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TREATMENT OF COAGULOPATHY IN PATIENTS WITH CRITICAL COVID-19

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Treatment of Coagulopathy in Patients with Critical COVID-19
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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This thesis is dedicated to my dear family, friends, and co-workers for putting up with my COVID-19 obsession.

POPULAR SCIENCE SUMMARY OF THE THESIS

Since December 2019, the viral disease COVID-19 has spread across the globe and caused an estimated 15 million deaths. In Sweden, 10,300 patients have become critically ill from COVID-19 and required intensive care. The reason for admission to intensive care was often respiratory failure, but also the serious complications of blood clots have been an area of particular concern. During the first wave, from February until July 2020, as many as 27.9% of patients treated in the ICU had complications of blood clots, most of them in the lungs.

Even before the COVID-19 pandemic, we knew that critically ill patients were at risk of blood clots. To combat the risk of undesirable blood clotting, blood-thinning medication is given to patients treated in intensive care. The recommended treatment is a low dose of a blood-thinner, so-called low-molecular-weight heparin. The same low dose of low-molecular-weight heparin was initially given to patients with critical COVID-19. However, it did not seem to be enough, as so many of them still developed blood clots. The purpose of this thesis is to understand if we can modify our prophylactic blood clot regime for patients with critical COVID-19, so that we can avoid blood clots also for them.

In most intensive care units worldwide, the doses of low-molecular-weight heparin were increased for COVID-19 patients to meet the higher risk of blood clots. With higher doses of low-molecular-weight heparin, there is an increased risk of adverse effects, especially bleeding. Therefore, we decided to study the consequences of the change in prophylactic dose of low-molecular-weight heparin. In our first study, we found that patients who received high doses of low-molecular-weight heparin survived to a greater extent than patients who received low doses. In this study, the results suggested that a high dose was also superior to an intermediate dose, albeit the difference was not statistically significant. At this time, a low dose was no longer used. Therefore, we collected data on more patients to compare the survival rate between patients who had received an intermediate dose or high dose. In this follow-up study, we could not find a difference in survival. In conclusion of both studies, we found a significant increase in survival among patients treated with a high dose compared to a low dose, but there was no difference for patients treated with high dose compared to intermediate dose of low-molecular-weight heparin.

Mid 2020, it was discovered that treatment with anti-inflammatory drugs, glucocorticoids, reduced mortality. The treatment effect was the greatest for patients requiring intensive care. Since it is established that inflammation can activate blood clot formation, we wanted to investigate whether glucocorticoids also reduced the risk of blood clots. In a large multi-center study with patients from several countries, patients were randomized to 12 or 6 mg daily of the glucocorticoid dexamethasone in order to compare survival. We used data from this study and collected supplementary data regarding blood clots and bleeding. In our analyses, we could not demonstrate any difference in the incidence of blood clots or bleeding between patients treated with 12 or 6 mg of dexamethasone daily.

The effect of a drug varies for different patients and may even vary for the same patient during different stages of a disease. This may be the reason why no trial has found a dosing regimen of low-molecular-weight heparin superior to any other. Factors that can influence include

weight, sex, how the drug is absorbed by the body, the presence of different cells and proteins that can bind the drug, and the metabolization and excretion, which largely depend on kidney function. Therefore, in our fourth study, we sought to investigate not the dose, but the effect of the given dose of low-molecular-weight heparin in patients. There is already a blood test, anti-Factor Xa, which measures low-molecular-weight heparin's ability to inhibit a protein, Xa, vital for the blood clotting process. However, it has previously been difficult to establish whether measured anti-Factor Xa can be correlated with a clinical response. We found that the patients with anti-Factor Xa samples indicating low activity of low-molecular-weight heparin had a higher risk of suffering a blood clot. We also found that patients with anti-Factor Xa samples indicating a high activity had an increased risk of death and bleeding. Our results suggest that measurement of anti-Factor Xa could be used to adjust dosage of low-molecular-weight heparin in the individual patient and thereby avoid both undertreatment, with an increased risk of blood clots, and overtreatment, with an increased risk of bleeding.

In summary, through our studies, we have gained new and improved knowledge about patients with critical COVID-19 by finding associations between different doses of blood-thinning treatment and survival during the first wave of the pandemic, by finding that higher, as opposed to lower, doses of anti-inflammatory medication did not change the risk of blood clots and that anti-Factor Xa may be a potential way to guide dosing of blood thinners in the individual patient, and thereby avoid blood clots and bleeding.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Sedan december 2019 har virussjukdomen COVID-19 spridit sig över världen och uppskattningsvis har cirka 15 miljoner dött. I Sverige har 10 300 blivit så svårt sjuka av COVID-19 att de har behövt intensivvård. Ofta har det varit lungsvikt som lett till att en patient behövt intensivvårdas, men sjukdomsförloppet har i många fall komplicerats av blodproppar. Av de patienter som intensivvårdades våren 2020 var den uppskattade risken att drabbas av blodpropp 27,9%.

Redan innan COVID-19-pandemin visste vi att svårt sjuka patienter har ökad risk för blodpropp. För att förhindra bildandet av blodproppar ges blodförtunnande läkemedel till i princip alla intensivvårdade patienter. Den rekommenderade blodförtunnande behandlingen är en låg dos av lågmolekylärt heparin. Samma låga dos av lågmolekylärt heparin gavs initialt till svårt sjuka COVID-19-patienter, men behandlingen verkade inte vara tillräckliga eftersom den önskade effekten uteblev då patienterna ändå fick blodproppar. Syftet med denna avhandling har varit att undersöka om det är möjligt att förändra den blodproppsförebyggande behandlingen, för att minska risken för blodproppar även hos COVID-19-patienter.

Runt om i världen höjdes doserna av lågmolekylärt heparin för att motverka den ökade risken för blodproppar. Men med högre doser lågmolekylärt heparin följer ökad risk för biverkningar, framför allt blödningar. Vi beslutade därför att studera den förändrade behandlingens konsekvenser. I vår första studie fann vi att patienterna som vi hade behandlat med hög dos lågmolekylärt heparin överlevde oftare än patienter som fått låg dos lågmolekylärt heparin. Det verkade också som att en hög dos var effektivare än en mellandos, men skillnaden var inte statistiskt säkerställd. Vi gick därför vidare med att samla in data från fler patienter för att jämföra skillnaden i överlevnad mellan patienter som fått mellandos och hög dos. I denna uppföljande studie kunde vi inte hitta någon skillnad. Slutsatsen i de två studierna blev att det under pandemins första våg gick bättre för de som blev behandlade med hög dos lågmolekylärt heparin jämfört med de som behandlats med låg dos men ingen skillnad kunde hittas mellan hög dos och mellandos.

Under sommaren 2020 upptäcktes att behandling med antiinflammatorisk medicin, 6 mg dagligen av glukokortikoiden dexametason, kunde minska dödligheten. Störst effekt hade behandlingen för patienter som intensivvårdades. Eftersom inflammation kan aktivera blodlevring så ville vi undersöka om glukokortikoider också minskar risken för blodpropp. I en stor internationell studie, där Södersjukhuset ingick, så lottades patienter till behandling med 12 eller 6 mg dexametason dagligen för att undersöka om den högre dosen kunde minska dödlighet ytterligare. Inom ramen för denna studie kunde vi också undersöka om det var skillnad avseende blodproppar och blödningar. Vår antagande var helt enkelt att om glukokortikoider minskar risk för blodpropp så kanske en högre dos minskade risken mer än en lägre dos. Studien genomfördes genom att komplettera data från huvudstudien med data avseende blodproppar och blödningar. När vi analyserade kunde vi inte påvisa någon säkerställd skillnad i frekvens av blodproppar mellan 12 eller 6 mg dexametason dagligen.

Vilken effekt ett läkemedel har skiljer sig mellan patienter och kan även variera för samma patient i olika stadier av en sjukdom. Faktorer som kan påverka effekterna av en behandling är

t ex vikt, kön samt hur läkemedlet kan tas upp av kroppen och sedan bryts ner och utsöndras. Även vilka celler och proteiner som läkemedlet binder kan skilja sig och det avgör hur stor det av läkemedlet som är funktionellt. Vårt antagande inför den fjärde studien var därför att det inte var dosen av blodförtunnande medicin som avgjorde hur det gick för patienterna utan vilken effekt de fick av den. Det finns redan ett blodprov, anti-Faktor Xa, som mäter hur hämmat ett protein i blodlevringsprocessen blir av blodförtunnande behandling med lågmolekylärt heparin. Dock har det varit svårt att få verifierat om resultatet av provet stämmer med det kliniska svaret hos patienten. Vi fann att de patienter med låga anti-Faktor Xa-prover som indikerade låg effekt av blodförtunning, hade en ökad risk att drabbas av blodpropp och att patienterna med höga anti-Faktor Xa-prover, som indikerade en hög effekt av blodförtunning, hade en ökad risk för att blöda eller dö. Våra resultat antyder att det här provet skulle kunna användas för att anpassa den blodförtunnande behandlingen med lågmolekylärt heparin till enskilda patienter för att undvika såväl underbehandling, som innebär risk för blodpropp, och överbehandling, vilket ger risk för blödningar.

Sammanfattningsvis har vi genom våra studier fått ny och förbättrad kunskap om patienter med intensivvårdskrävande COVID-19 genom att hitta associationer mellan olika doser blodförtunnande behandling och överlevnad under pandemins första våg, att högre jämfört med lägre doser glukokortikoider inte förändrade risken för blodpropp och att det finns potentiella möjligheter att styra blodförtunningsdosen för att undvika blodproppar och blödningar hos den individuella patienten.

ABSTRACT

Introduction: Thromboembolic complications affect a large proportion of patients with critical COVID-19, and it may be associated with an increased risk of death. It has been hypothesized that both the virus of SARS-CoV-2 itself and the inflammation caused by the infection puts patients in a pro-coagulative state. To reduce the risk of thromboembolism, low-molecular weight heparins are recommended as thromboprophylaxis for all patients in intensive care, including patients with critical COVID-19. The overall aim of this thesis was to investigate treatment of coagulopathy in patients with critical COVID-19. Specifically, studies I and II aimed to investigate the association with outcomes by different dosing of low-molecular-weight heparins, study III aimed to study the outcome depending on the dosage of glucocorticoids, and study IV explored outcomes associated with the monitored effect of low-molecular-weight heparins by anti-Factor Xa measurements. The overall goal was to find ways to mitigate the risk of death and thromboembolism in patients with critical COVID-19 without increasing their risk of bleeding.

Methods: Studies I, II, and IV were retrospective observational cohort studies, whereas study III was a *post hoc* analysis of an international, randomized, blinded trial. In all our studies, we included adult patients with critical COVID-19, defined as patients with polymerase chain reaction positive severe acute respiratory syndrome coronavirus 2, requiring intensive care due to respiratory failure. Patients were excluded if they already had the outcomes of thromboembolism or major bleeding at ICU admission. The four studies investigated patients during different time periods: studies I and II during the first wave, study III during the second wave, and study IV during both the first and second waves. The exposures in the first two studies were different doses of low-molecular-weight heparin: a low, intermediate, and high dose in study I, and an intermediate and high dose in study II. In study III, 12 versus 6 mg dexamethasone daily was investigated and in study IV, the activity of low-molecular-weight heparin by anti-Factor Xa was the exposure. Death, thromboembolism, and bleeding were the outcomes in all studies. To analyze the primary outcome, Cox regression was used in studies I, II and III, and logistic regression was used in studies III and IV. Multivariable models were used to adjust for pre-defined baseline variables with the potential to affect the outcome in studies I and II, for stratifying variables in study III, and for one potential confounder and interaction in study IV.

Results: In study I, high dose low-molecular-weight heparin was associated with a significant reduction of death at 28 days compared to low dose: adjusted hazard ratio 0.33 (95% CI 0.13 to 0.87). There was also a lower incidence of thromboembolism for patients treated with high (2.7%) versus intermediate (18.8%) and low dose low-molecular-weight heparin (17.9%) ($p = 0.04$) but no difference in the risk of bleeding ($p = 0.16$). When focusing on intermediate vs high dose low-molecular-weight heparin in study II, we found no differences in the risk of death at 90 days, thromboembolism or bleeding at 28 days, with hazard ratios of 0.74 (95% CI 0.36 to 1.53), 0.93 (95% CI 0.37 to 2.29), and 0.84 (95% CI 0.28 to 2.54), respectively. In study III, the incidence of the composite outcome death and thromboembolism during the ICU stay did not differ for patients randomized to 12 or 6 mg dexamethasone, odds ratio 0.93 (95% CI 0.58 to 1.49), nor were there any significant differences for the secondary outcomes of thromboembolism, major bleeding, or any bleeding complications. In study IV, when

analyzing anti-Factor Xa as a continuous variable in a spline model, associations were found between (1) lower peak anti-Factor Xa values and increased risk for thromboembolism and (2) higher trough anti-Factor Xa values and an increased risk of death and bleeding. When cut-off values of peak were investigated, patients with any value below 0.3 kIU/L had an associated odds ratio of thromboembolism of 5.1 (95% CI 1.8 to 14.4) compared to patients no values below 0.3 kIU/L. Trough values above 0.3 kIU/L were associated with an odds ratio of bleeding of 1.9 (95% CI 1.1 to 3.3), and trough values above 0.5 kIU/L were associated with an odds ratio of 2.4 (95% CI 1.0 to 5.6) of major bleeding, compared to patients with no values above these levels.

Conclusion: In the early days of the pandemic, we found that a high dose of low-molecular-weight heparin for thromboprophylaxis was associated with lower mortality compared to low dose. The results also suggested a benefit with high dose compared to intermediate dose, but no such association was found when including more patients and comparing only thromboprophylaxis with intermediate vs high dose low-molecular-weight heparin. A daily dose of 12 or 6 mg of dexamethasone did not result in a significant decrease in the composite outcome of death and thromboembolism, thromboembolism, or bleeding. Anti-Factor Xa values may be useful to guide thromboprophylaxis in patients with critical COVID-19.

LIST OF SCIENTIFIC PAPERS

Dosing of thromboprophylaxis and mortality in critically ill COVID-19 patients
Sandra Jonmarker, Jacob Hollenberg, Martin Dahlberg, Otto Stackelberg, Jacob Litorell, Åsa H Everhov, Hans Järnbert-Pettersson, Mårten Söderberg, Jonathan Grip, Anna Schandl, Mattias Günther, Maria Cronhjort
Critical Care, 2020 Nov 23;24(1):653

An observational study of intermediate or high-dose thromboprophylaxis for critically ill COVID-19 patients
Sandra Jonmarker, Jacob Litorell, Martin Dahlberg, Otto Stackelberg, Åsa H. Everhov, Mårten Söderberg, Johan Mårtensson, Rebecka Rubenson-Wahlin, Jacob Hollenberg, Mattias Günther, Eva Joelsson-Alm, Maria Cronhjort
Acta Anaesthesiologica Scandinavica, 2021 Mar;66(3):365–374

The effect of dexamethasone 12 mg vs 6 mg on thromboembolic and bleeding events in patients with critical COVID-19 - a *post-hoc* analysis of the blinded, randomized COVID STEROID 2 trial
Sandra Jonmarker*, Felix Alarcón*, Jacob Litorell, Anders Granholm, Eva Joelsson-Alm, Michelle Chew, Lene Russell, Sarah Weihe, Emilie Kabel Madsen, Nick Meier, Jens Wolfgang Leistner, Johan Mårtensson, Jacob Hollenberg, Anders Perner, Maj-Brit Nørregaard Kjær, Marie Warrer Munch, Martin Dahlberg, Maria Cronhjort, Rebecka Rubenson Wahlin
*Equal first authors
Annals of Intensive Care, 2023 Mar 2;13(1):12.

A retrospective, multicenter cohort study of the association between anti-Factor Xa and mortality, thromboembolism and bleeding in critically ill COVID-19 patients
Sandra Jonmarker, Jacob Litorell, Felix Alarcon, Kais Al-Abani, Sofia Björkman, Maria Farm, Jonathan Grip, Mårten Söderberg, Jacob Hollenberg, Rebecka Rubenson Wallin, Thomas Kander, Liivi Rimling, Johan Mårtensson, Eva Joelson-Alm, Martin Dahlberg, Maria Cronhjort
Manuscript

LIST OF SCIENTIFIC PAPERS BY THE AUTHOR BUT *NOT* INCLUDED IN THE THESIS

Fluid accumulation and major adverse kidney events in sepsis: a multicenter observational study.
Alessandro Mele, Emanuele Cerminara, Henrike Häbel, Borja Rodriguez-Galvez, Anders Oldner, David Nelson, Johannes Gårdh, Ragnar Thobaben, Sandra Jonmarker, Maria Cronhjort, Jacob Hollenberg, Johan Mårtensson
Annals of Intensive Care, 2022 Jul 4;12(1):62

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LIST OF ABBREVIATIONS

ACE2	angiotensin-converting enzyme 2
aFXa	anti-Factor Xa
aPL	antiphospholipid antibodies
ARDS	acute respiratory distress syndrome
APTT	activated partial thromboplastin clotting time
AT2	angiotensin 2
ATE	atrial thromboembolism
BMI	body mass index
CI	confidence interval
COVID	coronavirus disease
CRP	c-reactive protein
DIC	disseminated intravascular coagulopathy
DM	diabetes mellitus
DVT	deep venous thrombosis
EHR	electronic health records
GI	gastrointestinal
H-CoV	human coronavirus
HFNO	high flow nasal oxygen
HIT	heparin-induced thrombocytopenia
HR	hazard ratio
ICU	intensive care unit
IL-6	interleukin-6
IQR	interquartile range
IU	international units
JAK	janus kinase
LMWH	low-molecular-weight heparin
MERS-CoV	Middle East respiratory syndrome coronavirus
MI	myocardial infarction
MNP	mononuclear phagocytes
NET	neutrophil extracellular traps
OR	odds ratio
PE	pulmonary embolism
PT	pulmonary thrombosis
RAAS	renin-angiotensin-aldosterone system
RCT	randomized controlled trial

SAPS	simplified acute physiology score
SARS-CoV	severe acute respiratory syndrome coronavirus
TE	thromboembolism
TF	tissue factor
UFH	unfractionated heparins
VOC	variants of concern
VTE	venous thromboembolism
vWF	von Willebrand factor
WHO	World Health Organization

1 PREFACE

This thesis was meant to be about sepsis, septic cardiomyopathy, and fluids, but then the pandemic of coronavirus disease (COVID)-19 came upon us. COVID-19 changed the life of so many people, including mine. In hindsight, I was one of the lucky ones.
For me, it began with fear, not only for my loved ones of older age but also fear for my own life. When the reports came from China in January 2020, I was in the third trimester of pregnancy. Earlier epidemics with similar viruses had a high mortality rate among pregnant women, and I became obsessed by the difficult challenge approaching us. I harassed my superiors, trying to understand if we were prepared for this scenario. Devastating stories from Italy convinced me we were next. By this time, fortunately, there had been reports denying high mortality rates among healthy pregnant women. I was no longer afraid for my own life, but when our intensive care unit (ICU) filled up with COVID-19 patients fighting for their lives, I was not allowed to care for them. This thesis was born out of frustration caused by not being able to care for COVID-19 patients during the first wave. I was assigned to educate medical students, so my colleagues could be bedside. I was also involved in crash-courses for non-intensive care physicians at our hospital, who volunteered to help in the ICU when the ICUs were overwhelmed. I read a lot of scientific publications about COVID-19 and I listened to everything said at our doctors' meetings, but it was not enough. Together with my dear colleague and co-author, Jacob Litorell, we started to build a database with all the information about our patients and their treatment, with the aim of understanding more about this unknown disease. Many colleagues joined and helped us.

In May 2020, I got the most perfect baby who only slept, ate, and smiled. I could continue with my research during my parental leave almost full-time and, in fact, due to restrictions, there was not too much else to do when he slept. I was very glad to have something to do that seemed important. I could even join and present at conferences with the baby in my arms, as everything was on Zoom. During the winter 2020/2021, my baby was big enough to stay at home with my husband. As much as I appreciated being with my happy little baby, I could finally start taking care of COVID-19 patients. It was as frustrating, as sad, and as important as my colleagues had told me. During the pandemic we often took care of patients one generation younger than we previously used to do. Patients could be the same age as myself, and to speak with their kids the same age as mine, not knowing if their loved one would survive, was something I could never get used to. We all remember, and some patients we will remember forever. During the spring of 2021, I had a personal trauma of my own. The bone marrow of my then one and a half year old son shut down. I cannot prove it was directly caused by COVID-19, but it was certainly indirectly caused by COVID-19, as he had just started preschool during the time when the restrictions were lessened. Several other infections hit us massively as the herd immunity for viruses except COVID-19 was probably at an all-time low. After two weeks of intense investigations for different scary diagnoses, it turned out to be transient erythroblastopenia of childhood, a benign disorder suspected to be triggered by viral infections. Thankfully, the hemoglobin levels started rising again after a nadir of dangerously low values, and within three month he had recovered fully.

