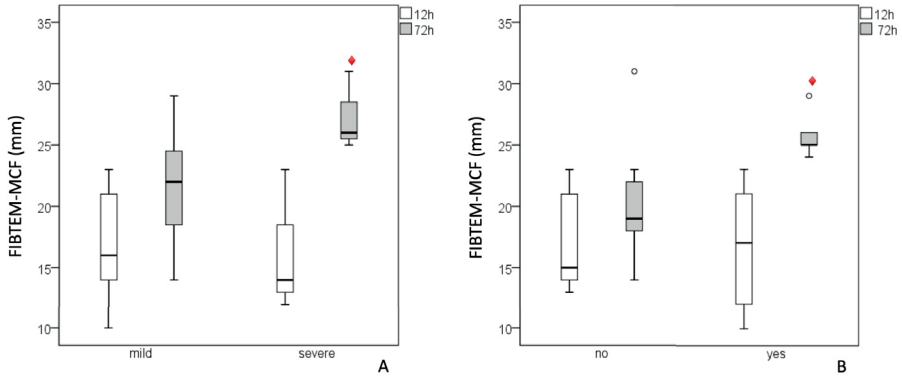

Figure 13. Association with FIBTEM-MCF and the incidence of early brain injury (A) and delayed cerebral ischemia (B) (IV). ✦p<0.05.

7 DISCUSSION

7.1 Anti-Xa levels with low-molecular-weight heparin thromboprophylaxis in critically ill patients (I)

In the systematic review, the reported median peak anti-Xa levels were between <0.1 to 0.35 IU/ml, and the mean peak anti-Xa ranged from 0.09 to 0.40 IU/ml. The reported median trough levels were generally low: undetectable to <0.1 IU/ml.

The interpretation of these results is challenging. Laboratory monitoring of prophylactic anticoagulation with anti-Xa level is not generally recommended since the association with clinical results – thrombosis or bleeding – is considered weak. At the moment, the measurement of anti-Xa is restricted to special cases where the anticoagulant effect of LMWHs is thought to be either unpredictable or uncertain, such as in obese patients and patients with renal insufficiency (Garcia et al., 2012; Nutescu et al., 2009). Nonetheless, in clinical research, the measurement of anti-Xa activity is widely used. There are no validated targets for prophylactic range and various arbitrary ranges have been implemented, complicating the interpretation of the results. Usually, the target levels are given for peak anti-Xa levels (Samama, 1995). It has been suggested that the target peak anti-Xa level should be decided based on the risk of thrombosis: 0.1–0.25 IU/ml for low risk and 0.2–0.5 IU/ml for high risk (Samama, 1995). The more recent trials conducted in postoperative patients receiving enoxaparin thromboprophylaxis have implemented higher peak anti-Xa targets: 0.2–0.4 IU/ml BID dosing and 0.3–0.5 IU/ml for OD dosing (Pannucci et al., 2017a; Pannucci et al., 2017b).

In the selected studies, the target levels set for anti-Xa level were most commonly given either as a constant target of 0.1–0.3 IU/ml or as peak level of 0.2–0.4 IU/ml (Table 6). These targets were reported irrespective of the type of LMWH or dosing. Interestingly, these targets were mainly based on the same study by Levine et al (Levine et al., 1989). In this substudy of RCT, 163 patients undergoing total hip replacement were evaluated. The patients received enoxaparin 40 or 60 mg OD subcutaneously. Anti-Xa levels were determined at 12 hours after previous enoxaparin injection on days 0, 1, 3, and 6 post-operatively. They observed that the cut-off anti-Xa level for hematoma was >0.2 IU/ml and for thrombosis <0.1 IU/ml

By using known pharmacokinetic principles of enoxaparin (Aiach et al., 1983), they further estimated that peak anti-Xa levels for thromboprophylaxis should be 0.1–0.4 IU/ml (Levine et al., 1989).

In a non-inferiority trial conducted by Leyvraz et al., thromboprophylaxis doses of UFH and nadroparin (41–62 IU/kg) were as effective in the prevention of proximal DVT after total hip replacement (Leyvraz et al., 1991). During the first three postoperative days, the mean peak anti-Xa levels after nadroparin were between 0.25 IU/ml and 0.29 IU/ml and between 0.33 IU/ml and 0.37 IU/ml during the fourth and 10th postoperative days. No association was seen between anti-Xa levels and DVT rate. Nevertheless, these results have been used as indirect evidence of adequate thromboprophylaxis (Dörffler-Melly et al., 2002).

However, if using the peak target of 0.2–0.4 IU/ml, only 8 out of 15 studies met this target partly, but only 4 out of 15 if it was an ICU cohort investigated (Table 5 and 6). The highest anti-Xa levels were seen in patients with renal insufficiency (Douketis et al., 2008; Rabbat et al., 2005) but also with increasing LMWH dose (Robinson et al., 2010; Robinson et al., 2013).

No sign of LMWH accumulation was seen in any of the studied cohorts. The low-anti-Xa levels seemed to be associated with a higher body weight, (Costantini et al., 2013; Droege et al., 2014; Mayr et al., 2002) and when weight-based dosing of LMWH was used, anti-Xa levels were higher (Robinson et al., 2013). A weak association was detected with anti-Xa and low AT level in two studies (Jochberger et al., 2005; Zenáhlíková et al., 2010).

Based on the systematic review, there was no clear association with clinical events and the anti-Xa level. In critically ill trauma patients, a low trough anti-Xa level (<0.1 IU/ml) was associated with an increased risk for VTE (Malinoski et al., 2010). Similar results have been observed in retrospective studies. Moreover, there is increasing evidence that peak anti-Xa level <0.3 IU/ml might be associated with post-operative VTE (Pannucci et al., 2017a). To reach anti-Xa levels > 0.3 IU/ml in critically ill patients, increasing the enoxaparin dose up to 40 mg bid or 1 mg/kg OD was required (Robinson et al., 2013).

As stated before, monitoring LMWH thromboprophylaxis is not generally recommended in critically ill patients (Garcia et al., 2012). Furthermore, the quality of the current literature was fair, and the selected studies were underpowered to detect clinical events (Downs et al., 1998; Hooper et al., 2008). Nevertheless, increasing dosing of LMWH thromboprophylaxis in critically ill patients might be beneficial.

7.2 Route for low-molecular-weight heparin anticoagulation (II and III)?

In study II, it was found that when enoxaparin thromboprophylaxis was delivered as a CII, the maximum concentration of anti-Xa was lower during the first 24 hours than was the case with SCB. The same was seen when the pharmacokinetic steady state of enoxaparin was reached.

It seems that administering 40 mg of enoxaparin as a SCB, the most commonly referred peak target for thromboprophylaxis in critically ill patients, i.e. 0.2–0.4 IU/ml (Samama, 1995), was reached within 72 hours. The $C_{max0–72\,h}$ in the CII group remained below this threshold. The most rigorous target for peak anti-Xa level, 0.3–0.5 IU/ml, was not reached in either of the groups. On the contrary, the constant target of anti-Xa 0.1–0.3 IU/ml (Leyvraz et al., 1991) was only reached with CII. Low trough levels have been observed by others when OD or BID dosing of LMWHs has been used (Lewis et al., 2018; Mayr et al., 2002; Rutherford et al., 2005; Vincent et al., 2011). There is also some evidence that low trough levels are associated with the incidence of VTE (Malinoski et al., 2010). Though the results from Study II imply more constant anticoagulation with CII, the clinical relevance remains unclear.

It has been speculated that low anti-Xa levels in critically ill patients might result from a reduced bioavailability of subcutaneously administered LMWH. Indeed, there is some evidence that both edema (Haas et al., 2005) and a concomitantly administered vasoconstrictor reduce plasma anti-Xa levels (Dörffler-Melly et al., 2002). In Study II, the used norepinephrine infusion did not affect the anti-Xa levels. Others have also failed to duplicate these results (Helviz et al., 2016).

In Study III, the CII resulted in significantly lower F1+2 levels *in vivo* at 51 and 72 hours in contrast to the SCB dosing. The level of thrombin activity cannot be directly measured. However, evaluation indirectly, for example by F1+2 can be used as a surrogate. Thus, these results suggest that CII controls the generation and activity of thrombin better than SCB.

At baseline, F1+2 levels were high in both groups as also observed by others (Gouya et al., 2012; Hoppensteadt et al., 2014). Within the 72 hours, F1+2 decreased in CII group, implying that there had been an improvement in fXa inhibition and thus improved anticoagulation. In medical patients, high F 1+2 levels have been associated with a risk of VTE, whereas a reduction in the F1+2 level has been

considered as a sign of adequate anticoagulation (Schutgens et al., 2004; Tripodi et al., 2008). In critically ill septic patients, standard enoxaparin prophylaxis did not correct the sustained increased levels of F1+2 (Gouya et al., 2012). Thus, it seems plausible that the standard SCB dosing of enoxaparin fails to suppress the high formation of F1+2 in critically ill patients. Furthermore, the results of Study III add to the previous results obtained in Study II, suggesting that the CII of enoxaparin may result in more reliable thromboprophylaxis.

In study III, the CII, but not the SCB, nearly normalized AT levels. Similar observations have been made previously in critically ill patients where low AT levels after subcutaneous enoxaparin thromboprophylaxis have been associated with reduced biological activity (Haas et al., 2011). Though continuous heparin infusion decreases AT concentration (Marciniak et al., 1977), and might be further associated with thromboembolic complications (Matthai et al., 1999) no previous data on AT concentration after CII of LMWH exist. Thus, the clinical relevance of AT concentration after CII remains unknown.

TGA-CAT is considered to reflect the overall coagulation capacity better than plasma anti-Xa level, especially in healthy subjects (Thomas et al., 2015), (Al Dieri et al., 2004). The evidence in critically ill patients is more limited (Gouya et al., 2012; Lindström et al., 2011) and it has not been previously investigated at steady state of LMWH thromboprophylaxis. In Study III, it was found that TGA-CAT was able to detect the anticoagulation response after subcutaneous enoxaparin, seen in prolonged lag time and tt-Peak. Gouya et al. previously obtained similar results during a shorter monitoring period (Gouya et al., 2012). At the final trough level, the peak thrombin activity was significantly lower when the drug was administered as a CII than as a SCB. However, the number of responders in the TGA-CAT measurement was only moderate. Hence, these results need to be interpreted with caution.

7.3 Coagulation changes after aneurysmal subarachnoid hemorrhage (IV)

In Study IV, it was found that at 72 hours after aSAH onset, the strength of blood clotting had increased as the EXTEM-MCF and FIBTEM-MCF increased, suggesting enhanced blood coagulability. The increase in FIBTEM-MCF was further associated with the incidence of EBI and DCI.

This is the first study where coagulation changes after aSAH have been evaluated with ROTEM. In a more recent study by Lauridsen et al., both EXTEM-MCF and FIBTEM-MCF were already elevated at admission (median 3 hours 39 min from the onset of aSAH symptoms) in comparison with healthy controls (Lauridsen et al., 2019). The median modified Fisher Scale was 4, implying a greater magnitude of blood on CT than in Study IV. This could explain the earlier detection of hypercoagulation. Similar results have also been obtained in moderate-to-severe aSAH patients with thromboelastography (TEG) if the coagulation status has been monitored over three days (Ramchand et al., 2016). In TEG trials, no difference has been observed (Frontera et al., 2017; Miao et al., 2018) with a monitoring period shorter than 72 hours in comparison with healthy controls. Interestingly, in Study IV, increased coagulation was observed with EXTEM and FIBTEM, but not with INTEM at 72 hours. Similar results have been obtained by others at 24 hours after aSAH (Lauridsen et al., 2019). Ramchand et al. found increased maximum amplitude with TEG when kaolin was used as an activator of the indirect pathway but only after three post-bleed days (Ramchand et al., 2016). The shorter monitoring period in Study IV could explain why no change in INTEM parameters was seen.

FIBTEM-MCF levels increased significantly after 72 hours from the onset of aSAH. This suggests that both fibrin formation and polymerization are significant contributors to the strength of clotting. To my knowledge, there are no data on fibrin function after aSAH. It is known that the plasma fibrinogen concentration with FIBTEM-MCF increases during many other critical illnesses (Sivula et al., 2009). However, at an early state of aSAH, other investigators have reported fibrinogen concentrations within normal limits (Ettinger, 1970; Fujii et al., 1997; Ilveskero et al., 2005; Larsen et al., 2012; Miao et al., 2018) or only slightly elevated levels (Ramchand et al., 2016), but no trials where changes in fibrinogen concentration after aSAH could be identified.

