

INVESTIGATION OF CLINICAL AND MECHANISTIC ASPECTS OF THROMBOINFLAMMATION IN COVID-19

Ph.D. Thesis

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“The struggle itself towards the heights is enough to fill a man's heart. One must imagine Sisyphus happy.”

- Albert Camus

TABLE OF CONTENTS

1.	LIST OF ABBREVIATIONS.....	5
2.	STUDENT PROFILE.....	7
2.1.	Vision and mission statement, specific goals.....	7
2.2.	Scientometrics.....	7
2.3.	Future plans.....	7
3.	SUMMARY OF THE THESIS.....	8
4.	GRAPHICAL ABSTRACT.....	9
5.	INTRODUCTION.....	10
5.1.	The COVID-19 Associated Coagulopathy.....	10
5.1.1.	Definition and Distinct Features.....	10
5.1.2.	The Pathophysiology of COVID-19 Associated Coagulopathy.	11
5.1.2.1.	From Viral Entry to Thromboinflammation.....	11
5.1.2.2.	The Thromboinflammatory Cascade.....	12
5.1.3.	Therapeutic Strategies and Their Impact on the Hemostatic System.....	15
5.1.3.1.	Anticoagulation Intensity.....	15
5.1.3.2.	Immunomodulation.....	15
5.1.4.	Knowledge gaps in COVID-19-associated coagulopathy.....	16
5.1.5.	Contributions of the present work.....	16
6.	OBJECTIVES.....	18
6.1.	Study I. – Higher Dose Anticoagulation Cannot Prevent Disease Progression in COVID-19 Patients: A Systematic Review and Meta-Analysis.....	18
6.2.	Study II. – Exploratory Analysis of Coagulation and Fibrinolysis Trajectories After IL-6 Antagonist Therapy in COVID-19: A Case Series.....	18
7.	METHODS.....	19
7.1.	Study I. - Higher Dose Anticoagulation Cannot Prevent Disease Progression in COVID-19 Patients: A Systematic Review and Meta-Analysis.....	19

7.1.1. Methodology and Protocol.....	19
7.1.2. Eligibility Criteria.....	19
7.1.3. Information Sources and Search Strategy and Data Extraction...	19
7.1.3.1. Subgroup Analyses.....	20
7.1.4. Risk of Bias and Quality of Evidence Assessment.....	21
7.1.5. Data Synthesis and Statistical Analysis.....	21
7.2. Study II. - Exploratory Analysis of Coagulation and Fibrinolysis Trajectories After IL-6 Antagonist Therapy in COVID-19: A Case Series.....	22
7.2.1. Study Design and setting.....	22
7.2.2. Ethics and Patient Consent.....	22
7.2.3. Participants.....	22
7.2.4. Variables and Data Sources.....	23
7.2.5. Outcomes.....	25
7.2.6. Statistical Methods.....	26
8. RESULTS.....	27
8.1. Study I. – Higher Dose Anticoagulation Cannot Prevent Disease Progression in COVID-19 Patients: A Systematic Review and Meta-Analysis.....	27
8.1.1. Study Search and Selection.....	27
8.1.2. Primary Outcome – All-cause mortality.....	33
8.1.2.1. Subgroup Analysis by Disease Severity and Anticoagulation Regimen.....	33
8.1.2.2. Subgroup Analysis by Baseline D-Dimer Levels.....	33
8.1.3. Secondary Outcomes.....	35
8.1.3.1. Any Thrombotic Events and Major Bleeding.....	35
8.1.3.2. Progression of Disease.....	38
8.1.4. Risk of Bias Assessment and Quality of Evidence.....	39
8.2. Study II - Exploratory Analysis of Coagulation and Fibrinolysis Trajectories After IL-6 Antagonist Therapy in COVID-19: A Case Series.....	45
8.2.1. Study Population.....	45
8.2.2. Primary Outcomes.....	47

8.2.3. Secondary Outcomes.....	49
8.2.3.1. Inflammation and Endothelium.....	49
8.2.3.2. Coagulation Initiation and Anticoagulant Activity.....	51
8.2.3.3. Clot Propagation and Strength.....	53
8.2.3.4. Fibrinolytic Activity.....	55
9. DISCUSSION.....	58
9.1. Summary of Findings.....	58
9.2. Study I - Higher Dose Anticoagulation Cannot Prevent Disease Progression in COVID-19 Patients: A Systematic Review and Meta-Analysis.....	59
9.2.1. Different Dosing Regimens and Disease Severity.....	59
9.2.2. Differences in the Degree of COVID-19-Associated Coagulopathy.....	60
9.3. Study II - Exploratory Analysis of Coagulation and Fibrinolysis Trajectories After IL-6 Antagonist Therapy in COVID-19: A Case Series.....	60
9.4. Strengths.....	62
9.5. Limitations.....	63
10. CONCLUSIONS.....	64
11. IMPLICATIONS FOR PRACTICE.....	65
12. IMPLICATIONS FOR RESEARCH.....	66
13. FUTURE PERSPECTIVES.....	67
14. REFERENCES.....	68
15. BIBLIOGRAPHY.....	78
15.1. Publications Related to the Thesis.....	78
15.2. Publications not Related to the Thesis.....	78
16. ACKNOWLEDGEMENTS.....	80

1. LIST OF ABBREVIATIONS

aPTT	Activated Partial Thromboplastin Time
CFT	Clot Formation Time
CLI-30/45	Clot Lysis Index at 30 or 45 min
COVID-19	Coronavirus Disease 2019
CRP	C-reactive protein
CT	Clotting Time
ECA-test	Ecarin Clotting Assay
eCRF	Electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
EX-test	Extrinsic Pathway Test
FDR	False Discovery Rate
FIBTEST	Fibrinogen Test
FiO₂	Fraction of Inspired Oxygen
IL-6	Interleukin-6
INR	International Normalized Ratio
IN-test	Intrinsic Pathway Test
LOT	Lysis Onset Time
LT	Lysis Time
MCF	Maximum Clot Firmness
ML	Maximum Lysis
PaCO₂	Partial Pressure of Carbon Dioxide
PaO₂	Partial Pressure of Oxygen in Arterial Blood
PAI-1	Plasminogen Activator Inhibitor-1

PAP	Plasmin–Antiplasmin Complex
PCR	Polymerase Chain Reaction
PCT	Procalcitonin
PLG	Plasminogen
RVVTEST	Russell Viper Venom Test
TAFI	Thrombin-Activatable Fibrinolysis Inhibitor
TAT	Thrombin–Antithrombin Complex
TPA	Tissue Plasminogen Activator
TPA-test	tPA-Enhanced Lysis Test
TT	Thrombin Time
VHA	Viscoelastic Hemostatic Assay
vWF	von Willebrand Factor
vWF:Act:	von Willebrand Factor Activity
vWF:Ag:	von Willebrand Factor Antigen

2. STUDENT PROFILE

2.1. Vision and mission statement, specific goals

My vision is to be a competent, careful intensivist who delivers evidence-based care to critically ill patients. Through this doctoral work I have developed the ability to critically appraise and synthesize medical literature, and my goal is to carry that skill into everyday clinical decision-making while staying involved in clinical research as a contributor where it supports better patient care.

2.2. Scientometrics

Number of all publications:	7
Cumulative IF:	24.7
Av IF/publication:	3.52
Ranking (SCImago):	D1: Q1:5 Q2:1 Q3: Q4:
Number of publications related to the subject of the thesis:	2
Cumulative IF:	9.2
Av IF/publication:	3.83
Ranking (Sci Mago):	D1: Q1:2 Q2:1 Q3: Q4:
Number of citations on Google Scholar:	21
Number of citations on MTMT (independent):	12
H-index:	2

The detailed bibliography of the student can be found on pages 73,74.

2.3. Future plans

I intend to build broad clinical competence in acute and intensive care medicine, with the longer-term aim of contributing to humanitarian medical work in resource-limited or crisis settings. I plan to keep the critical-appraisal skills developed during this work active in practice, applying evidence-based care in whatever environment I am working in.

3. SUMMARY OF THE THESIS

The COVID-19 pandemic highlighted a bidirectional relationship between inflammation and coagulation long recognized in critical illness, but never previously interrogated with such methodological urgency. COVID-19-associated coagulopathy (CAC) emerged as a distinct thrombo-inflammatory phenotype which is characterized by endothelial injury, IL-6-driven hyperfibrinogenemia, and in some cases fibrinolytic shutdown.

Two therapeutic strategies were concurrently proposed: escalation of anticoagulant intensity to mitigate downstream thrombotic consequences, and immunomodulation with IL-6 receptor antagonists to attenuate the upstream inflammatory driver thus mitigating coagulopathy.

Study I, a systematic review and meta-analysis of 14 randomized controlled trials enrolling 6,253 patients, demonstrated that higher-dose thromboprophylaxis significantly reduced thrombotic events (OR 0.58, 95% CI 0.44–0.76) but was counterbalanced by increased major bleeding (OR 1.64, 95% CI 1.25–2.16), yielding no overall mortality benefit (OR 0.83, 95% CI 0.54–1.28). Potential benefit appeared concentrated in non-critically ill patients with markedly elevated D-dimer, in whom an exploratory composite endpoint significantly favoured higher-dose anticoagulation (OR 0.46, 95% CI 0.21–0.67).

Study II provided an exploratory prospective longitudinal characterization of coagulation, fibrinolysis, and endothelial dynamics in 15 critically ill patients following IL-6 receptor blockade. Despite suppression of inflammatory axis, endothelial activation worsened, PAI-1 rose progressively, thrombin generation remained preserved, and antifibrinolytic pressure persisted, thus revealing a fundamental dissociation between cytokine attenuation and haemostatic normalization.

Taken together, both studies converge on a shared conclusion: targeting either arm of the thrombo-inflammatory axis in isolation produces incomplete haemostatic correction. Our study point at the endothelium as the insufficiently addressed common denominator.

4. GRAPHICAL ABSTRACT

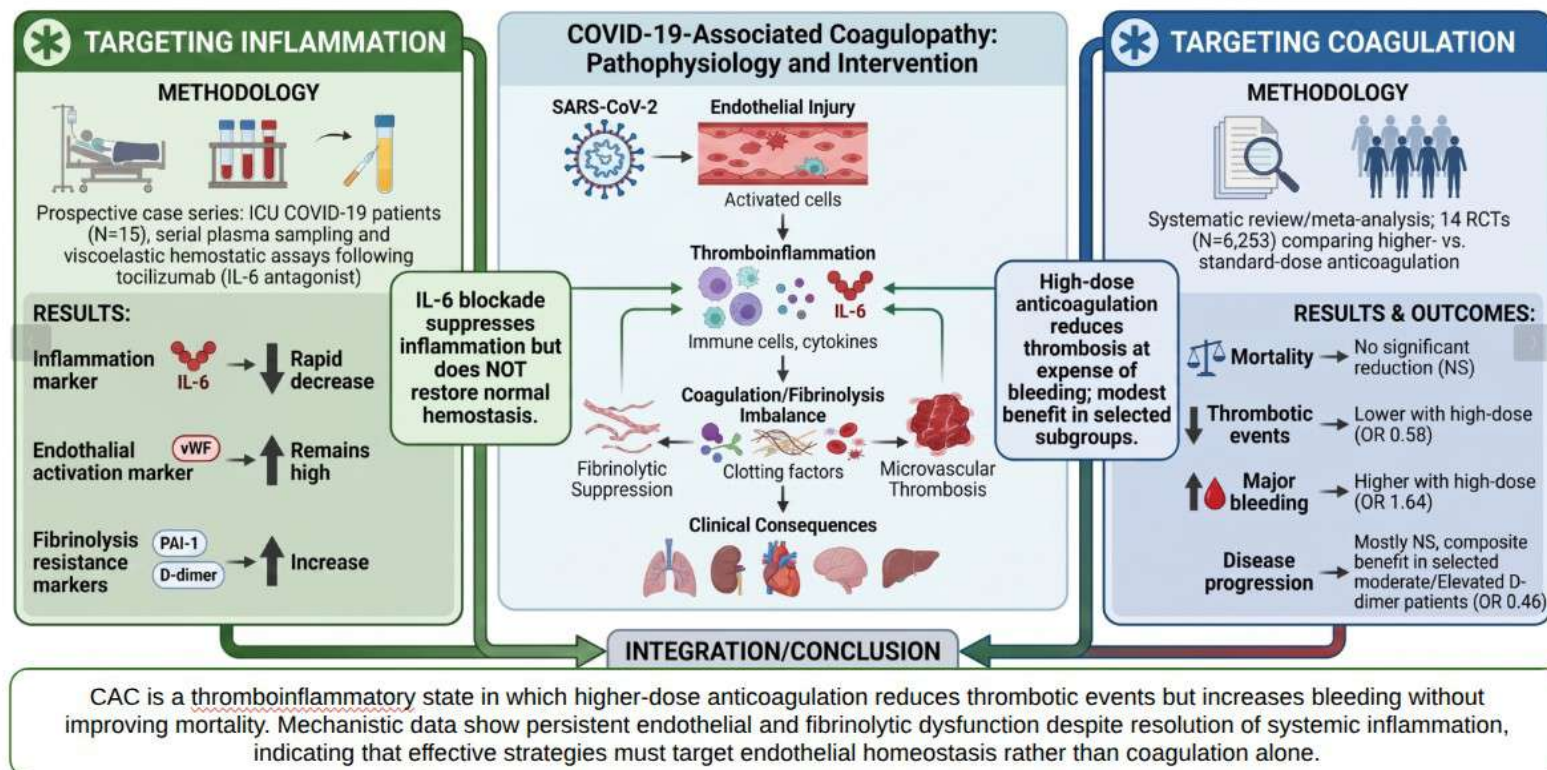


Investigation of Clinical and Mechanistic Aspects of Thromboinflammation in COVID-19