Now, finally, it seems like the pandemic is over, at least in our part of the world. All my loved ones are alive, even my two nearly one hundred-year-old grandparents who became infected already in April 2020. But I truly believe, in all the misery, we learnt a lot of things. COVID-19 patients made us question old truths. This was especially true when it came to thromboprophylaxis, where before the COVID-19 pandemic, we gave all patients the same regime regardless of the size, age or diagnosis of the patient. This is usually not the way to prescribe drugs in the intensive care unit.

COVID-19 showed us that the treatment of thromboprophylaxis may be more complicated than we previously thought. It is a treatment we expose so many patients to and, therefore, any improvement would mean a large reduction in the number of patients with complications of thromboembolism and bleeding. I hope our research can be a clue on how to proceed with thromboprophylaxis research. With the knowledge gained from our COVID-19 patients, we must perhaps ask the same questions when it comes to thromboprophylaxis in our “normal” intensive care patients.

2 LITERATURE REVIEW

2.1 THE ORIGIN AND DEFINITION OF COVID-19

COVID-19 is a highly contagious respiratory disease caused by the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) 2. SARS-CoV-2 is a member of the betacoronaviruses, which are large, enveloped, positive single-strand RNA viruses (Figure 1). Coronaviruses infect mammals, but natural reservoirs are bats, birds, and rodents (1). Seven coronaviruses are known to be pathogenic to humans. Four of them, human coronavirus (H-CoV) 229E, NL63, OC43, and HKU1, cause upper respiratory tract infections and are etiologic agents for about 15% of all common colds. The other three, SARS-CoV-1, Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and SARS-CoV-2, can infect the lower respiratory tract with the result of respiratory failure. SARS-CoV-1 and Middle East MERS-CoV have caused epidemics in the past two decades. SARS-CoV-1 emerged in China in 2002 and had an outbreak lasting between 2003 and 2004, with about 8,000 people infected. Most confirmed cases were in China and Southeast Asia, but SARS-CoV-1 also spread to North America and Europe. MERS was first identified in Saudi Arabia in 2012 and its spread has mostly been limited to the Arabian Peninsula. The mortality rates for these lower respiratory tract infections are high, 10% and 35%, for SARS-1 and MERS, respectively (2). The first case of SARS-CoV-2-infection causing COVID-19 was identified in Wuhan, China, in 2019 and has since spread around the globe. The most essential structures of SARS-CoV-2 are illustrated in Figure 1.

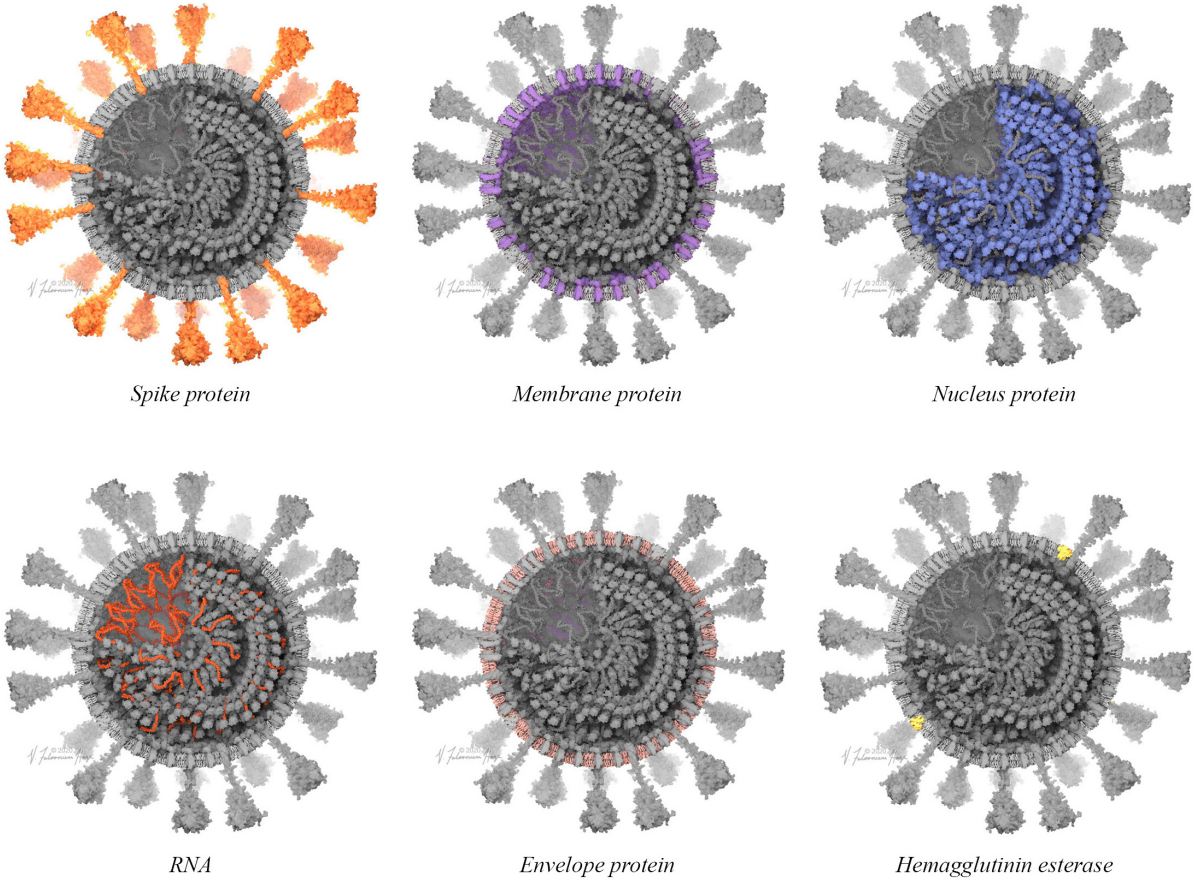


Figure 1: Severe acute respiratory syndrome coronavirus 2. The most essential structures are spike protein, membrane protein, nucleus protein, RNA, envelope protein, and hemagglutinin esterase. Illustrations by Veronica Falconieri/Falconieri Visuals, April 2020.

During the years of the pandemic, SARS-CoV-2 has mutated and given rise to new variants of concern. Several naming systems have been used for the variants circulating in different parts of the world during different time periods. The most commonly used are the World Health Organization's (WHO) labeling with a Greek letter and the Pango lineage naming system using Roman letters and numbers. The labeling and earliest documented sample of the most common variants can be seen in Table 1 (3, 4).

Table 1. SARS-CoV-2 variants

WHO label	Pango Lineage	Earliest documented sample
Wild type	A	December 2019 in China
Alpha	B.1.1.7	September 2020 in the United Kingdom
Beta	B.1.351	May 2020 in South Africa
Gamma	P.1	November 2020 in Brazil
Delta	B.1.617.2 and AY sub-lineages	October 2020 in India
Omicron	B.1.1.529 and BA sub-lineages	November 2021 in South Africa

In Sweden, four variants have given rise to the different waves in the pandemic. During the first wave in the spring of 2020, the dominating virus variant was the wild type. When the second wave hit us in October 2020, the wild type still dominated, but the wave gained new strength as the Alpha variant reached Sweden in December 2020. The Alpha variant dominated during the late winter and spring of 2021. The Delta variant took over in the summer, and during the fall, Delta slowly gave rise to the third wave. The third wave grew exponentially in December 2021 as Omicron infections started appearing in Sweden, and already by the Christmas holidays almost all cases were confirmed to be Omicron (5). With Omicron came an all-time high of confirmed cases in the beginning of 2022, over 250 000 infected patients per week. The third wave subsided during spring 2022. After February 2022 the incidence is difficult to appreciate, because testing is no longer recommended outside health care and nursing homes (6).

2.2 INCIDENCE

Up until February 1, 2023, 670,400,000 cases of COVID-19 have been diagnosed and has been attributed as the cause of 6,824,000 deaths (7). These numbers are only confirmed cases, and the real number of deaths has been estimated to be as high as 15,000,000 (8). In Sweden, about 2,693,000 COVID-19 cases have been diagnosed, out of which 23,300 of those infected have died, and 10,300 patients have been treated in an intensive care unit (ICU) (9). According to the Swedish Intensive Care Registry, Södersjukhuset, Stockholm, has had highest number of ICU admissions of critically ill COVID-19 patients in Sweden, with more than 1,100 admissions (10).

2.3 SYMPTOMS

Compared to SARS-1 and MERS, the clinical spectrum of symptoms of COVID-19 is much more diverse. For some people, the infection may be asymptomatic, but the most common symptoms include fever, dry cough, and fatigue. Other symptoms are loss of smell and taste, nasal congestion, conjunctivitis, sore throat, headache, muscle and joint pain, skin rash, nausea and vomiting, diarrhea, dyspnea, dizziness, and confusion (11, 12). Based on symptoms and the severity of these, COVID-19 infection can be classified into asymptomatic, mild, moderate, severe, or critical disease (13). Asymptomatic disease is when an infection is

verified by test, but no symptoms are present. Mild disease is when any of the various signs and symptoms of COVID-19 are present. The moderate form of COVID-19 includes dyspnea, as the infection has progressed to the lower respiratory tract, and the typical peripheral ground glass changes can often be seen on computed tomography. Usually, these patients do not need supplemental oxygen when resting. Patients with severe infection, defined by a saturation below 94% on room air, often require hospital admission and oxygen supplementation. With additional deterioration requiring intensive care, the term critical COVID-19 is used. Critical COVID-19 often includes bilateral interstitial pneumonitis causing acute respiratory distress syndrome (ARDS). In Sweden, in 2020, the mortality of patients requiring intensive care ICU due to COVID-19 was 27% (14).
After recovery from the acute COVID-19 infection, some patients, mostly children, can develop an unusual complication caused by autoantibody reactivity, a multisystemic inflammatory syndrome (12). This requires prompt and aggressive immunomodulation. A more common sequel to COVID-19 is long COVID. Both for multisystemic inflammatory syndrome and long COVID, the severity of the primary infection can be mild. For long COVID, the definitions and possible treatments are still under debate (15).

Multiple risk factors for a more severe infection and death have been identified, with older age being the most important. Other risk factors are male sex and also comorbidities, such as cardiovascular disease, chronic pulmonary disease, neurological disease, poorly controlled diabetes mellitus (DM), liver- and renal disease, obesity, late pregnancy, and immunocompromising treatments or diseases (12, 16). In addition, the risk of a more severe infection has been associated with immunological phenotypes, for example, imbalances of interferons, which play an important role in both the innate and adaptive response against viral infections (17, 18). Genotypes inherited from Neanderthals have also gained attention, as some genomic regions have been identified to increase the risk for severe infection and some may be protective (19, 20).

In addition to risk factors in the individual patient, we now know that the earlier variants, wild type, Alpha, Beta, Gamma, and Delta, had more severe symptoms, and a higher proportion of patients progressed to ARDS compared to the Omicron variant (21). Early variants also had more cardiovascular symptoms, including coagulopathy, causing thromboembolism and acute renal failure. In the less severe form, many patients experienced brain fog, and loss of smell and taste with the earlier variants. The omicron variant remains in the upper airways to a higher extent than the previous variants, hence are less likely to cause serious symptoms from the lower airways. During the omicron wave, more children required hospital admission, which could be due to children's smaller airways being more sensitive to upper airway infections compared to adults (22).

However, with time, the immunological protection, by the more than 13 billion doses of vaccine administered worldwide and from all previous infections, has played a fundamental role in decreasing the burden of disease of COVID-19 (7, 21). Since April 1, 2022, COVID-19 is no longer considered a disease dangerous to society in Sweden. Moreover, on February 1, 2023, for the first time since the beginning of the pandemic, no COVID-19 patients were cared for in the ICUs in Stockholm (10).

2.4 COAGULOPATHY OF COVID-19

Just like ARDS, coagulopathy has been a distinct feature of patients with critical COVID-19. This was recognized early in the pandemic, with reports of a high incidence of macro thrombotic complications, findings of micro thrombi in the lungs, and increased levels of coagulation markers (23-26). A meta-analysis, including studies published January 1 to July 31, 2020, showed that 17% of hospitalized COVID-19 patients and 27.9% of patients requiring intensive care were diagnosed with a venous thromboembolism (VTE) (27). Another meta-analysis showed that an event of VTE increased mortality by 74% compared to patients without this complication (28). In the absence of macro thrombosis, the micro thrombi were assumed to contribute to the severe hypoxia, by causing dead-space ventilation and thereby increasing the already existing mismatch of ventilation and perfusion caused by shunting due to damaged alveolar surface (29). Increased coagulation markers, in particular fibrin-D-dimer, have been reported as prognostic markers for more severe disease. Increased fibrin-D-dimer is produced by cleaved fibrin and indicates both an increased coagulation and fibrinolysis (30). Moreover, a distinct feature for COVID-19 patients is the disproportionally high incidence of pulmonary embolism (PE) compared to deep venous thrombosis (DVT) (31). The pathophysiology of PE is a thrombus arising from the veins, often in the legs, which then embolizes to the lungs. In non-COVID-19 patients, about 60% of all PE patients also have a DVT on ultrasound duplex (32). For COVID-19 patients, the absence of DVTs has led to speculations about *in-situ* thrombosis (33). Therefore, it may be more correct to refer to the mixture of PE or pulmonary thrombosis (PT).

Even though the coagulopathy in COVID-19 was unanticipated, it came as no surprise that infections can cause dysregulations of the coagulation. Many viral infections have been associated with coagulopathy. Viruses causing coagulopathy can be divided into non-hemorrhagic and hemorrhagic, arising from dysfunction in hemostasis, coagulation, and/or fibrinolysis. Respiratory viruses, such as influenza and coronaviruses, have been shown to increase the risk of thrombosis, while other viruses like Ebola and Dengue can cause bleeding (34). Severe inflammation and disease, regardless of trigger, can cause disseminated intravascular coagulopathy (DIC). DIC is characterized by “activation of coagulation with loss of localization”, leading to systemic clotting, reduced blood flow to the organs, and exhaustion of clotting factors, which may then lead to bleeding (35, 36). COVID-19 associated coagulopathy has been proposed to be a DIC with a thrombotic phenotype, not yet progressed into a consumptive coagulopathy (37).

In summary, the hypercoagulation caused by COVID-19 may be caused by virus-specific mechanisms, severe inflammation, or a mix of both. The following sections will try to untangle some of the pathways involved.

2.4.1 Virus-specific mechanisms causing coagulopathy

Three important virus-specific mechanisms for coagulopathy have been proposed:

(1) Interference with the Renin-Angiotensin-Aldosterone System (RAAS). SARS-CoV-2 has a spike surface glycoprotein that binds to the angiotensin-converting enzyme 2 (ACE2) for cellular entry. ACE2 is also an inhibitor of the RAAS, and the binding and translocation of ACE2 by SARS-CoV-2 thereby causes an imbalance. This imbalance

leads to an increase in the availability of angiotensin 2 (AT2), a prothrombotic, vasoconstrictive hormone which favors platelet aggregation, increases coagulation, and decreases fibrinolysis (38, 39). When infusing AT2 into a porcine model, a pathophysiological state resembling the clinical COVID-19 seen in human was induced (40). This indicates that the RAAS-imbalance is an important driver of the pathophysiology and could be a potential target of therapy. AT2 has previously been shown to increase blood pressure in patients with vasodilatory shock when conventional treatment was not enough (41). However, in the same study patients treated with AT2 had a higher risk of VTE, which adds to the hypothesis that high levels of AT2 causes a procoagulant milieu.

(2) Direct infection of pneumocytes, endothelial cells, and platelets. Pathogen-induced cell death can occur when infected by SARS-CoV-2. The most beneficial for the host is if the infected cell undergoes apoptosis or autophagy, causing no harm to the invironment. If the virus kills the cell in an uncontrolled way, this results in lysis of the cell with the escaping cytoplasmic content triggering thrombosis and microangiopathy (42). If and how SARS-CoV-2 infects other cell types involved in coagulation is under investigation. Platelets express ACE2, therefore, it is possible that SARS-CoV-2 can cause direct hyperactivation and thrombosis (43).

(3) Induction of auto-immunity. It is proposed that auto-immunity triggered by SARS-CoV-2 causes formation of antiphospholipid antibodies (aPL), which can contribute to thrombosis. aPL have been shown to be common among critically ill COVID-19 patients, but the antibodies are often transient (44). This has been seen in other viral infections, for example, parvovirus B19, herpes, hepatitis, and human immunodeficiency virus, but the clinical importance is controversial (34).

2.4.2 Inflammation causing coagulopathy

Inflammation is induced by pattern recognition receptors binding to pathogen- and damage-associated molecular patterns. These binding triggers intracellular signaling that leads to an immune response (43). Activation of inflammation and coagulation share many mutual pathways and may activate each other. When one activates the other, it is called immunothrombosis or thromboinflammation. Central to immunothrombosis is the loss of the normal antithrombotic and anti-inflammatory functions of endothelial cells (45, 46). Six different key players have been identified in immunothrombosis:

(1) Leukocytes: Both mononuclear phagocytes (MNP) and neutrophils, two types of leukocytes of the innate immune system, seem to play an important role in thrombosis formation. The levels of MNP found in the bronchoalveolar fluid are higher in COVID-19 patients compared to healthy controls and correlated to severity of disease (47). MNP produce cytokines and coagulation factor 3, commonly known as tissue factor (TF), which, in turn, is one of two ways to initiate the coagulation cascade (39, 43) (Figure 2). A special feature of neutrophils is that they can form neutrophil extracellular traps (NETs), which are found at high levels in COVID-19-patients (48). NETs are composed of DNA and histones and constitute an important part of the innate immune response by capturing and neutralizing pathogens. They are also

associated with thrombosis, possibly because they may also occlude vessels when formed intravascularly (49).

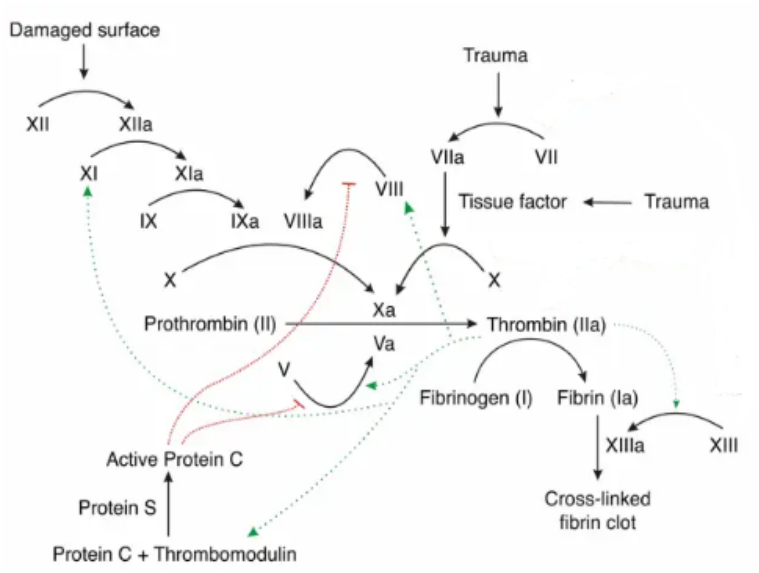


Figure 2: The coagulation cascade. This picture illustrates the two ways to activate the coagulation cascade, via damaged cell surface causing contact activation and via trauma, which triggers tissue factor pathway. Both pathways activate factor X, leading to the common pathway and resulting in a cross-linked fibrin clot. Activated protein C can inhibit the common pathway. Illustrations by Internet Book of Critical Care.

(2) Cytokines: COVID-19 causes a profound inflammation with high levels of cytokines. Interleukin-6 (IL-6) has gained much attention for being an important driver of the COVID-19 associated inflammation (50). IL-6 has many proposed prothrombotic characteristics. IL-6 can downregulate thrombomodulin, which debilitates the anticoagulating protein C-system (Figure 2). An increase of IL-6 in patients induces acute phase proteins, of which fibrinogen (coagulation factor I) and factor VIII are two of the most important for coagulation (Figure 2) (51). In clinical trials preceding COVID-19, IL-6 was found to induce expression of TF in inflamed tissues and to promote the synthesis of coagulation factors. The purpose of IL-6 mediated coagulation activation is suggested to counteract viral infections by trapping viruses in the fibrin network but may easily lead to thrombus formation if activated extensively (52).

(3) Complement system: Viral infections activate the complement system, which is a vital part of the host defense. Respiratory viruses, like SARS-CoV-2, seem to have an enhanced complement activation via the lectin pathway, which can cause acute lung injury and in severe cases be associated with thrombotic microangiopathy (43, 52). Increased complement activation has been confirmed by findings of elevated levels of complement components in plasma obtained from severely ill COVID-19 patients (53, 54).

(4) Kallikreins: Kallikreins have been suggested to cause excessive inflammation in COVID-19. Low levels of the precursor, prekallikrein, reflecting consumption, predict a poor outcome in critically ill COVID-19 patients (54). The main effects of plasma

kallikrein are prothrombotic by liberating kinins, which activate inflammation and trigger and amplify the contact activation pathway in the blood cascade. However, kallikreins also stimulate fibrinolysis, resulting in high levels of fibrin degradation products (Figure 3).