High FIBTEM-MCF at 72 hours after the onset of aSAH was further associated with the incidence of severe EBI and DCI. No association was detected with EXTEM-MCF. Previously, there has been some evidence that higher fibrinogen concentrations might be associated with the incidence of DCI (Fujii et al., 1997). In TEG trials, the association with hypercoagulability and DCI are inconsistent (Frontera et al., 2017; Ramchand et al., 2016). The pathophysiology of DCI is complex and still not fully understood. However, the activation of blood coagulation, that further predisposes cerebral microthrombosis is thought to be one of the key elements (Macdonald, 2014). Based on the results of study IV, it seems plausible that increasing the formation of fibrin and its polymerization might be a contributor to the pathophysiology. However, this needs to be confirmed in a larger clinical study.

The incidence of DVT was 11.8% (n=2), which is within the limits of previous trials where ultrasonography screening has been applied (Ray et al., 2009). Both DVTs were clinically meaningful since, after the diagnosis of DVT, both patients were further diagnosed with a PE. No association was detected with any of the parameters measured in the ROTEM analysis. This is in contrast, to a single report examining aSAH, where hypercoagulation detected with TEG was associated with the DVT incidence (Miao et al., 2018). According to a meta-analysis (including nine cohort studies and one RCT, with a total of 1056 patients) by Dai et al, TEG is not considered accurate enough in to predict post-operative VTE (Dai et al., 2009). In the current guidelines starting early pharmacological prophylaxis after aSAH is highly addressed (Steiner et al., 2013). This is in line with the results of increased blood coagulability after aSAH in Study IV.

7.4 Limitations

These studies have some limitations which need to be addressed.

7.4.1 Study I

First, majority of the included studies were observational studies with small sample sizes. Second, the included studies were highly heterogeneous with respect to the type of ICU, the patient population and the type, and dose of LMWH limiting the

generalization of the results. Finally, methods and designs for anti-Xa monitoring were not identical.

7.4.2 Study II and III

First, Studies II and III were based on the same population with a small sample size and no conclusion on clinical relevance can be drawn. Second, some protocol violations were encountered that led to a smaller PP group than expected. Nevertheless, the results of the ITT and PP groups did not differ. Third, the stratified block randomization independently in each study site meant that there was a different number of patients in each study group. Fourth, the study period of 72 hours was too short to detect the accumulation of enoxaparin (Douketis et al., 2008). Fifth, to avoid bias, patients with acute kidney injury and obesity were excluded. This was decided due to the preliminary nature of the study design. Sixth, because no initiation dose of enoxaparin was given in the CII group, this probably affected the results within the first 24 hours. Finally, because of the repeated measures of same individuals over the study period, using linear mixed model, not included in the predetermined statistical plan, might have improved the reliability of results (Pietrzak et al., 2010).

Some additional limitations concerning Study III. First, because of laboratory technical reasons, the TGA-CAT analysis was only conducted in 18 patients. Moreover, the number of non-responders in the TGA-CAT measurement was surprisingly high due to the preanalytical properties of the blood samples (e.g. shade of plasma in pancreatitis) (Lindström et al., 2011).

7.4.3 Study IV

First, the study was conducted in an emergent situation without the ability to obtain real baseline values from aSAH patients. Therefore, it was decided to use neurosurgical patients as a surrogate for the reference range. Though these patients represent the same patient population, the aSAH patients were younger, and the number of smokers was higher than in control group. It is not known how these differences have contributed to the ROTEM results. Second, the ROTEM was monitored only for 72 hours. In TEG trials, the hypercoagulability state has continued to evolve up to day 10 (Ramchand et al., 2016). Third, a decrease in the hemoglobin level (from 145 g/l ±13 to 127 g/l ± 12.6, $p<0.05$) might have increased

the EXTEM-MCF levels. However, the effect is most likely to be minimal (Nagler et al., 2013). Fourth, the measurement of fibrinogen level or platelet function testing were not done. Thus, the exact contribution of fibrinogen and platelets on increased coagulation remains unknown. Finally, the sample size was too small for clinical endpoints that limit the interpretation of these results. Furthermore, the influence of other relevant confounders on the incidence of EBI and DCI could not be tested, nor was it possible to investigate the role of different operative interventions on blood coagulation.

7.5 Clinical implications and future perspectives

The reduced bioavailability of LMWHs in critically ill patients has been suspected for over 15 years (Freedman, 2003). Indeed, the risk for VTE has remained in a range of 5 to 10% despite the provision of LMWH thromboprophylaxis. When planning future studies, there are several aspects that need to be considered. First, the association of plasma anti-Xa level and clinical endpoints should be studied in critically ill patients. Currently, anti-Xa levels are widely used both in research and clinical settings without adequate validation. Second, it is unsure how best to evaluate the incidence of DVT in clinical research. Screening protocols are often used, but the clinical guidelines have placed less emphasis on these results because of the potential bias on clinical endpoints (i.e. earlier DVT detection might influence PE rate). Third, there is a need for a large multicenter RCTs where different modalities of LMWH thromboprophylaxis are studied. The study raises some questions to be answered:

1. Should a larger dose of thromboprophylaxis be used in all critically ill patients?
2. Should a weight-based dosing of thromboprophylaxis be applied in critically ill patients?
3. Should LMWH thromboprophylaxis be administrated as a CII administration of LWMH instead of a SCB in critically ill patients?

Nonetheless, in order to answer these questions, a sample size of around 3000 patients would be warranted.

However, the results from Studies II and III are promising about the benefits of LMWH thromboprophylaxis delivered as CII in critically ill patients. This should be considered especially for patients who have both an increased risk for thrombosis and bleeding e.g. in DIC. In the future, because of the known pathophysiology of DIC, it would be especially interesting to conduct an in-depth investigation of CII thromboprophylaxis in this patient population.

Neurological impairment after aSAH is a significant contributor to the functional recovery. The initial bleeding itself is beyond the scope of critical care, but one of the primary reasons for a poor neurological recovery is DCI, which usually develops between 3 to 14 days after the onset of aSAH, offering a window for treatment. The pathophysiology of DCI is still not fully understood; however, it is reasonable to assume that improving the understanding in the pathophysiology would lead to interventional clinical trials in the future. In Study IV, it was observed that the coagulation changes after aSAH could be detected with ROTEM. Furthermore, this increment in overall coagulation seemed to have an association with the incidence of DCI. This finding needs to be confirmed in an observational study where the sample size would be adequate for evaluation of the incidence of DCI. If one wishes to diagnose the coagulation changes more precisely, the study period should also be longer and have more specific coagulation markers than only ROTEM.

8 CONCLUSIONS

The following conclusions can be drawn based on this thesis:

1. When the literature on anti-Xa levels after LMWH thromboprophylaxis in critically ill patients was systematically reviewed, the reported median anti-Xa levels were between <0.1 to 0.35 IU/ml. No association was detected between clinical events and anti-Xa levels. The overall quality of the pharmacokinetic studies examining the anti-Xa level with LMWH thromboprophylaxis was moderate.
2. When enoxaparin thromboprophylaxis was given as a continuous intravenous infusion, the maximal concentrations of anti-Xa remained lower within 24 and 72 hours in comparison with subcutaneous dosing. However, the trough level at 72 hours was higher, suggesting more constant anticoagulation.
3. A continuous infusion of enoxaparin thromboprophylaxis reduced prothrombin fragment 1+2 levels in plasma significantly more as compared with subcutaneous dosing. The continuous infusion of enoxaparin normalized the AT levels, whereas this did not occur with the subcutaneous dosing of the drug. This novel finding suggests that continuous intravenous infusion of enoxaparin might be more beneficial than SCB in providing thromboprophylaxis in critically ill patients.
4. After 72 hours from the onset of aSAH, the blood coagulation was activated. The increase in coagulation could be detected with ROTEM EXTEM-MCF and FIBTEM-MCF analyses. This suggests that fibrin formation and polymerization have significant contributions to the strength of clotting.

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Full Length Article

Plasma anti-FXa level as a surrogate marker of the adequacy of thromboprophylaxis in critically ill patients: A systematic review

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ABSTRACT

Background: Critical care patients are prone to venous thromboembolism (VTE) and, thus, pharmacological thromboprophylaxis is generally advised. Low-molecular weight heparins (LMWHs) have become the drug of choice in ICU patients, since their predictable and reproducible dose response. Monitoring their pharmacological effect is not usually necessary except in special occasions (i.e. with obese or renal failure patients), where anti-FXa level measuring is recommended. However, there is neither recommendation of adequate anti-FXa levels in critically ill patients nor is it known whether peak or trough level should be measured. The aim of this systematic review was to evaluate the recommended LMWH doses, and the reasons to monitor anti-FXa levels.
Methods: We searched MEDLINE, Scopus, Cochrane Central Register of Controlled Trials and ClinicalTrials.com to identify all potentially relevant studies. Prospective studies done in critically ill patients were included if at least one anti-FXa level (i.e. peak or trough) after any specified LMWH thromboprophylaxis dose was measured.
Results: Total 18 eligible studies including 1644 patients were included. There was a wide variation in the median peak anti-FXa levels (<0.1–0.35 IU/ml). Trough levels were generally low. Of note, none of the studies detected any correlation with bleeding events and anti-FXa levels. Low trough level increased incidence of DVT in one study only.
Conclusion: Based on the current literature, no definite conclusions can be drawn on targeted anti-FXa level in critically ill patients when using LMWH thromboprophylaxis.

1. Introduction

Critically ill patients are at increased risk of venous thromboembolism (VTE) because of several additive risk factors [1,2], with the critical illness itself acting as a hypercoagulable state [3,4]. In addition, withholding anticoagulant prophylaxis because of an elevated bleeding risk may predispose to VTE [5]. Therefore, the incidence of deep vein thrombosis (DVT) varies widely, from 10% to almost 100% [2,6]. Hospital mortality rates in patients with VTE have been reported to be relatively high, up to 28% [7]. In addition, VTE has been associated with increased risks of longer durations of mechanical ventilation and hospitalization [1].

Current guidelines of the American College of Chest Physicians (ACCP) recommend the use of low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH) to prevent VTE in critically ill patients [8]. However, data supporting the thromboprophylactic effects of LMWH and UFH are mainly based on studies in medical and surgical ward patients, in which LMWHs were superior to UFH in preventing VTE [9]. LMWHs have also become the drug of choice for patients in the intensive care unit (ICU) owing to their more predictable and reproducible dose responses without the need for monitoring [10]. Furthermore, LMWHs have a lower risk of heparin-induced thrombocytopenia than UFH [11]. However, despite the almost routine use of pharmacological prophylaxis, the incidence of VTE has remained relatively high, between 5% and 16% [11,12], raising questions about the adequacy of current recommendations in heterogeneous high-risk groups of ICU patients.

The antithrombotic effects of LMWHs are mainly owing to their enhancement of the inhibitory effects of the intrinsic anticoagulant antithrombin III (AT III) on activated factor X (FXa) and thrombin (FIIa). Each LMWH has its own pharmacological profile (i.e., molecular weight distribution and anti-FXa/anti-FIIa activities), which must be considered when interpreting laboratory results [13]. As all LMWHs are predominantly cleared by the kidneys, patients with renal insufficiency may be predisposed to bleeding. Therefore, monitoring the pharmacological effects of LMWHs by measuring anti-FXa levels has been recommended only in patients with renal insufficiency or with other special circumstances (e.g., morbid obesity) [10]. To date, recommendations for adequate anti-FXa levels in critically ill patients have not been proposed, nor is it known whether the peak or trough level should be measured. Peak levels are generally regarded as reflecting thromboprophylactic effect, whereas trough levels are regarded as reflecting accumulation. However, it is unclear whether high peak levels predispose to bleeding

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or low trough levels to thrombosis. This systematic review was designed to determine anti-FXa levels in blood of critically ill patients after prescribed LMWH thromboprophylaxis and to evaluate whether clinically relevant events (e.g., VTE or bleeding) correlate with anti-FXa levels.

2. Methods

This systematic review is based on the methodology recommended by the Cochrane Collaboration. PRISMA Statement and Moose reporting recommendations were used. The protocol was published on the PROSPERO register (www.crd.york.ac.uk/PROSPERO, number CRD42015025744) before final data extraction.

2.1. Data sources and search strategy

MEDLINE, SCOPUS, the Cochrane Library, and the ClinicalTrials.com databases were searched in collaboration with a librarian from the University of Helsinki, using the terms Venous Thrombosis/thromboprophylaxis/Venous Thromboembolism AND Critical Care/Intensive Care Unit/Critical Illness AND LMWH. There were no restrictions on language, date, or type of publication. The initial search was completed in May 2015. The reference lists of all retrieved articles were manually reviewed to identify any potentially relevant studies. Details of the search strategies are shown in Supplemen 1.

2.2. Study selection

Predefined inclusion criteria were used. Studies had to be prospective in design, performed in adult (age >18 years) critical care patients, include more than 10 patients, use any LMWH thromboprophylaxis, and include at least one anti-FXa measurement at a known time point after specified LMWH administration. All abstracts and titles were screened independently by two reviewers (AV and AK). The full text of every identified article was read. Agreement between the two reviewers on article inclusion was high (kappa value 0.89), with any disagreements resolved through discussions.