Ph.D. Thesis

Translational Medicine Program, Pharmaceutical Sciences and Health Technologies Division, Semmelweis University

Supervisors: Prof. Zsolt Molnár, Dr. Krisztián Tánccs, M.D



5. INTRODUCTION

5.1. The COVID-19 Associated Coagulopathy

5.1.1. *Definition and Distinct Features*

First identified in Wuhan, China in December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) exerted a major disruptive global impact, prompting prolonged quarantine protocols as a vast portion of the world population became affected (1). According to official data reported to the World Health Organization (WHO), this culminated in 779,231,447 confirmed cases and a staggering cumulative total of 7,115,339 deaths worldwide by June 2026 (2).

Patients with severe SARS-CoV-2 infection were presenting a case of viral pneumonia with high incidence of thrombotic and embolic complications (3,4). Data from early pooled cohorts showed that the incidence of venous thromboembolism reached 28% in critically ill intensive care unit (ICU) patients compared to 10% in non-ICU patients. (5)

Early post-mortem analyses reinforced this clinical observation, revealing a specific microvascular pattern characterized by severe endothelial injury, widespread microangiopathic thrombosis, and an alveolar capillary microthrombi prevalence nine times greater than that observed in influenza-associated respiratory failure, accompanied by a 2.7-fold increase in intussusceptive angiogenesis (6).

Iba et al. proposed a formal diagnostic definition for COVID-19 Associated Coagulopathy (CAC) based on a confirmed viral test paired with distinct hematological and clinical shifts, specifically tracking significant elevations in D-dimer and fibrinogen, prolonged prothrombin times, preserved yet declining platelet counts, and the objective manifestation of microvascular or macrovascular thrombosis (7). This overwhelming rate of clotting forced the medical community to recognize that CAC was fundamentally different from traditional systemic coagulopathies like sepsis or trauma (8). See Table 1 for comparison of different coagulopathies. (9–11).

Table 1: Characteristics of COVID-19 -associated-, Sepsis-induced and Trauma-induced Coagulopathy

	COVID-19 Coagulopathy (CAC)	Sepsis induced Coagulopathy (SIC)	Trauma induced Coagulopathy (TIC)
Primary Initiators	Viral PAMPs & NET-derived DAMPs binding TLRs.	Bacterial PAMPs (LPS) & cell-injury DAMPs triggering PRRs.	Massive DAMP release (mtDNA, HMGB1) from cellular shock & necrosis.
Pathophysiology	Localized pulmonary immunothrombosis via IL-6 endothelialitis.	Disseminated intravascular clotting via systemic tissue factor.	Systemic hypocoagulability via glycocalyx shedding & protein C.
Localization	Localized pulmonary vessels; high micro/macro thrombosis risk.	Systemic microvascular network; Bimodal	Systemic vascular bed; severe life-threatening hemorrhage risk.
Fibrinogen	Markedly elevated	Depleted	Rapidly Consumed
Platelets	Preserved to Mildly Decreased	Severe Thrombocytopenia	Diluted & Dysfunctional
Fibrinolysis	Severe Shutdown	Variable spectrum (Delayed shutdown to acute hyperfibrinolysis).	Hyperfibrinolysis
Antithrombin	Preserved / Intact	Severely Depleted	Impaired

Abbreviations used: DAMPs: Damage-Associated Molecular Patterns DIC: Disseminated Intravascular Coagulation DVT: Deep Vein Thrombosis HMGB1: High-Mobility Group Box 1 IL-6: Interleukin-6 LPS: Lipopolysaccharide mtDNA: Mitochondrial DNA NETs: Neutrophil Extracellular Traps PAI-1: Plasminogen Activator Inhibitor-1 PAMPs: Pathogen-Associated Molecular Patterns PE: Pulmonary Embolism PRRs: Pattern Recognition Receptors SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2;TLRs: Toll-Like Receptors tPA: Tissue Plasminogen Activator

5.1.2. The Pathophysiology of COVID-19 Associated Coagulopathy

5.1.2.1. From Viral Entry to Thromboinflammation

Horizontal viral transmission occurs predominantly via respiratory droplets or airborne aerosols expelled during coughing or sneezing episodes. During the early phase of infection, the high density of angiotensin-converting enzyme 2 (ACE2) receptor localized within the oral cavity and lingual mucosa facilitates initial viral inhalation. SARS-CoV-2 specifically targets host structures including the nasal epithelium, bronchial mucosa, and alveolar pneumocytes using its structural surface spike (S) glycoprotein to bind directly to host membrane-bound ACE2. This cellular internalization is structurally catalyzed by host type 2 transmembrane serine protease (TMPRSS2), which cleaves the ACE2 receptor and primes the viral S protein, thereby mediating fusion and endocytosis into the target cells (4,12).

As viral replication accelerates during advanced stages of the disease, the structural integrity of the epithelial-endothelial barrier is severely disrupted. Direct viral infection of the

pulmonary capillary endothelium worsens local inflammatory kinetics, inducing a massive chemotactic influx of circulating monocytes and polymorphonuclear neutrophils. This physical breakdown of the endothelial barrier, combined with a severe impairment of alveolar-capillary gas exchange dynamics, constitutes the primary pathophysiological hallmark of severe COVID-19 (4,13,14).

5.1.2.2. *The Thromboinflammatory Cascade*

The uncontrolled overproduction of early phase immunomodulatory molecules drives a systemic hyperinflammatory response or "cytokine storm". Circulating levels of IL-1 β , IL-2, IL-6, IL-10, interferon- γ (IFN- γ), granulocyte-macrophage colony-stimulating factor (GM-CSF), and monocyte chemoattractant protein-1 (MCP-1) increase exponentially, mirroring clinical disease severity and accelerating the risk of diffuse vascular hyperpermeability, multi-system organ failure, and lethality. This massive accumulation of circulating damage-associated molecular patterns (DAMPs), paired with an unmitigated immune response, inflicts direct aggression upon the vascular architecture, leading to the structural degradation of the endothelial glycocalyx as a primary target in virus-induced coagulopathy. Simultaneously, intense activation of the complement pathway worsens endothelial cell injury and lysis, prompting vascular denudation and exposing the underlying thrombogenic subendothelial basement membrane. This exposure further drives the reciprocal activation of both the intrinsic and extrinsic clotting cascades, creating a direct bridge between acute systemic inflammation and immediate procoagulant activation (15–17).

At the cellular level, infiltrating, activated neutrophils discharge massive quantities of histotoxic metabolites, including reactive oxygen species and neutrophil extracellular traps (NETs), which physically stabilize microthrombi within the microvasculature, while inflammatory activation prompts monocytes to express high levels of membrane-bound tissue factor (TF) to drive substantial thrombin generation.(18) This prothrombotic flux is poorly balanced by endogenous tissue factor pathway inhibitor (TFPI) and is further accelerated by the pathologically increased exposure of phosphatidylserine on damaged cellular membranes. Concurrently, endothelial-associated anticoagulant regulatory mechanisms, such as the protein C system, are suppressed by circulating cytokines. Following activation by interleukins and TNF, the endothelium expresses P-selectin, von Willebrand factor (vWF), and fibrinogen, leading to massive platelet capture and adhesion. These recruited platelets

release vascular endothelial growth factor (VEGF), which upregulates endothelial tissue factor expression, creating a vicious cycle of fibrin generation and persistent microvascular clotting (14,19–21).

Central to this sustained hemostatic imbalance is the pleiotropic role of cytokines such as IL-6, which enhances coagulation and drives fibrinolytic shutdown through multiple concurrent pathways (22). Specifically, IL-6 upregulates the hepatic synthesis of fibrinogen, increases the expression of Factor VIII and tissue factor, and downregulates the natural regulatory anticoagulants antithrombin and protein S. Additionally, it drives antifibrinolytic activity by stimulating endothelial cells to produce plasminogen activator inhibitor-1 (PAI-1) via trans-signaling pathways (22,23). The cumulative effect of this elevated IL-6 axis shifts the systemic equilibrium toward a highly procoagulant, hypofibrinolytic state, resulting in localized alveolar inflammation, diffuse microvascular thrombosis, mural edema, and microhemorrhagic changes. This pathology establishes a clear link between localized bronchoalveolar coagulation/fibrinolytic imbalances and the clinical progression of acute respiratory distress syndrome (ARDS), which accelerates intrapulmonary fibrin deposition. Although the respiratory architecture represents the primary site of injury, systemic viral dissemination can induce identical thromboinflammatory conditions across multiple vascular beds (6,24,25).

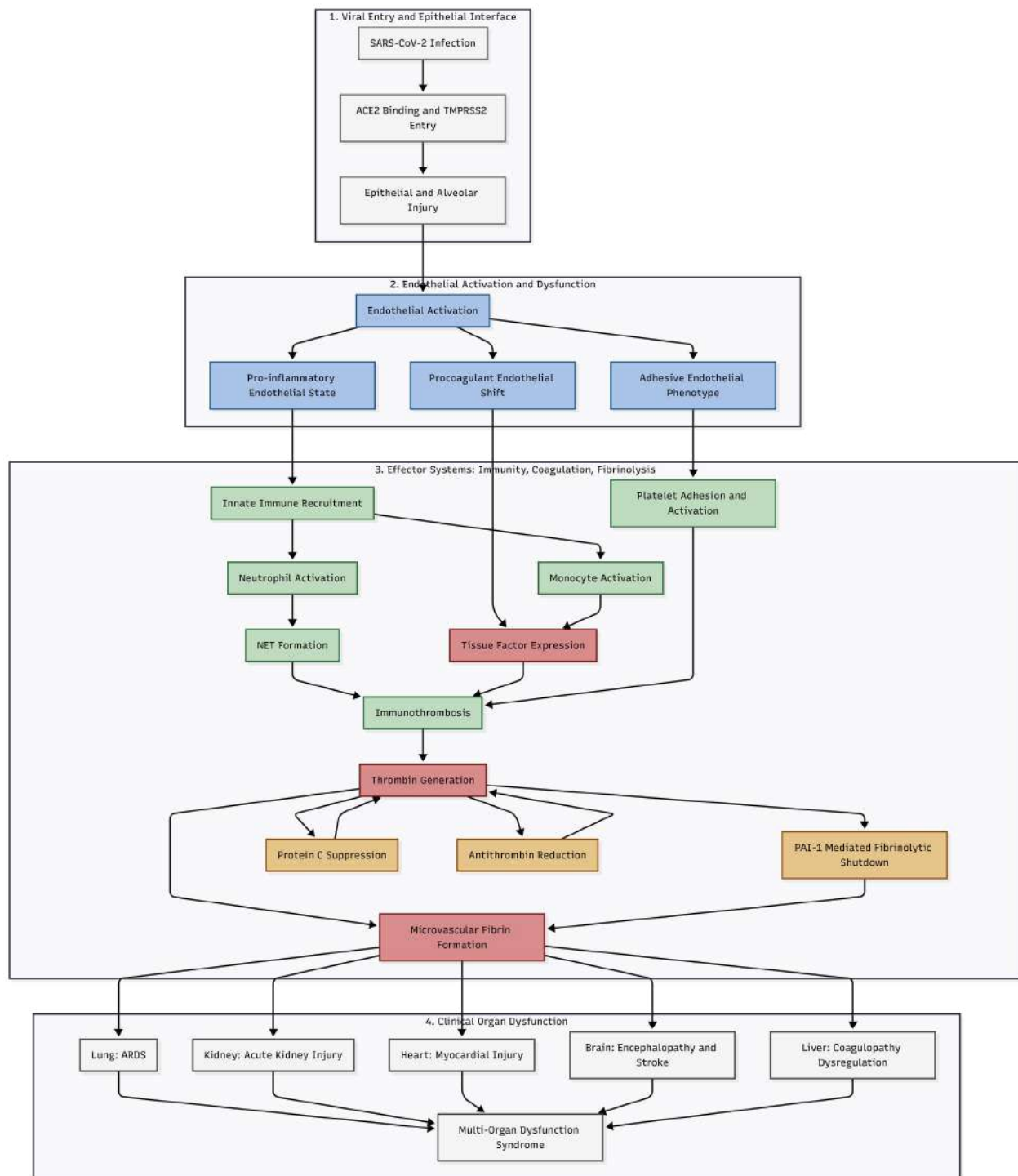


Figure 1. Pathophysiological mechanisms underlying COVID-19-associated coagulopathy

Abbreviations used in the table: ARDS: acute respiratory distress syndrome; NET: neutrophil extracellular trap; PAI-1: plasminogen activator inhibitor-1; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

5.1.3. *Therapeutic Strategies and Their Impact on the Hemostatic System*

To diminish the morbidity of severe COVID-19, therapeutic strategies are broadly categorized into direct antiviral interventions and host-directed therapies. Host-directed strategies can be further divided based on their primary biological target: those directly modulating the immune system such as corticosteroids and cytokine inhibitors and those targeting the downstream hemostatic and fibrinolytic systems to mitigate thrombotic complications (26–28).