(5) Fibrinolysis: A suppression in fibrinolytic activity has been reported in COVID-19 patients, which can be due to an increased fibrin resistance to fibrinolysis and also downregulation of profibrinolytic proteins, for example, urokinase (Figure 3) (30). This has been documented in a meta-analysis of viscoelastic testing in COVID-19 patients, where the common findings were increased clot strength mainly due to excessive fibrinogen components and impaired fibrinolysis (55).

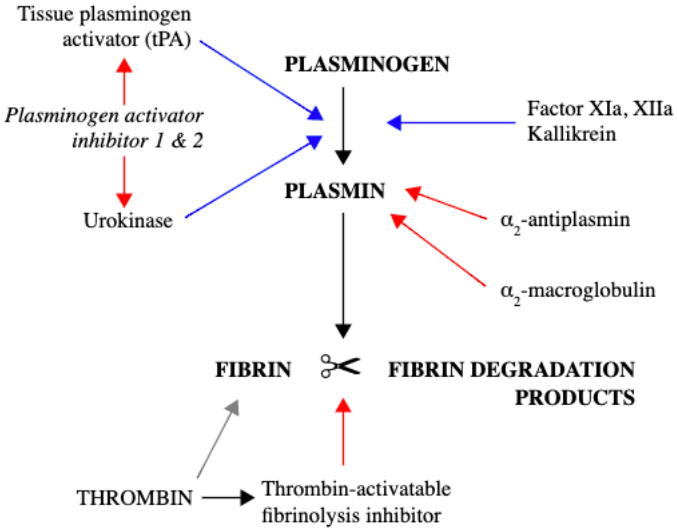


Figure 3: Fibrinolysis is the process where blood clots are broken down into degradation products. Its main purpose is to keep coagulation localized to where it is needed. As seen in severely ill COVID-19 patients, the procoagulant state puts great demands on the fibrinolysis to prevent blood clots from growing. This can be seen by the high plasma levels of fibrin-D-dimer, one of the degradation products of fibrin. Fibrinolysis is stimulated by tissue plasminogen activator, urokinase, fXIa, fXIIa, and kallikrein. Illustration by Jacob De Wolff.

(6) Platelets: Most COVID-19-patients have a mildly elevated platelet count, but the platelet count can also decrease. An elevated platelet count can be explained by high levels of cytokines stimulating the proliferation of the parent cells of platelets, megakaryocytes (56, 57). A trend of increasing platelet count has actually been associated with improved survival and reduced thrombotic risk (58). Low platelet count is associated with poor outcomes. This suggests an activation and consumption of platelets, which may be due to a progression from COVID-19 associated coagulopathy to classical DIC (37, 59-61).

In the presence of von Willebrand factor (vWF), platelets can attach to the endothelium and to each other. vWF-complexes are released from the endothelium in response to trauma or inflammation. Under normal conditions, a protein called ADAMTS-13 cleaves vWF-complexes to avoid excessive platelet clotting. Inflammation, as in COVID-19, causes an imbalance with high levels of vWF-complexes compared to ADAMTS-13, which promotes hypercoagulability and formation of microthrombosis

(Figure 4). This is similar to the pathophysiology in thrombotic thrombocytopenic purpura, which is caused by a severe deficiency in ADAMTS-13 (62).

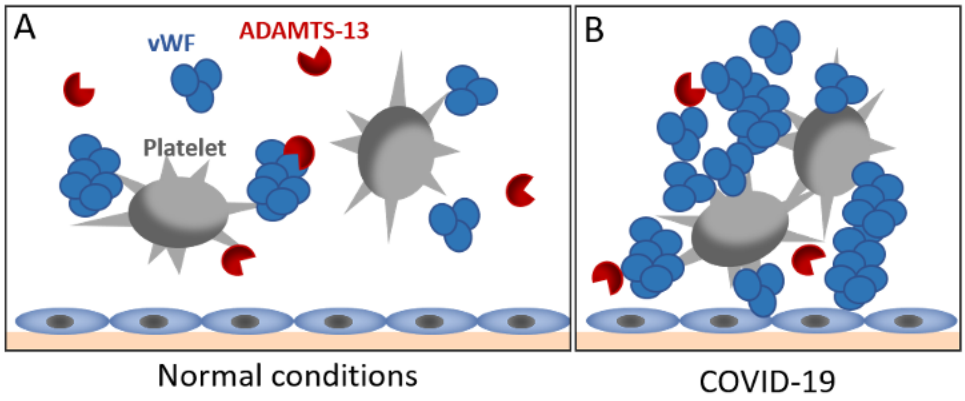


Figure 4: The endothelium releases von Willebrand factor-complexes in response to trauma or inflammation. The vWF-complexes act as a kind of glue between platelets when building a clot. In normal conditions, ADAMTS-13 cleaves the vWF-complexes, which change the conformation, so the platelet binding domains are not exposed. However, in patients with COVID-19 associated inflammation, excess release of vWF-complexes causes an imbalance, and platelet aggregation cannot be prevented. Illustrations by Diapharma.

2.5 PREVENTION OF COVID-19

2.5.1 Vaccine

The most important drugs for lowering the burden of disease of COVID-19 are vaccines. For all variants of concern (VOC), two doses of the vaccines mostly used in Europe (messenger RNA vaccines from Pfizer® and Moderna®, adenovirus-based vaccines from AstraZeneca® and Janssen®) have been shown to dramatically lower the risk for severe disease and death (63-66). Even though infections are still common after vaccination, especially with the Omicron variant, the symptoms of a post vaccination infection are often considered mild. The risk of severe disease and death is even lower after a vaccine booster. Moreover, the viral load becomes lower, which could attenuate the rate of spread (8, 67).

2.6 TREATMENT OF COVID-19

When infection has occurred, treatment is guided by symptoms and risk factors of the individual patient. For patients without risk factors and mild or moderate disease, treatment is usually not required.

Early in the disease course during the virus replicating phase, antiviral medication can be used for patients with already severe disease or critical disease or risk factors to develop severe or critical disease. In the later inflammatory phase, if the disease has progressed, immunomodulating treatment is strongly suggested by the guidelines. In addition to the specific drugs recommended for severe and critical COVID-19, organ supportive treatment with respiratory support and thromboprophylaxis is highlighted as important (12). In contrast to influenza, secondary infections are uncommon at hospital admission (68). Therefore, antibiotics should be avoided if possible. For patients with severe or critical disease, where empiric antibiotics cannot be withheld, it is recommended to be guided by microbiology testing, and if the results are negative, then the antibiotic treatment can be halted (12).

2.6.1 Antiviral medication

Remdesivir, molnupiravir, and nirmatrelvir (in combination with ritonavir) inhibit the replication of SARS-CoV-2. Antivirals have a documented effect to prevent clinical deterioration from mild to severe disease in the early phase of infection, which is of clinical significance for vulnerable patient groups. However, a convincing clinical benefit for patients that have already progressed to severe disease has only been shown if therapy is started within five to seven days of symptoms (69-72). Given the requirement of early administration, these drugs are usually not prescribed, as most patients are admitted seven to ten days after symptoms debut. However, they can be considered at a later time point in immunocompromised patients, where virus replication is thought to be prolonged (12).

Monoclonal antibodies have previously been recommended for patients who are seronegative, both as a prophylaxis and as a treatment. Currently, monoclonal antibodies are not recommended, as the majority of the Omicron variants are resistant to neutralization of the available alternatives (12).

Imbalance of interferons has been identified as a risk factor for severe disease, and different interferon treatments have been tested. Promising results of interferon lambda for preventing hospital admission have been published but interferon treatment not is recommended for critical COVID-19 (73). Passive transfer of immunoglobulins by giving plasma from patients that have recovered from COVID-19 has also been tested, but is currently not recommended (12).

2.6.2 Immunomodulating treatment

The second phase, if COVID-19 progresses to a more severe stage, is characterized by hyperinflammation. Several immunomodulating drugs have shown to improve survival. This contrasts with other virus pneumonites (influenza, SARS-CoV-1, and MERS) where the most used immunomodulating drug, glucocorticoids, have been associated with worse outcomes (74-76).

By mid 2020, the glucocorticoid dexamethasone, 6 mg daily for up to 10 days, was recommended to patients with oxygen supplementation, as a result from the publishing of the Recovery trial (77). The results showed a 2.9% absolute risk reduction in mortality for all patients admitted to hospital and 12.1% absolute risk reduction in mortality for patients requiring invasive ventilation. However, for patients not requiring oxygen supplement, and for patients early in the disease course (< 7 days), no benefit was seen. One explanation could be that an immunomodulating drug may increase the virus load if the infection is still in the virus replicating state and thereby cause a more severe disease.

The optimal dose of glucocorticoids for COVID-19 patients on oxygen supplementation has been investigated. For pneumonia, ARDS and/or sepsis due to bacterial infection, higher doses than 6 mg daily of dexamethasone have been shown to improve the outcome (78-83). For these, non-COVID-19 patients, glucocorticoids reduced the length of stay in the ICU and accelerated weaning from invasive ventilation when given in doses between 7.5–28 mg dexamethasone, or equivalent, for seven to ten days. In addition, some studies also showed a reduced mortality (78, 81-83). For COVID-19 patients with oxygen supplementation of more

than 10 L/minute, 12 mg vs. 6 mg was investigated in a large multicenter study, but no significant difference was shown (84, 85). However, all estimates pointed toward a benefit in the 12 mg group, and no safety concerns were raised. Other studies investigating higher doses have not found any difference in mortality compared to dexamethasone 6 mg daily, but one study found a difference in the secondary outcome of days on mechanical respiratory support, which were fewer in the high dose group (86-88).

Currently, guidelines recommend 6 mg dexamethasone or equivalent to patients requiring oxygen supplementation, but because higher doses may be beneficial, guidelines leave it up to clinicians to judge if an individual patient needs a higher dose.

Inhaled glucocorticoids have also been studied and have reduced health care contacts and duration of symptoms (89-92). However, the results have not been deemed sufficiently convincing to motivate recommendations of inhalation glucocorticoids in the guidelines (12).

Drugs inhibiting interleukin-6 (IL-6) and janus kinase (JAK) have shown to improve the outcome for patients with critical COVID-19. IL-6-inhibitors have shown to lower mortality, with the largest effect when studied in an ICU population with a high degree of inflammation, defined by C-reactive protein (CRP) above 75 mg/L (93, 94). Treatment with baricitinib, a JAK-inhibitor, in combination with remdesivir, showed a significantly shorter time to clinical improvement for patients treated with high flow oxygen and non-invasive ventilator (95). Moreover, when most of the patients were treated concomitantly with glucocorticoids, a decrease in mortality was shown in all or subgroups of COVID-19 patients (96-98). Guidelines conclude that inhibition of either IL-6 or JAK should be considered as an adjunct to glucocorticoids in patients who are in an early critical phase of COVID-19, combined with a high degree of inflammation (12).

2.6.3 Respiratory support

Respiratory support of varying degrees is often needed for hospitalized COVID-19 patients. Patients admitted to the ICU frequently require high flow nasal oxygen, non-invasive ventilation, and/or invasive ventilation.

Treatment for severe hypoxia with prone positioning, both for awake and sedated patients, is extensively used. Improved outcome by prone positioning for severe and critical COVID-19 patients is in concordance with the results from non-COVID patients with severe ARDS (99-101). However, the optimal timing of intubation and the usage of different ventilatory strategies have varied and are still debated (102-106). In the beginning of the pandemic, many patients were intubated early due to the hypoxia being critical already at admission and also to decrease the risk of spreading the virus (107, 108). Later on in the pandemic, early intubation was questioned. If intubation could be postponed, this would shorten the time with invasive mechanical ventilation, or even avoid it, and thereby decrease the risk of ventilator associated complications. Guidelines now recommend treating hypoxia with supplemental oxygen and non-invasive respiratory support initially, and to advance to intubation only if there is a persistent need for a high fraction of inspired oxygen, an increasing work of breathing, decline in mental status, hemodynamic instability, and/or other organ failure (106). Ventilatory strategies used for classical ARDS, recognized by low compliance, were questioned as early

reports classified COVID-19-ARDS as having a high compliance (109). However, the latest guidelines recommended management in accordance with evidence-based ARDS-strategies with low tidal volume and high positive end-expiratory pressure, as COVID-19 lungs may also stiffen even if the initial compliance is high (110).

2.6.4 Thromboprophylaxis

As early as 1856, Richard Virchow recognized a triad of factors increasing the risk of venous thrombi: hypercoagulability, stasis, and endothelial injury/dysfunction. Patients with critical disease are often exposed to hypercoagulability because of inflammation, infection, and drugs, exposed to stasis as a result of immobilization, positive pressure ventilation, and high body mass index, and exposed to endothelial injury due to trauma, surgery, indwelling catheters, and underlying cancer diagnoses (111). Most of these risk factors have been confirmed when studied in an intensive care setting (112).

Pharmacologic thromboprophylaxis for ICU patients was introduced based on the results of two randomized controlled trials (RCT): one in 1982 and one in 2000. Both studies found a lower rate of DVT in the groups with thromboprophylaxis compared to placebo (113, 114). Current guidelines strongly recommend pharmacological thromboprophylaxis for critically ill patients in the absence of contraindications (115, 116). Absolute contraindications are ongoing intracranial or other life-threatening bleeding, recent surgery or trauma, or platelet numbers below 30 x 10^9/L. The most common relative contraindications are congenital or acquired bleeding disorders, renal and/or liver failure, treatment with other drugs that affect hemostasis, and/or uncontrolled hypertension (117). Although thromboprophylaxis has been broadly implemented in the ICU, the incidence of VTE, prior to COVID-19, was still reported to be between 2–27%, depending on the clinical setting and the study design (118-122).

Critically ill patients are not only exposed to the risk of VTE but also the risk of bleeding, which can be assumed to increase even more with pharmacologic thromboprophylaxis. Non-major bleeding, for example, bruising after peripheral venous catheter or hematuria after insertion of urine catheter, is reported frequently, up to 90% (123). The main reasons for anemia during intensive care are bleeding, decreased erythropoiesis, and frequent blood sampling. As many as 97% of patients are anemic after one week in the ICU (124-126). The incidence of major bleeding is reported to be around 5–7% and doubles the risk for mortality (120, 127-129).

Heparins are the recommended drugs for thromboprophylaxis in the ICU. Besides being anticoagulant, heparins have also been suggested to have anti-inflammatory and antiviral effects (Figure 5) (130, 131). Heparins are negatively charged glycosaminoglycan polysaccharide polymers, extracted from mast cell granules from the small intestine mucosa of swine. The two forms of heparins used are unfractionated heparin (UFH) and the depolymerized form, low-molecular-weight heparin (LMWH). The anticoagulant effect is caused by heparin binding and potentiating antithrombin, which inhibits fXa and thrombin, two factors essential for the coagulation cascade (Figure 2). The anticoagulant characteristics of UFH were discovered in the 1930s by Erik Jorpes at Karolinska Institutet. By the 1950s, it was used clinically to prevent postoperative VTE (132). In 1960, a landmark trial established

that heparin could reduce mortality from pulmonary embolism (133). In the 1980s, Ulf Lindahl at Uppsala University further modified UFH to LMWH.

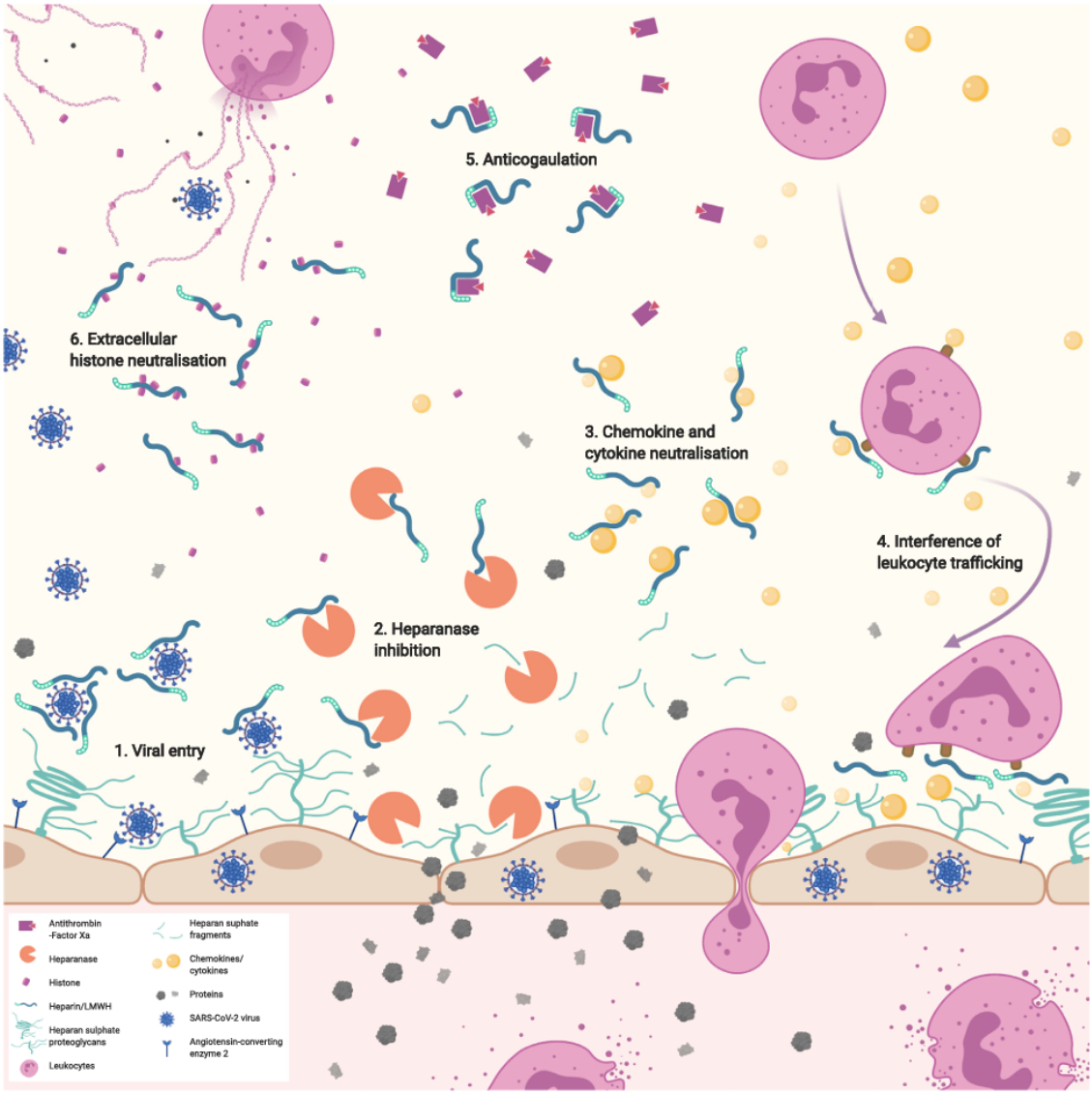


Figure 5. Proposed mechanisms for heparins. 1. Direct inhibition of pathogens 2. Inhibition of heparinase activity to protect the integrity of the glycocalyx. 3. Neutralizing of chemokines and cytokines 4. Interference of leukocyte trafficking and ability for adhesion 5. Anticoagulation by binding to antithrombin 6. Neutralization of histones in neutrophil extracellular traps. Illustration from review by Buijsers et al., published in eBioMedicine.

The PROTECT trial, the largest RCT to compare UFH and LMWH in an ICU setting, included 3,764 patients, of whom 90% were mechanically ventilated (120). No difference was found between the two classes of heparins regarding rates of DVT or bleeding, but there was a lower incidence of PE with LMWH compared to UFH. In a recent meta-analysis of 13 RCTs, LMWH was shown to also reduce the incidence of DVT compared to UFH (117). This has resulted in guidelines recommending LMWH in lieu of UFH with the exception of some patient populations, for whom UFH is preferred (134, 135). Important clinical features and differences between UFH and LMWH are summarized in Table 2 (136, 137).
The longer half-life of LMWH makes the dosing intervals less frequent and therefore easier to administer and brings fewer episodes of discomfort for the patient. But a short half-life can also be preferred if handling a patient where careful control of the anticoagulation is needed. For example, in a patient with a bleeding risk, both a faster weaning of drug effect after

administration and the full reversibility with protamine sulfate are the desired characteristics of UFH. The shorter half-life of UFH and the partly non-renal clearance also decrease the risk of accumulation and are, therefore, the reason for choosing UFH in patients with severe renal failure (134, 135).