2.3. Data extraction and quality assessment

The selected studies were independently reviewed by the same two investigators (AV and AK), and data were extracted using predefined criteria, including the study design, patient population, anti-FXa results, and clinical outcomes (i.e., DVT, symptomatic DVT, pulmonary embolism (PE), symptomatic PE, and major or minor bleeding based on the original trial definition). The quality of eligible studies was assessed using the Downs and Black checklist [14], and any disagreement was resolved by consensus. Quality assessment is presented as total Downs and Black score (maximum, total score 27), as well as by subgroup scores (reporting, external validity, internal validity-bias, internal validity-confounding). Because of the high heterogeneity of clinical outcomes in included studies, quantitative analyses could not be performed.

3. Results

The initial search identified 5206 citations, after duplicates were removed. Of these, 31 studies were retrieved for more detailed evaluation and 18 studies, including 1644 patients, were included in the final systematic review (Fig. 1). These studies were mainly observational, with only two randomized clinical trials (RCTs) identified. Trial characteristics are presented in Table 1. Most of these studies (10 observational studies and two RCTs) tested the effects of different dosages of enoxaparin [15–26], whereas four observational studies assessed dalteparin [27–30], and one each tested certoparin [31] and nadroparin [32]. No studies testing tinzaparin were identified. The median peak anti-FXa levels in ICU patients varied widely, from <0.1 IU/mL [31] to 0.35 IU/mL [27]. The median trough anti-FXa levels were reported in four studies, and they varied between undetectable and <0.1 IU/mL [27,31,18,20].

The quality of the studies according to the Down and Black score is shown in Table 2. The median total score (interquartile range, IQR) was 19 [15–20]/27. The median subscores (IQR) were 8.5 [7–10]/11 for reporting, 1 [1–2]/3 for external validity, 5 [4–5]/7 for internal validity-bias, and 3 [2–5]/7 for internal validity-confounding and power.

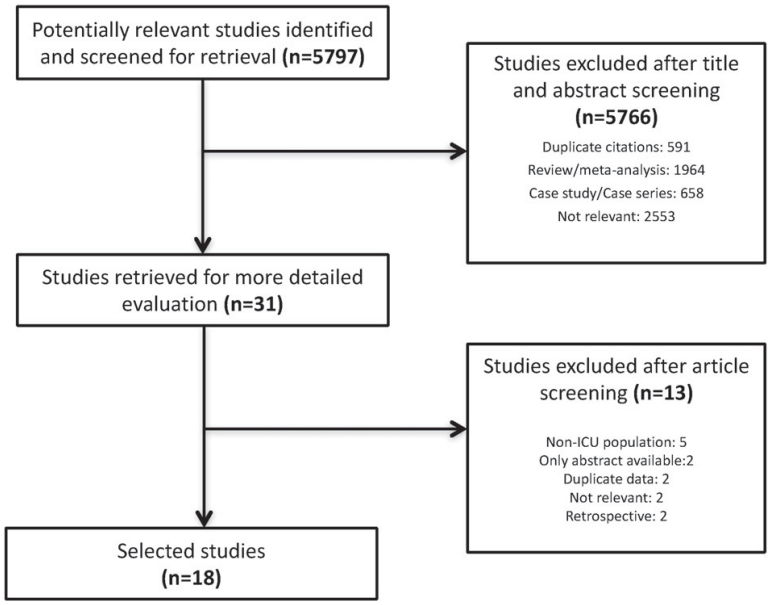

Fig. 1. Flow chart of the study selection.

3.1. Certoparin

The only observational study of certoparin included 62 critically ill patients. Treatment with 3000 IU OD certoparin resulted in a median peak anti-FXa level that was undetectable <0.1 (IQR <0.1–0.2) IU/mL. Only 28% of patients were within the antithrombotic range using the study definition of target value. When the dosage was doubled to 3000 IU bid, the median peak anti-FXa level did not change but 47% patients were within the recommended range. Median trough levels in patients administered 3000 IU OD and bid certoparin were undetectable <0.1 (IQR <0.1–0.17) and <0.1 (IQR <0.1–0.26) IU/mL, respectively. One patient in the lower dose group experienced severe PE, but the anti-FXa concentration of this patient was not reported. There was no correlation between bleeding and high anti-FXa levels (>0.3 IU/mL) [31].

3.2. Dalteparin

Four studies were identified. The first study, involving patients in the ICU with renal insufficiency (creatinine clearance "CrCl" < 30 mL/min) administered 5000 IU OD dalteparin, reported median peak anti-FXa levels 4 h (hrs) after treatment to be 0.29 (IQR 0.20–0.42) IU/mL after 3 days and 0.34 (IQR 0.27–0.45) IU/mL after 17 days. Trough levels were undetectable. The incidence of major bleeding was 7.2% and two patients died of bleeding complications. However, there was no correlation between anti-FXa levels and deaths [27]. Similar results were observed in a small observational study of patients with renal insufficiency (CrCl < 30 mL/min) [29].

The third study was a before and after study, in which dalteparin dosage was doubled if the anti-FXa level at 12 h was below <0.1 IU/mL. The protocol reduced the total incidence of VTE (12.8% vs. 7.0%, p = 0.009). Moreover, if the median anti-FXa level was below <0.1 IU/mL at 12 h, the rates of VTE (14.4% vs. 5.4%, p = 0.05) and DVT (14.4% vs. 3.2% p = 0.01) were significantly higher [28].

In the fourth study, the pharmacokinetics of dalteparin were assessed in edematous and non-edematous ICU patients. There were no between-group differences in mean ± standard deviation (SD) peak (0.15 ± 0.05 vs. 0.14 ± 0.06 IU/mL) and trough (0.05 ± 0.06 vs. 0.02 ± 0.02 IU/mL) anti-FXa concentrations [30].

3.3. Enoxaparin

Median peak anti-FXa levels tended to be lower in ICU than in medical ward patients: 0.16 (IQR 0–0.22) vs 0.2 (IQR 0.15–0.27) IU/mL [16]. Similar results were observed when the area under curve $(AUC)_{0-12\ h}$ was measured (SD 2.63 ± 1 vs. 4.26 ± 1.7 IU/mL/h) [21]. Two RCTs also showed positive correlations between enoxaparin dosage and median peak and mean anti-FXa levels [22,23]. Five studies reported trough levels, which were generally low (0–0.10 IU/mL) and in one study the low trough level correlated with the incidence of DVT (37 vs. 11%, p = 0.026) [19]. Median trough levels were generally low or undetectable [18,20]. Nine studies reported adverse events, with none of these studies detecting any correlation between bleeding events and anti-FXa levels [15,18–20,22,23,24,33,26].

3.4. Nadroparin

Only one observational study was found, in which nadroparin 2850 IU OD was compared in three different patient groups: ICU patients on vasopressors (n = 15), ICU patients not on vasopressors (n = 15), and surgical ward patients (n = 15). The mean peak anti-FXa levels at 3 h were 0.09 (95% confidence interval (CI) 0.05–0.10), 0.23 (95% CI 0.18–0.27), and 0.28 (95% CI 0.23–0.31) IU/mL respectively. Trough levels were not measured, and clinical outcomes were not reported [32].

4. Discussion

This systematic review, which included 16 observational studies and two RCTs, found a lack of evidence regarding optimal targeted anti-FXa levels in critically ill patients. Median peak anti-FXa levels (<0.1–0.35 IU/mL) [31,27] and mean anti-FXa levels (0.09–0.40 IU/mL) [32,23] varied widely, depending on the type and dose of LMWH and on the study population. In addition, the trough levels were consistently low [27,31,18,20,29,30,24,33]. Data on peak and trough anti-FXa levels after LMWH thromboprophylaxis in ICU patients are sparse, and more research is certainly needed. Irrespective of LMWH drug and dose, peak and trough anti-FXa levels were generally low throughout the studies, but correlations between anti-FXa levels and clinically relevant outcomes, such as DVT, PE, and bleeding, remain uncertain. However, one study found that a low trough anti-FXa level significantly increased the incidence of DVT (37% vs. 11%, p = 0.026) [19].

These findings may have several explanations. First, AT III levels in critically ill patients were consistently low, correlating with low anti-FXa levels [31,21]. Low AT III levels may be due to elevated levels of other heparin-binding proteins (e.g., fibronectin and vitronectin), as observed in an animal endotoxin model [34]. Second, the absorption of subcutaneously administered LMWH may be reduced in patients treated with a vasopressor, reducing systemic bioavailability [32]. Third, critically ill patients often receive excessive amounts of intravenous fluids, resulting in peripheral edema, which may affect LMWH pharmacokinetics and reduce its bioavailability [17].

Administration of a standard prophylactic dosage of LMWH to obese ICU patients results in lower anti-FXa activity than in non-obese individuals [28,20]. However, enoxaparin dose may be positively correlated with anti-FXa levels, with adequate peak anti-FXa levels achieved using a weight-adjusted dose of enoxaparin (1 mg/kg once daily) [23].

Because of the lack of good quality RCTs [35], evidence of the effectiveness of LMWH thromboprophylaxis in ICU patients is mainly based on measurements of plasma levels of anti-FXa, as anti-FXa is a surrogate marker for anticoagulant effects. Correct anti-FXa levels have been determined only in patients undergoing orthopedic surgery [36,9]. It has not yet been determined whether peak or trough level should be measured. The generally recommended peak anti-FXa level for thromboprophylaxis is 0.1–0.3 IU/mL in medical and surgical patients [37]. The rates of VTE in patients undergoing hip surgery with trough (12 h after administration of enoxaparin) anti-FXa activity of >0.1 IU/mL, <0.1 IU/mL, and <0.05 IU/mL were reported to be 6.3%, 14.6%, and 18.8%, respectively. Anti-FXa levels >0.2 IU/mL were associated with hematomas [36]. These values are also generally used as references for anticoagulant effects in ICU patients in the observational studies included in this review.

Two of the observational studies were conducted in critically ill patients with renal insufficiency. Administration of a prophylactic dose of dalteparin to patients with impaired renal function (CrCl < 30 mL/min) did not alter median peak anti-FXa levels [29,27] nor was there evidence of an increased risk of bleeding, despite a mean ± SD CrCl of 18.9 ± 6.5 mL/min [27]. Lower serum creatinine and urea levels (not specified) were associated with a greater clearance of LMWH (certoparin), as measured by lower plasma anti-FXa levels [31].

Renal insufficiency is one of the most frequent reasons for omission of pharmacological thromboprophylaxis or for preferring UFH over LMWH [5]. Current research evidence does not support this practice, but these data are observational and limited. Moreover, urinary CrCl is an imprecise estimate of glomerular filtration rate in critically ill patients [38]. A meta-analysis that included patients with severe renal insufficiency (CrCl < 30 mL/min) found that only when therapeutic doses of enoxaparin were administered were anti-FXa levels elevated and the risk of major bleeding increased [39]. Prophylactic administration of enoxaparin but not of tinzaparin showed similar accumulation findings in elderly medical patients with impaired renal function (CrCl 34 ± 11.4 mL/min) [40].

Table 1
The characteristics of the selected studies.