5.1.3.1. *Anticoagulation Intensity*

The high incidence of thrombotic complications observed in hospitalized patients with COVID-19 led to widespread interest in the use of anticoagulation regimens exceeding standard thromboprophylactic doses. Several trials subsequently evaluated intermediate- and therapeutic-dose anticoagulation strategies, producing heterogeneous and sometimes conflicting results. While randomized control studies such as the multiplatform ATTACC, ACTIV-4a, and REMAP-CAP trials, together with RAPID and HEP-COVID, suggested benefit in selected non-critically ill populations, similar advantages were not consistently observed in critically ill patients. Conversely, trials such as ACTION and INSPIRATION failed to demonstrate improved outcomes with intensified antithrombotic strategies (29–33).

Consequently, despite an unprecedented volume of clinical research, uncertainty remains regarding the overall effectiveness of higher-dose anticoagulation in COVID-19. Differences in disease severity, patient selection, thrombotic risk, concomitant therapies, and outcome definitions have contributed to persistent discrepancies across studies. As a result, the balance between potential thrombotic benefit and bleeding risk remains incompletely defined, highlighting the need for a comprehensive synthesis of the available evidence to better identify patient populations most likely to benefit from anticoagulation intensification.

5.1.3.2. *Immunomodulation*

Given the central role of inflammatory pathways in the development of CAC, immunomodulatory therapies rapidly emerged as an alternative strategy to mitigate

downstream hemostatic disturbances. Among these, IL-6 receptor antagonists (Tocilizumab) demonstrated significant clinical benefit in large randomized trials, introducing tocilizumab in the standard of care for selected patients with severe COVID-19 (34,35).

However, although these studies clearly demonstrated improvements in clinically relevant outcomes, they were not designed to investigate the biological effects of IL-6 blockade on coagulation and fibrinolytic pathways (34).

Consequently, the mechanisms through which immunomodulation influences the hemostatic system remain incompletely understood. In particular, little is known regarding the temporal evolution of hypercoagulability and fibrinolytic shutdown following IL-6 receptor inhibition, or whether correction of inflammation is accompanied by measurable restoration of hemostatic homeostasis.

5.1.4. Knowledge gaps in COVID-19-associated coagulopathy

COVID-19-associated coagulopathy represents a distinct thrombo-inflammatory phenotype in which both anticoagulation intensity and immunomodulation have been proposed as therapeutic strategies, yet their optimal biological and clinical impact remains uncertain. Evidence regarding higher-dose anticoagulation is heterogeneous across disease severities, with unresolved balance between thrombotic prevention and bleeding risk (36).

In parallel, although IL-6 blockade improves clinical outcomes, its effects on functional coagulation and fibrinolysis trajectories remain insufficiently characterized. Defining how these interventions modify dynamic hemostatic states is essential to move from empiric treatment strategies toward mechanism-based patient stratification (37,38).

5.1.5. Contributions of the present work

The first part of this work, focusing on higher-dose anticoagulation in COVID-19, aims to clarify the overall clinical value of intensified antithrombotic strategies across different severity strata. By synthesizing available evidence, it may help better define the balance between thrombotic prevention and bleeding risk, and support more precise identification of patient subgroups who may benefit from escalated anticoagulation.

The second part investigates the biological effects of IL-6 receptor blockade on coagulation and fibrinolysis dynamics. By characterizing longitudinal hemostatic trajectories following tocilizumab administration, it provides mechanistic insight into whether immunomodulation

leads to restoration of functional coagulation balance beyond its known anti-inflammatory effects. Together, these studies contribute to a more mechanism-informed framework for managing thrombo-inflammation in critically ill patients.

6. OBJECTIVES

6.1. Study I. – Higher Dose Anticoagulation Cannot Prevent Disease Progression in COVID-19 Patients: A Systematic Review and Meta-Analysis

This systematic review and meta-analysis evaluates whether higher-dose thromboprophylaxis, compared with standard-dose regimens, improves clinical outcomes in hospitalized COVID-19 patients while maintaining an acceptable safety profile.

6.2. Study II. – Exploratory Analysis of Coagulation and Fibrinolysis Trajectories After IL-6 Antagonist Therapy in COVID-19: A Case Series

This exploratory observational study investigates the effects of IL-6 receptor blockade on coagulation and fibrinolytic dynamics in critically ill COVID-19 patients. Using serial ClotPro® viscoelastic measurements and circulating biomarkers, we characterize longitudinal haemostatic changes following treatment and explore their relationship with systemic inflammation and endothelial injury.

7. METHODS

7.1. Study I. - Higher Dose Anticoagulation Cannot Prevent Disease Progression in COVID-19 Patients: A Systematic Review and Meta-Analysis

7.1.1. Methodology and Protocol

We conducted a systematic review and meta-analysis according to the Cochrane Handbook for Systematic Reviews of Interventions. The study protocol was prospectively registered in PROSPERO (CRD42021284808) (39).

7.1.2. Eligibility Criteria

We included randomized controlled trials enrolling adult patients with clinically or laboratory-confirmed COVID-19 that compared higher-dose and standard-dose thromboprophylaxis strategies.

The primary outcomes were mortality, organ support-free days (defined as days without respiratory, inotrope/vasopressor support), length of hospital stay, thrombotic and bleeding complications, and transfusion requirements. Secondary outcomes included respiratory and organ support parameters, disease progression, adverse events, and changes in inflammatory and coagulation biomarkers.

Given the rapidly evolving research landscape during the COVID-19 pandemic, the final analysis was adapted to the outcomes reported by eligible studies. Accordingly, we evaluated the incidence of invasive mechanical ventilation and progression to acute respiratory distress syndrome (ARDS) where available. In addition, a post hoc composite outcome comprising death, pulmonary embolism, and invasive mechanical ventilation was explored as a marker of severe pulmonary disease progression.

7.1.3. Information Sources and Search Strategy and Data Extraction

We performed the systematic search on 18 October 2021 and updated it on 23 May 2022. Searches were conducted in MEDLINE, Embase, and CENTRAL using predefined terms related to COVID-19 and anticoagulation. To capture the most recent evidence, we

additionally searched the medRxiv preprint archive for studies published between 1 January and 23 May 2022.

Study selection was performed independently by two reviewers (E.H.K. and C.T.) through title, abstract, and full-text screening using reference management software. Inter-rater agreement was assessed using Cohen's kappa at each stage of the selection process, and disagreements were resolved by a third reviewer (Z.M.).

Data were extracted using a standardized data collection form. In addition to the predefined outcomes, we collected information on study characteristics (title, first author, year of publication, country, study design, and eligibility criteria), patient demographics, anticoagulation regimens, and interventions. Data extraction was performed independently by two reviewers (E.H.K. and B.Y.L.), with discrepancies adjudicated by a third reviewer (F.D.).

7.1.3.1. Subgroup Analyses

Prespecified subgroup analyses were conducted according to disease severity, anticoagulation intensity, and baseline D-dimer concentration to explore potential sources of heterogeneity.

Disease severity was defined according to the level of care required. Severe disease cohorts included patients requiring ICU-level care, whereas moderate disease cohorts comprised hospitalized patients who did not require organ support or ICU admission.

Standard-dose anticoagulation was defined as prophylactic thromboprophylaxis. Higher-dose anticoagulation included intermediate- and therapeutic-dose regimens according to the 2021 American Society of Hematology recommendations. Therapeutic-dose anticoagulation corresponded to enoxaparin 1 mg/kg twice daily or 1.5 mg/kg once daily (or equivalent regimens), whereas intermediate-dose anticoagulation corresponded to enoxaparin 0.5 mg/kg once daily or 40 mg twice daily (or equivalent regimens)(40).

Subgroup analyses according to baseline coagulation status used a D-dimer threshold of 1 µg/mL (or 1 mg/L), selected based on early reports demonstrating a substantially increased mortality risk above this level. As most studies reported median baseline D-dimer values,

cohorts were categorized according to whether more than 50% of enrolled patients had baseline D-dimer concentrations above or below 1 µg/mL.

7.1.4. Risk of Bias and Quality of Evidence Assessment

The risk of bias assessment was performed independently by two review authors (E.H.K. and C.T.) in accordance with Cochrane Collaboration recommendations (41). Disagreements were resolved by a third reviewer (F.D.). Risk of bias in randomized controlled trials was evaluated using the RoB 2 tool(42), while the GRADE-Pro(43) framework was applied to assess the overall quality of evidence. Publication bias was examined through visual inspection of funnel plots.

7.1.5. Data Synthesis and Statistical Analysis

Statistical analyses were performed in R (v4.1.1; R Foundation for Statistical Computing, Vienna, Austria) using the **meta** (v5.2.0) and **dmetar** (v0.0.9000) packages for computation and visualization (44–46).

Effect sizes were expressed as odds ratios (ORs) with 95% confidence intervals (95% CI) for dichotomous outcomes, based on the number of events and total participants extracted from each study (47–49).

Meta-analyses were conducted using a random-effects model. The Hartung–Knapp adjustment was applied when more than five studies were available for a given outcome (50,51). Pooled estimates were calculated using the Mantel–Haenszel method without continuity correction to account for zero-event cells. Between-study variance (τ^2) was estimated using the Paule–Mandel method, with confidence intervals derived via the Q-profile method (52,53).

Statistical heterogeneity was assessed using Cochran’s Q test and the I^2 statistic, with $p < 0.1$ considered indicative of significant heterogeneity (54).

Publication bias was evaluated by visual inspection of funnel plots plotting log-transformed effect sizes against standard errors. Outlier and influence diagnostics were performed according to established methodological recommendations (Harrer et al., 2021; Viechtbauer and Cheung, 2010) (55,56).

7.2. Study II. - Exploratory Analysis of Coagulation and Fibrinolysis Trajectories After IL-6 Antagonist Therapy in COVID-19: A Case Series

7.2.1. Study Design and setting

We conducted a prospective, exploratory, multicenter case series involving three Hungarian intensive care units (See Table 2.). The protocol has been published previously and was registered on ClinicalTrials.gov (NCT05218369) (57).

Table 2. Type and main characteristics of the participating centres

Participating site	Progressivity level	No. of beds
Department of Anesthesiology and Intensive Care, Szent György Hospital Fejér County (district general hospital)	III.	31
Department of Anesthesiology and Intensive Care , Flór Ferenc Hospital of Pest County (district general hospital)	III.	1
Department of Anesthesiology and Intensive Care, Semmelweis University (university hospital)	III.	41
Department of Anesthesiology and Intensive Care, University of Pécs (university hospital)	III.	25

*Progressivity level is measured from 1 to 3, where 3 is the institution with most facility and personal thus can take care of a wide variety of pathology and 1 are institution with reduced personel and facilities.