Table 2: Difference between unfractionated heparin and the low-molecular-weights heparin enoxaparin, dalteparin, and tinzaparin (138).

	unfractionated heparin	enoxaparin	dalteparin	tinzaparin
Half-time (hours)[1]	1.5	4 to 5	3 to 4	3 to 4
Clearance	By reticulo-endothelial system, endothelial cells, and by renal excretion	By renal excretion	By renal excretion	By renal excretion
Dose adjustments for renal impairment	If GFR[2] is less than 15 ml/min	If GFR[2] is less than 30 ml/min	If GFR[2] is less than 30 ml/min	If GFR[2] is less than (20 to) 30 ml/min
Reversal agent	Full and rapid reversal with protamine sulfate	About 60% reversal with protamine sulfate[3]	About 60% reversal with protamine sulfate[3]	About 60% reversal with protamine sulfate[3]
Molecular weight (Dalton)	Mean 15,000	4,200	6,000	6,800
Proteases mainly inhibited, ratio fXa/fIIa	1	3.9	2.5	2.0
Risk of heparin induced thrombocytopenia (%)	2.6	0.2	0.2	0.2

[1] after subcutaneous administration
[2] glomerular filtration rate
[3] based on evidence from animal studies and small retrospective studies (139).

UFHs, compared to LMWHs, have a larger mean size, which increases the affinity to plasma proteins. This is illustrated by the inhibition of thrombin being greater with the UFH-antithrombin complex compared to LMWH-antithrombin complex, because of the LMWH-antithrombin complex being too small to bind to thrombin as efficiently (Figure 6). But the increased affinity of the larger UFH to various plasma proteins also affects bioavailability. This explains why the anticoagulation effect of UFH is more unpredictable than the effect of LMWH, especially in highly inflamed patients (140, 141). In some situations, a resistance to heparin is observed, where a high dose of heparin is needed to achieve target levels of anticoagulation. However, the definition of high dose is not exact (142). The more inflamed and hypercoagulable a patient is, the more heparin can bind to proteins and cells of the immune and coagulation system (Figure 5) (131). This is believed to be one of the major contributors to heparin resistance. Two other causes of heparin resistance can be antithrombin deficiency and heparin binding to platelets. Platelet binding can prompt the release of platelet factor 4 and thereby trigger auto-immunity and heparin-induced thrombocytopenia (HIT) with thrombosis and thrombocytopenia. If HIT develops, non-heparin alternatives for thromboprophylaxis, such as fondaparinux, bivalirudin, or argatroban, may be used. Prior to the COVID-19 pandemic, heparin resistance was mostly discussed for treatment with UFH, but has now been reported also for LMWH in patients with COVID-19 (143).
UFH can be monitored, and the dosing guided by algorithms using functional assays, such as activated partial-thromboplastin time (APTT) and activated clotting time, or using chromogenic assays, such as anti-Factor Xa (aFXa) assays. In contrast to the UFH, guidelines often recommend a fixed dose of LMWH for thromboprophylaxis in the ICU without monitoring (144). However, monitoring may be used when the elimination of LMWH is impaired, in extremely overweight patients, or if there is an unexpected clinical response

possible due to a deviation from predicted pharmacokinetics (145-147). As for UFH, aFXa assays can be used to measure the activity of LMWH. However, associations between the clinical outcome and the value of aFXa have been hard to establish and the use of aFXa is therefore intensively debated, especially when it comes to thromboprophylaxis (145, 148-151). Recommended peak and trough target values for treatment of VTE with LMWH are 0.6 to 1.0 kIU/L and 0.2 to 0.6 kIU/L, respectively (152). Target values in thromboprophylaxis treatment are not agreed upon (153, 154). A systematic review from 2015 found a lack of evidence regarding the optimal aFXa levels in critically ill patients (154). However, in critically ill trauma and surgery patients, low trough aFXa levels significantly increased the incidence of DVT (155). Two studies report an inverse correlation between severity of critical disease (size of burns and multiple organ dysfunction score) and aFXa when given the same dose of thromboprophylaxis (156, 157). This could indicate heparin resistance with LMWH.

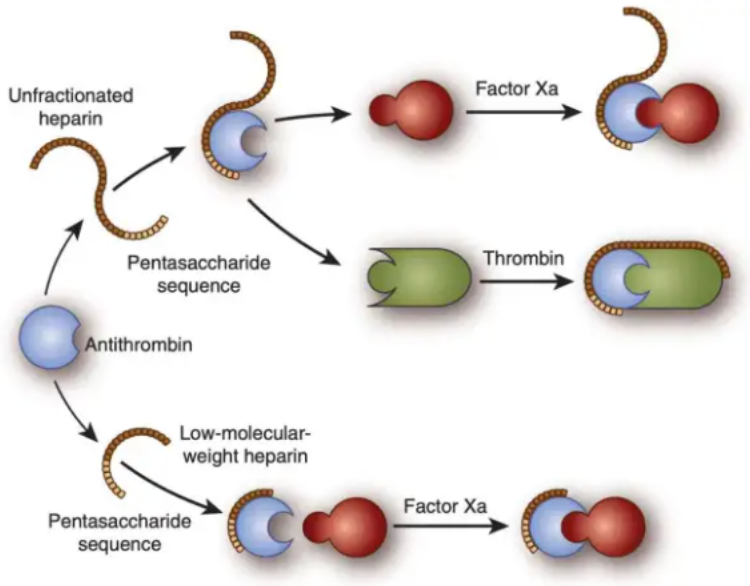


Figure 6: Both UFH and LMWH bind to antithrombin. The complex of UFH-AT binds and inhibits both factor Xa and thrombin. LMWH-antithrombin complex inhibits factor Xa but is too small to inhibit thrombin as efficiently. Illustration by Internet Book of Critical Care.

The pharmacodynamics of LMWH, with a half-life of 3–5 hours, make dosing once daily debatable (158). When measuring aFXa in critically ill patients with different dosing intervals of enoxaparin, a flatter profile of aFXa was seen when dosing twice daily compared with once daily (159). However, if this is clinically meaningful has yet to be proven. A meta-analysis from 2020 could not find a difference in the risk of VTE and major bleeding when comparing once daily versus twice daily regimes, but the authors state that the quality of evidence may not have been sufficiently high to support the conclusion since only one of the six included trials was a RCT (160).

2.6.4.1 Thromboprophylaxis in patients with COVID-19

Targeting primary hemostasis with antiplatelet drugs has not been shown to reduce mortality or progression to invasive mechanical ventilation in hospitalized COVID-19 patients, but it may be associated with a small increased chance of being discharged alive within 28 days (161). For patients treated in the ICU, antiplatelet drugs, in combination with high doses of anticoagulation, increase bleeding (162). Guidelines state that patients on chronic use of

antiplatelet drugs for a prior cardiovascular indication can continue treatment. For patients with no prior antiplatelet treatment, it should only be added in selected cases of patients with critical COVID-19, and only in combination with low dose thromboprophylaxis (163, 164).

To prevent hypercoagulability in COVID-19 patients, the main focus has been to find the optimal dose of LMWH. The first studies attempting to answer this question reported a beneficial effect with high doses of LMWH. An early randomized controlled trial, HESACOVID, found that therapeutic doses of LMWH improved the gas exchange in 20 mechanically ventilated COVID-19 patients compared to the standard dose. The study was not designed to detect a difference in mortality or TE (165). Observational studies, including our first study (study 1), have favored a higher dose of thromboprophylaxis compared to standard low dose (166-172), but there are also studies showing no difference (173, 174). No larger RCTs have identified a benefit with intensified thromboprophylaxis compared to the standard or usual care dose in the critically ill COVID-19 patients. Two open label RCTs randomized patients to receive low or intermediate dose thromboprophylaxis. In the largest of the two, the INSPIRATION-trial, the drugs were given once daily, and there were no differences in the composite outcome of death, VTE, arterial thrombosis, or treatment with extracorporeal membrane oxygenation (175). The study by Perepu also failed to detect any differences in the incidence of death, TE, and bleeding between the groups (176). The multi-platform trial, consisting of the trials REMAP-CAP, ACTIV-4, and ATTACC, compared high dose thromboprophylaxis to usual care (177). The intervention arm with a high dose was discontinued after an interim analysis due to pre-specified criteria of suspected futility for the high dose group. The subsequent analysis could not show any difference in the hospital survival or days free of organ support between the groups. They did, however, report a lower incidence of VTE in the high dose group. This could be due to the trial not being blinded and, therefore, an unwillingness by clinicians to refer patients in the high dose groups for an examination that would not alter their current treatment, since the high dose was the same dose used for VTE treatment. Notably, in these pragmatic platform trials, usual care was defined as institutional standard care. This resulted in the majority of the controls being treated with an intermediate dose, which makes the interpretation of the results more difficult. However, subgroup analysis, according to sites using different regimes for the comparator groups, did not alter the results. HEP-COVID investigated high dose thromboprophylaxis compared to standard/intermediate dose in the subgroup of patients with fibrin-D-dimer above four times the upper limit. For patients stratified as having critical COVID-19 needing ICU care, no difference was seen between groups in the primary outcome, a composite of VTE, arterial thromboembolism (ATE), and death (178).

In contrast, when the multi-platform trial reported their results from patients with non-critical COVID-19, high compared to low or intermediate dose of LMWH demonstrated benefits, and these results have been replicated by other investigators (178-181). This is contradictory, as one may think that the highest dose of LMWH would be of most benefit for the ICU patients who have the highest risk of TE. The multi-platform investigators speculate that if microvascular thrombosis is an important contributor to the burden of organ injury, then high dose thromboprophylaxis may provide the most benefit early on, before the injury becomes excessive. Patients admitted to the ICU may already have irreversible organ injuries that can no longer be remedied by higher doses of thromboprophylaxis. Furthermore, it may be

possible that the anti-inflammatory and possible anti-viral properties may be of different importance in the different stages of the disease (131, 182-184).

It is not only severity, but also the disease stage that is likely to be of importance for coagulopathy. TE seems to be an earlier complication than bleeding for patients with critical COVID-19 (185, 186). This could be due to a decreased need for anticoagulation in the later stages of intensive care, when inflammation and the proposed immunothrombosis have been mitigated and, therefore, the sensitivity to heparins is increased.

One consistent conclusion from the above-mentioned RCTs is an overall low risk of bleeding, no matter what dosing regimen was used. However, it must be taken into account that criteria excluding patients with a high risk of bleeding were applied in all studies. It is also possible that some bleeding may occur as pulmonary alveolar hemorrhage secondary to micro thrombosis, and this is mainly a diagnosis made by the pathologists in the autopsy lab and therefore difficult to detect (187).

A striking difference between the results from the RCTs compared to earlier observational studies is the change in VTE incidence (Table 3). In the RCTs, both in the intervention arms and for the controls, the incidence of VTE was low, 3.3–11.1%. This contrasts with the initial reports, with the most important being summarized in a meta-analysis concluding a TE incidence of 27.9% of patients with critical COVID-19. The low incidence in the RCTs is also comparable to critically ill patients in the pre-COVID-era (8.2%) (27, 120). One potential explanation for the variation in TE incidence among COVID-19 patients could be differences in concomitant treatment with glucocorticoids, which can be seen in Table 3. Glucocorticoids became the standard of care in the mid 2020, after the initial reporting of high VTE incidence and before or just around the time when most of the large RCTs started recruiting. A potential explanation could be that treatment with glucocorticoids may attenuate the immunothrombosis.

To conclude, until this day, no dose of thromboprophylaxis has shown a survival benefit for patients with critical COVID-19. Some studies have shown a decreased incidence of TE when treating patients with intensified thromboprophylaxis. However, this has been outweighed by the numerically higher risk of major bleeding with higher compared to lower doses of LMWH, even though the absolute risk is still low (188). Guidelines conclude that the standard dose of LMWH should be used for thromboprophylaxis (164). The Swedish Society of Thrombosis and Hemostasis recommends the standard dose of LMWH, both to patients with critical and non-critical COVID-19, even though higher doses have been shown to be associated with a decreased risk of disease progression and a lower mortality for the non-critical patients (189). They state that with the now circulating Omicron variant and a frequent prescription of glucocorticoids, the number of patients progressing to respiratory failure requiring mechanical ventilation is much lower than before and, therefore, the added effect of intensified thromboprophylaxis to non-critically ill patients can be questioned. This statement may also be supported by vaccination, which has been shown to lowering the risk for VTE in hospitalized patients with COVID-19, since 86.4% of the adult Swedish population is as of March 2023 vaccinated with at least two doses (190, 191).

Table 3. Comparison of the incidence of death, thromboembolism, and bleeding between studies by severity of disease, dose of thromboprophylaxis, and concomitant treatment with glucocorticoids.

	Meta-analysis by Jimenez (27)		Our first study at Södersjukhuset **(168)**			Our second study at Södersjukhuset **(192)**		Perepu **(176)**		INSPIRATION **(175)**		Multi-platform trial of REMAP-CAP, ATTACC, ACTIVE-4 **(177, 179)**				RAPID **(180)**		HEP-COVID **(178)**		PROTECT (55) (non-COVID-19)
Study design	Systematic review and meta-analysis of 49 observational prospective and retrospective studies		Retrospective observational study			Retrospective observational study		Randomized controlled trial		Randomized controlled trial		Randomized controlled trial				Randomized controlled trial		Randomized controlled trial		Randomized controlled trial
Number of patients included	18,093		152			165		176		592		2219		1098		465		83		3764
Time period for inclusion	January 1 to July 31, 2020		March 6 to April 30, 2020			March 6 to July 15, 2020		April 26, 2020 to January 6, 2021		July 29 to November 19, 2020		April 21, 2020 to January 22, 2021				May 29, 2020 to April 12, 2021		April 21 to December 19, 2020		2006-2010
Patients included	Ward	ICU	ICU			ICU		ICU or coagulopathy		ICU		Ward		ICU		Ward and increased fibrin-D-dimer		Ward, ICU and increased fibrin-D-dimer		ICU
Type of thromboprophylaxis	NA		tinzaparin			tinzaparin		enoxaparin		enoxaparin		UFH or LMWH				UFH or LMWH		UFH or LMWH		UFH or LMWH
Dose of thromboprophylaxis	NA	Different regimes[1]	Low	Intermediate	High	Intermediate	High	Low	Intermediate	Low	Intermediate	Usual care	High	Usual care[2]	High	Low	High	Low	High	Low[3]
Death (%)	NA		38.8 (28 days)	25.0 (28 days)	13.5 (28 days)	19.6 (90 days)	19.2 (90 days)	21 (30 days)	15 (30 days)	40.9 (30 days)	43.1 (30 days)	8.2 (hospital)	7.3 (hospital)	35.5 (hospital)	37.3 (hospital)	7.6 (28 days)	1.8 (28 days)	25.0 (30 days)	19.4 (30 days)	23.3 (hospital)
TE (%)	VTE 7.1 (hospital)	VTE 27.9 (hospital)	17.9	18.8	2.7	14.1 (28 days)	11.0 (28 days)	VTE 7 ATE 3	VTE 8 ATE 6	VTE 3.5	VTE 3.3	2.7	1.4	11.1	7.2	VTE 2.5 ATE 0.4	VTE 0.9 ATE 0	29.0	10.9	9.0
Major bleeding (%)	3.9 (hospital)		4.5	4.1	0	3.2 (28 days)	1.4 (28 days)	2	2	1.4	2.5	0.9 (14 days)	1.9 (14 days)	2.3 (14 days)	3.8 (14 days)	1.7	0.9	1.6	4.7	5.6
Concomitant treatment with glucocorticoids (%)	NA		37.3[4]	45.8[4]	29.7[4]	30.4	19.2	78	72	91.6	94.9	63.3	60.6	82.5	81.6	68.4	70.6	75.6	87.4	NA

Follow up periods for TE and major bleeding are the same as follow up periods for death within the same study unless indicated otherwise. [1] In four studies less than 40% received thromboprophylaxis, in 19 studies more than 70% received a low dose thromboprophylaxis, and in eight studies patients received an intermediate or a high dose thromboprophylaxis. [2] For patients with usual care 51% received intermediate dose, 41% low dose, and 8% subtherapeutic or therapeutic dose of thromboprophylaxis. [3] Randomized to a low dose of LMWH or UFH. [4] All systemic glucocorticoids during ICU stay, including glucocorticoids not prescribed for COVID-19

3 RESEARCH AIMS

The overall aim of this thesis was to investigate the treatment of coagulopathy in patients with critical COVID-19.

The specific aims were:

- To evaluate the association between the initial dose of thromboprophylaxis and the risk of death, thromboembolism, and bleeding in patients with critical COVID-19.

- To investigate if 12 mg versus 6 mg dexamethasone daily reduced the risk of death, thromboembolism, and bleeding in patients with critical COVID-19.

- To study the activity of LMWH, measured by anti-Factor Xa, and the association with death, thromboembolism, and bleeding in patients with critical COVID-19.

4 MATERIALS AND METHODS

4.1 ETHICAL CONSIDERATIONS

All studies were approved by the Swedish Ethical Review Authority and were performed in accordance to the Declaration of Helsinki. The three main principles of the declaration are as follows: the welfare of the patients should always be prioritized over the interest of science and society, all individuals participating in research should be informed and give their consent, and that confidential patient data must be handled in the most careful way to minimize the impact on the patients' physical and mental integrity and personality.

Studies I, II, and IV had no intervention affecting the care of the patients and the retrospective nature of the study made it impossible to collect informed consent for using patients' data. Therefore, the studies were approved with a waiver of informed consent, as the trials were deemed to have a minimal risk for the patients.

Study III was a *post-hoc* analysis of a drug intervention, and patients were required to provide informed consent before entering the study. For studies including patients with a severe condition, for example patients with critical COVID-19, the emphasis on "informed" cannot be underlined enough, as it is very difficult to understand and process information during this time. This puts high demands on judgment of the enrolling investigator/clinician.
The reference numbers for ethical approvals and amendments are outlined in the respective studies.

4.2 STUDY DESIGN, EXPOSURE AND OUTCOME

Table 4. Study design and outcome for Studies I to IV.

	Study I	Study II	Study III	Study IV
Design	Retrospective observational cohort study	Retrospective observational cohort study	*Post-hoc* analysis of international, randomized controlled trial	Multicenter retrospective observational cohort study
Study period	March to April 2020	March to July 2020	August 2020 to May 2021	March 2020 to May 2021
Setting	Two intensive care units at Södersjukhuset, Stockholm	Two intensive care units at Södersjukhuset, Stockholm	Intensive care units at 17 Danish and two Swedish hospitals	Intensive care units at Södersjukhuset, Stockholm, Karolinska University Hospital, Stockholm, and Lund University Hospital, Lund
Number of patients included	152 patients	165 patients	357 patients	408 patients
Exposure/intervention	High, intermediate, or low dose low-molecular-weight heparin as thromboprophylaxis	High or intermediate dose low-molecular-weight heparin as thromboprophylaxis	12 or 6 mg dexamethasone daily	Acquired levels of anti-Factor Xa
Outcome	Death, thromboembolism, bleeding, and major bleeding within 28 days	Death within 90 days, thromboembolism, bleeding, and major bleeding within 28 days	Death or thromboembolism, thromboembolism, major bleeding, and bleeding during intensive care stay	Death within 90 days, thromboembolism, bleeding, and major bleeding within 28 days
Statistical analyses	Kruskal-Wallis Fisher's exact test Log rank test Cox proportional hazards regression	Kruskal-Wallis Fisher's exact test Log rank test Cox proportional hazards regression	Kruskal-Wallis Fisher's exact test Logistic regression Cox proportional hazards regression	Logistic regression Analysis of variance Chi-square test Spearman's correlation test

4.3 TRIAL SITES AND PARTICIPANTS

All four studies included patients from Södersjukhuset. In studies I and II, patients from the two ICUs at Södersjukhuset were included, while studies III and IV were multicenter studies. Study III was a *post hoc* study on an international, multicenter study with patients from both Sweden and Denmark, and study IV included patients from three hospitals in Sweden (Table 5).

Table 5. Hospital, city, and country of patients included in the studies.