Study	Population	Study design	Thromboprophylaxis	Anti-FXa timing	Peak anti-FXa levels IU/ml	Trough anti-FXa levels IU/ml	Adverse events	Anti-FXa assay
Certoparin								
[31]	Mixed ICU	Single-center, prospective, open label study (n = 30 + 32)	Certoparin 3000 IU s.c. OD and 3000 IU s.c. bid	At 0, 4, 12 and 24 h	Median (IQR) at 4 h < 0.1 (<0.1–0.2) vs. <0.1 (<0.1–0.28)	Median (IQR) <0.1 (<0.1–0.17) vs. <0.1 (<0.1–0.26)	One PE in certoparin 3000 IU OD group	Coamatic Heparin©Coamatic Heparin©
Dalteparin								
[27]	ICU patients with renal insufficiency (CrCl <30 ml/min)	Multi-center, single-arm, open label study (n = 138/156)	Dalteparin 5000 IU s.c. OD	At 0, 1, 2, 4, 8, 12, 20 and 24 h and on days 3, 10 and 17	Median (IQR) at 4 h: after 3 days 0.29 (0.20–0.42), 10 days 0.35 (0.24–0.43), 17 days 0.34 (0.27–0.45)	Median (IQR) <0.10 (<0.10– < 0.10) (n = 120)	5.1% DVT, 7.2% major bleeding, 1.4% HIT. Two patients died with bleeding.	Stachrom-Heparin/Spectrolyze Heparin Xa/Electrochrome Beckman Coulter©
[28]	TICU	Single-center, before and after, prospective study (n = 190/785)	Before: dalteparin 5000 IU s.c OD, after: dalteparin 5000 IU s.c. OD if anti-Xa <0.1 IU/ml 5000 IU s.c. bid	At 12 h	Not measured	Not measured	Pre vs. post protocol: overall VTE 12.8% vs. 7.0% (p = 0.009)	Not reported
[29]	Mixed ICU patients with renal insufficiency (CrCl <30 ml/min)	Single-center, prospective, cohort study (n = 19)	Dalteparin 5000 IU s.c. OD	At 4 h and 22–23 h	Mean (95% CI) at 4 h 0.30 (0.27–0.33)	Only 3/19 patients had levels above detection threshold (>0.1)	One catheter-related thrombus, two macroscopic bleeding	Rotochrom Heparin Kit©
[30]	Mixed ICU	Two-center open label study, edematous (n = 7) vs. nonoedematous (n = 7)	Dalteparin 2500 IU s.c. OD	At 0, 3, 4, 6, 8, 12 and 24 h	Mean (SD) at 3 h: 0.15 (0.05) vs. 0.14 (0.06)	Mean (SD) 0.05 (0.06) vs. 0.02 (0.02)	Not reported	Spectrolyze Heparin Xa©
Enoxaparin								
[15]	SICU (trauma ISS 24 ± 10.9)	Single-center, prospective, open label study (n = 61). LMWH dosing was increased if peak anti-FXa was <0.2 IU/ml	Enoxaparin 30 mg s.c. bid (initial dose) up to 60 mg s.c. bid	At 4 h on the 3rd dose and before the 4th dose	At 4 h sub-therapeutic (<0.2) in 70.5% (n = 43)	Not reported	4.9% VTE, did not correlate to anti-FXa levels. No bleeding events.	HemosillL©

(continued on next page)

Table 1 (*continued*)

Study	Population	Study design	Thromboprophylaxis	Anti-FXa timing	Peak anti-FXa levels IU/ml	Trough anti-FXa levels IU/ml	Adverse events	Anti-FXa assay
[16]	MICU vs. general medical ward	Single-center, prospective, controlled open label study (n = 15 + 16)	Enoxaparin 40 mg s.c. OD	at 0, 1, 3, 6 and 12 h	Median (IQR) 3 h 0.16 (0–0.22) vs. 0.2 (0.15–0.27)	Not measured	Not reported	Rotachrom HBPM/LMWH©
[17]	TICU (ISS > 10)	Single-center, prospective cohort study, nonoedematous (n = 11/14) vs. edematous (n = 10/11).	Enoxaparin 30 mg s.c. bid	At 0, 5, 1, 2, 3, 4, 6, 8 and 12 h	Median 0.27 vs. 0.12. IQR not reported	Not reported	Not reported	Chorm Z Heparin Kit©
[18]	MICU	Single-center, prospective, observational study (n = 55)	Enoxaparin 40 mg s.c. OD	At 0, 4, 12 and 24 h	Median (IQR) at 4 h 0.22 (0.17–0.26)	Median (IQR) 0 (0–0.03)	12.7% DVT, 1.8% PE. No bleeding complications	STA-STACLOT Heparin©
[19]	SICU	Single-center, prospective, observational study (n = 54)	Enoxaparin 30 mg s.c. bid	At 4 h and 1 h before the 4th dose	Mean (SD) at 4 h low trough group 0.17 (0.1) vs. normal trough group 0.27 (0.1)	Low trough (≤0.1) n = 27 vs. normal trough (>0.1) n = 27	Low through anti-FXa level correlated to incidence of DVT (37 vs. 11%, p = 0.026)	Not reported
[20]	Mixed ICU	Single-center, prospective, observational study (n = 89)	Enoxaparin 40 mg s.c. OD	At 0, 4, 12 and 24 h	Median (IQR) at 4 h 0.18 (<0.1–0.52)	Median (IQR) <0.1 (<0.1–0.43)	No thromboembolic complications	Coamatic Heparin©
[21]	Mixed ICU vs. medical ward patients	Single-center, prospective, controlled open label study (n = 16 + 13)	Enoxaparin 40 mg s.c. OD	At 0, 1, 3, 6, 12 h on day 1 and at 3 h on days 2–5	Mean AUC (SD) at 0–12 h 2.63 (1) vs. 4.26 (1.7)	Not measured	Not reported	Rotachrom HBPM/LMWH©
[22]	Mixed ICU	Single-center, prospective, randomized double blind study (n = 18 + 16 + 20 + 18)	Enoxaparin 40, 50, 60 or 70 mg s.c. OD	At 0, 4, 12 and 24 h	Median at 4 h 0.13, 0.14, 0.27 vs. 0.29. IQR not reported	Not reported	One minor nosebleed	Coamatic Heparin©
[23]	Mixed ICU	Single-center, prospective, randomized double blind study (n = 20 + 20 + 19 + 19)	Enoxaparin 40 mg s.c. OD, 30 mg s.c. bid, 40 mg s.c. bid or 1 mg/kg s.c. OD	At 0, 4, 12, 16, 24 h daily for 3 days	Mean at 4 h on the 1st day: 0.20, 0.08, 0.17, 0.34 on the 3rd day: 0.13, 0.14, 0.33, 0.40. SD not reported	Not reported	No adverse events	Coamatic Heparin©
[24]	TICU (ISS > 10)	Single-center, prospective, open label, cohort study (n = 17)	Enoxaparin 40 mg s.c. OD	At 4 and 24 h (on day 3)	Mean (SD) at 4 h 0.19 (0.09)	Mean (SD) 0.04 (0.04)	One DVT, no bleeding	Not reported
[33]	Mixed ICU	Single-center, prospective, open label study (n = 36)	Enoxaparin 30 mg s.c. bid	At 0, 3, 6, 9 and 12 h	Median (IQR) 3 h 0. 24 (0.19)	Median (IQR) 0.10 (0.09)	One DVT, one bleeding, one thrombocytopenia	Stago Rotachrom Heparin kit©
[26]	MICU sepsis patients	Single-center, prospective, open label study (n = 16)	Enoxaparin 40 mg s.c. OD	At 4 h on days 1, 2, 3, 6, 9, 12 and 15	Mean (SD) at 4 h 0.17 (0.17)	Not measured	One DVT, two bleeding events	Coamatic Heparin©
Nadroparin								
[32]	ICU vs. general surgery ward	Single-center, ICU patients on vasopressors (n = 15) vs. ICU patients not on vasopressors (n = 15) vs. surgical ward patients (n = 15)	Nadroparin 2850 IU s.c. OD	At 0, 3, 4, 6, 8 and 12 h, LMWH given at least 3 days	Mean (95%CI) at 3 h: 0.09 (0.18–0.37) vs. 0.23 (0.18–0.27) vs. 0.28 (0.23–0.31)	Not measured	Not reported	Coamatic Heparin©

Abbreviations
ICU, indicates intensive care unit; CrCl, creatinine clearance; TICU, trauma intensive care unit; SICU, surgical intensive care unit; MICU, medical intensive care unit; ISS, injury severity score; LMWH, low-molecular weight heparin; s.c., subcutaneous; OD, once daily; bid, twice daily; hrs, hours; h, hour; IQR, interquartile range; CI, confidence interval; SD, standard deviation; AUC, area under curve; PE, pulmonary embolism; DVT, deep venous thrombosis; HIT, heparin induced thrombocytopenia; VTE, venous thromboembolism.

Table 2
Down and Black checklist.

Study	Total score (27)	Reporting (10)	External validity (3)	Internal validity-bias (7)	Internal validity-confounding and power (7)
[15]	8	6	1	0	1
[32]	15	8	1	4	2
[27]	20	10	1	6	3
[28]	13	7	2	4	0
[16]	19	9	1	5	4
[17]	15	6	1	5	3
[31]	19	8	1	5	5
[18]	22	10	3	5	4
[19]	17	7	3	4	3
[20]	20	9	1	5	5
[21]	16	7	1	5	3
[29]	14	8	0	4	2
[23]	26	10	2	7	7
[22]	23	9	1	7	6
[30]	21	10	1	5	5
[24]	14	8	0	4	2
[33]	20	11	1	5	3
[26]	20	10	1	5	4
Median (IQR)	19 (15–20)	8.5 (7–10)	1 (1–2)	5 (4–5]	3 [2–5)

IQR indicates interquartile range.
Reporting
1. Is the hypothesis/aim/objective of the study clearly described?
2. Are the main outcomes to be measured clearly described in Sections 1 and 2?
3. Are the characteristics of the patients included in the study clearly described?
4. Are the interventions of interest clearly described?
5. Are the distributions of principal confounders in each group of subjects to be compared clearly described?
6. Are the main findings of the study clearly described?
7. Does the study provide estimates of the random variability in the data for the main outcomes?
8. Have all important adverse events that may be a consequence of the intervention been reported?
9. Have the characteristics of patients lost to follow-up been described?
10. Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?

External validity
1. Were the subjects asked to participate in the study representative of the entire population from which they were recruited?
2. Were those subjects who were prepared to participate representative of the entire population from which they were recruited?
3. Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive?

Internal validity — bias
1. Was an attempt made to blind study subjects to the intervention they have received?
2. Was an attempt made to blind those measuring the main outcomes of the intervention?
3. If any of the results of the study were based on "data dredging", was this made clear?
4. In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case–control studies, is the time period between the intervention and outcome the same for cases and controls?
5. Were the statistical tests used to assess the main outcomes appropriate?
6. Was compliance with the intervention/s reliable?
7. Were the main outcome measures used accurate (valid and reliable)?

Internal validity — confounding (selection bias) and power.
1. Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case–control studies) recruited from the same population?
2. Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case–control studies) recruited over the same period of time?
3. Were study subjects randomized to intervention groups?
4. Was the randomized intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable?
5. Was there adequate adjustment for confounding in the analyses from which the main findings were drawn?
6. Were losses of patients to follow-up taken into account?
7. Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%?

The critical illness itself often acts as a hypercoagulable state (e.g., sepsis or trauma) [3]. In addition, the severity of critical illness (e.g., multiple organ dysfunction score or severity of burn injury) was reported to correlate with lower anti-FXa [41,20]. Whether the latter reflects an insufficient anticoagulant effect or the nature of anti-FXa as a surrogate marker requires further investigations.

These findings of low levels of anti-FX activity and several conditions that can alter LMWH bioavailability in intensive care patients have raised the question of the adequacy of thromboprophylaxis with dosing schemas applied to medical and surgical patients. Although VTE events have decreased with LMWH prophylaxis, thromboembolism has occurred, despite recommended dosing of LMWHs and even within recommended anti-FXa levels [19]. In addition to the reasons described above, uneventful thrombosis may result from a fear of bleeding, resulting in pauses and delays in thromboprophylaxis [5].

4.1. Strengths and limitations

To our knowledge, this is the first systematic review of the role of anti-FXa monitoring during LMWH thromboprophylaxis in critically ill patients. However, this study had several limitations. First, all included studies were relatively small, with most being observational and underpowered for clinical endpoints, and of low quality. Second, as the type of ICUs varied among studies (i.e., mixed, medical, trauma, and surgical) and patients had various degrees of renal failure, the study population was quite heterogeneous. The benefits and adverse effects of LMWH may vary in different subgroups of ICU patients. Third, the type and dose of LMWH varied among studies; because LMWHs have different pharmacological properties, it is unclear whether anti-FXa levels can be generalized. Fourth, there were methodological limitations concerning anti-FXa measurements, as the protocols of the included studies were not identical (e.g., times samples were obtained after LMWH administration, blood sample collecting procedures, and delay from sampling to analysis) and the analytic methods and reference levels differ among laboratories. Moreover, the reporting of measured anti-Xa activity concentrations in the studies varied (e.g., median, mean or AUC), making data interpretation difficult. Fifth, the secondary outcomes should also be considered a study limitation because the definitions of these adverse events (e.g., DVT, symptomatic DVT, PE, symptomatic PE, and minor or major bleeding) varied across studies. Sixth, the literature search was updated in May 2015; thus, more recent data may be missing.

5. Conclusion

No definite conclusions can be drawn regarding target anti-FXa levels in critically ill patients administered LMWHs for thromboprophylaxis. No recommendations can be made on the timing of anti-FXa monitoring or for dose adjustments for individual patients.

Acknowledgments

AV, SV, VP and AK planned and designed the study. AV and AK acquired and reviewed the data and assessed risk of bias. All authors revised the manuscript critically with a contribution and gave final approval of the version to be published. No external funding was received.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.thromres.2015.12.016.

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Full Length Article

Plasma anti-FXa concentration after continuous intravenous infusion and subcutaneous dosing of enoxaparin for thromboprophylaxis in critically ill patients. A randomized clinical trial☆

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ABSTRACT

Introduction: In intensive care unit (ICU) patients, subcutaneous low-molecular weight heparin thromboprophylaxis results in lower plasma anti-factor Xa (anti-FXa) levels compared to general ward patients. The aim of this study was to examine whether enoxaparin thromboprophylaxis given as a continuous intravenous infusion (CII) results in more constant and predictable anti-FXa concentration than standard subcutaneous bolus (SCB) administration.