7.2.2. Ethics and Patient Consent

The study was conducted in accordance with the amended Declaration of Helsinki and approved by the Hungarian Medical Research Council (1405-3/2022/EÜG). As a non-interventional observational study, participation did not interfere with standard clinical care, which followed Hungarian national guidelines for the management of severe COVID-19 (58).

Written informed consent was obtained from all participants or their legally authorized representatives prior to inclusion, with the opportunity to ask questions. Participants retained the right to withdraw at any time without justification.

7.2.3. Participants

We included adult patients with PCR- or antigen-confirmed SARS-CoV-2 infection who developed acute respiratory failure requiring immunomodulatory therapy (see Table 3 for detailed eligibility criteria). Indications for treatment included invasive or non-invasive

ventilation or high-flow nasal oxygen therapy ($\text{FiO}_2 > 0.4$; flow > 30 L/min) in the presence of systemic inflammation ($\text{CRP} > 75$ mg/L).

Table 3: Eligibility criteria

Inclusion criteria:	Exclusion criteria:
- Adults (>18 years old) - Clinical diagnosis of SARS-CoV2 infection with rtPCR confirmation - Disease severity that has the indication of immunomodulation therapy with interleukin-6 antagonist: acute respiratory failure that requires invasive, noninvasive ventilation, Noninvasive O2 therapy or high flow nasal oxygen therapy with the following parameters: $\text{FiO}_2 > 0.4$, flow > 30 L/min AND $\text{CRP} > 75$ mg/L	- The patient had previously been administered one of the following immunomodulating drugs: anakinra, tocilizumab, sarilumab - Presence of any condition or drug in the medical history that can lead to immunosuppression - Suspicion of infection (active tuberculosis, bacterial, viral, fungal) or level of procalcitonin higher than 0,5 ng/ml at the enrollment of the patient - The number of thrombocytes lower than $50 \times 10^9/\text{L}$ - More than >120 hours passed between the admission to the intensive care unit and the administration of an interleukin-6 antagonist - Administration of any of the following drugs the week before or during the study: fibrinolytic therapy, factor products (PCC, ATIII, FVIIa, FXIII), fibrinogen, desmopressin, tranexamic acid, blood products (fresh frozen plasma, thrombocyte concentrate) - Pregnancy - The patient or his legal guardian does not sign the consent

Abbreviations used in the table: rtPCR: Reverse transcription polymerase chain reaction, FiO_2 : fraction of inspired oxygen, CRP: C reactive protein, PCC: prothrombin complex concentrate, ATIII: antithrombin III concentrate, FVIIa: factor VIIa concentrate, FXIII: factor XII concentrate

7.2.4. Variables and Data Sources

Prospective data collection included demographics, comorbidities, clinical status, administered therapies, microbiological results (including blood cultures), routine laboratory parameters, and viscoelastic hemostatic assays (VHA). Data were entered into standardized electronic case report forms (eCRFs) within a centralized database, and all patients were de-identified using unique study identifiers.

Blood sampling was performed at baseline prior to tocilizumab administration (T0) and at 24 h (T1), 48 h (T2), 120 h (T3), and 168 h (T4) thereafter (see Table 4).

VHA were performed using the ClotPro® system (Haemonetics Corporation, Boston, MA, USA), including extrinsic (EX), intrinsic (IN), fibrinogen (FIB), tissue plasminogen

activator-enhanced (TPA), Russell viper venom (RVV), and ecarin clotting (ECA) assays. Derived parameters included indices of clot initiation (clotting time, CT), propagation and firmness (CFT, MCF), and fibrinolysis (maximum lysis, ML; clot lysis index at 30 and 45 min, CLI-30/45; lysis onset time, LOT; and lysis time, LT).

Routine laboratory testing (complete blood count, CRP, procalcitonin, ferritin, INR, aPTT, thrombin time, fibrinogen, and D-dimer) was performed daily.

In addition, plasma samples were stored at -80°C for centralized ELISA-based quantification of inflammatory, endothelial, and fibrinolytic biomarkers, including IL-6, syndecan-1, von Willebrand factor antigen and activity, antithrombin, plasminogen, $\alpha 2$ -antiplasmin, thrombin-activatable fibrinolysis inhibitor, thrombin-antithrombin complex, plasmin-antiplasmin complex, plasminogen activator inhibitor-1, and tissue plasminogen activator. These analytes were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource, San Diego, CA, USA) at the Szentágotthai Research Center (University of Pécs), according to the manufacturer's instructions, with detection ranges as follows: TAFI (2.5–160 ng/mL), TAT (31.2–1000 pg/mL), PAP (0.78–50 ng/mL), syndecan-1 (1.56–100 ng/mL), PAI-1 (0.312–20 ng/mL), and tPA (0.156–10 ng/mL). Biomarkers limited by logistical constraints were measured at T0, T2, and T4.

Table 4.: Timeline of the assesment of inflammatory and coagulation parameters relative to the administration of IL-6 antagonist

Day	Conventional laboratory parameters	ClotPro tests	Blood sample
0	inflammatory parameters, clinical biochemistry and conventional coagulation parameters	EX, IN, FIB, TPA, RVV, ECA tests	Plasma, Serum
	Administration of IL-6 antagonist		
24h	inflammatory parameters, clinical biochemistry and conventional coagulation parameters	EX, IN, FIB, TPA, RVV, ECA tests	
48h	inflammatory parameters, clinical biochemistry and conventional coagulation parameters	EX, IN, FIB, TPA, RVV, ECA tests	Plasma, Serum
5. day	inflammatory parameters, clinical biochemistry and conventional coagulation parameters	EX, IN, FIB, TPA, RVV, ECA tests	
7. day	inflammatory parameters, clinical biochemistry and conventional coagulation parameters	EX, IN, FIB, TPA, RVV, ECA tests	Plasma, Serum

Abbreviations used in the table: EX-test: extrinsic coagulation pathway test, FIB-test functional fibrinogen test, IN-test intrinsic coagulation pathway test, TPA-test:tissue plasminogen activator testRVV-test Russell's viper venom reagent test and ECA-test: ecarin clotting assay test.

7.2.5. Outcomes

The primary outcome was VHA-derived fibrinolysis dynamics assessed by lysis time (LT) and lysis onset time (LOT) using TPA- and ECA-tests, as well as maximum lysis (ML) and clot lysis index at 45 min (CLI-45) derived from the EX-test, measured at T0 and serial timepoints (T1–T4).

Secondary outcomes included longitudinal trajectories of plasma biomarkers of coagulation (plasminogen, plasmin–antiplasmin complex, thrombin-activatable fibrinolysis inhibitor, plasminogen activator inhibitor-1, α 2-antiplasmin, and tissue plasminogen activator; T0, T2, T4), inflammatory markers (procalcitonin, CRP, ferritin, and LDH), endothelial injury markers (syndecan-1 and von Willebrand factor antigen/activity), and conventional coagulation parameters (fibrinogen, antithrombin, thrombin–antithrombin complex, and platelet count), as well as their associations with VHA-derived indices.

7.2.6. *Statistical Methods*

All statistical analyses were performed using R (version 4.5.1; R Core Team, 2021).(59) Given the exploratory nature of the study and limited sample size, analyses focused on descriptive statistics and longitudinal pattern exploration rather than causal inference.

Categorical variables were summarized as counts and percentages, and continuous variables as mean $\pm$ standard deviation or median (interquartile range), as appropriate. No imputation, winsorization, or outlier exclusion was applied, and analyses were conducted on available cases.

Longitudinal comparisons across timepoints were performed using the Wilcoxon signed-rank test, with Hodges–Lehmann estimates and corresponding 95% confidence intervals. P-values were adjusted for multiple testing using the false discovery rate (FDR) method.

For visualization, box-and-jitter plots were generated for each biomarker and timepoint, displaying individual patient values stratified by survival status (survivors: grey circles; non-survivors: red triangles). Due to right-skewed distributions, predefined upper display limits were applied for graphical clarity; values exceeding these thresholds were truncated at the axis limit and indicated with asterisks, without affecting statistical analyses.

For fibrinolysis-specific analyses, patients were stratified according to fibrinolytic resistance defined by a TPA-test lysis time (LT) threshold of 312 s at admission, as previously described by Coupland et al (60).

Temporal evolution was additionally illustrated using spaghetti plots with individual trajectories, overlaid with median trends and interquartile ranges.

Given the prospective design and complete in-hospital follow-up, loss to follow-up did not occur. Statistical significance was set at $p < 0.05$.

8. RESULTS

8.1. Study I. – Higher Dose Anticoagulation Cannot Prevent Disease Progression in COVID-19 Patients: A Systematic Review and Meta-Analysis

8.1.1. Study Search and Selection

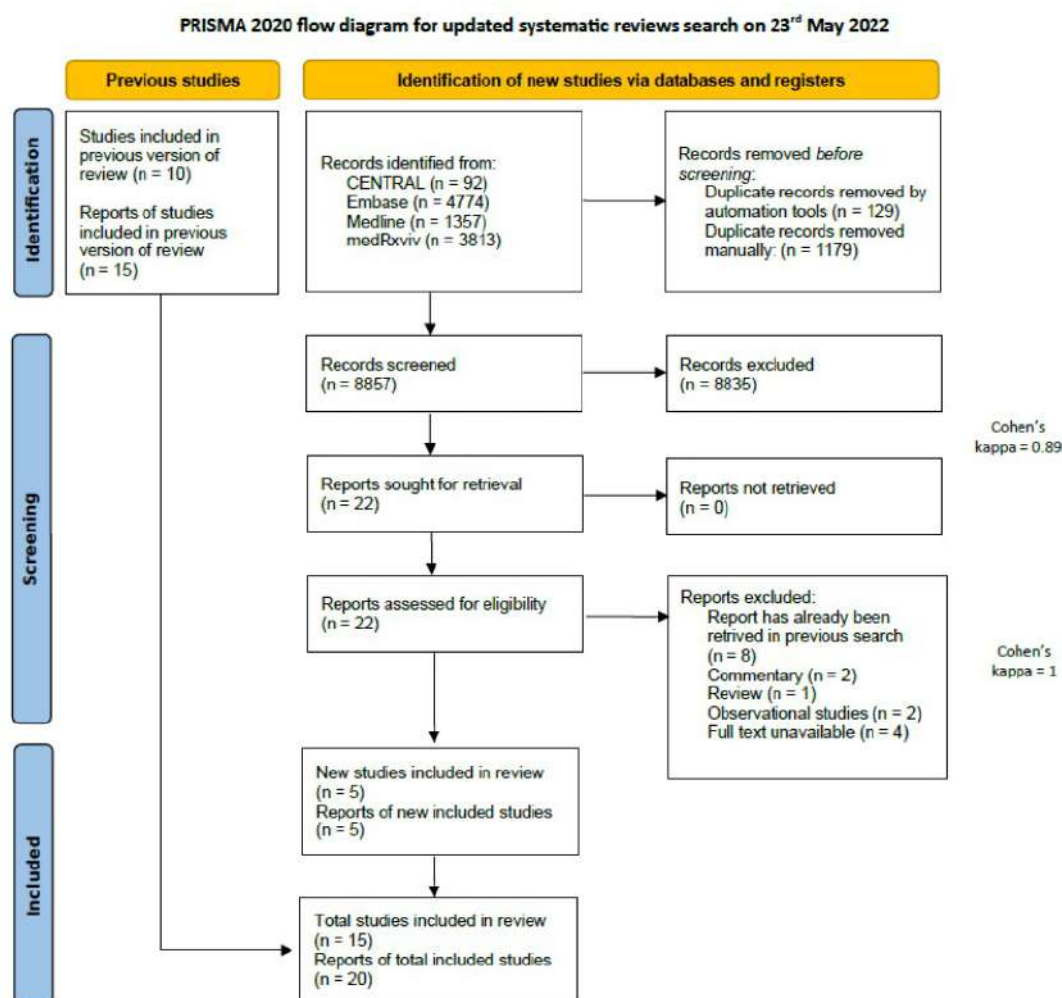


Figure 2. PRISMA 2020 flow diagram of the updated search.

Our systematic search identified 17,114 records (8,749 from the 18 October 2021 search and 8,365 from the 23 May 2022 update). Following study selection, 14 articles were included in the meta-analysis (30,32,33,61–71) and 15 in the systematic review (72). The study selection process is summarized in the PRISMA 2020 flow diagram (Figure 2).

The included open-label studies were conducted between April 2020 and September 2021 across 15 countries, enrolling a total of 6,253 adult patients with a mean age above 50 years. Seven studies included patients with severe disease (33,61,66,68–71), seven with moderate disease at baseline, and one with mild disease (30,32,63,64,67,69,72).