Hospital name	Number of patients in Study I (%)	Number of patients in Study II (%)	Number of patients in Study III (%)	Number of patients in Study IV (%)
Södersjukhuset, Stockholm, Sweden	152 (100)	165 (100)	52 (15)	132 (32)
Linköping University Hospital, Linköping, Sweden			5 (1.4)	
Karolinska University Hospital, Stockholm, Sweden				227 (56)
Lund University Hospital, Lund, Sweden				49 (12)
Bispebjerg Hospital, Copenhagen, Denmark			9 (2.5)	
Herlev Hospital, Copenhagen, Denmark			53 (15)	
Regional hospital Gœdstrup, Herning, Denmark			2 (0.6)	
Nordsjællands Hospital, Hillerœd, Denmark			19 (5.3)	
Regional Hospital of north Jutland, Hjœrring, Denmark			1 (0.3)	
Holbæk Hospital, Holbæk, Denmark			1 (0.3)	
Hvidovre Hospital, Copenhagen, Denmark			19 (5.3)	
University Hospital of Zealand, Kœge, Denmark			44 (12)	
Kolding Hospital, Kolding, Denmark			31 (8.7)	
Nykœping Falster Hospital, Nykœping Falster, Denmark			3 (0.8)	
Odense University Hospital, Odense, Denmark			24 (6.7)	
Sœnderjylland Hospital, Aabenraa, Denmark			1 (0.3)	
Aalborg University hospital, Aalborg, Denmark			54 (15)	
Rigshospitalet, Copenhagen, Denmark			18 (5.0)	
University hospital of Zealand, Roskilde, Denmark			1 (0.3)	
Slagelse Hospital, Slagelse, Denmark			18 (5.0)	
Aalborg University hospital, Thisted, Denmark			2 (0.6)	
Total number of patients	152	165	357	408

The inclusion criteria in all studies were adult patients (≥ 18 years of age), with a positive polymerase chain reaction for SARS-CoV-2, admitted to the ICU because of critical COVID-19 with available data. In studies I and II, patients were excluded if treatment with LMWH was not started on the first day of the ICU admission. Moreover, patients needed to be treated for at least one day, excluding patients with a very brief ICU stay. For inclusion in study I, patients with an initial low, intermediate, or high dose LMWH were included. In study II, only patients with intermediate and high doses were included. In study III, LMWH treatment was not a requirement. In study IV, LMWH treatment in steady state at the time of aFXa sampling was required. Since TE and bleeding were outcomes, we excluded patients already at ICU-

admission diagnosed with TE or major bleeding (and bleeding disorders in study II). We defined the diagnoses as being present at admission if it was made before or during the first ICU day. We chose this since a diagnosis made the same date as admission date was probably at least part of the reason for ICU admission, even if not discovered prior to admission. Study III, being a *post hoc* study of an RCT, had additional criteria for inclusion: ongoing treatment for hypoxia with at least 10 L/min of oxygen, non-invasive mechanical ventilation, continuous positive airway pressure, or invasive mechanical ventilation; for exclusion: previously randomized to the COVID STEROID 2 trial, already received glucocorticoids for COVID-19 for more than four consecutive days, treatment with glucocorticoids in doses higher than 6 mg dexamethasone for an indication other than COVID-19, a diagnosis of active tuberculosis or active fungal infection, hypersensitivity to dexa-/betamethasone, or if they were pregnant. Also, study III differed slightly from studies I, II and IV, as we included patients from randomization in ICU and not ICU admission, although the median difference between the two was only one day.

4.4 BLOOD ANALYSES

In all studies, we have presented the results of blood samples at ICU admission. All blood samples were analyzed as routine tests in the respective hospital laboratories. In study II, patients were stratified by fibrin-D-dimer less than or equal to or greater than twice the upper limit of normal. The reference value used in our laboratories for fibrin-D-dimer was less than 0.5 in patients younger than 50 years of age and 0.01 times age (in years) in patients 50 years or older. In study IV, the results of all aFXa during the ICU stay, or a maximum of 28 days from ICU admission, were collected. The aFXa-assay is illustrated in Figure 7.

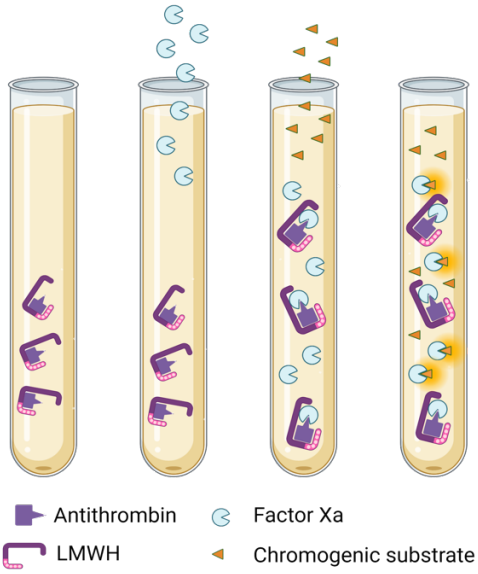


Figure 7. Factor Xa inhibition by heparin. The blood sample contains the heparin administered to the patient and endogenous antithrombin. Factor Xa is added and will be inhibited by the heparin-antithrombin complex. The more heparin-antithrombin complex in the sample, the greater proportion of the added factor Xa will be inhibited. To measure the proportion of the active factor Xa remaining, a chromogenic substrate is added. Binding to active factor Xa leads to the hydrolysis of the chromogenic substrate, activating its chromogenic properties. The activated chromogenic substrate is measured by chromogenicity at 405 nm and the amount is inversely proportional to the heparin-antithrombin activity. By calibrating the analysis for the drug of interest, low-molecular-weight heparin, the value of aFXa will be presented as the activity of low-molecular-weight heparin. Illustration made in BioRender for this thesis.

The first aFXa to be analyzed for each patient was sampled after at least four doses of LMWH, when a steady state was assumed. aFXa was sampled at various times. Peak values were defined as blood sampled at 3 (± 1) hours after administration of subcutaneous LMWH, and trough values were defined as blood sampled at 12 (± 2) hours after subcutaneous administration of LMWH. Values after diagnosis of TE or major bleed were excluded.

Within the categories of peak and trough, samples of aFXa were used to generate a minimum and maximum value during the ICU stay and a median value during the first 14 ICU days for each patient. If a patient only had one value, this was classified as both minimum and maximum, and also median, if sampled during the first 14 ICU days. With this categorization, all patients in the study had between two (minimum and maximum for either peak and trough) and six aFXa values (minimum, median, and maximum for both peak and trough).

4.5 DOSING OF THROMBOPROPHYLAXIS

Due to the reports and the experience from the beginning of the pandemic, new local guidelines were established, encouraging intensified thromboprophylaxis. These guidelines continued to develop during the first half of 2020 in the study hospitals. In all four studies, patients were categorized according to initial dose; low, intermediate, or high dose of LMWH.

Low dose is also referred to in the literature as the standard thromboprophylactic dose and high dose as the therapeutic since it is the same dose as is used when treating thromboembolism. Intermediate dose is defined as everything in between, but in the guidelines, it is often a standard low dose, somewhat adjusted for weight, administered twice daily, instead of only once daily. Because of the differences between the types of LMWH seen in Table 2, these are not considered fully interchangeable. The categorization for what is considered low/intermediate/high dose is based on dosage recommendations for other indications, as provided by the pharmaceutical companies producing the different LMWH. In studies I, II, and IV, the LMWH doses were classified according to Table 6.

Table 6. Classification of thromboprophylaxis with different low-molecular-weight heparins by daily dose.

	low dose	intermediate dose	high dose
tinzaparin, IU[a]	2500- 4500	>4500 IU but <175 /kg of body weight	≥175 /kg of body weight daily
dalteparin, IU[a]	2500- 5000	>5000 IU but <200 /kg of body weight daily	≥200 /kg of body weight daily
enoxaparin, mg	≤ 40	>40 mg but <2 /kg of body weight	≥2 /kg of body weight

[a]*International units*

For study III, the data from our local database was merged with the Danish data. The Danish sites used a different classification for what was considered a high dose for enoxaparin, 1 mg/kg daily, but the same definition for a high dose of tinzaparin and dalteparin. For low and intermediate doses, the definitions were in agreement for all types of LMWH.
Initially, at both ICUs at Södersjukhuset, the standard low dose LMWH was used for all patients, including COVID-19 patients. In April 2020, the recommendations were altered to an intermediate dose for COVID-19 patients and then finally, to a high dose thromboprophylaxis. In one of the ICUs a high dose was only used for one week, and then recommendations were altered to intermediate dose thromboprophylaxis again. At Karolinska University Hospital and

Lund University Hospital, thromboprophylaxis with LMWH was intensified from low to intermediate dosing also in April 2020. Danish patients received the standard low dose LMWH up until April 20, 2020, after which there were new national guidelines recommending intermediate or high dose LMWH.

Furthermore, the guidelines at Södersjukhuset, Karolinska University Hospital, and Lund University Hospital had recommendations for monitoring the activity of LMWH by measuring aFXa. At one of the ICUs at Södersjukhuset ICU and at Lund University Hospital, aFXa values for all patients were to be monitored, and the LMWH dose should be adjusted if the aFXa was not within target. For patients at Södersjukhuset´s other ICU (medical) and at Karolinska University, aFXa monitoring was recommended only if the treating clinician suspected either the dose was too low, or drug accumulation. Target values and time between the administration of LMWH and blood sampling for both peak and trough values were defined locally and are summarized in Table 7.

Table 7. Target anti-Factor Xa values for thromboprophylaxis following local guidelines from Södersjukhuset, Lund University Hospital, and Karolinska University Hospital.

	Södersjukhuset ICU	Lund University Hospital	Södersjukhuset medical ICU and Karolinska University Hospital
Time between sc administration of LMWH and sampling of aFXa for peak values (hours)	NA	2 to 4	3 to 4
Target peak value (kIU/L)	NA	0.3 to 0.6	0.3 to 0.5
Time between sc administration of LMWH and sampling of aFXa for trough values (hours)	10-14	NA*	NA*
Target trough value (kIE/L)	0.4 to 0.6	NA	0.1 to 0.3

*A trough value is sampled before the next dose. So even if not defined, this would mean after 12 hours with a two-dose regime.

The definition of treatment with glucocorticoids differed between study I and studies II–IV. During the first spring of the pandemic, glucocorticoids were not yet a recommended treatment. Therefore, no patients in study I and most patients in study II were not treated with glucocorticoids. If they were, this was done at the clinician's discretion or for another indication than COVID-19, for example, as an adjuvant to a vasopressor for a secondary bacterial sepsis. In study I, any glucocorticoid treatment during the ICU stay for any reason was included. However, in study II, we looked at individual dosing for every patient and only adjusted for glucocorticoids if it was given in concordance with what was later recommended for all COVID-19 patients needing respiratory support, defined as treatment with 6 mg or more of dexamethasone or equivalent glucocorticoid started within 7 days of admission to the hospital.

4.6 OUTCOMES

Studies I, II, and IV had death as the primary outcome. The follow-up period for death was only 28 days in the first study, since we felt it was important to publish our results fast, given the superior survival rate with one of the treatments. In studies II and IV, we used death within 90 days. Study III also had death as the primary outcome but in composite with thromboembolism. We chose a composite outcome, since the number of patients eligible for the *post hoc* study was smaller than expected, and we wanted to increase statistical efficiency.

The follow-up period for the primary endpoint in study III also differed from the other studies, as we only had complete data on patients during the ICU stay.

The secondary outcome was TE, bleeding, and major bleeding in all studies. PE/PT, DVT, and ischemic stroke, verified by computed tomography or ultrasound, were defined as TE in all studies. In study I, we also included other thrombotic events (defined as acute peripheral ischemia) and in study III, other thrombotic events and myocardial infarction (MI) were included. The reason for choosing only PE/PT, DVT, and ischemic stroke in studies II and IV was pragmatic, the diagnoses were more objective and therefore reduced the risk for misinterpretations when registered from the electronic health records (EHR). However, during the time period for inclusion in studies I and II, computed tomography was not easily available because of the fear of spreading the virus and also due to the lack of personnel, equipment, and experience to transport patients to the radiology suite safely. Therefore, in a few cases, PE/PT diagnosis was defined by a strong clinical suspicion including findings of acute strain of the right heart on echocardiography, combined with fibrinolytic treatment for PE/PT. In study III, we adapted the Swedish data to the available, but limited, Danish data, by rechecking the EHR so the definition of TE was consistent between the databases (193).

In studies I, II, and IV, we used the WHO bleeding scale to define and grade the severity of bleeding (194-196). The WHO bleeding scale grades bleeding from 1 to 4 as follows: (1) petechiae, tissue hematoma, oropharyngeal bleeding, (2) mild blood loss, hematemesis, macroscopic hematuria, hemoptysis, joint bleeding, bleeding at invasive sites, (3) gross blood loss requiring red blood cell transfusion and/or hemodynamic instability, and (4) debilitating blood loss, severe hemodynamic instability, fatal bleeding, or central nervous system bleeding. The WHO bleeding scale scores 1 to 4 defined the category “any bleeding” and scores 3 and 4 defined “major bleeding.” This definition with only two types of bleeding, any and major, was used as outcomes when merging with the Danish data in study III. This agreed well, since the Danish database had used a similar definition: any bleeding event or major bleeding events, defined as bleeding requiring transfusion of at least two units of red blood cells and/or intracranial bleeding and/or bleeding resulting in the need for a major therapeutic intervention (193).

All secondary outcomes in studies I, II, and III were followed for 28 days from the ICU admission since TE and bleeding later than that was deemed unlikely to be associated with the LMWH treatment in the ICU for most patients. Secondary outcomes in study III were followed only during the ICU stay for the same reason as this was the follow-up period for the primary outcome.

4.7 STATISTICAL ANALYSIS

Descriptive statistics were used to summarize the baseline and follow-up data with numbers and proportions (%) for categorical data, and medians and interquartile range (IQR) for continuous data.

When analyzing differences in continuous variables between groups, non-parametric tests were used, Mann-Whitney U-test when comparing two groups and Kruskal-Wallis test when

comparing three groups. When analyzing the differences between the categories of nominal data, Chi-square was used when it was judged to be a sufficient number of observations, otherwise, Fisher's exact test was used.

In studies I, II, and III, survival analysis was used. Kaplan-Meier curves illustrated the cumulative risk with log rank tests to compare the groups, and Cox proportional hazard was used as it enables adjusting for pre-defined confounders and baseline characteristics. The results of the Cox proportional hazard were presented both as crude and adjusted hazard ratios. In studies I and II, patients were right-censored at death or on day 28/90, whatever occurred first. In study III, patients were right-censored at death, when discharged from the ICU, or when withdrawing consent from study participation, whatever occurred first. Testing for violations of assumptions was done. We added splines to check for nonlinearity of included variables and by regressing scaled Schoenfeld residuals against survival time to check if hazards were proportional. Although no formal evidence was found for violation of nonlinearity in any study, indications resulted in continuous variables being transformed into categorical variables. If indication for non-proportional hazards was found, as in study I, the follow-up time was split, and an interaction term between exposure and follow-up time was included. Wald's test was used to test if interactions were significant.

Stratified analyses were done by dividing patients into subgroups using clinical factors that could theoretically impact the effect of the exposure, for example, level of respiratory support, time period of admission, and coagulation and inflammatory markers. Furthermore, sensitivity analyses were done to check the stability of the results, for example, when excluding patients who had a change in exposure, and when adding additional confounders, for example, glucocorticoids and renal function.

Study III was an RCT, and analysis was done with an intention to treat. Logistic regression with the results presented as odds ratios was used, in addition to Cox proportional regression. Odds ratios were presented both with and without adjusting for the variables used for stratification in the randomization (age and use of invasive mechanical ventilation at inclusion). Important laboratory values and their interactions were investigated in separate models, including splines, as no assumption was made of the nature of the association (linear vs. nonlinear).

Also, in study IV, logistic regression with splines was used because of not knowing if the assumption of linearity would be violated. The regression generated coefficients for the splines. The whole model was analyzed using ANOVA, which checked if any of the spline coefficients showed an association between exposure and outcomes. In study VI, only one possible confounder was identified. To investigate if the confounder was at all associated with the exposure, the non-parametric, Spearman's correlation test was done. This was chosen as neither the exposure nor the possible confounder were normally distributed.

Missing data did not require specific handling in studies II, III, and IV. In study I, we imputed data in a sensitivity analysis, since the BMI was missing for 6 patients. Imputation was done using chained iterations, which is a process when data is generated through an iterative series of predictive models using other variables in the data set.

Two-sided p-values of less than 0.05 were considered statistically significant throughout the thesis. Three statistical programming software suites were used for the analysis: STATA 13.1 (StataCorp), R v. 3.5.1 and v. 4.4.4 (R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.), and SPSS Statistics v. 28.0.0.0 (190) (IBM, 2021).

5 RESULTS

The detailed results are presented in the studies I–IV. The purpose of this section is to highlight the main results of each study.

5.1 STUDY I

Of 165 patients with critical COVID-19, 152 who were treated with different doses of LMWH remained after exclusion: 67 in the low dose group, 48 in the intermediate dose group, and 37 in the high dose group, according to the initial dose at ICU admission. The three groups did not differ in demographic or clinical characteristics.

5.1.1 Primary outcome

Mortality on day 28 was 38.8%, 25.0%, and 13.5% in the low, intermediate, and high dose group, respectively, p-value of 0.02 (Table 8).

Table 8. Outcome by initial dosing of thromboprophylaxis

	Total (n=152)	Low dose LMWH (n=67)	Intermediate dose LMWH (n=48)	High dose LMWH (n=37)	P-value
28-day mortality, no. (%)	43 (28.3)	26 (38.8)	12 (25.0)	5 (13.5)	0.02
Thromboembolism <28 days, no. (%)	22 (14.5)	12 (17.9)	9 (18.8)	1 (2.7)	0.04
Time to thromboembolism, median (IQR), days	8 (6 to 17)	8 (6 to 20)	8 (6 to 10)	11 (11 to 11)	0.61
Bleeding <28 days, no. (%)	16 (10.5)	8 (11.9)	7 (14.6)	1 (2.7)	0.16
Time to bleeding, median (IQR), days	13 (8 to 18)	16 (6 to 20)	11 (10 to 20)	1 (1 to 1)	0.36

The cumulative proportion of death differed between the groups, p-value of 0.02 (Figure 8).

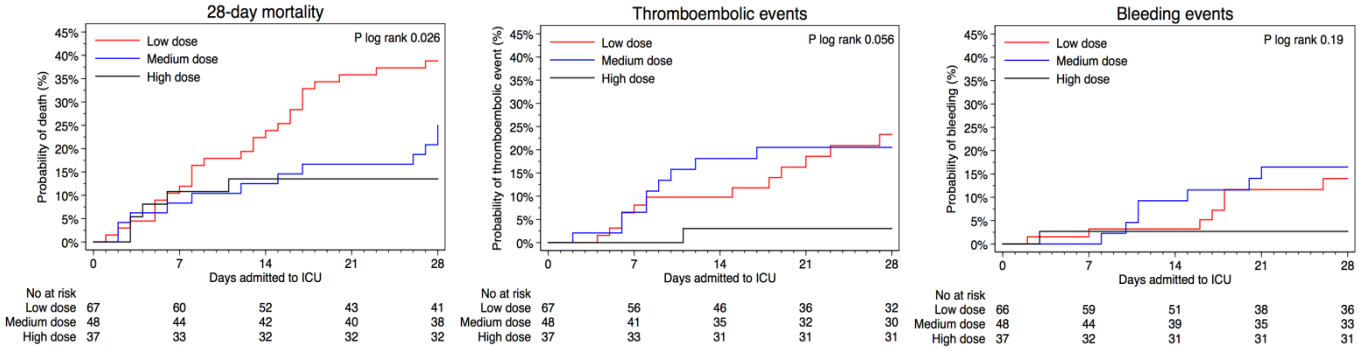


Figure 8. Kaplan-Meier-plots mortality, thromboembolic events, and bleeding events within 28 days by initial dosing.

The hazard ratios for death were 0.33 (95% CI 0.13–0.87) for the high dose group and 0.88 (95% CI 0.43–1.83) for the intermediate group compared to the low dose group with the multivariate model, adjusting for sex, age (continuously), body-mass index (</≥30 kg/m^2 and

missing [n=6]), invasive mechanical ventilation (yes/no), and Simplified Acute Physiology Score III (continuously), see Table 9. The high dose group had a lower hazard ratio for death compared to the low dose group, both in the crude analysis and the sensitivity analysis with glucocorticoids included in the model.

Table 9. Risk of death by initial dosing of thromboprophylaxis

		HR (95% CI) of death ≤28 days			
Initial dosing strategy of LMWH	No. of patients	Univariable model	Multivariable model	Multivariable model with glucocorticoids	Multivariable model among those who did not change dose (n=86)
High dose	37	0.31 (0.12 to 0.82)	0.33 (0.13 to 0.87)	0.32 (0.12 to 0.85)	0.33 (0.11 to 1.00)
Intermediate dose	48	0.59 (0.30 to 1.16)	0.88 (0.43 to 1.83)	0.83 (0.39 to 1.73)	1.15 (0.38 to 3.47)
Low dose	67	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)

5.1.2 Secondary outcomes

The proportion of thromboembolism was 17.9% with low dose, 18.8% with medium dose, and 2.7% with high dose, p-value of 0.04, see Table 8, with the cumulative risks displayed in Figure 8. The proportion of bleeding events did not differ significantly between the groups, 11.9%, 14.6%, and 2.7% in the low, intermediate, and high dose group, respectively (p = 0.16), see Table 8, with the cumulative risks displayed in Figure 8.

Of the 152 patients, 69 (45.4%) had at least one change in dose during the ICU stay (Figure 9). When excluding patients with a change in dose, high dose was still associated with an increased survival compared to low dose, HR 0.33 (95% CI 0.11–1.00), see Table 9.