Materials and methods: This was a prospective, single-blind, multicenter, randomized controlled trial where ICU patients requiring thromboprophylaxis received enoxaparin either 40 mg as a SCB once daily or 40 mg as a CII over 24 h for three consecutive days.

The primary outcome was maximum serum anti-FXa concentration ($C_{max24\ h}$) within the first 24 h; the secondary outcome was anti-FXa area under the curve $(AUC)_{(0–24\ h)}$. Trough level was measured at 72 h.

Results: Thirty-nine patients were included in the intention to treat analysis. The median anti-FXa $C_{max24\ h}$ was 0.05 (interquartile range, IQR, 0.05–0.18) IU/ml in the CII group and 0.18 (IQR, 0.12–0.33) IU/ml in the SCB group ($p = 0.05$). Median anti-FXa $AUC_{(0–24\ h)}$ was 1.20 (IQR, 0.98–2.88) in the CII and 1.54 (IQR, 1.22–4.12) in the SCB group ($p = 0.095$). After 72 h, 66.7% of patients in the CII group had a detectable anti-FXa concentration of > 0.1 IU/ml, compared with 16.7% in the SCB group ($p = 0.019$).

Conclusions: Continuous infusion of enoxaparin led to lower anti-FXa $C_{max24\ h}$ than standard SCB administration. No difference in anti-FXa $AUC_{0–24\ h}$ was detected.

1. Introduction

Despite pharmacologic thromboprophylaxis, venous thromboembolism (VTE), including deep vein thrombosis (DVT) and pulmonary embolism (PE), are common complications of critical illness, and substantially increase morbidity and mortality [1,2]. Low-molecular-weight heparins (LMWHs) have become the drug of choice for thromboprophylaxis, as they have a more predictable and reproducible dose response than low-dose unfractionated heparin. The monitoring of anticoagulant effect is not generally recommended when using LMWHs [3]. Nonetheless, the measurement of plasma anti-factor Xa (anti-FXa) concentration has been described, although its efficacy as a means of monitoring therapeutic effect and association with clinical thromboembolic events is thought to be inadequate [4].

There is growing evidence that critically ill patients have lower anti-FXa concentration than general ward patients after the initiation of standard LMWH thromboprophylaxis [5,6]. It has been proposed that the bioavailability of subcutaneous LMWH is impaired in critically ill patients, due to low cardiac output, impaired peripheral blood flow, concomitant use of vasoconstrictors [6] and subcutaneous edema [7]. In support of this hypothesis, subcutaneous LMWH thromboprophylaxis in ICU patients receiving vasopressor therapy has been shown to result in substantially lower anti-FXa activity than in patients not receiving vasoconstrictors [6].

To investigate whether the current practice of subcutaneous bolus (SCB) LMWH thromboprophylaxis is suitable for critically ill patients,

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we compared SCB therapy with a continuous intravenous infusion (CII) in this randomized clinical trial (RCT).

2. Materials and methods

2.1. Trial design

This prospective, randomized, single-blind clinical trial was conducted in two Finnish university hospital mixed ICUs at Tampere University Hospital and Meilahti University Hospital. The trial was conducted in accordance with the amended Declaration of Helsinki. The study design was approved by the local ethics committee of Pirkanmaa, Finland and the Finnish Medicines Agency, and it was registered in the Clinical Trials database (ClinicalTrials.gov; NCT02095509). Before enrolment, written informed consent was obtained from each patient, or his or her legal representative.

2.2. Study population

Adult ICU patients aged between 18 and 80 years with an indication for pharmacologic thromboprophylaxis, a body mass index (BMI) 18–30 kg/m^2 and an expected ICU stay ≥ 72 h were eligible. Exclusion criteria were: indications for anticoagulant therapy other than thromboprophylaxis; intracranial hemorrhage or central neurosurgical operation within 3 months of ICU admission; diagnosis of disseminated intravascular coagulation according to International Society on Thrombosis and Haemostasis criteria [8]; known heparin-induced thrombocytopenia (HIT); hypersensitivity to enoxaparin or heparin; blood platelet count < 20 × 10^9/l, prothrombin time (PT) < 20% or International Normalized Ratio (INR) > 1.7; major hemorrhage within the last week unless definitively treated; glomerular filtration rate < 50 ml/min/1.73 m^2 estimated from serum creatinine concentration by applying the Cockcroft-Gault equation [9] or chronic dialysis; known HIV, hepatitis B or hepatitis C infection; pregnancy; and known liver disease. A patient who had received LMWH thromboprophylaxis within 24–72 h of ICU admission could be included if measured anti-FXa concentration was < 0.1 IU/ml at the time of randomization. Basic patient characteristics, comorbidities and Acute Physiology and Chronic Health Evaluation (APACHE II) score were also recorded at baseline.

2.3. Study intervention

Patients were randomized to receive 40 mg enoxaparin (Klexane®, Sanofi-Aventis, Helsinki, Finland) either as an SCB every 24 h or as a CII over 24 h for three consecutive days. Block randomization into two groups was performed using sequentially numbered, sealed envelopes that were stratified according to the use of a vasopressor (yes or no). The SCB dose was administered once daily from a prefilled single-dose syringe containing 40 mg enoxaparin. The CII (40 mg enoxaparin diluted in 100 ml 0.9% sodium chloride solution) was prepared by a pharmacist or ICU nurse, divided in two syringes of 50 ml and infused intravenously (via either a central or peripheral venous catheter) over 24 h via an automatic pump. Any discontinuations of the study drug were recorded; if the infusion was stopped for > 2 h, the patient was excluded from the final analysis. Mechanical thromboprophylaxis was undertaken according to normal clinical practice. The study period was 72 h, after which thromboprophylaxis was continued according to routine clinical practice in the ICUs.

Plasma anti-FXa concentration was determined at 0, 3, 6, 9, 12, 15, 18, 24, 27, 48, 51 and 72 h after the beginning of the study, where 24, 48 and 72 h samples represented trough concentrations and 27 and 51 h peak concentrations for SCB dosing. Additional samples were obtained from patients in the CII group at 1.5 and 4.5 h. The total dose of norepinephrine was documented daily. Blood chemistry, serum C-reactive protein concentration, platelet count, INR and PT were checked daily. All blood samples were drawn from an arterial catheter that did not contain any heparin. Anti-FXa activity was measured in fresh blood samples in the core laboratory of each study hospital using a validated chromogenic assay (STA-Liquid anti-Xa, Diagnostica Stago, Asnières-sur-Seine, France).

2.4. Outcome measurements

The primary outcome measure was maximum plasma anti-FXa concentration within 24 h after initiation ($C_{max24\ h}$). The secondary outcomes were maximum anti-FXa C_{max} within 72 h ($C_{max72\ h}$), area under the time-concentration curve at 24 and 72 h ($AUC_{(0–24\ h)}$ and $AUC_{(0–72\ h)}$) determined by standard pharmacokinetic procedures. The trough level was evaluated by anti-FXa concentration after the study period at 72 h. The influence of norepinephrine infusion (yes/no) and total norepinephrine dose on anti-FXa $C_{max24\ h}$ and $AUC_{0–24\ h}$ were also examined.

Clinically relevant complications were defined as follows: major hemorrhage (requiring > 2 units transfusion of red blood cells, intracranial bleeding, or bleeding requiring major therapeutic intervention, causing hemodynamic compromise or resulting in death), minor hemorrhage (any other bleeding), DVT (confirmed by compression ultrasound, if clinically suspected), PE (confirmed by chest computed tomography angiography if clinically suspected) and HIT [10]. During the study period, the duration of mechanical ventilation and daily Sequential Organ Failure Assessment score were recorded, as well as the length of ICU stay and all-cause mortality at day 90 after ICU admission.

2.5. Statistical analysis

Standard sample size calculations indicated that at least 20 patients would be needed in each group to detect a clinically meaningful 33% reduction (from 0.30 to 0.20, standard deviation 0.11) in peak anti-FXa concentration, assuming a power of 80% and a significance level of 5%.

The distribution of data was assessed with the Shapiro-Wilk test. Non-normally distributed data are presented as the median (interquartile range, IQR). All comparisons between the study groups were performed with the Mann-Whitney *U* test, the χ^2 test, Fisher's test and Spearman's correlation coefficient as appropriate. All statistical analyses were performed using the SPSS statistical software program (version 23.0; IBM, Armonk, NY).

3. Results

Forty patients were randomized between March 2014 and July 2016. One patient did not receive the study drug because of infusion pump failure, and was excluded from the modified intention to treat (ITT) analysis. There were four randomization errors and three protocol violations, leaving 32 patients in the per protocol (PP) analysis (Fig. 1). Baseline characteristics and laboratory values are shown in Table 1; the study groups were well balanced.

3.1. Outcomes

In the ITT analysis, the median $C_{max24\ h}$ was 0.05 (IQR, 0.05–0.18) IU/ml in the CII group and 0.18 (IQR, 0.12–0.33) IU/ml in the SCB group (p = 0.05). The median $AUC_{(0–24\ h)}$ was 1.20 (IQR, 0.98–2.88) IU/l/h in the CII group and 1.54 (IQR, 1.22–4.12) IU/l/h in the SCB group (p = 0.095). Per protocol analysis did not change the results (Table 2). After the study period of 72 h, the trough anti-FXa concentration was 0.12 (IQR, 0.05–0.17) IU/ml in the CII group and 0.05 (IQR, 0.01–0.05) IU/ml in the SCB group (p = 0.021), leaving only 16.7% (two out of 10) patients with detectable anti-FXa concentration > 0.1 IU/ml in the SCB group compared with 66.7% (10 out of 15) in the CII group (p = 0.019).

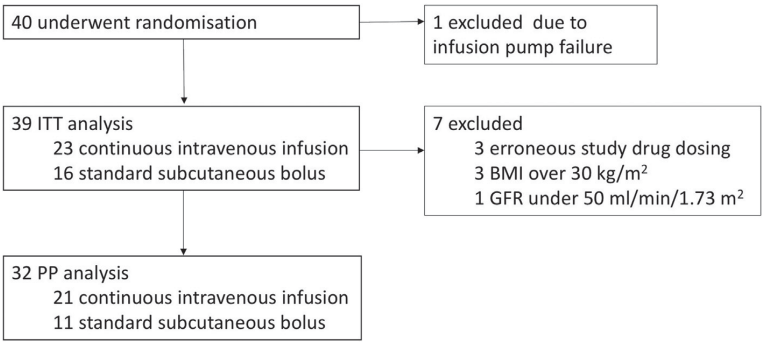

Fig. 1. Diagram of enrollment. One patient from the CII group was excluded prior the study start due to infusion pump failure leaving 39 patients in the ITT group. From the ITT group 7 patients were excluded (3 erroneous study drug dosing, 3 BMI over 30 kg/m^2 and 1 GFR under 50 ml/min/1.73 m^2) leaving 32 patients in the PP group.
Abbreviations: ITT, intention to treat; CII, continuous intravenous infusion; SCB, standard subcutaneous bolus; BMI, body mass index; GFR, glomerular filtration rate; PP, per protocol analysis.

No correlations between the first ICU day total norepinephrine dose and $C_{max24\ h}$ or $AUC_{(0–24\ h)}$ were observed: Spearman's correlation coefficients were −0.176 for $C_{max24\ h}$ ($p = 0.291$) and −0.171 for $AUC_{(0–24\ h)}$ ($p = 0.305$). Norepinephrine infusion at randomization did not affect significantly the anti-FXa concentration: $C_{max24\ h}$ was 0.05 (IQR, 0.05–0.20, $p = 0.93$), mean difference + 0.001 [95% confidence interval, CI, −0.08, +0.008] and 0.13 (IQR, 0.05–0.28, $p = 0.174$), mean difference + 0.08 [95% CI −0.03, +0.20] in the CII and SCB groups, respectively. And 0.13 (IQR, 0.05–0.28, $p = 0.174$) in the CII and SCB groups, respectively. The effect of the baseline norepinephrine infusion on $AUC_{(0–24\ h)}$ is shown in Fig. 2.

The follow-up data are provided as Supplementary material (Supp. 1). Three PEs were diagnosed; two in the CII group and one in the SCB group. Four patients (three in the CII group and one in the SCB group) had minor bleeding. None of the adverse events were assessed to be associated with the anti-FXa concentration (data not shown). The 90-day mortality was 12.8% ($n = 5$); all deaths were judged to be independent of the study drug.

4. Discussion

We found that continuous intravenous enoxaparin infusion as thromboprophylaxis therapy resulted in lower plasma anti-FXa concentration than standard subcutaneous administration during the first 24 h. No associations between anti-FXa concentration and clinical endpoints (bleeding or thromboembolic complications) were detected. This result supports our previous findings [4].

In medical and surgical patients, the recommended peak anti-FXa concentration for thromboprophylaxis is 0.1–0.3 IU/ml [11], but there are no recommendations on adequate anti-FXa concentration in

Table 1
Baseline characteristics and laboratory values according to route of enoxaparin administration.