Elevated D-dimer levels above the upper limit of normal or a disseminated intravascular coagulation (DIC) score ≥ 3 were used as eligibility criteria in ten studies (30,32,61,62,64–66,68–71). Four studies compared intermediate-dose with standard-dose thromboprophylaxis (33,63,68), including one three-arm trial that also evaluated therapeutic anticoagulation (65). The remaining studies compared therapeutic-dose anticoagulation with standard care, predominantly using enoxaparin (30,32,61–63,66,67,69–71). One trial evaluated high-dose rivaroxaban versus standard LMWH (32), while a three-arm study compared therapeutic unfractionated heparin with therapeutic and standard-dose LMWH (70). Additional trials assessed different dosing regimens of bemiparin and tinzaparin, respectively (64, 65). Study-level characteristics are summarized in Table 5.

Table 5. Characteristics of included studies

<u>Study</u>	<u>Design</u>	<u>Enrollment period</u>	<u>Country</u>	<u>N</u>	<u>Patients admitted to</u>	<u>Intervention arms</u>	<u>Follow-up period (days)</u>
HESACOVID 2020(66)	Open-label, single center RCT	From April 2020 to July 2020.	Brazil	20	100% ICU	Therapeutic: enoxaparin 1 mg/kg twice daily or UFH adjusted to aPTT 1.5-2 Standard: enoxaparin 40 mg once daily or UFH 5000 three times a day ^a	28
Goligher et al. 2021(61)	Open-label, adaptive, multiplatform RCT	From April 21 2020 to December 19 2020	United States, Canada, the United Kingdom, Brazil, Mexico, Nepal	1103	100% ICU	Therapeutic: enoxaparin 1,5 mg/kg once daily, UFH titrated to have 1,5-2,5 aPTT, dalteparin 200 UI once daily, Tinzaparin 175 UI/kg once daily Standard: enoxaparin 40 mg once daily , dalteparin 5000 UI once, tinzaparine 4500 UI, fondaparinux 2,5 mg, UFH 5000 UI/ 8-12h ^b	21
Lawler et al. 2021(67)	Open-label, adaptive, multiplatform RCT	From April 21 2020 to January 22 2021	United States, Canada, the United Kingdom, Brazil, Mexico, Nepal	2231	100% ward	Therapeutic: enoxaparin 1,5 mg/kg once daily, UFH titrated to have 1,5-2,5 aPTT, dalteparin 200 UI once daily OR 100UI twice daily tinzaparin 175 UI/kg once daily Standard: enoxaparin 40 mg once daily, dalteparin 5000 UI once, tinzaparine 4500 UI, fondaparinux 2,5 mg UFH 5000 UI/ 8-12h ^b	21
Perepu et al. 2021(68)	Open-label,	From April 26	USA	173	38% ward	Intermediate: enoxaparin 1 mg/kg daily	30

Table 5. Characteristics of included studies

<u>Study</u>	<u>Design</u>	<u>Enrollment period</u>	<u>Country</u>	<u>N</u>	<u>Patients admitted to</u>	<u>Intervention arms</u>	<u>Follow-up period (days)</u>
	multicenter RCT	2020 to January 6 2021			62% ICU	Standard: enoxaparin 40 mg daily ^b	
X-COVID 2021(63)	Open-label, multicentre RCT	From April 30 2020 to April, 25 2021	Italy	183	100% ward	Intermediate: enoxaparin 40 mg twice daily Standard: enoxaparin 40 mg once daily	30
COVID-HEP 2022 (69)	Open-label, multicentre RCT	From April 2020 to June 2021	Switzerland	159	45% ward 26% intermediate care 28% ICU	Therapeutic: enoxaparin 1 mg/kg twice daily or UFH with anti-Xatitration Standard: enoxaparin 40 mg once daily or UFH 5000 IU twice daily at ward enoxaparin 40 mg twice daily or UFH 5000 three times daily ^c	30
HEP-COVID 2020 (62)	Single-blinded, multicentre RCT	From May 8 2020, to May 14 2021	USA	253	67% ward 32% ICU	Therapeutic: enoxaparin 1 mg/kg twice daily Standard dose: enoxaparin 30 or 40 mg once or twice daily ^d	30
RAPID 2021 (30)	Open-label, adaptive, multicentre RCT	from May 29 2020 to April 12 2021	Brazil, Canada, Ireland, Saudi Arabia, United Arab Emirates,	465	100% ward	Therapeutic: enoxaparin 1.5 mg/kg once daily, dalteparin 200 UI/kg once daily, tinzaparin 175 IU/kg once daily, or UFH titrated according to aPPT Standard: 40 mg enoxaparin once daily, dalteparin 5000	28

Table 5. Characteristics of included studies

<u>Study</u>	<u>Design</u>	<u>Enrollment period</u>	<u>Country</u>	<u>N</u>	<u>Patients admitted to</u>	<u>Intervention arms</u>	<u>Follow-up period (days)</u>
			United States of America			UI daily, tinzaparin 4500 UI daily, UFH 5000 UI two or three times a day ^a	
ACTION 2021 (32) ^l	Open-label, pragmatic, multicentre RCT	From June 24 2020 to Feb 26 2021	Brazil	615	93% ward 6% ICU	Therapeutic: Rivaroxaban 20 mg once daily, enoxaparin 1mg/kg twice daily, UFH titrated until anti-Xa 0.3-0.7 Standard: local prophylatic guidelines	30
Oliylyk et al. 2021 (70)	Open-label RCT	From July 2020 to 1 March 2021	Ukraine	126	100% ICU	Therapeutic: group 1: enoxaparin of 100 antiXa IU/kg twice daily group 2: UFH titrated to aPTT 40-70s Standard: enoxaparin of 50 antiXa IU/kg once daily	28
INSPIRATION 2021 (33)	Open-label, multicenter RCT	From July 29, 2020 to November 19, 2020	Iran	562	100% ICU	Intermediate: enoxaparin 1 mg/kg daily Standard: enoxaparin 40 mg daily	30
Rashidi et al. 2021 (71)	Open-label, pilot RCT	From September 2020 to April 2021	Iran	10	100% ICU	Therapeutic dose: UFH 5000 IU every 8 hourse Standard: UFH titrated until aPTT 5-70	30
BEMICOP 2022 (64)	Open-label, multicenter, RCT	From October 2020 to May 2021	Spain	65	100% ward	Therapeutic: bemiparin 115 UI/kg daily Standard: bemiparin 3500 UI daily	30

Table 5. Characteristics of included studies

<u>Study</u>	<u>Design</u>	<u>Enrollment period</u>	<u>Country</u>	<u>N</u>	<u>Patients admitted to</u>	<u>Intervention arms</u>	<u>Follow-up period (days)</u>
PROTHROMCOVID 2022 (65)	Open-label, multicenter RCT	From February 1st 2021 to September 30th 2021	Spain	300	100% ward	Therapeutic: tinzaparin 175 UI/kg Intermediate: tinzaparin 100 UI/kg Standard: tinzaparin 4500 UI daily	30

Abbreviations used in this table: RCT: randomised controlled trial, BMI: body mass index, CrCl: creatinine clearance, UFH: unfractionated heparine, UI: international units

Explanations: a adjusted to age, BMI, and CrCL; b adjusted to BMI; c adjusted to patient's weight; d adjusted for CrCl

8.1.2. Primary Outcome – All-cause mortality

To assess the effectiveness of higher-dose (HD) compared with standard-dose (SD) anticoagulation, data on 21–30-day all-cause mortality were extracted from 12 included RCTs (30,32,33,62–66,68–71). This outcome occurred in 232/1525 patients in the HD group and 235/1405 patients in the SD group, demonstrating no significant difference between treatment strategies (OR 0.83, 95% CI 0.54–1.28; $p = 0.3748$; $I^2 = 41\%$, $p = 0.06$).

8.1.2.1. Subgroup Analysis by Disease Severity and Anticoagulation Regimen

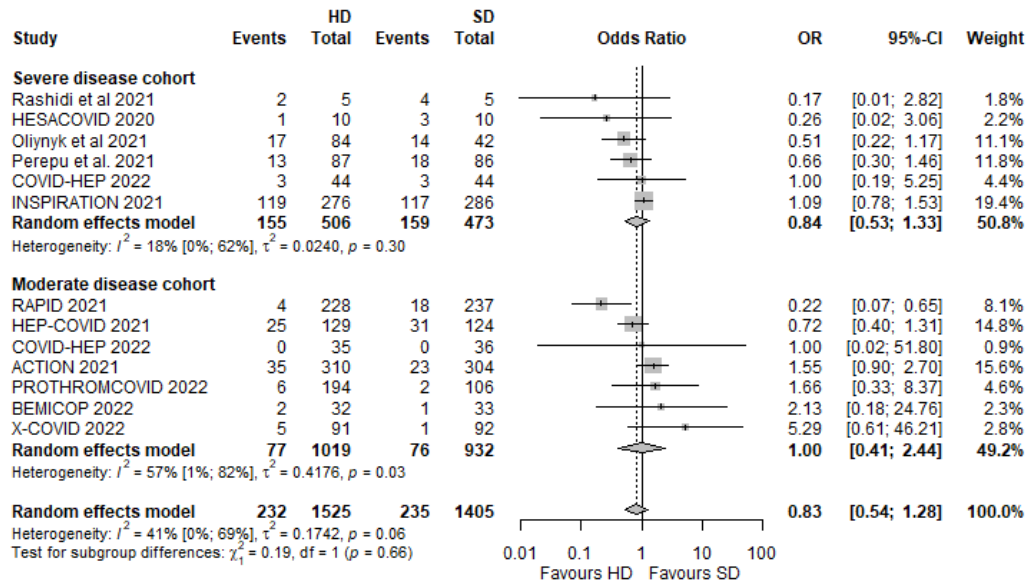
No relevant differences between HD and SD anticoagulation were observed in subgroup analyses stratified by disease severity, either in severe (OR 0.84, 95% CI 0.53–1.33) or moderate disease cohorts (OR 1.00, 95% CI 0.41–2.33) (Figure 3A).

Similarly, no effect of HD compared with SD was identified when stratified by anticoagulation intensity. Intermediate-dose regimens yielded an OR of 1.08 (95% CI 0.44–2.65), whereas therapeutic-dose regimens were associated with an OR of 0.72 (95% CI 0.40–1.31). The pooled effect estimate across dosing strategies was OR 0.85 (95% CI 0.55–1.31; $p = 0.4237$) (Figure 3B).

8.1.2.2. Subgroup Analysis by Baseline D-Dimer Levels

In studies with baseline D-dimer levels $<1 \mu\text{g/mL}$, no significant difference was observed between HD and SD anticoagulation (OR 1.19, 95% CI 0.67–2.11). In contrast, studies enrolling patients with D-dimer $>1 \mu\text{g/mL}$ showed a non-significant trend toward benefit with HD anticoagulation (OR 0.56, 95% CI 0.31–1.00). The overall pooled estimate across D-dimer strata indicated no significant difference between groups (OR 0.83, 95% CI 0.51–1.32; $p = 0.3914$) (Figure 4).