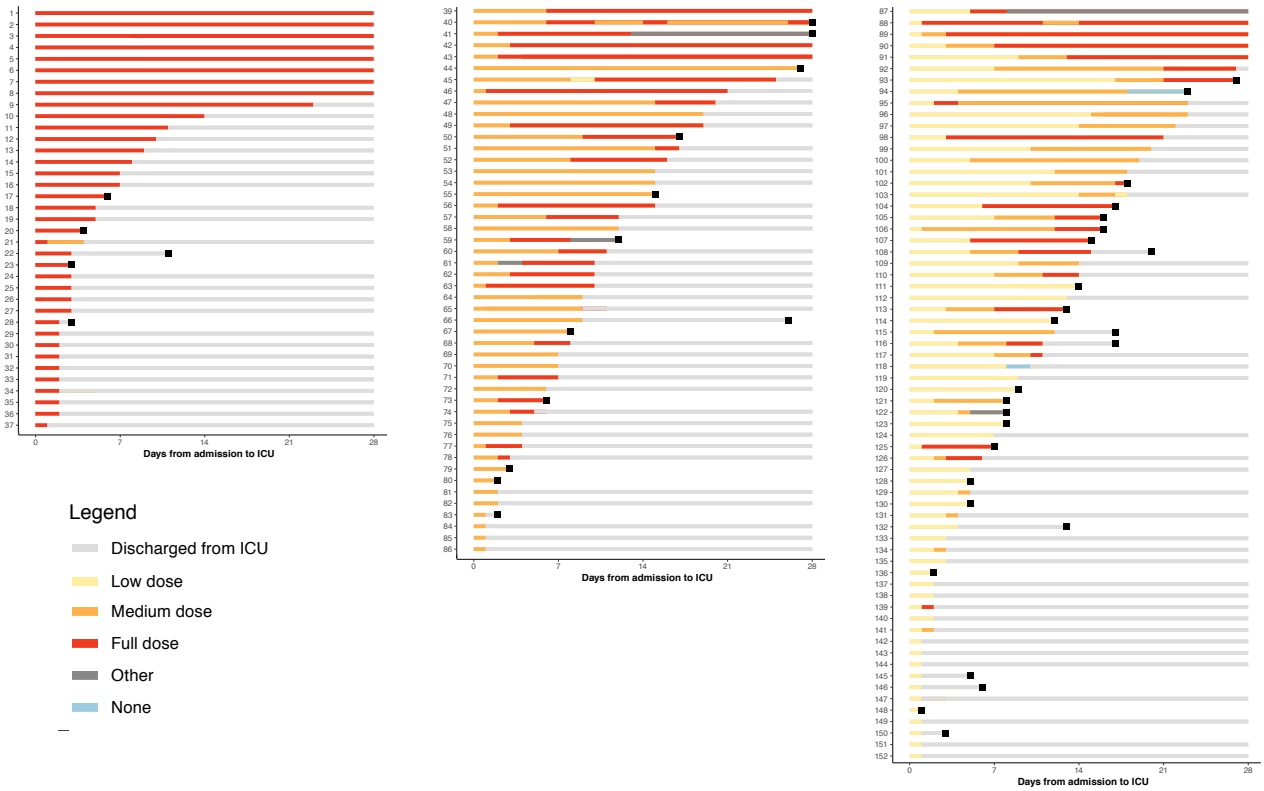


Figure 9. Dosing of low-molecular-weight heparin for all patients in study1 during the intensive care or maximum 28 days. Every line indicates a patient. Black squares indicate a patient's death.

5.2 STUDY 2

Out of 257 patients, 165 remained after exclusion: 92 in the intermediate dose group and 73 in the high dose group, according to the initial dose at ICU admission. The groups did not differ in demographic and clinical characteristics.

Table 10. Outcome by initial dosing of thromboprophylaxis

	Intermediate dose LMWH (n=92)	High dose LMWH (n=73)	P-value
90-day mortality, no. (%)	18 (19.6)	14 (19.2)	1.00
Thromboembolism <28 days, no. (%)	13 (14.1)	8 (11.0)	0.64
Time to thromboembolism, median (IQR), days	8 (6 to 10)	8 (2 to 19)	0.79
Bleeding <28 days, no. (%)	12 (13.0)	6 (8.2)	0.45
Time to bleeding, median (IQR), days	13 (10 to 18)	13 (3 to 15)	0.64

5.2.1 Primary outcome

Mortality at day 90 was 19.6% and 19.2% in the intermediate and high dose groups, respectively, p-value of 1.00 (Table 10). The cumulative proportion of death did not differ between the groups, p-value of 0.95 (Figure 10).

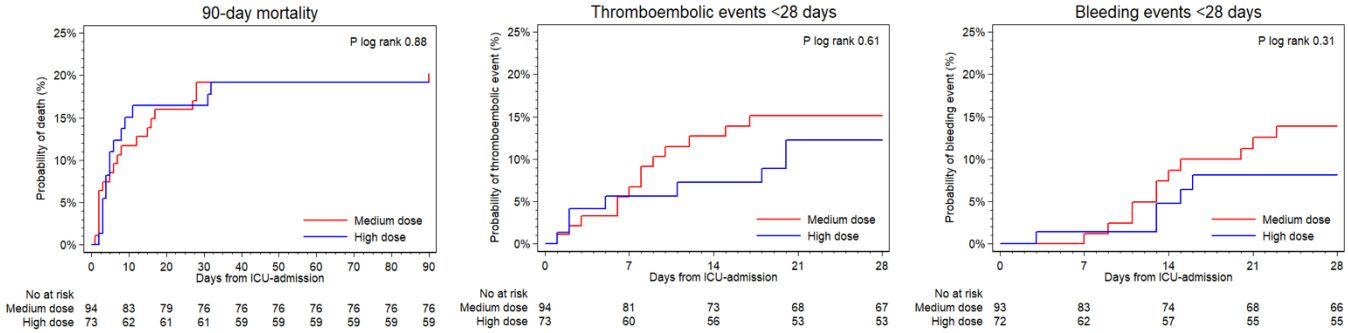


Figure 10. Kaplan-Meier plots of 90-day mortality, thromboembolic events, and bleeding events within 28 days, by initial dosing.

The HR did not differ between the groups in the univariable analysis, in the multivariable analysis when adjusting for sex, age (continuously), body mass index (</≥ 30 kg/m^2), invasive mechanical ventilation (yes/no), and Simplified Acute Physiology Score III (continuously), or when also adjusting for treatment with glucocorticoids for COVID-19 (Table 11).

Table 11. Risk of death by initial dosing of thromboprophylaxis

		HR (95% CI) of death ≤90 days			
Initial dosing strategy of LMWH	No. of patients	Univariable model	Multivariable model	Multivariable model with glucocorticoids for COVID-19	Multivariable model among those who did not change dose (n=135)
High dose	73	0.98 (0.49 to 1.97)	0.74 (0.36 to 1.53)	0.77 (0.36 to 1.65)	0.52 (0.23 to 1.20)
Intermediate dose	92	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)

There was a significant interaction between dosing of thromboprophylaxis and admission date, p = 0.047. In the stratified analysis, the adjusted HR for patients admitted before April 30, 2020 was 0.42 (0.15 – 1.15) and after April 30, 2020, it was 1.15 (0.33 – 3.99), for high dose compared to intermediate dose (Table 12). Even though no formal evidence was found for the interaction between dosing of thromboprophylaxis and fibrin-D-dimer (p = 0.09), a stratified analysis was done, resulting in an adjusted HR for patients with a fibrin-D-dimer below twice the upper limit of normal of 1.65 (0.52 – 5.24) and above twice the upper limit of normal of 0.36 (0.12 – 1.06) for high dose compared to intermediate dose.

Table 12. Risk of death in stratified analyses by initial dosing of thromboprophylaxis

	HR (95% CI) of death ≤90 days			
Initial dosing strategy of LMWH	Multivariable model for patients admitted before April 30, 2020 (n= 81)	Multivariable model for patients admitted after April 30, 2020 (n=84)	Multivariable model for patients with fibrin-D-dimer <2 times above reference (n=81)	Multivariable model for patients with fibrin-D-dimer ≥2 times above reference (n=80)
High dose	0.42 (0.15 to 1.15)	1.15 (0.33 to 3.99)	1.65 (0.52 to 5.24)	0.36 (0.12 to 1.06)
Intermediate dose	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)

5.2.2 Secondary outcomes

No significant difference was found in any of the secondary outcomes, absolute risk, cumulative proportions, or in the adjusted HRs (Table 10 and Figure 10). All investigations were done at the discretion of the treating clinician, and this resulted in 37% of the patients undergoing a computed tomography pulmonary angiography, 18% undergoing computed tomography for the brain, and eight percent undergoing ultrasound duplex.
Of the 165 patients included, 30 patients (18.2%) changed doses during the ICU stay (Figure 11).

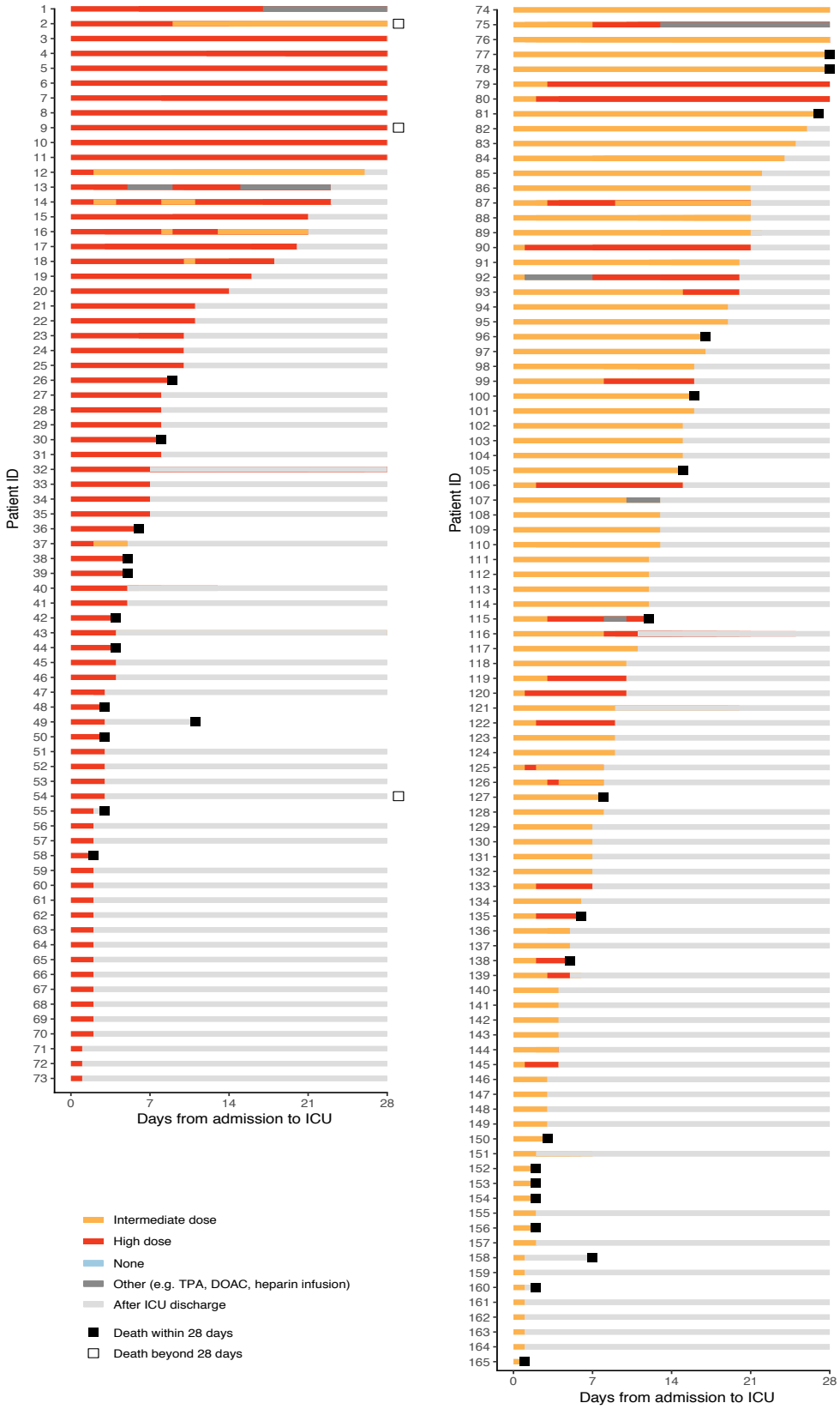
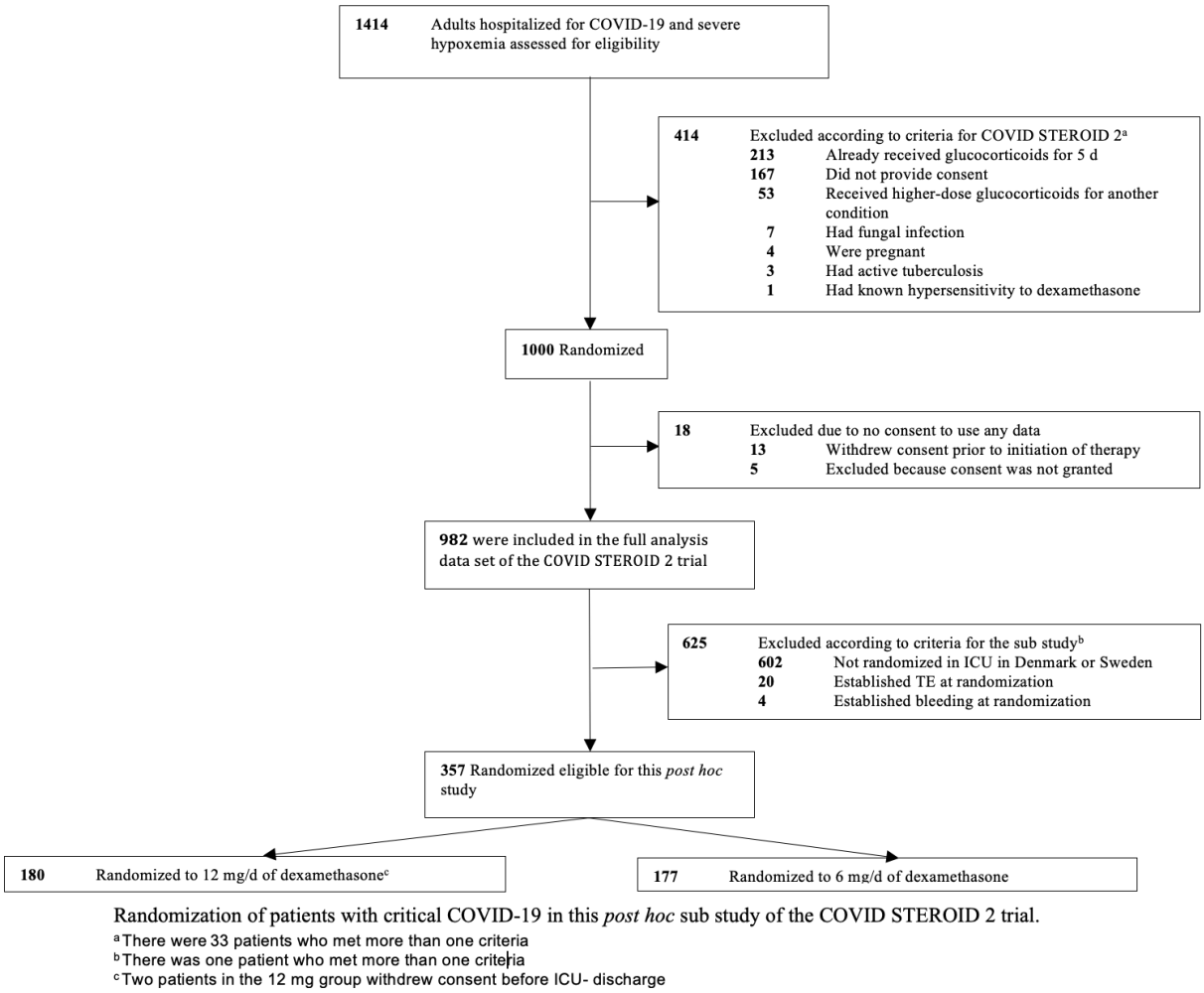


Figure 11. Dosing of LMWH for all patients during the intensive care or within a maximum of 28 days in study II.

5.3 STUDY III

Patients eligible for study III were those randomized in the COVID STEROID 2 trial for whom we had additional data on TE and bleeding, a total of 357 patients (Figure 12).



Randomization of patients with critical COVID-19 in this *post hoc* sub study of the COVID STEROID 2 trial.

[a] There were 33 patients who met more than one criteria
[b] There was one patient who met more than one criteria
[c] Two patients in the 12 mg group withdrew consent before ICU- discharge

Figure 12. Flow chart for patients included in study III

5.3.1 Primary outcome

The primary outcome, the composite of death and thromboembolism during the ICU stay, was met by 53 patients (29%) in the 12 mg group and 53 patients (30%) in the 6 mg group, p = 1.00 (Table 13).

Table 13. Outcome by daily dose of dexamethasone

Outcome during ICU stay	12 mg of dexamethasone (n=180)	6 mg of dexamethasone (n=177)	Absolute differences (%)	95% CI (%)	P-value
Death or thromboembolism, no. (%)	53 (29)	53 (30)	-0.50	-10 to 9.5	1.00
Death, no. (%)	40 (22)	40 (23)	-0.38	-9.4 to 8.7	1.00
Thromboembolism, no. (%)	18 (10)	18 (10)	0.17	-6.6 to 6.2	1.00
Major bleeding, no. (%)	10 (5.6)	10 (5.6)	-0.09	-5.0 to 4.8	1.00
Any bleeding, no. (%)	35 (19)	41 (23)	-3.7	-13 to 5.3	0.47

The cumulative proportions of death or TE did not differ, as displayed in Figure 13.

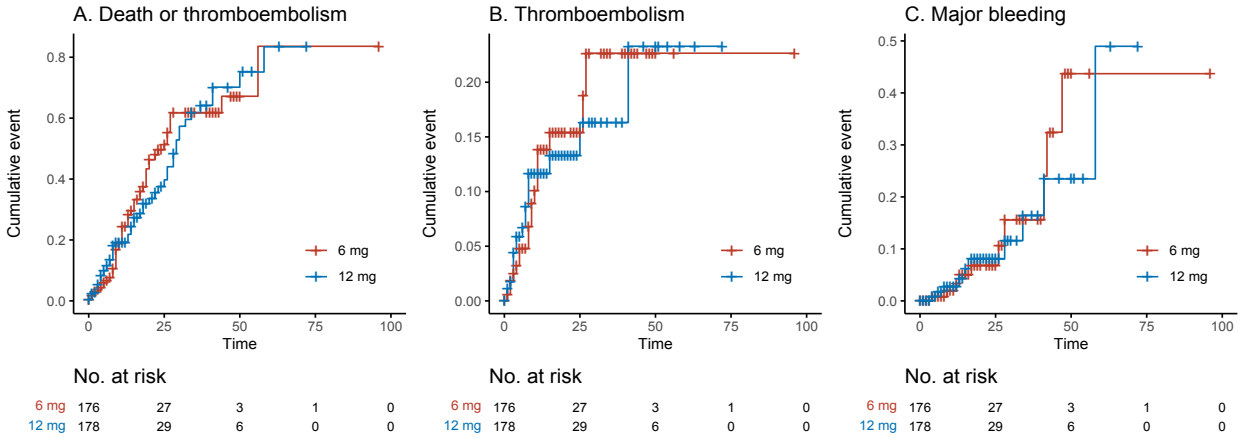


Figure 13. Kaplan-Meier plots of A. Death or thromboembolism, B. Thromboembolism, and C. Major bleeding during intensive care, by daily dose of dexamethasone.

When adjusting for stratification variables, age and invasive ventilation, the OR was 0.93 (95% CI 0.58 – 1.49) for death or thromboembolism (Table 14).

Table 14. Risk of death or thromboembolism, thromboembolism, major bleeding, and any bleeding during intensive care, by daily dose of dexamethasone.

		OR (95% CI) of outcomes during ICU stay			
Dose of dexamethasone	No. of patients	Death or thrombo-embolism	Thrombo-embolism	Major bleeding	Any bleeding
12 mg/d	180	0.93 (0.58 to 1.49)	0.97 (0.48 to 1.94)	0.97 (0.39 to 2.42)	0.78 (0.46 to 1.30)
6 mg/d	177	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)

5.3.2 Secondary outcomes

No differences were found between the 12 and 6 mg group for any of the secondary outcomes (Table 13, Figure 13, and Table 14). The most common TE was PE/PT for both groups, with an incidence of 7.8% and 9.6%, respectively, in the 12 and 6 mg group. Only two patients were diagnosed with MI, one with ischemic stroke (all in the 12 mg group), and no patients were diagnosed with DVT in either group. As with TE, bleeding sites were also similar between the groups, with the most common being upper airways, 7.2% and 9.0%, followed by upper gastrointestinal (GI), 5.0% and 5.1%, and iv lines and catheters, 5.0% and 4.5%, in the 12 and 6 mg group, respectively.

The continuous variables CRP and fibrin-D-dimer were investigated as possible interactions, and no significant differences were found (Figure 14).