Patient characteristics	Continuous intravenous infusion $n = 23$	Standard subcutaneous bolus $n = 16$			p-Value
Male sex	13	56.5	14	87.5	0.076
Age, years	52	45–59	56	42–64	0.646
BMI, kg/m^2	26.8	22.9–29.5	26.5	23.9–29.6	0.940
Active cancer	2	8.7	2	12.5	1.000
Diabetes	4	17.4	2	12.5	1.000
Smoking	13	56.5	5	31.3	0.119
Alcohol abuse	11	47.8	8	50.0	0.894
Hypertension	9	39.1	6	37.5	0.918
APACHE II	16.0	14–20	17.5	11.3–25.8	0.536
Creatine clearance, ml/min	121.4	83.5–152.0	121.5	94.3–162.0	0.848
Norepinephrine	8	34.8	7	43.8	0.571
Sepsis/septic shock					
Sepsis	7	30.4	7	43.8	0.692
Septic shock	2	8.7	1	6.3	
Primary admission diagnosis					0.991
Cardiovascular	1	4.3	0	0.0	
Respiratory	10	43.5	6	37.5	
Gastrointestinal	7	30.4	6	37.5	
Neurologic	1	4.3	0	0.0	
Infection	2	8.7	2	12.5	
Trauma	1	4.3	1	6.3	
Metabolic	1	4.3	1	6.3	
LMWH prophylaxis before inclusion	11	47.8	5	31.3	0.301
Length of ICU stay before LMWH, h:mm	18:56	10:18–26:47	17:33	13:55–24:55	0.471
Mechanical ventilation	15	65.2	12	75.0	0.726
Platelet count, 10^9/l	195	153–319	142	122–192	0.135
WBC count, 10^9/l	13.4	7.6–16.5	11.6	9.9–21.3	0.848
CRP mg/l	236	140–295	262	190–393	0.848
Hct%	0.36	0.30–0.38	0.34	0.29–0.43	0.399
Bilirubin μmol/l	12	7–20	13	9–15	0.646

Data are presented as n (%) or median (interquartile range).
Abbreviations: BMI, body mass index; APACHE, Acute Physiology and Chronic Health Evaluation; VTE, venous thromboembolism; LMWH, low-molecular weight heparin; WBC, white blood cell; CRP, C-reactive protein.

Table 2
Maximum enoxaparin concentration and area under the curve by route of administration.

	Pharmacological parameters	Continuous intravenous infusion		Standard subcutaneous bolus		*p*-Value
		Median	Q_1–Q_3	Median	Q_1–Q_3	
ITT	Anti-FXa $C_{max\ 24\ h}$	0.05	0.05–0.18	0.18	0.12–0.33	0.005
	Anti-FXa $C_{max\ 72\ h}$	0.14	0.05–0.22	0.23	0.20–0.38	0.009
	$AUC_{(0–24\ h)}$	1.20	0.98–2.88	1.54	1.22–4.12	0.095
	$AUC_{(0–72\ h)}$	5.37	3.60–10.90	7.74	5.89–11.67	0.427
PP	Anti-FXa $C_{max\ 24\ h}$	0.05	0.05–0.16	0.19	0.11–0.34	0.008
	Anti-FXa $C_{max\ 72\ h}$	0.11	0.05–0.19	0.22	0.18–0.34	0.006
	$AUC_{(0–24\ h)}$	1.20	0.79–2.67	1.61	1.26–4.77	0.113
	$AUC_{(0–72\ h)}$	4.10	1.83–7.91	5.70	3.06–7.98	0.506

Abbreviations: FXa, activated Factor X; C_{max}, maximum concentration; AUC, area under the curve; ITT, intention-to-treat; PP, per-protocol.

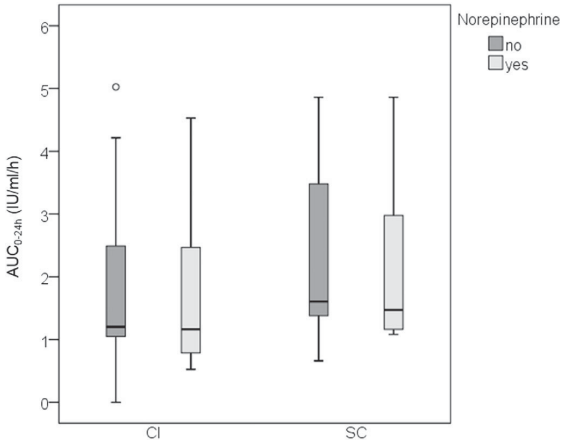

Fig. 2. The effect of the baseline norepinephrine infusion on anti-FXa $AUC_{(0–24\ h)}$.
Abbreviations: AUC, area under the curve; CI, continuous intravenous infusion; SC, subcutaneous bolus.

critically ill patients, nor is it known whether peak or trough concentration should be measured. In previous studies, ICU patients have had lower peak anti-FXa concentration than ward patients [5,6]. However, when administering enoxaparin 40 mg once daily or 30 mg twice daily subcutaneously, peak anti-FXa concentration reportedly reaches the lower target concentration of 0.1 IU/ml [7,12–19], consistent with our findings in the SCB group. In the CII group, $C_{max24\ h}$ remained < 0.1 IU/ml and the target concentration was not reached until 72 h. This could likely have been avoided by giving a loading dose.

To our knowledge this is the first RCT to have examined continuous dosing of thromboprophylactic LMWH in ICU patients. Previous studies have found that trough anti-FXa concentration tends to be low in these patients [14,17,18,20]. In this trial, anti-FXa concentration remained low in the CII group, but by the end of the study period (at 72 h) most of the patients in the CII group had a measurable anti-FXa concentration, whereas the concentration in the SCB group was comparable with those reported in previous trials. This supports our hypothesis that CII could offer more consistent anticoagulation; however, the clinical relevance of this finding is unclear. Furthermore, there is no consensus whether the laboratory target for LMWH prophylaxis should be a high peak anti-FXa concentration or a constant anti-FXa concentration.

The findings of previous trials suggest that suboptimal anti-FXa concentration in ICU patients could be explained by reduction in the bioavailability of subcutaneous LMWH, due to subcutaneous edema [7] or concomitant vasopressor therapy [6]. Moreover, the procoagulant effect of exogenous epinephrine is well established in historic trials, and there is some evidence that norepinephrine infusion (0.15–0.75 μg/kg/min) promotes platelet aggregation [21]. Johansson and colleagues have demonstrated that even though higher endogenous norepinephrine concentration was associated with increased endothelial activation and fibrinolysis, exogenous norepinephrine infusion did not further potentiate these changes in patients with septic shock [22]. Our trial did not identify any significant effect of norepinephrine infusion on anti-FXa concentration in either of the study groups. However, it must be remembered that the choice and dose of LMWH used here differed from the study design of Dörffler-Melly and colleagues, who used nadroparin 2850 IU instead of enoxaparin 40 mg [6]. In a more recent trial in which the enoxaparin dose was similar to ours, norepinephrine did not have any effect on peak or trough anti-FXa concentration [23]. Additionally, despite lacking statistical significance our findings cannot refute a clinically meaningful effect size (upper confidence interval for difference in means antiFXa 0.20) of norepinephrine in subcutaneous dosing of enoxaparin.

The clinical relevance of anti-FXa concentration to bleeding or VTE is unclear; however, in one study a low trough anti-FXa concentration increased the risk of DVT [13]. In this trial, three patients were diagnosed with PE irrespective of the anti-FXa concentration; screening ultrasound was not undertaken in these patients in line with current guidelines [24]. The therapeutic benefits of mechanical thromboprophylaxis for these thrombotic events are uncertain. Nearly all our patients had compression stockings and/or pneumatic compression devices.

Our study had some limitations. First, the PP group was smaller than planned. Nevertheless, the results of the ITT and PP groups were broadly comparable. Second, our decision to stratify groups with norepinephrine administration in two different study sites led to different number of patients in each study group. However, differing numbers in the study groups do not compromise statistical testing and we considered it important to stratify according to norepinephrine dosing due to preliminary reports suggesting marked confounding influence of norepinephrine [6]. Third, as the study period was only 72 h, it is possible that we missed the opportunity to observe enoxaparin accumulation and its consequences. Fourth, we excluded patients with acute kidney injury and obesity to avoid bias. Fift, the sample size was not designed to detect clinically relevant endpoints. Finally, we acknowledge that anti-FXa concentration is only a surrogate marker for thromboprophylaxis, and ignores the anticoagulant effect of enoxaparin on other plasma proteins (such as thrombin and platelet factor 4).

5. Conclusions

Continuous intravenous infusion of enoxaparin as thromboprophylaxis led to lower 24 h anti-FXa C_{max} compared with standard SCB administration. No difference in anti-FXa $AUC_{0–24\ h}$ was detected. Our findings could not confirm or refute a clinically relevant effect of norepinephrine infusion on antiFXa levels using subcutaneous dosing of

enoxaparin. Further studies scrutinizing enoxaparin infusion with a loading dose in critically ill patients are warranted.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.thromres.2017.08.014.

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Activation of Blood Coagulation After Aneurysmal Subarachnoid Hemorrhage: A Prospective Observational Trial of Rotational Thromboelastometry

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■ OBJECTIVE: **Aneurysmal subarachnoid hemorrhage (aSAH) has been reported to actuate blood coagulation. Rotational thromboelastometry (ROTEM) is a dynamic hemostatic test that can differentiate various coagulation abnormalities. For example, increased coagulation activity can be detected as a wider amplitude of tracing (maximal clot firmness [MCF]). ROTEM had not been used to evaluate coagulation changes after aSAH. We evaluated the ongoing coagulation process in patients with aSAH in a prospective, observational study to compare their ROTEM assay results with the control values obtained from patients undergoing clipping of nonruptured aneurysms.**

■ METHODS: **ROTEM analyses were performed at 12, 24, 48, and 72 hours after the onset of aSAH and compared with the preoperative analyses from the control group. A total of 17 patients with aSAH treated in the intensive care unit and 16 control patients were enrolled.**

■ RESULTS: **At 72 hours, EXTEM-MCF was significantly greater in patients with aSAH compared with the baseline values of the control group (68.0 mm [interquartile range (IQR), 66.0–71.0] versus 64.5 mm [IQR, 59.5–66.8]; $P = 0.024$). This was mainly due to increased fibrin formation and fibrin polymerization. The same comparison in the FIBTEM-MCF analysis yielded similar results (aSAH group, 23.0 mm [IQR, 19.0–25.0] vs. control group, 15.4 mm [IQR, 12.5–17.8], respectively; $P = 0.001$).**

■ CONCLUSIONS: **Blood coagulation is activated at 72 hours after aSAH onset, which can be detected by ROTEM EXTEM-MCF analysis. Also, the FIBTEM-MCF was elevated, implying that the relative contribution of fibrin formation and fibrin polymerization is essential.**

INTRODUCTION

The annual incidence of aneurysmal subarachnoid hemorrhage (aSAH) has been decreasing[1,2]; however, its 1-year mortality has remained at nearly 50%.[3] Among the survivors of aSAH, both early brain injury (EBI) and delayed cerebral ischemia (DCI) are major risk factors for poor neurological outcomes[4,5] and increased mortality.[6] EBI is defined as early neurological deterioration caused by transient direct toxic effects from an initial hemorrhage.[7] In contrast, DCI is delayed brain injury presenting as either clinical deterioration or cerebral infarction.[8] However, the pathophysiology of these 2 entities is not fully understood.[9,10]

Blood coagulation and fibrinolytic systems seem to activate during the acute phase of aSAH,[11] and increased coagulation can be detected within minutes after the initial hemorrhage.[12] However, the association of cerebral microthrombosis with EBI or DCI has not been unequivocally determined in the clinical setting.[9]

Key words
- Aneurysmal subarachnoid hemorrhage
- Blood coagulation
- Intensive care unit
- Neurosurgery
- Thromboelastometry

Abbreviations and Acronyms
aSAH: Aneurysmal subarachnoid hemorrhage
CFT: Clot formation time
CT: Clotting time
DCI: Delayed cerebral ischemia
DVT: Deep venous thrombosis
EBI: Early brain injury
ICU: Intensive care unit
IQR: Interquartile range
MA: Maximum amplitude
MCF: Maximal clot firmness
PE: Pulmonary embolism
ROTEM: Rotational thromboelastometry
TEG: Thromboelastography
VTE: Venous thromboembolism

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Viscoelastic point-of-care coagulation tests (e.g., rotational thromboelastometry [ROTEM]) are thought to be advantageous compared with conventional laboratory tests when analyzing the increased coagulation.[13] No studies have investigated the ongoing coagulation process after aSAH using ROTEM. Our main aim was to assess the ROTEM measurements after aSAH and analyze the role of platelets and fibrinogen on clot formation. We also examined the association of ROTEM assay results with clinical events such as EBI and DCI.