2A)



2B)

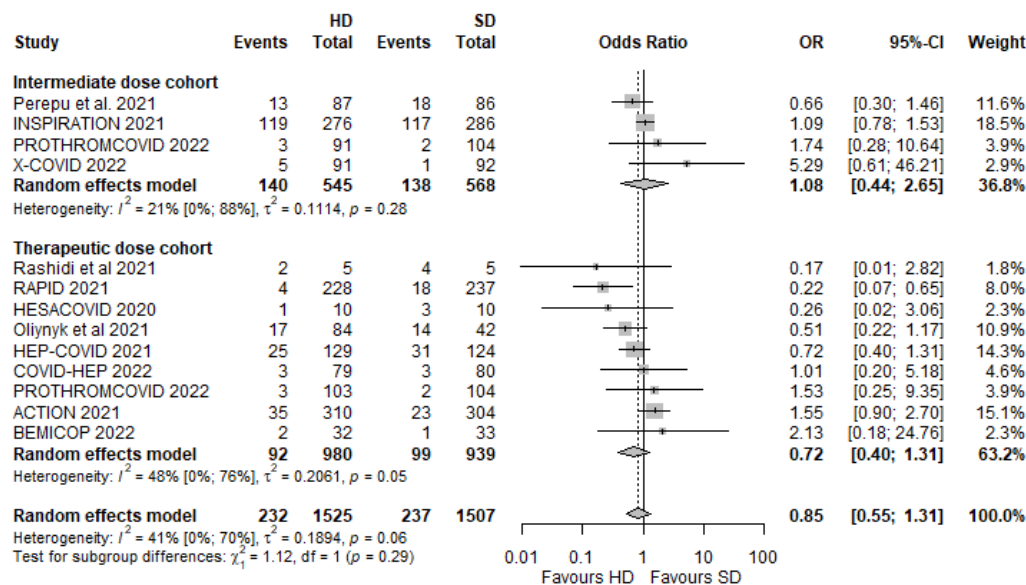


Figure 3. All-cause mortality in different dosing regimens in moderate and severe disease cohorts [19,21–25,50,52–56]. (A) All-cause mortality in severe and moderate disease cohorts; (B) all cause mortality in cohorts where intermediate or therapeutic dose was used in the HD group. Abbreviations: HD: high dose; SD: standard dose

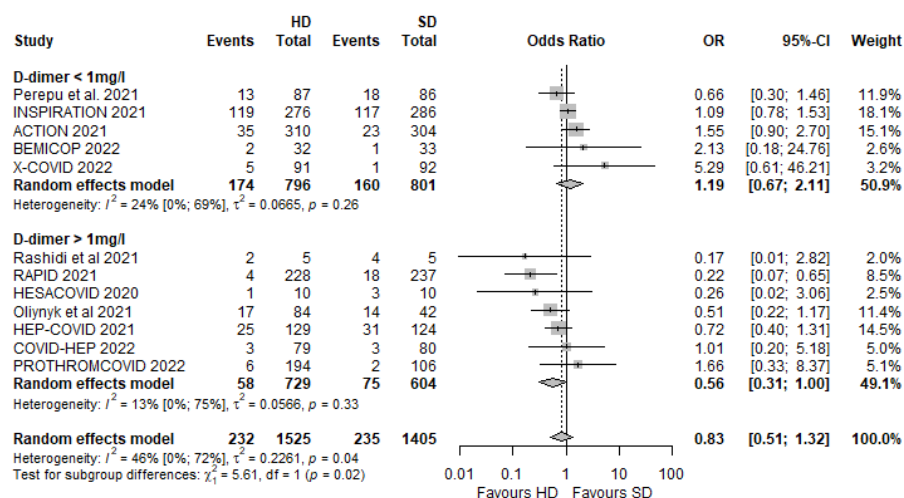


Figure 4. All-cause mortality in cohorts with different baseline D-dimer levels [19,21–25,50,52–56]. Abbreviations: HD: higher-dose; SD: standard dose

8.1.3. Secondary Outcomes

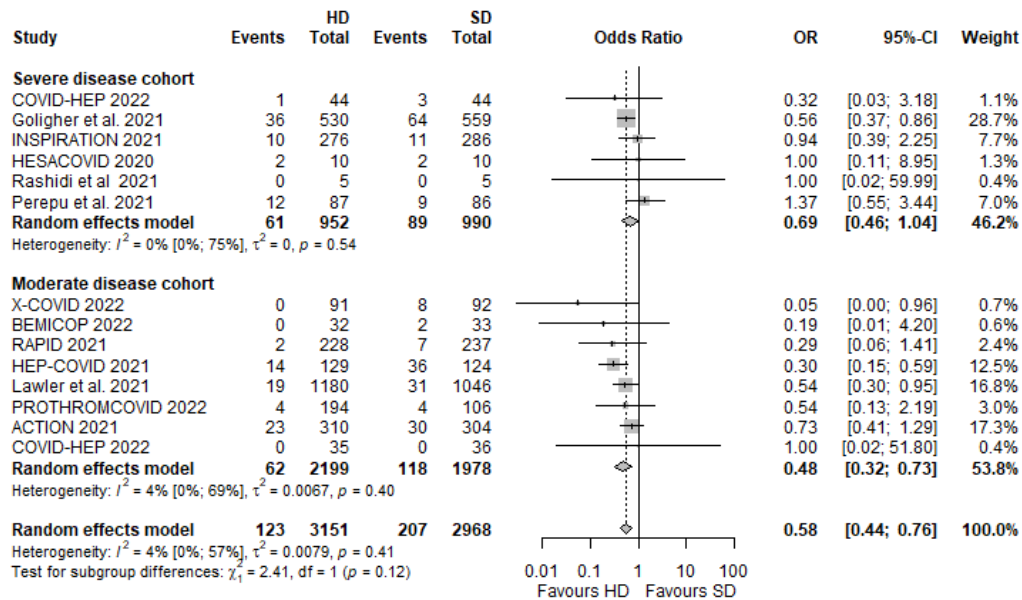
8.1.3.1. Any Thrombotic Events and Major Bleeding

A total of 13 RCTs (30,32,33,61–69,71) reporting any thrombotic events (venous or arterial) were included, comprising 6119 patients. Thrombotic events occurred in 123/HD and 207/SD patients, corresponding to a significantly lower risk in the HD group (OR 0.58, 95% CI 0.44–0.76; $p < 0.001$; Figure 5A).

In subgroup analyses, a non-significant reduction in thrombotic events was observed in the severe disease cohort (OR 0.69, 95% CI 0.46–1.04), whereas a significant reduction was found in the moderate disease cohort (OR 0.48, 95% CI 0.32–0.76). Stratification by anticoagulation intensity showed a non-significant effect in the intermediate-dose subgroup (OR 0.71, 95% CI 0.13–4.00), while therapeutic-dose anticoagulation was

associated with a significant reduction in thrombotic events (OR 0.52, 95% CI 0.41–0.66) (Figure 5B).

4A)



4B)

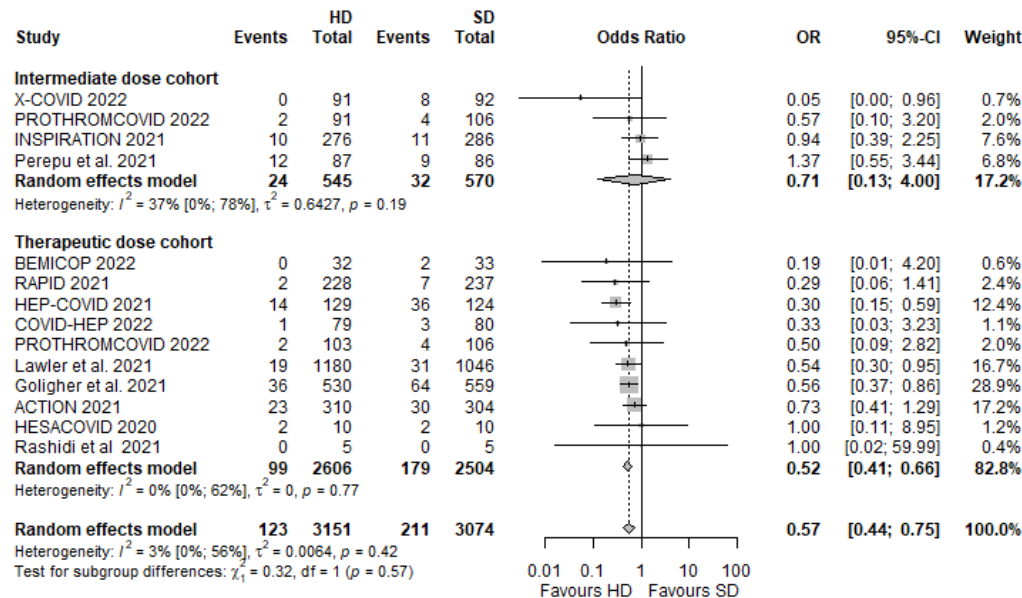
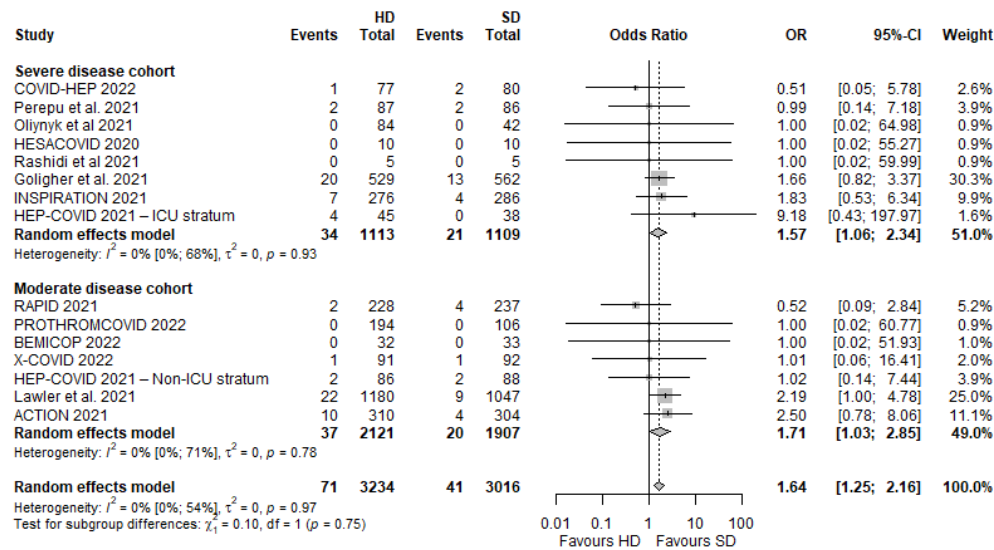


Figure 5. Any thrombotic events in different dosing regimens in moderate and severe disease cohorts [19–25,51–56]. (A) Any thrombotic events in severe and moderate

disease cohorts; (B) any thrombotic events in cohorts where an intermediate or therapeutic dose was used in the HD group. Abbreviations: HD: higher-dose; SD: standard dose

Fourteen RCTs including 6250 patients (30,32,33,61–71) reported major bleeding outcomes. HD anticoagulation was associated with a significantly increased risk of major bleeding compared with SD (OR 1.64, 95% CI 1.25–2.16; $p < 0.0017$; Figure 6).

5A)



5B)

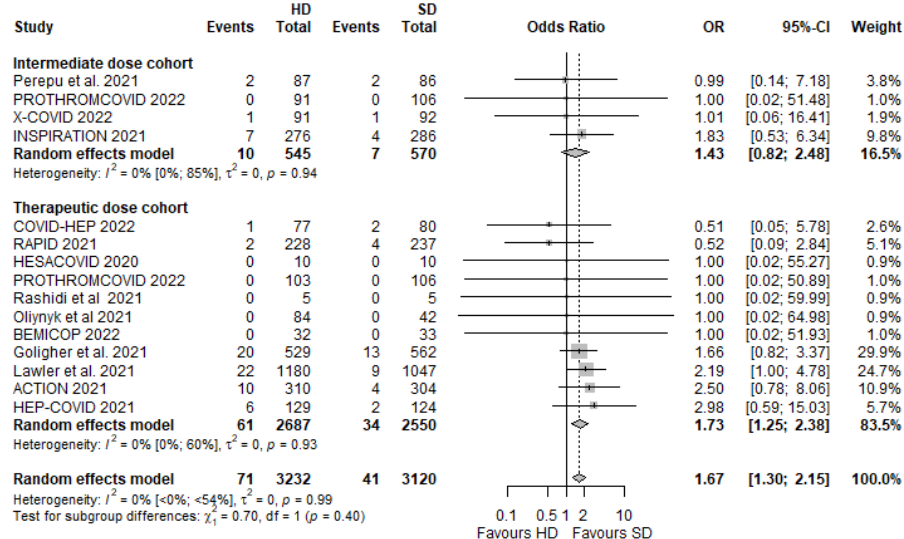


Figure 6. Major bleedings in different dosing regimens in moderate and severe disease

cohorts . (A) Major bleeding events in severe and moderate disease cohorts; (B) major bleeding events in cohorts where intermediate or therapeutic dose was used in the HD group. Abbreviations: HD: higher-dose; SD: standard dose

Subgroup analyses showed consistent findings across disease severity strata, with increased bleeding risk in both severe (OR 1.57, 95% CI 1.06–2.34) and moderate disease cohorts (OR 1.71, 95% CI 1.03–2.85) (Figure 6A). When stratified by anticoagulation intensity, the association did not reach statistical significance in the intermediate-dose subgroup (OR 1.43, 95% CI 0.82–2.48), whereas therapeutic-dose regimens were associated with a significant increase in major bleeding (OR 1.73, 95% CI 1.25–2.15) (Figure 6B).

8.1.3.2. Progression of Disease

To evaluate the effect of anticoagulation intensity on disease progression, we assessed the need for invasive mechanical ventilation, ICU admission, progression to ARDS, and an exploratory composite outcome including death, pulmonary embolism, and invasive mechanical ventilation.