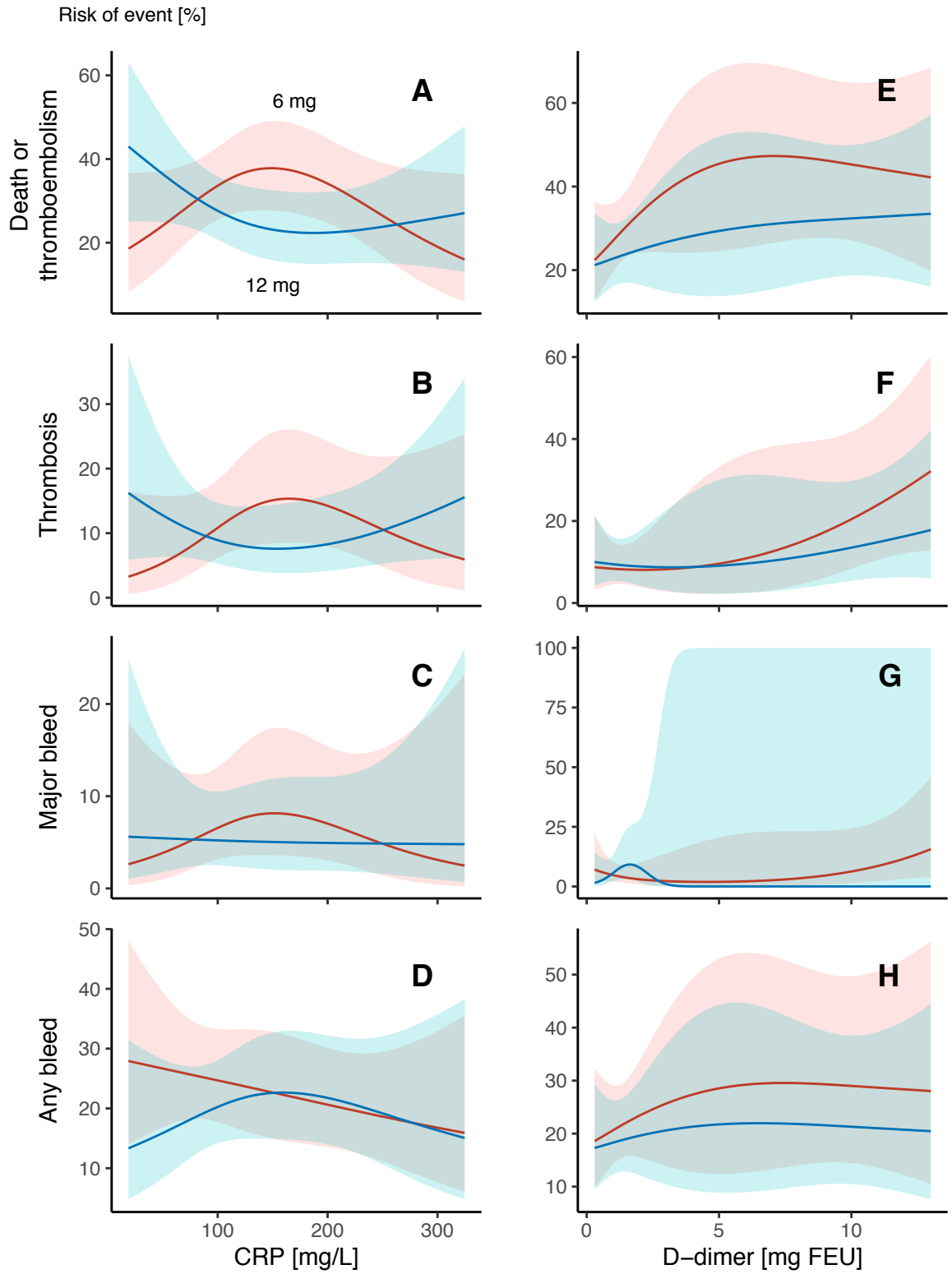


Figure 14. Outcome by C-reactive protein to the left and fibrin-D-dimer to the right as interaction with dose of dexamethasone. Red line indicating 6 mg of dexamethasone daily, and blue line indicating 12 mg of dexamethasone daily and the shaded area 95% confidence intervals.

5.4 STUDY IV

Out of 1,140 eligible patients with 7,302 collected aFXa values, 408 patients had values meeting our pre-defined definition for a valid peak or trough value (Figure 15). Patients with valid aFXa values had a longer ICU stay compared to patients with no valid values (Table 15).

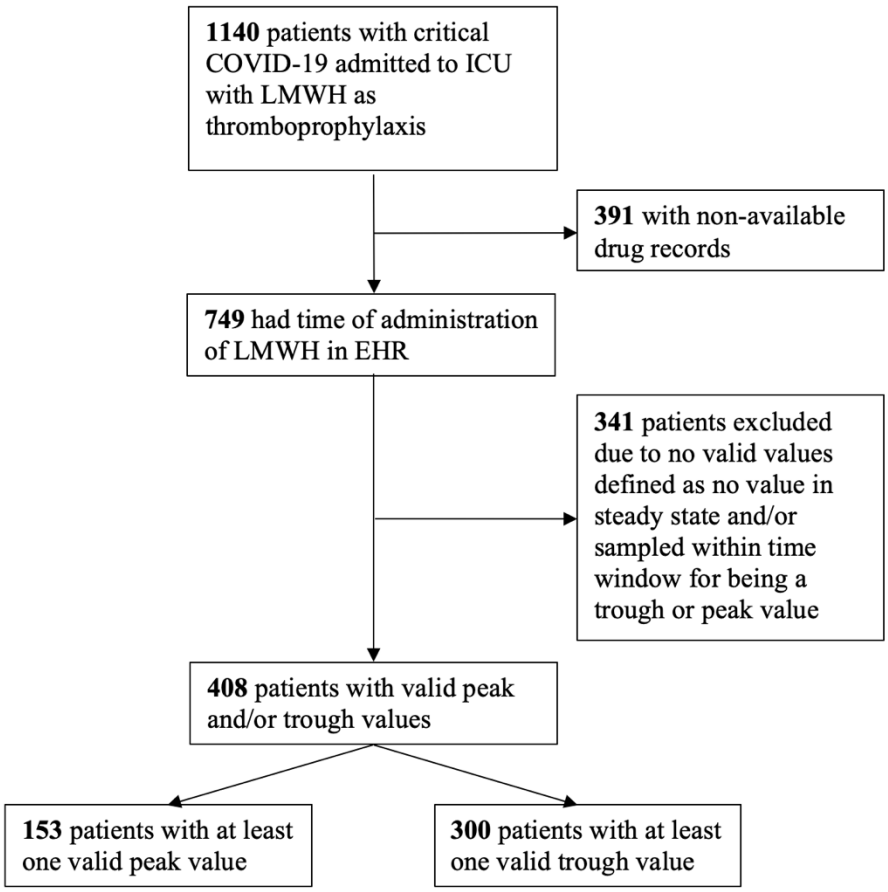


Figure 15. Flow chart for patients included in study VI.

5.4.1 Primary outcome

On day 90, 36% of the patients with peak values and 32% of the patients with trough values had died (Table 15). Peak values were not, but patients' median and maximum trough values were, associated with death, with a higher trough value increasing the risk, p-values of 0.03 and 0.002 respectively (Figure 16). When adding eGFR into the model, the association between the maximum trough value was still significant, but not the median value, p-values of 0.02 and 0.16.

Table 15. Outcomes for patients with no valid anti-Factor Xa values, patients with peak values, and patients with trough values.

Outcomes	Patients treated with thromboprophylactic LMWH (n=1140)	Patients with peak values (n=153)	Patients with trough values (n=300)
Duration of ICU stay, median (IQR), days	11 (5 to 22)	19 (13 to 30)	17 (9 to 28)
Death within 90 days, no. (%)	314 (28)	55 (36)	95 (32)
Thromboembolism within 28 days, no. (%)	160 (14)	17 (11)	37 (12)
Major bleeding within 28 days, no. (%)	72 (6.3)	15 (9.8)	23 (7.7)
Any bleeding within 28 days, no. (%)	288 (25)	48 (31)	100 (33)

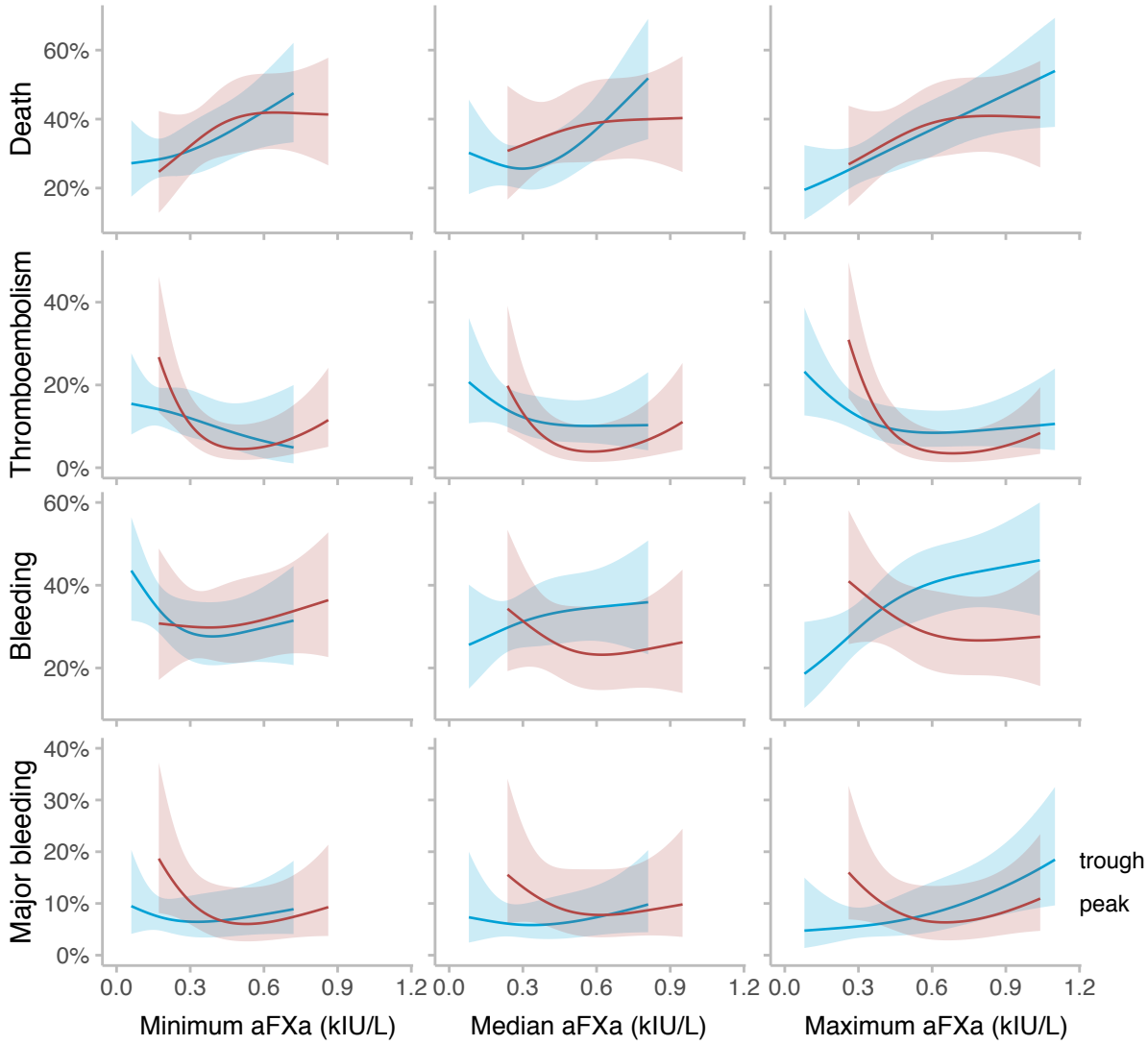


Figure 16. The association between anti-Factor Xa and death, thromboembolism, bleeding and major bleeding. Red lines represent peak values, and blue lines represent trough values, and the shaded area 95% confidence intervals. The figures illustrate anti-Factor Xa values when summarized as a minimum during intensive care (153 peak values and 300 trough values), median during the first 14 days of intensive care (126 peak values and 266 trough values), and maximum during intensive care (153 peak values and 300 trough values).

5.4.2 Secondary outcomes

Lower minimum, median, and maximum peak values were all associated with a higher risk of TE, p-values of 0.005, 0.01, and 0.001 (Figure 16). The association was stable when adding eGFR to the model for maximum peak values, but not for minimum and median peak values, p-values 0.004, 0.05, and 0.07, respectively. Testing of different cut-off values is presented in Table 16 and Figure 17. Patients with a minimum peak value at any point below 0.3 kIU/L had an OR of 5.1 (95% CI 1.8 to 14.4) for TE compared to patients with no value below 0.3 kIU/L.

Table 16: Minimum peak value and risk of thromboembolism

aFXa-cut-off (kIU/L)	Patients ever below out of 153, no	Odds ratios of thromboembolism if ever below cut-off, compared to patients with no values below (95 % CI)
<0.1	0	[a]
<0.2	15	3.5 (0.97 to 12.6)
<0.3	40	5.1 (1.8 to 14.4)[b]
<0.4	71	2.3 (0.81 to 6.6)
<0.5	98	2.0 (0.60 to 6.3)
<0.6	124	0.73 (0.22 to 2.4)
<0.7	133	0.67 (0.17 to 2.6)

Odds ratios for different cut-off-values of minimum peak aFXa for 153 patients admitted to the ICU due to critical COVID-19.
[a] No patients in one group [b] P-value = 0.003

Trough values were not associated with TE, and no cut off value could be identified which separates patients into different risk categories for TE.

For peak values, there were no associations with bleeding or major bleeding (Figure 16). Higher maximum trough values were associated with an increased risk of both bleeding and major bleeding, p-values of 0.01 and 0.02. The results were stable when adjusting for eGFR, p-values of 0.04 and 0.03. When investigating different cut-offs for maximum trough values, the odds for bleeding doubled if ever a value above 0.3 kIU/L, 0.4 kIU/L, 0.5 kIU/L, and 0.6 kIU/L and for major bleeding if ever above 0.5 kIU/L, 0.6 kIU/L, and 0.7 kIU/L compared to no value above these cut-offs (Table 17 and Figure 17).

Table 17. Maximum trough value and risk of bleeding and major bleeding

aFXa-cut-off (kIU/L)	Patients ever above out of 300, no	Odds ratio of bleeding if ever above cut-off compared to no value above (95 % CI)	Odds ratio of major bleeding if ever above cut-off compared to no value above (95 % CI)
>0.1	283	1.7 (0.53 to 5.3)	1.4 (0.17 to 10.7)
>0.2	246	1.5 (0.79 to 3.0)	2.4 (0.55 to 10.7)
>0.3	193	1.9 (1.1 to 3.3)[a]	2.8 (0.93 to 8.5)
>0.4	139	1.9 (1.2 to 3.1)[b]	1.9 (0.79 to 4.5)
>0.5	99	2.1 (1.3 to 3.4)[c]	2.4 (1.0 to 5.6)[e]
>0.6	62	2.1 (1.2 to 3.7)[d]	2.7 (1.1 to 6.6)[f]
>0.7	51	1.7 (0.90 to 3.1)	2.9 (1.2 to 7.3)[g]

Odds ratios for different cut-off-values of maximum trough aFXa for 300 patients admitted to the ICU due to critical COVID-19.
[a] P-value = 0.01 [b] P-value = 0.009 [c] P-value = 0.004 [d] P-value = 0.01 [e] P-value = 0.04 [f] P-value = 0.05 [g] P-value = 0.04

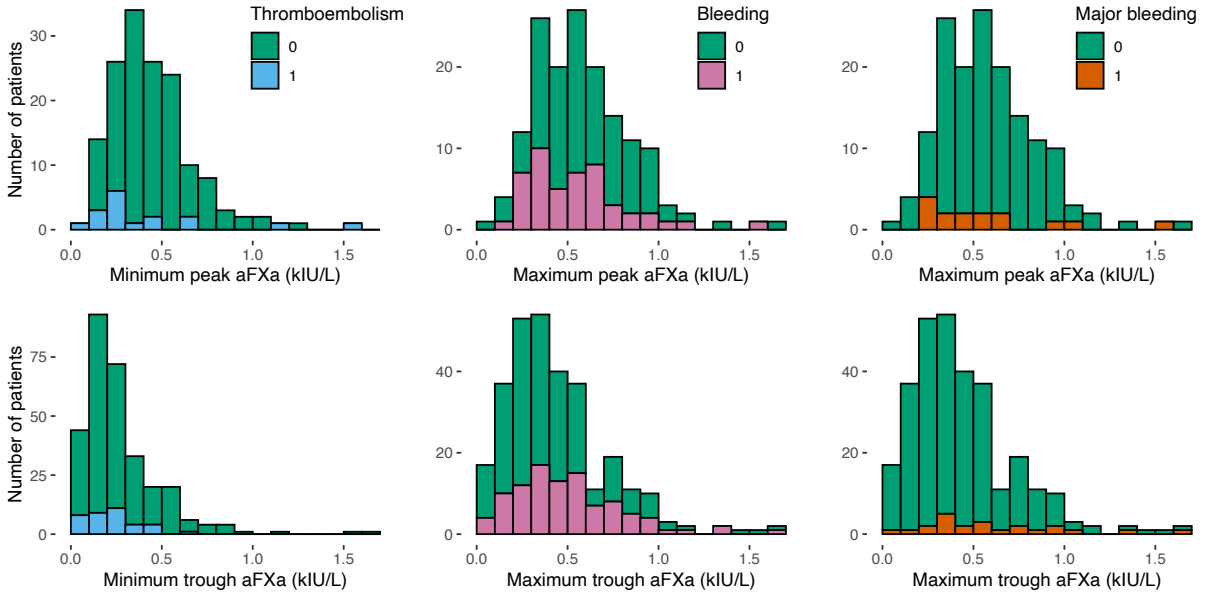


Figure 17. Distribution of patients' values of peak and trough for anti-Factor Xa and the outcomes of thromboembolism, bleeding, and major bleeding. Lowest values are visualized against the event of thromboembolism and the highest values against the event of bleeding and major bleeding. Green color indicates no event, blue color indicates thromboembolism, pink color indicates bleeding, and orange color indicates major bleeding, all within 28 days of intensive care admission.

6 DISCUSSION

The discussion of this thesis will first focus on the methodological consideration, with the emphasis on the limitation of the observational, retrospective study design used in studies I, II and IV and the *post hoc* analysis of the RCT in study III. This will be followed by a section of general discussion on the findings in each study and, finally, my interpretation of the main findings.

6.1 METHODOLOGICAL CONSIDERATIONS

6.1.1 Missing data

Even though we had missing data, there were very few missing data for the variables included in the statistical models. For studies I, II, and IV, we had continuous access to the EHR and could therefore go back and retrieve data if a specific data point was missing. This was done specifically in study II, when we needed to re-categorize the treatment with glucocorticoids, as prescribed according to the recommendations for COVID-19 or not. Also, in study IV, we could find additional values for baseline creatinine since the automatic extraction was not complete. However, in study I, we did impute missing data in a sensitivity analysis, since the BMI was missing for six patients, but as expected, this did not change the results.

6.1.2 Participants

6.1.2.1 Number of patients

Since all studies were retrospective, no power calculations were performed. Instead, we included all patients available to us at the time of the study. In the beginning of the pandemic, there were no networks for COVID-19 research, but as the studies progressed, so did the collaborations. Therefore, we could include more patients, also from other hospitals than Södersjukhuset, for each subsequent study.

The consequence of including too few patients can be a type II error, that is, there is a difference, but we could not find it. Neither in study II nor in study III did we find a significant difference in the results, but since the CIs are wide, we must consider that absence of evidence is not evidence of absence. However, in study III, the small differences in point estimate indicated that if there is a difference, it is probably small.

6.1.2.2 Inclusion and exclusion criteria

All inclusion and exclusion criteria must be carefully considered, as each can possibly lead to a selection bias and affect the generalizability.

We included adult patients with a positive polymerase chain reaction for SARS-CoV-2 admitted to the ICU because of critical COVID-19. The definition of critical COVID was a COVID-19 pneumonitis, with the symptom of respiratory failure. We carefully excluded patients infected by COVID-19, but for whom COVID-19 was not the reason for ICU admission.

In studies I and II, we excluded patients if they were discharged from the ICU on the same day as the ICU admission. This was done, as the most commonly used regime of LMWH was twice daily dosing and, therefore, the categorization of low, intermediate, or high dose was difficult if they had not received two doses.

During the pandemic, ICUs had to expand. To lessen the burden, intermediate care wards were developed. The definition of intensive care might have differed, depending on the organization of individual hospitals to meet the increased demand. When intermediate care beds were made available, the treatments that could be performed outside ICU were extended, for example, respiratory support with high flow nasal oxygen (HFNO). In normal, non-pandemic conditions, this may be a treatment most commonly used in the intensive care unit. Therefore, there is a possible selection bias, excluding patients, who, in some hospitals, would have been admitted to the ICU, but in our hospitals may have been treated in an intermediate care ward. In studies III and IV, to better define the state of our patients with critical COVID-19 and ease the comparison with other cohorts of COVID-19 patients admitted to the ICU, we collected baseline data for oxygen requirement, in the form of partial pressure of oxygen/fraction of inspired oxygen (PaO2/FiO2).