METHODS

The present prospective, observational clinical study was conducted in the intensive care unit (ICU) and neurosurgical department of Tampere University Hospital (Tampere, Finland) from October 2015 to June 2016. The trial was conducted in accordance with the amended Declaration of Helsinki. The local ethics committee of Pirkanmaa approved the study design (approval no. 230215-1), and the study was registered in the ClinicalTrials.gov database (ClinicalTrials.gov identifier, NCT02540005). All the patients or their next of kin provided written informed consent before study enrollment.

Study Subjects

Consecutive patients with acute aSAH who were admitted to the ICU within 12 hours of the onset of aSAH symptoms (defined as a sudden severe headache or loss of consciousness) and expected to stay in the ICU for ≥72 hours, were considered eligible. Subarachnoid bleeding was diagnosed from the non–contrast-enhanced computed tomography scan findings of the brain. A ruptured aneurysm as a source of hemorrhage was confirmed by either computed tomography angiography or digital subtraction angiography. The exclusion criteria were age <18 years, pregnancy, anticoagulant medication in regular use, and known active cancer. Only acetylsalicylic acid (<150 mg daily) was allowed as an antithrombotic medication. Patients undergoing elective nonruptured intracranial aneurysm clipping were chosen as the control group because they had the same disease entity and thus offered the closest surrogate values for the bleeding state before the occurrence of aSAH. The control group also served as a local reference group for ROTEM.

Clinical Management

All patients with aSAH received neurointensive care in accordance with international guidelines.[14,15] The therapy included thromboprophylaxis therapy (tinzaparin, 4500 IU subcutaneously, once daily) after occlusion of the ruptured aneurysm by either endovascular coiling or surgical clipping.[16] After tinzaparin was started, no mechanical thromboprophylaxis was used.[17] All control patients were treated in accordance with perioperative protocols.

In addition to our standard neurointensive care, bilateral compression ultrasonography of the lower extremity veins was performed to exclude asymptomatic deep venous thrombosis (DVT) by the radiologist once within days 3–5. When necessary, computed tomography pulmonary angiography was performed to rule out pulmonary embolism (PE).

Blood Sampling

Blood samples for ROTEM analysis were retrieved from patients with aSAH at 12, 24, 48, and 72 hours after the onset of aSAH symptoms and compared with the preoperative samples of the control group (i.e., baseline). Complete blood, platelet, and leukocyte counts, serum C-reactive protein, and international normalized ratio concentrations were measured daily during the study period. All blood samples from the aSAH and control groups were taken from a heparin-naive arterial line.

Thromboelastometry

All ROTEM assays were performed in the central laboratory of Tampere University Hospital using a ROTEM delta analysis system (TEM Innovations GmbH, Munich, Germany). The following parameters were measured: clotting time (CT; which represents the interval to initiation of clot formation), clot formation time (CFT, which represents stabilization of the clot), and maximum clot firmness (MCF, which represents the maximum clot strength). Each analysis was performed using single-use reagents. The EXTEM (tissue factor activated, citrated, and recalcified) analysis measures coagulation activated by the extrinsic pathway. The FIBTEM (tissue factor plus platelet inhibitor cytochalasin D-activated, citrated, and recalcified) analysis measures formation of fibrin-based clots after platelet inhibition by cytochalasin D to block the function of glycoprotein IIb/IIIa receptor. The INTEM (contact-activated, citrated, and recalcified) analysis measures clot formation via the contact phase. In general, a hypercoagulable state can be detected if the MCF is elevated.[18] Using different reagents, the effect of platelets and fibrinogen (EXTEM-MCF) and fibrin formation and its polymerization (FIBTEM-MCF) on clots can be distinguished.

Our primary outcome was the EXTEM-MCF value in the aSAH group compared with the baseline value from the control group. The secondary outcomes were other ROTEM parameters (i.e., EXTEM-CT, EXTEM-CFT, FIBTEM-MCF, INTEM-MCF, INTEM-CT, and INTEM-CFT) compared with the baseline values from the control group. To identify the platelet contribution to clot strength, the difference between EXTEM-MCF and FIBTEM-MCF was calculated.

Clinical and Outcome Measures

The clinical severity of aSAH at admission was reviewed retrospectively using the Hunt–Hess grade, from which the EBI severity was classified as severe (Hunt–Hess score, 4–5) or mild (Hunt–Hess score, 1–3).[19] The severity of bleeding was evaluated from the primary head computed tomography scan using the Fisher scale[20] and defined as moderate to severe if the scale score was ≥3. Moreover, DCI was evaluated retrospectively from the ICU database (Centricity Critical Care Clinisoft; GE Healthcare, Barrington, Illinois, USA) at 24 hours to 14 days from the onset of aSAH symptoms using the criteria presented by Vergouwen et al.[8] In brief, DCI was defined as neurological deterioration (a reduction in the Glasgow coma scale score by ≥2 points) for ≥1 hour, a new neurological symptom for ≥1 hour that could not be explained by other features, or a new ischemic episode on neuroimaging data that was not related to the primary aSAH or neurosurgery.

Table 1. Baseline Patient Characteristics

Characteristic	aSAH Group (n = 17)	Control Group (n = 16)	P Value
BMI (kg/m^2)	30 (25–33)	28 (25–31)	0.363
Age (years)	49 (40–60)	62 (56–67)	0.037
Male sex	6 (35.3)	7 (43.8)	0.728
Smoking	10 (58.8)	6 (37.5)	0.003
Alcohol abuse	2 (12.5)	0 (0)	0.227
HTN	7 (41.2)	11 (68.8)	0.166
Diabetes	0 (0)	3 (18.8)	0.103
Cancer in remission	1 (5.9)	1 (6.3)	1.000
Low-dose aspirin	1 (5.9)	5 (31.3)	0.085
Aneurysm location			
ACA	6 (35.3)	0 (0)	
BA	2 (11.8)	0 (0)	
ICA	4 (23.5)	0 (0)	
MCA	4 (23.5)	15 (93.8)	
PCA	1 (5.9)	0 (0)	
PA	0 (0)	1 (6.3)	

Data presented as median (quartile 1 to quartile 3) or n (%).
aSAH, aneurysmal subarachnoid hemorrhage; BMI, body mass index; HTA, hypertension; ACA, anterior communicating artery; BA, basilar artery; ICA, internal carotid artery; MCA, middle cerebral artery; PCA, posterior cerebral artery; PA, pericallosal artery.

Clinically significant events representing the hypercoagulable state were evaluated (e.g., venous thromboembolism [VTE], including DVT and PE). The extended Glasgow outcome scale score,[21] including mortality, was registered on day 90.

Statistical Analysis

Statistical analyses were performed using the SPSS statistical software program, version 23.0, released 2015 (IBM Corp., Armonk, New York, USA). Depending on the distribution of the variables, comparisons between the continuous variables were performed using either the Mann-Whitney U test or Student t test. For categorical variables, univariate analysis with Fisher's exact test was performed.

Using standard sample size calculations, ≥16 patients in each group were needed to detect a clinically significant increase in MCF, from 65 to 70 mm (standard deviation, 5) using the EXTEM S reagent, assuming a power of 80% and a significance level of 5%.

RESULTS

We enrolled 17 patients with aSAH and 16 control patients. The groups did not differ in sex, comorbidities, or body mass index. However, the proportion of smokers was greater and the patients were younger in the aSAH group (Table 1). Moreover, the ruptured aneurysm was more commonly located in the anterior communicating artery in the aSAH group. In contrast, most aneurysms in the control group were located in the middle cerebral artery (Table 1). In most of the patients with aSAH, bleeding was classified as moderate to severe, and the aneurysm was repaired by endovascular coiling in 13 of the patients (76.5%; Table 2). On day 90, nearly 60% of the patients with aSAH showed good neurological recovery; the mortality was 5.9%. More detailed demographic information on the aSAH patient group is presented in Table 2.

Table 2. Characteristics of Patients with Aneurysmal Subarachnoid Hemorrhage

Parameter	Value
Hunt–Hess score	2.4 ± 1
1	2 (11.8)
2	10 (58.8)
3	1 (5.9)
4	4 (23.5)
Fisher scale score	3 ± 0.9
1	0 (0)
2	6 (35.3)
3	5 (29.4)
4	6 (35.3)
Treatment	
Clipping	4 (23.5)
Coiling	13 (76.5)
TXA	1 (5.9)
LMWH during ICU	15 (88.2)
GOSe score at 90 days	6.7 ±1.9
Death	1 (5.9)
Vegetative state	0 (0)
Lower severe disability	0 (0)
Upper severe disability	0 (0)
Lower moderate disability	1 (5.9)
Upper moderate disability	3 (17.6)
Lower good recovery	3 (17.6)
Upper good recovery	7 (41.2)

Data presented as mean ± standard deviation or n (%).
TXA, tranexamic acid; LMWH, low-molecular weight heparin; GOSe, Glasgow outcome scale, extended.

At 72 hours, the EXTEM-MCF was significantly greater in the patients with aSAH compared with the baseline value from the control group (68.0 mm [interquartile range (IQR), 66.0–71.0] versus 64.5 mm [IQR, 59.5–66.8], respectively; P = 0.024). FIBTEM-MCF was also significantly increased at 72 hours compared with the baseline value from the control group (23.0 mm [IQR, 19.0–25.0] vs. 15.4 mm [IQR, 12.5–17.8], respectively; P = 0.001). The difference between EXTEM-MCF and

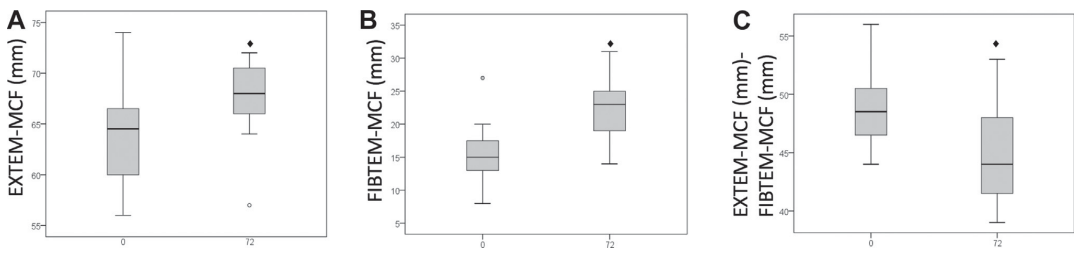

Figure 1. Change in rotational thromboelastometry (ROTEM) analysis from baseline value of the control group to 72 hours after the onset of aneurysmal subarachnoid hemorrhage symptoms. (**A**) EXTEM-maximal clot firmness (MCF): baseline median, 64.5 mm (interquartile range [IQR], 59.5–66.8); at 72 hours, 68.0 mm (IQR, 66.0–71.0; $P = 0.024$). (**B**) FIBTEM-MCF: baseline median, 15.0 mm (IQR, 12.5–17.8); at 72 hours, 23.0 mm (IQR, 19.0–25.0; $P = 0.001$). (**C**) Difference between EXTEM-MCF and FIBTEM-MCF represents the effect of platelets on clot formation Baseline median, 48.5 mm (IQR, 46.3–50.8); at 72 hours, 44.0 mm (IQR, 41.0–48.0; $P = 0.027$). Black diamonds indicate statistical significance ($P < 0.05$).

FIBTEM-MCF represents the platelet contribution to clot formation. This decreased significantly during the first 72 hours (from 48.5 mm [IQR, 46.3–50.8] to 44.0 mm [IQR, 41.0–48.0]; P = 0.027) compared with the baseline values from the control group (Figure 1). The absolute platelet concentration remained unchanged (221 ± 66 10^9/L at 72 hours vs. 244 ± 88 10^9/L at baseline for the control group; P = 0.426). Compared with the aSAH value at 72 hours, the EXTEM-CFT had decreased significantly from the baseline value for the control group (96.5 seconds [IQR, 81.3–120.5] vs. 74.0 seconds [IQR, 65.0–89.0]; P = 0.015). No differences in INTEM-MCF, EXTEM-CT, INTEM-CT, or INTEM-CFT were observed at 72 hours compared with the baseline values from the control group (Table 3). Other time comparisons (12, 24, and 48 hours after the onset of aSAH symptoms) with the baseline values of the control group are shown in Table 3.

Furthermore, DCI was observed in 7 of the 17 patients with aSAH (41%). At 72 hours, the FIBTEM-MCF was significantly greater in the patients who developed DCI compared with those who did not (25.0 mm [IQR, 24.8–26.8] vs. 19.0 mm [IQR, 16.5–22.5]; P = 0.012; Figure 2). No differences were detected in EXTEM-MCF (68.5 mm [IQR, 66.8–69.8] vs. 67.0 mm [IQR, 64.5–71.5]; P = 0.698) or INTEM-MCF (66.0 mm [IQR, 36.0–70.5] vs. 68.0 mm [IQR, 65.5–71.5]; P = 0.606). Four patients had severe EBI. In these patients, FIBTEM-MCF was significantly greater at 72 hours compared with those patients with mild EBI (26.0 mm [IQR, 25.0–26.0] vs. 22.0 mm [IQR, 18.3–24.8]; P = 0.031; Figure 2). EXTEM-MCF was unchanged at 72 hours (data not shown).