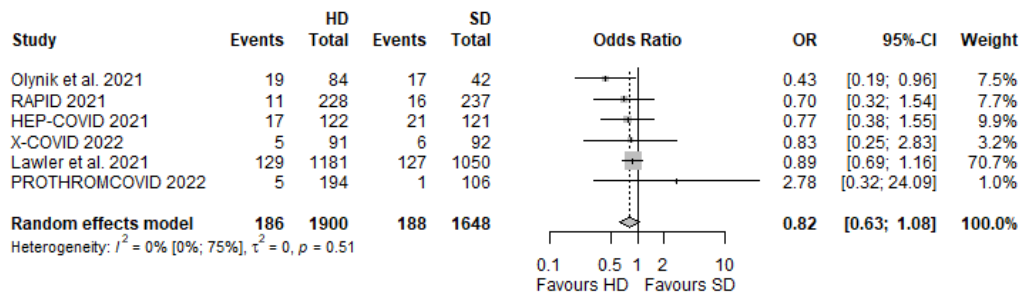


Figure 7. Need for invasive mechanical ventilation. Abbreviations used: HD: higher-dose; SD: standard dose

Six RCTs (30,32,62,63,65,70) comprising 3548 patients reported the need for invasive mechanical ventilation. No significant difference was observed between HD and SD anticoagulation, although a non-significant trend towards a lower risk was noted in the HD group (OR 0.82, 95% CI 0.63–1.08; $p = 0.1214$) (Figure 7).

ICU admission was reported in four RCTs (30,64,65,69), with no significant difference between groups (OR 0.98, 95% CI 0.65–1.45; $p = 0.9047$).

Three RCTs (62,63,65) reported ARDS progression, occurring in 16/412 patients in the HD group and 10/319 in the SD group, corresponding to a non-significant effect (OR 1.22, 95% CI 0.35–4.24; $p = 0.7563$).

An exploratory composite outcome was constructed to capture overall disease progression, comprising death, pulmonary embolism, and invasive mechanical ventilation, in order to increase sensitivity for detecting a potential aggregated treatment effect across clinically relevant severe outcomes not uniformly powered or consistently reported across individual trials. This outcome was reported in four RCTs (30,62,63,70). The pooled analysis demonstrated a significantly lower risk in the HD group (OR 0.46, 95% CI 0.21–0.67; $p < 0.0001$).

8.1.4. Risk of Bias Assessment and Quality of Evidence

The risk of bias was assessed using the RoB 2 tool (42). Overall risk of bias was low in three RCTs, high in one trial due to missing outcome data, and of some concern in the remaining studies (Figure 8). Certainty of evidence assessment GRADE can be found in detail in Table 6.

	Risk of bias domains					
	D1	D2	D3	D4	D5	Overall
Perepu et al. 2021	+	+	+	-	+	-
RAPID 2021	+	+	+	+	+	+
HEP-COVID 2021	+	-	+	+	+	-
Goligher et al. 2021	+	-	-	-	+	-
Lawler et al. 2021	+	+	-	-	+	-
INSPIRATION 2021	+	-	-	+	+	-
ACTION 2021	+	-	+	+	+	-
HESACOVID 2020	+	+	+	-	-	-
X-COVID 2022	+	+	+	+	+	+
BEMICOP 2022	-	+	X	-	-	X
PROTHROMCOVID 2022	-	+	+	-	-	-
COVID-HEP 2022	+	+	+	+	+	+
Oliynyk et al. 2021	-	-	+	-	-	-
Rashidi et al 2021	-	-	+	-	-	-

Domains:

D1: Bias arising from the randomization process.
D2: Bias due to deviations from intended intervention.
D3: Bias due to missing outcome data.
D4: Bias in measurement of the outcome.
D5: Bias in selection of the reported result.

Judgement

X High
- Some concerns
+ Low

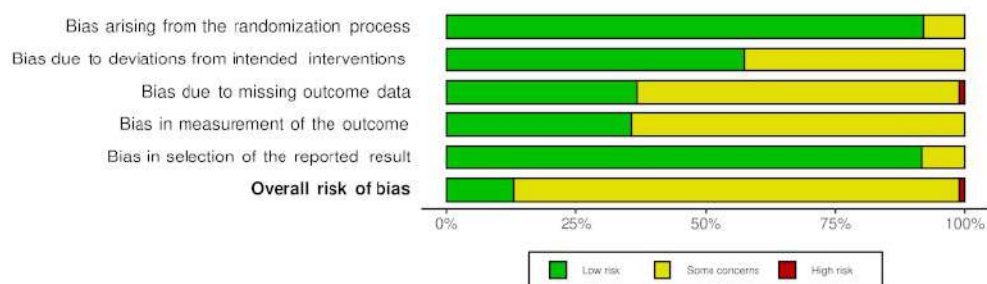


Figure 8. Risk of bias assessment for included randomized controlled trials.

Table 6. GRADE assessment of the included studies.

Certainty assessment							N ₂ of patients		Effect		Certainty
N ₂ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	HD group	SD group	Relative (95% CI)	Absolute (95% CI)	
All-cause mortality (follow-up: 21-30 days)											
12	randomised trials	not serious ^a	not serious	not serious	serious ^b	none	232/1525 (15.2%)	235/1405 (16.7%)	OR 0.83 (0.54 to 1.28)	24 fewer per 1,000 (from 69 fewer to 37 more)	⊕⊕⊕○ Moderate
Any thrombotic events (follow-up: 21-30 days)											
13	randomised trials	serious ^a	not serious	not serious	serious ^c	none	123/3151 (3.9%)	207/2968 (7.0%)	OR 0.58 (0.44 to 0.76)	28 fewer per 1,000 (from 38 fewer to 16 fewer)	⊕⊕○○ Low
Major bleeding											
13	randomised trials	not serious ^a	not serious	not serious	serious ^c	none	71/3234 (2.2%)	41/3016 (1.4%)	OR 1.69 (1.17 to 2.44)	9 more per 1,000 (from 2 more	⊕⊕⊕○ Moderate

Certainty assessment							No of patients		Effect		Certainty
No of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	HD group	SD group	Relative (95% CI)	Absolute (95% CI)	
										to 19 more)	
Need for invasive mechanical ventilation											
6	randomised trials	serious ^a	not serious	serious ^d	serious ^c	none	186/1900 (9.8%)	188/1648 (11.4%)	OR 0.82 (0.63 to 1.08)	19 fewer per 1,000 (from 39 fewer to 8 more)	⊕○○○ Very low
ICU admission											
4	randomised trials	serious ^a	not serious	serious ^d	serious ^b	none	61/489 (12.5%)	57/412 (13.8%)	OR 0.98 (0.65 to 1.45)	2 fewer per 1,000 (from 44 fewer to 51 more)	⊕○○○ Very low
Progression to ARDS											
3	randomised trials	serious ^a	very serious ^e	serious ^d	very serious ^b	none	16/412 (3.9%)	10/319 (3.1%)	OR 1.22 (0.35 to 4.24)	7 more per 1,000 (from 20 fewer to 89 more)	⊕○○○ Very low

Certainty assessment							№ of patients		Effect		Certainty
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	HD group	SD group	Relative (95% CI)	Absolute (95% CI)	
Composite outcome of death, pulmonary embolism and need for invasive mechanical ventilation											
4	randomised trials	serious ^a	not serious	serious ^d	serious ^c	none	108/532 (20.3%)	145/495 (29.3%)	OR 0.46 (0.31 to 0.67)	133 fewer per 1,000 (from 179 fewer to 76 fewer)	⊕○○○ Very low

Explanations:

a The included studies are open-label studies, some of them with blinded adjudication. All-cause mortality and major bleeding are objective outcomes are thus less influenced by a lack of allocation concealment.

b Some studies included a small sample size and low number of events. The 95% confidence interval is wide and crosses the line of no effect.

c Some of the studies included a small sample size and low number of events.

d There were some differences in outcome measures. Need for invasive mechanical ventilation, ICU admission, progression to ARDS, and the composite outcome included a subjective clinical decision—this and the open-label design could have influenced the measurement of the outcomes.

e There were wide differences in point estimates across studies.

Abbreviations used in this table: ARDS: acute respiratory distress syndrome; CI: confidence interval; HD: higher-dose (anticoagulation); ICU: intensive care unit; OR: odds ratio; SD: standard-dose (anticoagulation)

8.2. Study II - Exploratory Analysis of Coagulation and Fibrinolysis Trajectories After IL-6 Antagonist Therapy in COVID-19: A Case Series

8.2.1. Study Population

Between January 2022 and January 2023, 15 critically ill COVID-19 patients receiving IL-6 receptor blockade were included across three Hungarian ICUs. Enrollment was terminated early due to a decline in national case numbers; results are therefore presented as an exploratory case series.

The study population was predominantly male, with a mean age of 68.3 ± 8.6 years and a high burden of cardiovascular and metabolic comorbidities, including universal hypertension. Disease severity was reflected by a mean APACHE II score of 11.2 ± 3.4 and severe hypoxemic respiratory failure at baseline. All patients received standard-of-care treatment including corticosteroids and anticoagulation, with most also receiving antiviral therapy.

All patients completed follow-up; in-hospital mortality at day 7 and day 30 was 1/15 and 3/15, respectively, and 5 patients were discharged from the ICU during follow-up. Baseline characteristics are summarized in Table 7.

Table 7: General characteristics of the study population at admission

Characteristic	Value
Study population	15
Male	12/15 (80%)
Age	68.3 (8.6) ; 69.0 [64.0, 74.0]
BMI	32.2 (6.2) ; 30.0 [27.2, 35.4]
Hypertension	15/15 (100.0%)
Ischemic heart disease	6/15 (40.0%)
Atrial fibrillation	4/15 (26.6%)
Diabetes Type II	7/14 (50.0%)
Chronic Respiratory Disease	2/15 (13.3%)
Autoimmune disease	2/15 (13.3%)
Malignant Disease	1/15 (6.7%)
Renal disease	1/15 (6.7%)
Neurological or psychiatric disease	3/15 (20.0%)
Obesity	7/15 (46.7%)
APACHE II	11.2 (3.4) ; 12.0 [8.2, 13.0]
Clinical frailty scale	3.5 (1.2) ; 3.0 [3.0, 4.0]
Severe organ failure at admission	7/15 (46.7%)
Vasopressor support	4/15 (26.7%)
Currently on oxygen or ventilated	15/15 (100.0%)
Type of respiratory support	
High Flow Nasal Cannula	4/15 (26.7%)
Invasive Mechanical Ventilation	6/15 (40.0%)
Non Invasive Ventilation	3/15 (20.0%)
Non-rebreather mask	2/15 (13.3%)
Prone position	7/15 (46.7%)
Neuromuscular relaxant	2/15 (13.3%)
PaO ₂	74.4 (21.2) ; 69.2 [60.4, 80.2]
FiO ₂	0.9 (0.2) ; 1.0 [0.8, 1.0]
PaO ₂ /FiO ₂ (Horowitz index)	91 (40) ; 74 [65, 114]
PaCO ₂ (mmHg)	39.4 (6.4) ; 36.6 [35.1, 43.9]
Ferritin (ug/L)	1351.2 (1930.1) ; 673.0 [602.8, 1105.2]
CRP (mg/l)	183.7 (58.6) ; 180.9 [150.8, 211.0]
LDH (U/l)	841.4 (396.1) ; 759.5 [531.2, 1176.0]
Leucocytes (G/l)	10 (5) ; 8 [7, 13]
Hemoglobin (g/l)	127 (20) ; 120 [111, 146]
Hematocrite (%)	37 (5) ; 36 [33, 42]
Thrombocytes (G/l)	209 (81) ; 195 [180, 218]
ASAT / GOT (U/l)	76.3 (45.6) ; 62.0 [42.0, 109.5]
ALAT / GPT (U/l)	47.7 (33.1) ; 31.0 [26.5, 64.5]
APTT (sec)	39.0 (6.5) ; 40.4 [36.0, 44.7]
Thrombin time (sec)	18.8 (2.9) ; 18.1 [17.4, 21.6]
Fibrinogen (g/l)	5.6 (1.5) ; 5.5 [5.1, 6.4]
D dimer (ug/L)	10674.9 (33130.4) ; 1125.5 [837.5, 2347.5]
Tocilizumab dose (mg)	704.0 (98.9) ; 720.0 [600.0, 800.0]
Corticosteroids	15/15 (100.0%)
Antiviral medication (acyclovir, remdesivir or	14/15 (93.3%)

both)	
Blood products or derivatives	0/15 (0.0%)
Immunglobulin	0/15 (0.0%)
Antiplatelet agents	8/15 (53.3%)
Aspirin	8/15 (53.3%)
Clopidogrel	2/15 (13%)
Anticoagulants	13/15 (86.7%)
DOAC	1/13 (7.7%)
LMWH	12/13 (92.3%)
Daily dose of LMWH (IU)	11500.0 (5125.7) ; 12000.0 [8000.0, 13000.0]
Patient outcome at the end of the study period (7 days)	
Deceased	3/15 (20.0%)
Remained in ICU	8/15 (53.3%)
Discharged from ICU and transferred to ward	4/15 (26.7%)

Continuous: Mean (SD) ; Median [IQR] ; Binary: n/N, (%).