We excluded all patients with the outcome of TE or major bleeding already at the ICU admission, since we wanted to study thromboprophylaxis and not treatment of TE or patients being withheld thromboprophylaxis. However, TE at ICU admission for COVID-19 patients is not uncommon. The prevalence of PE/PT at hospital admission, when all patients were investigated with CTPA, was 15/106 (14%) patients in one study, including five patients directly admitted to the ICU, of whom one had PE/PT (197). When comparing this with the TE prevalence in our cohorts, our prevalence was lower. In studies I, II, and III, four (2.4%), ten (3.9%), and 20 (5.2%) patients were excluded from the analysis due to TE at ICU admission, respectively. In study IV, this was not fully investigated. However, for the cohort of patients with critical COVID-19 from Södersjukhuset and Karolinska University Hospital's preliminary results from a study investigating the differences in prognosis by PE/PT in different stages of COVID-19, the prevalence at ICU admission was 7.0% (80/1143 patients). Since PE/PT seems to be the dominant manifestation of TE, the prevalence of total TE can be estimated to be similar. The fact that so few patients in study I were excluded because of TE at admission is probably due to a lack of investigation, rather than no patients having TE, and might therefore introduce a surveillance bias. For study I, this could potentially be part of the explanation for why a high dose was superior to a low dose, since a proportion of patients with undiagnosed TE got a high, therapeutic dose even though their diagnosis was missed. Consequently, when interpreting the incidence of outcomes in our studies, this must be done recognizing that a proportion of patients with diagnosed TE already at ICU admission are not included. Since TE has been shown to increase the risk of death, this may explain why the mortality in our cohorts differs from other reports on patients with critical COVID-19 (28).

6.1.3 Exposure and statistical methods

The exposure in studies I and II was the initial dose of LMWH, which was prescribed by the treating clinician. This was also the case for study III, even though LMWH was not the exposure of interest. This is the first and foremost limitation of this thesis for four reasons:

(1) The initial dose may have changed during the ICU stay and, therefore, changed the exposure. We investigated the extent of dose changing by registering and presenting all changes in the dose for every patient during the ICU stay or a maximum of 28 days in studies I and II. In both studies, we also performed sensitivity analyses when excluding patients who had a change in dose, and the results were stable. However, as illustrated in Figure 9 and 11 almost all patients who had a change in dose, had an increase of LMWH. This meant a decrease of the differenes in exposure between groups, and, therefore, the findings in study I can be interpreted as even more remarkable. In study III, we did not have access to dosing data during the entire ICU stay, however, the dose of LMWH was presented mainly to describe the patients, and the categorization had no impact on the conclusion.

(2) Dosage may have been individualized due to patients' risk factors. For example, a lower dose prescribed for patients with a high risk of bleeding and a high dose prescribed if there was a clinical suspicion of a patient having a TE. In study I, we trusted clinicians to follow guidelines, but in study II, we tried to investigate this matter further. We looked at each individual patient to see if the dose recommended by the guideline applicable for that time, was prescribed or not. We found a total of 16 patients who were prescribed low or no dose thromboprophylaxis, even though they were admitted when higher doses were recommended. The motivation for three patients was bleeding or high risk of bleeding; for two, urgent surgery; and for two, accumulation/overdose of vitamin-K-antagonists at ICU admission. For the other patients, no reason could be found in the EHR. Since these patients were not included in the analysis, it may have caused an underestimation of the bleeding risk in the population. As in study I, we did not control if individual factors affected the decision to prescribe the dosing variants recommended at the time at ICU admission, but trusted clinicians to follow the guidelines.

(3) Dosage changed over time, but so did many other things pertaining to COVID-19 patients. Intensified dosing regimens were implemented in April 2020. It is possible, even likely, that we modified the treatment and care of patients with critical COVID-19 in other ways, also affecting the outcome. The mortality-lowering treatment of glucocorticoids was implemented in the beginning of June 2020, but it may be possible that clinical experience had proceeded the guidelines and therefore made treatment with glucocorticoids more common toward the end of the study period when more patients also had a higher dose of LMWH. Therefore, we chose to include glucocorticoids in the model to adjust for possible baseline differences between the dosing groups. An attempt to adjust for all confounders caused by time, identifiable as well as unknown, was done by dividing the time period in the first and second half and analyzing them separately. In study I, the HR for death with a high dose was still lower than for the low dose group, however, the results were no longer statistically significant. This was expected due to the few patients with a high dose in the first half of the period and with a low dose in the second half of the period.

(4) In studies I and II, the two ICUs at Södersjukhuset had different dosing guidelines, where one ICU recommended high doses and the other ICU recommended intermediate doses. Even though the cooperation between the two ICUs was robust, with weekly doctors' meetings, it is possible that patient selection and/or other

differing components of the intensive care, in addition to different LMWH dosing, could explain the results.

The exposure for patients in study III was a randomization to 12 or 6 mg dexamethasone daily. For the Swedish sites, dexamethasone was replaced by betamethasone, as the two drugs are likely equipotent (198).

The exposure for patients in study IV was the activity of LMWH, measured by aFXa. Since there could be many values per patient, the values were summarized as minimum, median, and maximum. Minimum and maximum values could be any value during the ICU stay, as we assumed that the risk of TE and/or bleeding associated with these values did not change while in the ICU. The median value during the first 14 days was chosen as we hypothesized that if mortality was associated with aFXa, it would be due to the LMWH activity early on during the ICU stay. Also, for the exposure of study IV, it is worth noting that the aFXa values were a result of treatment with different LMWHs. *In vitro* equivalent values of aFXa, as a result of adding tinzaparin or enoxaparin, were associated with different levels of APTT and clot time (199). This may be a consequence of tinzaparin being a larger molecule that inhibits factor IIa (FIIa) to a larger extent compared to enoxaparin, and this is not measured by aFXa assays (138). Possible differences between patients treated with different LMWH are illustrated in Table S2 and Figure S5, as found in study IV. The possible differences in the clinical outcomes, because of the different types of LMWH resulting in different levels of anticoagulation at the same aFXa value, could not be compared. This was due to the treatment, according to local guidelines, resulted in higher peak values for patients treated with enoxaparin compared to patients on tinzaparin or dalteparin and higher trough values for tinzaparin compared to dalteparin (Table S2, study IV).

In studies I and II, we mistakenly adjusted for age twice, both separately and when adjusting for SAPS, since age is already incorporated in SAPS. However, it is unlikely that this would have changed the conclusion, as the unadjusted and adjusted results were the same in both studies, and also SAPS and age did not differ between the groups, p-values of 0.25 and 0.39 in study I and 0.22 and 0.49 in study II.

6.1.4 Outcomes

6.1.4.1 Primary outcomes

In all four studies, we chose death, alone or in composite, as the primary outcome. We chose death, as it was the most objective outcome. Also, micro thrombi in the lungs were reported as an important part of the pathophysiology. Micro thrombi could not be investigated with the modalities usually used for diagnosing TE, therefore, the burden of micro thrombi could perhaps be more accurately reflected by the risk of death.

6.1.4.2 Secondary outcomes

In all four studies, all TEs were considered a binary outcome. In the ICU, diagnosing TE may be difficult in patients with critical COVID-19. TE can be obvious as a life-threatening pulmonary embolus or a small thrombosis on a central venous catheter and these, of course, have different consequences for patients. The categorization of PE can be confusing, since the

nomenclature, for example, “massive” or “submassive,” refers to the clot size and proportion of the pulmonary circulation affected, while the therapy should be guided by the symptoms of the patient, stratified as “low,” “intermediate,” or “high” risk pulmonary embolism (200). Whether routine screening for TE should be done for ICU patients is under debate. For some clots, an early diagnosis and treatment is important, as they can be detrimental if they embolize or grow, and some may be small or distal and, therefore, it could even be possible that the treatment may be of a higher risk than the clot itself. This is recognized in the guidelines, in that treatments differ substantially depending on the type and symptoms of the diagnosed TE, ranging from thrombolysis for high-risk patients to surveillance for patients diagnosed with subsegmental PE without risk factors and absence of DVTs in the lower extremities (201). Altogether, the uncertainty of the true incidence of TE in our COVID-ICU populations must be recognized, especially when it comes to smaller TE. We limit ourselves by choosing TE as objectively as possible, but as it is still a binary outcome, we cannot rule out that the different exposures in the studies were associated with TE of different risks. Therefore, in future studies, the outcome of TE should be addressed in more detail.

In all studies, except for study III, we graded bleedings according to the WHO bleeding scale. There are many alternative scales, including the often-used bleeding scale, according to the International Society on Thrombosis and Hemostasis. However, we chose the WHO scale, since it was less complex, and it also agreed well when converting to any (grades I–IV) and major bleedings (grades III and IV). A concern for us when using the WHO bleeding scale was the distinction between grade I and II. To us, some bleedings were very minor, for example, transient macroscopic hematuria after a difficult placement of a urine catheter, as a consequence of the urethral trauma. However, according to the WHO scale, this was considered a grade II. Also, a bleed at an invasive site was considered grade II, and this could, for example, be an oozing from a new tracheostomy, which may even be considered normal in the first hours after this procedure. Therefore, to grade the bleedings as correct as possible, borderline cases were discussed at investigator meetings in order for there to be a consensus decision.

Even if it was not a result interpreted in the studies, we found in studies I, II, and III that TE was an earlier ICU complication compared to bleeding. The number of days from ICU admission to TE in study I were 8 (IQR 6 to 20), 8 (IQR 6 to 10), and 11 (IQR 11-11) in the low, intermediate, and high dose groups, and in study II, 8 (IQR 6 to10) and 8 (IQR 2 to 19) in the intermediate, and high dose groups, respectively. The number of days from ICU admission to bleeding in study II were 16 (IQR 6 to 20), 11 (IQR 10 to 20), and 1 (IQR 1 to 1) in the low, intermediate, and high dose groups, and in study II, 13 (IQR 10 to 18) and 13 (IQR 3-15) in the intermediate and high dose groups, respectively. In study III this is illustrated by the Kaplan-Meier curves with a larger proportion of patients having events earlier on the timeline of TE compared to major bleeding. As discussed in the literature overview, this may be a result of patients being less resistant to heparins in the later stages of intensive care.

6.2 STUDY I

The main finding in study I was an association between treatment with a high dose of LMWH and an improved 28-day survival compared to low dose LMWH. Moreover, a high dose was associated with a lower incidence of TE without an increase in bleeding.

The limitations of study I are thoroughly discussed in the section on methodological considerations, but the lack of evidence at this early age of the pandemic justified our study. We were in desperate need of treatments to improve the outcome for patients with critical COVID-19 and, therefore, felt obliged to report this clinical finding. The results of our study have not been confirmed in later RCTs. However, higher doses of low-molecular-weight heparins may very well have been beneficial in the ICU early in the pandemic when patients were not treated systematically with glucocorticoids, and at the same time were exposed to heavy sedations, fluids restriction, and usage of classic ARDS ventilator strategies, all of which may have contributed to hypercoagulation and stasis, according to Virchow's triad.

6.3 STUDY II

In study II, we could not find a difference between patients treated with intermediate dose or high dose LMWH. The low dose group was excluded, since this was no longer a recommended treatment. This also minimized the time confounder, since intermediate and high dosing were prescribed during the same time period. During the time of working on this study, the results from RCTs, including the multi-platform trial, were published and no thromboprophylactic regimes could show a benefit over another (175, 177). The findings illustrated that the optimal dose for the individual patient is complex and perhaps should be tailored to include other factors than just level of care. In an attempt to address this, we also analyzed different subgroups of patients. Patients were stratified according to low or high fibrin-D-dimer at ICU admission, use of invasive ventilation during ICU stay, and date of admission. The results, for fibrin-D-dimer, showed point estimates, indicating a lower 90-day mortality with a high dose for patients with high fibrin-D-dimer and the opposite if a high dose was prescribed for patients with low fibrin-D-dimer, HR of 0.36 (0.12 to 1.06) and 1.65 (0.52 to 5.24), respectively. Patients treated with either invasive ventilation or other respiratory support had point estimates of HR just below 1, suggesting no difference between the groups. Just as fibrin-D-dimer, splitting the group into patients admitted before or after April 30, 2020, suggested a benefit with a high dose in the early period and the opposite if admitted in the later period, 0.42 (0.15 to 1.15) and 1.15 (0.33 and 3.99), respectively. None of these results were statistically significant. However, we did find a significant interaction between time of admission and high dose, indicating that patients did have different effects of the exposure in the early, as opposed to the later period, p-value of 0.047.

Maybe the most interesting question would have been to explore the relationship between the dose of LMWH and glucocorticoids. For the patients in study II, 30.4% in the intermediate group and 19.2% in the high dose group had glucocorticoid treatment in the regime recommended for COVID-19. By including an interaction or stratifying patients by glucocorticoid treatment for COVID-19, this would investigate the hypothesis if an improved survival with higher doses of LMWH in the first wave of COVID-19 could be due to the anti-inflammatory effect of the heparin, later not needed due to routine use of glucocorticoids. However, just as for the analyses stratified for fibrin-D-dimer, invasive ventilation, and time of admission, even if there were a true difference, this would probably not have been statistically significant, given the small sample size.

6.4 STUDY III

In study III, the *post hoc* analysis of COVID STEROID 2, we could not find a significant difference in the risk of death and TE, TE alone, or bleeding for patients with critical COVID-19 treated with 12 or 6 mg dexamethasone daily.

Our hypothesis was that increasing the intensity of anti-inflammatory treatment with glucocorticoids would attenuate immunothrombosis and thereby decrease the risk of TE. There might be many reasons why we could not find a difference. Firstly, we were limited by the low number of patients and events leading to wide confidence intervals, indicating that substantial effects in both directions were possible. Another explanation might be that glucocorticoids act on other pathways, some of which may cause an increase in the risk of TE resulting in an equilibrium of the anti-thrombotic properties by attenuating immunothrombosis and the possible pro-thrombotic effects caused by glucocorticoids (202). Furthermore, it is possible that already 6 mg of dexamethasone will result in a ceiling effect of attenuations of immunothrombosis. The underlying hypothesis we tried to investigate in study III, namely whether glucocorticoids attenuate immunothrombosis, is unlikely to ever be answered, since it is not possible to randomize patients to a placebo, given the now well-established mortality lowering effect of glucocorticoids.

In study III, we included more types of TE, for example, MI. We did this to synchronize with the Danish data. As expected, the number of MI was extremely small, with only two patients out of 357. However, this may be a consequence of the difficulty in diagnosing MI in an intubated COVID-19 patient with severe ARDS straining the heart.

6.5 STUDY IV

The main finding in study IV was the association between an increased risk of TE in patients with low peak aFXa values and the increased risk of death and bleeding in patients with high aFXa trough values. For TE and bleeding, we could also identify cut-off values when the associated risk became significantly increased. Future studies investigating aFXa-guided thromboprophylaxis could use these cut-off values when structuring a treatment algorithm.

As discussed earlier in this thesis, aFXa-guided LMWH treatment has many limitations, with one being the possibility that patients treated with different LMWH may have different degrees of anticoagulation with the same values of aFXa, since aFXa does not measure the concurrent inhibition of fIIa. Perhaps there are even better alternatives to monitor thromboprophylaxis in the ICU, such as viscoelastic methods or thrombin generation methods (199, 203). However, as concluded in the discussion on aFXa-monitoring, it is a widely available functional assay, indicating LMWH activity. This should be maybe preferred to empiric fixed dosing given to our heterogeneous population treated in the ICU (151).

6.6 INTERPRETATION OF OVERALL FINDINGS IN THIS THESIS

Patients early in the pandemic had an association with a better outcome if treated with intensified thromboprophylaxis compared to standard low dosage. If this was an effect of the anticoagulation, the anti-inflammatory characteristics of LMWH, a consequence of other unknown temporal changes in the treatment of critical COVID-19 during the first wave, or

random variation, we will never know. Even if glucocorticoids may attenuate immunothrombosis in patients with critical COVID-19, no additive effect was shown by increasing the dose from 6 to 12 mg dexamethasone. If this means glucocorticoids do not mitigate immunothrombosis, or if 6 mg is enough to reach a ceiling effect, we will also never know as it is not possible to compare glucocorticoids to a placebo, with glucocorticoids already being an established mortality-lowering treatment. However, we can conclude, when prescribing drugs to a patient with critical COVID-19 and risk for TE and/or bleeding, choosing a high or a low dose of glucocorticoids is unlikely to considerably increase or decrease the risk further. Low peak values and high trough values of aFXa are associated with the clinical outcomes of TE and bleeding in patients with critical COVID-19. These promising results must be investigated further, also in other critically ill populations. Since thromboprophylaxis is one of the most prescribed treatments for the critically ill, an improvement in dosing would have a large effect on reducing the number of TE and bleeding in ICUs worldwide.

7 CONCLUSIONS

The specific conclusions for each study are as follows:

- Among critically ill COVID-19 patients with respiratory failure, high-dose thromboprophylaxis was associated with a lower risk of death and a lower cumulative incidence of thromboembolic events compared with lower doses.
- A difference in 90-day mortality between intermediate and high-dose thromboprophylaxis could neither be confirmed nor rejected due to small sample size.
- Among patients with critical COVID-19, 12 mg versus 6 mg of dexamethasone daily did not result in a statistically significant difference in the composite outcome of death or thromboembolism. However, uncertainty remains due to the limited number of patients.
- Measuring anti-Factor Xa activity may be relevant for dosing low-molecular-weight heparin to patients with critical COVID-19. Lower peak values were associated with increased risk of thromboembolism and higher trough values were associated with increased risk of death and bleeding. Prospective studies are needed to confirm these results.

8 POINTS OF PERSPECTIVE

When executing these studies, we constructed an extensive database containing more than 1,200 patients with critical COVID-19 in Sweden, from March 2020 to May 2021. The database is currently used for further studies and will, therefore, continue to bring new knowledge. The ongoing and planned COVID-19 studies are summarized as follows.

We study if different ventilation modes and levels of respiratory support are associated with a change in gas exchange and barotrauma. The hypothesis is that non-traditional ARDS ventilator modes, with a high PEEP and spontaneous breathing, may be superior in improving oxygenation for patients with severe respiratory failure compared to traditional ventilation. However, since this can result in high airway pressures, the associated risk of barotrauma must also be investigated. Barotrauma was a common complication among patients with critical COVID-19, not only pneumothorax but also pneumomediastinum, which was new to us. Whether barotrauma is associated with the level of respiratory support, ventilation modes, or a complication of COVID-19 itself will, therefore, be investigated.

Many patients were transported between ICUs because of a shortage of beds. This was frustrating both for the staff, the relatives of the patients, and probably, most of all, for the patients who were aware. We recognize transportation as a situation of risk, both due to the complications during the actual transportation but also caused by information gained by the caretakers getting lost as new personnel take over. The hypothesis in this study is, when forced to transfer, we chose patients who were suitable, and therefore had a low risk for worse outcome as a result of the transportation.

In studies I-IV we have analyzed the outcomes of death and events of coagulopathy, however, for the surviving patient, heath related quality of life may be more important. We are currently analyzing if exposure to different doses of LMWH for patients admitted March to July 2020 was associated to differences in physical and emotional well-being measured by a questionnaire, RAND-36.

As mentioned in the thesis, the outcome of TE and especially, PE/PT is probably not a binary. A study is planned with the aim of exploring the size and place of PE/PT in COVID-19-patients as it may differ from other critically ill patients. The study will also investigate if the timing of PE/PT, before or during the ICU stay, is associated with a different outcome compared to patients without PE/PT.

The knowledge gained in this thesis regarding the treatment of coagulopathy in patients with critical COVID-19 must be seen in the light of other critically ill patients. To me, this was an eye-opener, how little we know when it comes to the optimal thromboprophylaxis for ICU patients with the possible heterogenous treatment effects, not only between patients but also for the same patients during different stages of disease. Therefore, we must proceed by investigating coagulopathy outside the COVID-19 population. Currently, we are involved in the following studies:

At Södersjukhusets ICU, we are planning a prospective study to investigate coagulopathy in patients with septic shock. The aim is to improve the identification of coagulopathy, which may enable earlier treatment. In this study, a novel method of analyzing viscoelastic testing will be used. With repeated testing, we will follow the coagulopathy during the phases of sepsis.

As a part of an international intensive care research group, Collaboration for Research in Intensive Care, CRIC, we are planning to participate in a randomized controlled trial investigating different thromboprophylactic strategies. Precise interventions have not been determined, but a survey has been distributed to clinicians in 20 hospitals all over the world, including Södersjukhuset, to investigate the current practice. Based on the results from this thesis, I will strive to investigate whether aFXa-guided thromboprophylaxis can decrease the complications of TE and bleeding in critically ill patients with a high risk of coagulopathy.

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