Two DVTs were detected in patients with aSAH, and both patients also developed a PE. In these patients, EXTEM-MCF was not greater compared with that of the other patients with aSAH at 72 hours (61.5 mm [IQR, 57.0–61.5] vs. 69.0 mm [IQR, 66.5–71.5]; P = 0.076). No thromboembolic complications were observed in the control group.

DISCUSSION

The present clinical, observational trial examined the ongoing coagulation process after aSAH using ROTEM analysis. We observed that at 72 hours after the onset of aSAH, the strength of the formed blood clot had increased, as shown by the greater EXTEM-MCF and FIBTEM-MCF values. Greater FIBTEM-MCF levels were associated with the incidence of DCI and EBI. To the best of our knowledge, our study is the first to use ROTEM to examine the changes in coagulation after aSAH.

In the present study, EXTEM-MCF was increased at 72 hours after the onset of aSAH, suggesting overall coagulability was increased. In previous trials, the hemostatic changes after aSAH were investigated by another viscoelastic point-of-care coagulation test, such as thromboelastography (TEG), in which the overall coagulation state is evaluated by the maximum amplitude (MA), which is analogous to the MCF value in ROTEM.[22,23] The results of these studies are consistent with ours for patients with moderate to severe aSAH, with onset of the hypercoagulable state observed at 3 days after bleeding, with the greatest MA levels found on day 10.[24] When the monitoring period was shorter than 72 hours, no change in the MA value was observed in the overall aSAH population.[22,23] Interpretation of these previous results is difficult,[22,23] because the exact timing of the blood samples is not known. Moreover, because blood coagulation has been activated by different reagents in previous trials, a direct comparison between results would be challenging. We chose to use the extrinsic pathway because it most accurately mimics rupture of an aneurysm and the release of tissue factors to initiate blood coagulation. However, these data suggest that the hypercoagulation state develops gradually after aSAH and can be detected by viscoelastic point-of-care coagulation test 3 days after the bleeding event.

We noted that at 72 hours after the onset of aSAH bleeding, the FIBTEM-MCF levels had significantly increased. This implies that fibrin formation and polymerization exert a major contribution on clot strength. To the best of our knowledge, no previous trials have investigated fibrin function after aSAH. In general, FIBTEM-MCF is a surrogate marker for plasma fibrinogen concentration, and they are both known to increase the recovery phase of many acute illnesses (e.g., severe sepsis).[25] When measured at the early phase of aSAH, the fibrinogen concentration will reportedly be within normal limits[11,23,26-29] or slightly elevated.[24] However, we have not found any trials in which the change in fibrinogen concentration has been studied. It is known that higher values

Table 3. Laboratory Results and Rotational Thromboelastometry Assays

Parameter	Reference Range	Baseline of Control Group		From Onset of aSAH (hours)							
				12		24		48		72	
		Median	Q_1-Q_3	Median	Q_1-Q_3	Median	Q_1-Q_3	Median	Q_1-Q_3	Median	Q_1-Q_3
EXTEM											
MCF (mm)	50–72	64.50	59.5–66.8	64.0	62.0–69.5	68.0	63.0–70.0	66.0	65.5–69.0	68.0*	66.0–71.0
CT (seconds)	38–79	48	45–58	53	48–57	54	45–61	51	47–62	52	45–61
CFT (seconds)	34–159	96.50	81.3–120.5	100.0	71.5–109.5	101.0	68.5–111.5	86.0†	69.0–92.0	74.0‡	65.0–89.0
INTEM											
MCF (mm)	50–72	65.50	61.3–68.0	65.0	62.5–71.5	68.0	64.0–71.5	67.0	65.5–70.0	67.5	65.8–71.3
CT (seconds)	100–240	158	149–170	142	129–167	145	129–167	149	138–167	149	143–159
CFT (seconds)	30–110	66.00	58.5–78.5	72.0	51.5–82.5	65.0	51.0–74.5	61.0	52.5–79.0	59.0	50.5–66.8
FIBTEM											
MCF (mm)	9–25	15.40	12.5–17.8	15.00	13.5–21.5	16.00	14.5–22.5	19.0§	16.5–23.0	23.0¶	19.0–25.0
Platelet count (10^9/L)	150–360	244 ± 88	NA	NA	NA	240 ± 50	NA	217 ± 66	NA	221 ± 66	NA
Leukocyte count (10^9/L)	3.3–8.2	7.2 ± 1.6	NA	NA	NA	13.7 ± 3.9	NA	13.5 ± 3.9	NA	12.3 ± 3.5	NA
Hemoglobin (g/L)	134–167	145 ± 13	NA	NA	NA	132 ± 15.2‖	NA	124 ± 12.5#	NA	127 ± 12.6**	NA
CRP (mg/L)	<10	23.7	13.0–36.8	NA	NA	8.3	3.7–13.2	19.5	9.2–45.5	34.0	6.2–88.5

Data presented as median and first to third quartile or range and mean ± standard deviation.
All statistical comparisons were between baseline values of control group and different evaluation points for aSAH group.
aSAH, aneurysmal subarachnoid hemorrhage; Q_1, first quartile; Q_3, third quartile; MCF, maximum clot firmness; CT, clotting time; CFT, clot formation time; NA, not applicable; CRP; C-reactive protein.
*$P = 0.024$.
†$P = 0.023$.
‡$P = 0.015$.
§$P = 0.004$.
¶$P = 0.001$.
‖$P = 0.010$.
#$P < 0.001$.
**$P < 0.001$.

of the fibrin degradation product, D-dimer, after aSAH are associated with poor neurological outcomes.[30] This is in accordance with our results, because a greater D-dimer value implies that fibrin formation is increased, as does a higher FIBTEM-MCF. Additional studies are needed to confirm these results and determine whether FIBTEM-MCF continues to increase after 3 days.

We noticed that the absolute and relative differences between EXTEM-MCF and FIBTEM-MCF both decreased after 72 hours and the absolute platelet concentration remained unchanged. This shows that the functional effect of platelets on the formation of clot strength declines and outlines the functional effect of fibrin. In previous TEG trials, the clot strength was solely evaluated by the MA, and thickening of the clot was concluded to reflect activation of platelets only.[22,24] In a recent trial, activation and aggregation of platelets was observed after aSAH.[31] Nevertheless, it is known that both platelet activation and fibrin formation and crosslinking are needed for clot formation. The relative contribution of platelets and fibrin to clot strength in patients with aSAH is unknown. In healthy individuals, fibrinogen accounts for 25% of clot strength, and in trauma patients, the contribution increases ≤44% after 72 hours of insult.[32,33] From our results, it appears that the relative contribution of fibrinogen also increases after aSAH, from 23.5% to 33.3% at 72 hours after the onset of aSAH.

We found significantly greater FIBTEM-MCF at 72 hours in patients who had severe EBI or further developed DCI. We did not observe any association between DCI and EXTEM-MCF, although the absolute increase in EXTEM-MCF was statistically significant. To the best of our knowledge, no trial has investigated this previously. Fujii et al.[26] found that greater fibrinogen levels predicted the incidence of DCI at 6 days after aSAH; however, the baseline fibrinogen concentration has not shown an association with poor neurological outcomes.[34] From the results of previous TEG trials, the association of MA levels with EBI or DCI has been

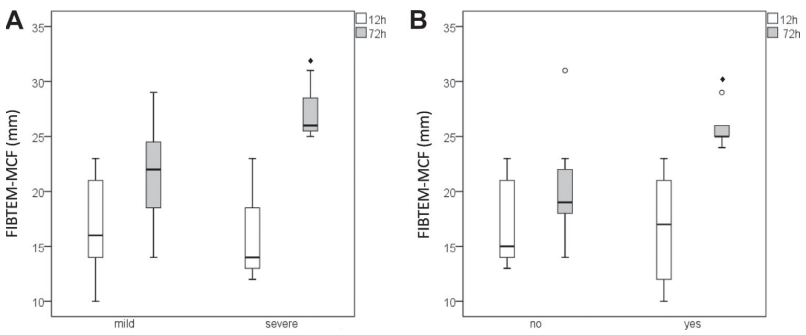

Figure 2. Association with fibrin formation and polymerization (FIBTEM-MCF) during 72 hours after onset of aneurysmal subarachnoid hemorrhage symptoms with the incidence of early brain injury (EBI) and delayed cerebral ischemia (DCI). (**A**) In patients with severe EBI ($n = 4$), FIBTEM-MCF was significantly greater at 72 hours compared with that in patients with mild EBI (26.0 mm, [interquartile range (IQR), 25.0–26.0] versus 22.0 mm [IQR, 18.3–24.8]; $P = 0.031$). (**B**) In patients with DCI ($n = 7$), FIBTEM-MCF at 72 hours was 25.0 mm (IQR, 24.8–26.8) compared with 19.0 mm (IQR 16.5–22.5) for patients without DCI ($n = 10$; $P = 0.12$). Diamonds indicate statistical significance ($P < 0.05$).

inconsistent. Some studies have reported that greater MA levels increase the likelihood of severe EBI and the risk of developing DCI.[22] In contrast, others did not observe any association with DCI, although hypercoagulability was associated with poor neurological outcomes.[24]

These inconsistent results might be due to the widely varied definitions used for DCI.[8] Moreover, the pathophysiology of DCI is complex and not completely understood. It is known that aneurysm rupture causes platelet activation, which results in thrombin generation, additional fibrin cleavage from fibrinogen, and formation of cerebral microthrombi. Ultimately, this might contribute to the pathophysiology of DCI.[35] From our results, it seems that increasing fibrin formation and polymerization might play a role in the pathophysiology of DCI. In clinical setting, antiplatelet therapy has failed to prevent this increased fibrin formation or reduce the incidence of DCI or mortality after aSAH.[36] Nonetheless, a recent retrospective trial showed promising results when dual antiplatelet therapy was used.[35]

The overall incidence of VTE in our study was high: 11.8% for both DVT and PE. In previous trials, the incidence of DVT among patients with aSAH varied from 2% to 24%, depending on whether a screening method was used.[37,38] Although weak evidence exists that the earlier onset of clot formation might increase the risk of DVT,[23] in our present trial, we did not observe shortened EXTEM-CT or elevated EXTEM-MCF among the 2 patients with VTE. Moreover, EXTEM-CT and INTEM-CT remained unchanged during the study period for the whole aSAH group, indicating that initiation of clotting and thrombin formation and the start of fibrin polymerization were unaffected. In general, the predictive value of TEG for VTE diagnostics is highly inconsistent.[39] Thus, a much larger trial is required to show any association between the changes in coagulation factors and the incidence of VTE after aSAH. Altogether, this supports the current practice to start pharmacological thromboprophylaxis as early as is safe.[15]

The present study had limitations. First, we had a 12-hour delay from the onset of initial bleeding to the first ROTEM measurement. However, this delay would be inevitable when performing clinical research on this patient population. Second, we monitored ROTEM measurements for only 72 hours. In previous TEG trials, coagulation continued to increase from day 3 to day 10, with a clear hypercoagulability state identified on day 5.[24] Third, although neurosurgical patients undergoing elective aneurysmal clipping represent the same patient population, the patients with aSAH were younger and the proportion of smokers was higher. This is unsurprising, because smoking is known to be 1 of the major risk factors for aneurysm rupture.[40] However, it is not known whether smoking would affect the ROTEM results. Fourth, a decrease in the hemoglobin level after aSAH might have paradoxically increased the measured EXTEM-MCF. However, the magnitude of this would have most likely been minimal.[41] Also, the precision of FIBTEM-MCF measurement is known to be better in anemic patients.[42] Fifth, our sample size was underpowered for some clinical endpoints (e.g., DCI, EBI, and VTE). Thus, these results must be interpreted with caution. Furthermore, owing to small sample size, we were unable to perform multivariate testing on other clinically relevant confounders (e.g., Hunt–Hess or Fisher score), which might have influence on the incidence of EBI and DCI. Finally, with the present trial design, we were unable to differentiate the effects of the different operative interventions on blood coagulation.

CONCLUSIONS

Our study has shown that blood coagulation appears to increase at 72 hours after the onset of aSAH, and for the first time, this change can be detected by ROTEM EXTEM-MCF analysis. At the same time, the FIBTEM-MCF was also elevated, suggesting that

the relative contributions of fibrin formation and fibrin polymerization to clot strength are essential. Furthermore, FIBTEM-MCF was greater in patients with DCI and EBI. Thus, it seems that formation and polymerization of fibrin might influence the pathophysiology of DCI and EBI. Further clinical trials are warranted to confirm these results.

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