Abbreviations used in the table: ALAT / GPT, alanine aminotransferase / glutamate pyruvate transaminase; APACHE II, Acute Physiology and Chronic Health Evaluation II; APTT, activated partial thromboplastin time; ASAT / GOT, aspartate aminotransferase / glutamate oxaloacetate transaminase; BMI, body mass index; CRP, C-reactive protein; DOAC, direct oral anticoagulant; FiO₂, fraction of inspired oxygen; ICU, intensive care unit; LDH, lactate dehydrogenase; LMWH, low-molecular-weight heparin; PaCO₂, partial pressure of carbon dioxide; PaO₂, partial pressure of oxygen

8.2.2. Primary Outcomes

Paired Wilcoxon analyses identified nominally significant changes in four parameters: ECA-test LOT (T0 vs. T2) and EX-test CLI-45 (T0 vs. T2, T3, and T4) ($p < 0.05$; Table 7). None remained significant after false discovery rate adjustment; therefore, interpretation focused on temporal biological trajectories rather than isolated p-values.

Table 8 :Paired Wilcoxon Analysis With Hodges–Lehmann Estimates and FDR Correction for primary outcomes

Variable	Comparison	n_pairs	Median_T0	Median_Tn	Delta_med	HL_shift	HL_CI	p_value	p_adj	Sig	FDR_BH
TPA-test LT (s)	T0 vs T1	13	340.0 [245.0–353.0]	313.0 [225.0–380.0]	-20	17.5	[-141.5, 84.0]	0.53	0.85		
TPA-test LT (s)	T0 vs T2	14	321.5 [245.8–353.0]	325.0 [268.2–370.0]	-25.5	6.5	[-594.5, 76.5]	0.9	0.94		
TPA-test LT (s)	T0 vs T3	13	340.0 [248.0–353.0]	263.0 [182.0–333.0]	-98	96.5	[-21.5, 151.5]	0.11	0.31		
TPA-test LT (s)	T0 vs T4	12	321.5 [247.2–353.0]	306.5 [196.5–382.0]	-44	40.4	[-91.5, 95.5]	0.48	0.85		
TPA-test LOT (s)	T0 vs T1	14	91.0 [82.8–97.0]	88.5 [74.5–96.5]	0	-0.5	[-36.0, 16.0]	0.94	0.94		
TPA-test LOT (s)	T0 vs T2	13	92.0 [85.0–97.0]	95.0 [85.0–97.0]	0	-2.5	[-25.0, 10.0]	0.69	0.86		
TPA-test LOT (s)	T0 vs T3	12	94.5 [84.2–97.8]	82.0 [80.0–88.2]	-11.5	7.5	[-5.0, 20.0]	0.21	0.52		
TPA-test LOT (s)	T0 vs T4	11	92.0 [83.5–98.5]	87.0 [76.0–96.0]	-8	8.7	[-8.0, 23.0]	0.29	0.63		
ECA-test LOT (s)	T0 vs T1	14	249.0 [230.5–277.0]	299.0 [215.5–360.0]	-20	8	[-58.0, 66.0]	0.78	0.86		
ECA-test LOT (s)	T0 vs T2	14	241.5 [230.0–267.2]	224.5 [210.0–275.8]	-30	30.5	[4.0, 109.5]	0.04	0.23	*	
ECA-test LOT (s)	T0 vs T3	13	235.0 [230.0–265.0]	247.0 [210.0–303.0]	-20	13.5	[-31.5, 53.0]	0.55	0.85		
ECA-test LOT (s)	T0 vs T4	12	241.5 [229.5–265.8]	251.5 [212.5–316.0]	-8.5	6.5	[-40.5, 43.0]	0.67	0.86		
EX-test ML (%)	T0 vs T1	10	5.5 [3.2–7.0]	5.5 [5.0–7.0]	0	-0.5	[-3.5, 2.0]	0.73	0.86		
EX-test ML (%)	T0 vs T2	7	6.0 [3.5–7.0]	6.0 [2.5–6.5]	-2	1.5	[-3.0, 3.0]	0.39	0.79		
EX-test ML (%)	T0 vs T3	8	5.5 [3.8–7.2]	3.0 [2.0–5.0]	-2.5	2.2	[-0.0, 5.0]	0.11	0.31		
EX-test ML (%)	T0 vs T4	8	5.5 [3.0–7.2]	2.0 [1.8–3.2]	-3	2.7	[-0.5, 5.0]	0.06	0.23		
EX-test CLI-45 (%)	T0 vs T1	12	97.0 [96.0–99.0]	97.5 [96.8–99.2]	0	-0.5	[-2.0, 2.0]	0.67	0.86		
EX-test CLI-45 (%)	T0 vs T2	11	97.0 [96.0–99.0]	99.0 [98.0–100.0]	1	-2	[-3.5, 0.0]	0.05	0.23	*	
EX-test CLI-45 (%)	T0 vs T3	11	97.0 [96.5–99.0]	99.0 [98.5–100.0]	1	-2	[-2.5, -1.5]	0.02	0.23	*	
EX-test CLI-45 (%)	T0 vs T4	10	97.5 [96.2–99.0]	99.0 [99.0–100.0]	1.5	-2	[-3.0, -1.0]	0.03	0.23	*	

Variable, biomarker or viscoelastic parameter analyzed; Comparison, timepoint contrast (Tn vs T0); n_pairs, number of paired observations; Median_T0, median at baseline; Median_Tn, median at follow-up; Delta_med, median change (Median_Tn – Median_T0); HL_shift, Hodges–Lehmann shift estimator of the median paired difference; HL_CI, 95% confidence interval of the Hodges–Lehmann estimator; p_value, two-sided p-value from the Wilcoxon signed-rank test; p_adj, p-value after False Discovery Rate correction (Benjamini–Hochberg); Sig, significance coding (* = p<0.05, ** = p<0.01, *** = p<0.001*); FDR, False Discovery Rate adjustment procedure. Abbreviations: CLI-45, clot lysis index at 45 minutes; ECA-test, ecarin chromogenic assay (or ecarin clotting assay) test; EX-test, extrinsic pathway test; LOT, lysis onset time; LT, lysis time; ML, maximum lysis; s, seconds; TPA-test, tissue plasminogen activator test.

8.2.3. Secondary Outcomes

8.2.3.1. Inflammation and Endothelium

At admission, IL-6 (49.6 [16.2–79.2] pg/mL) and CRP (180.9 [150.8–211.0] mg/L) were markedly elevated. Following IL-6 receptor blockade, CRP decreased rapidly, with >90% reduction within five days, while IL-6 showed a transient increase at 48 h before declining during follow-up.

Procalcitonin demonstrated a gradual decrease following an early transient rise, whereas ferritin increased within the first 24 h and subsequently stabilized (826 µg/L at 168 h), suggesting delayed resolution of macrophage activation. Leukocyte counts increased during follow-up, while LDH declined but remained above the reference range.

Markers of endothelial injury were markedly elevated at baseline, with vWF activity (351.7 [341.8–438.0]%) and antigen (449.3 [321.6–595.3]%) further increasing to 483.9% and 630.0% at 168 h, respectively. Syndecan-1 was generally low or undetectable, with intermittent peaks suggestive of episodic glycocalyx shedding (Figure 9).

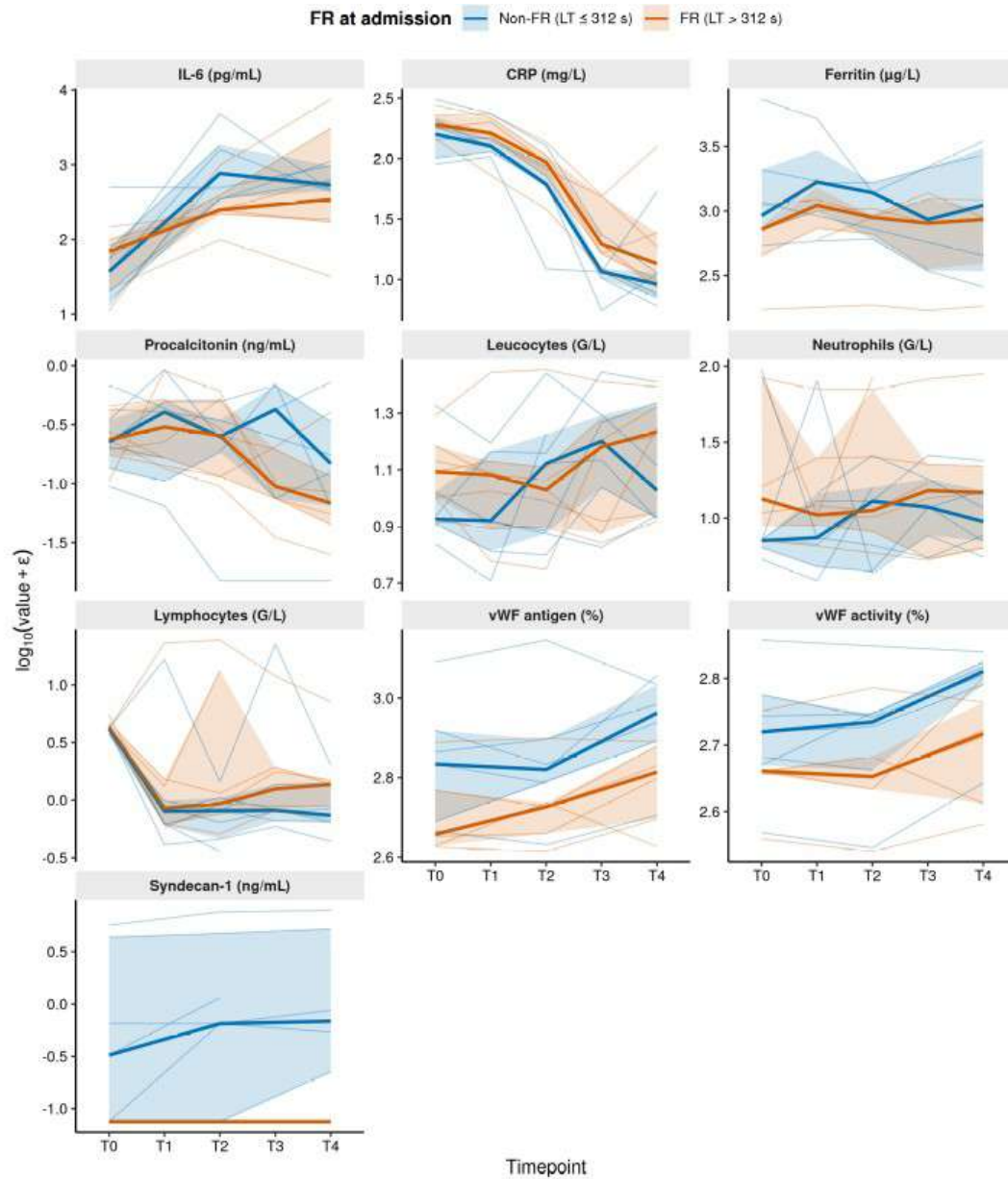


Figure 9: Ribbon plots (log10 scale) depict the temporal evolution of key inflammatory (IL-6, CRP, Ferritin, Procalcitonin, Leucocytes, Neutrophils, Lymphocytes) and endothelial injury biomarkers (vWF antigen, vWF activity, Syndecan-1) across timepoints T0–T4. Median trajectories are shown as solid lines, with shaded ribbons denoting the interquartile range (IQR). Thin lines represent individual patient trajectories. Values were transformed using a $\log_{10}(\text{value} + \epsilon)$ scaling to account for skewed distributions and zero-inflation. Patients were stratified by fibrinolytic resistance (FR) at admission, defined as TPA-test lysis time (LT) > 312 s. Non-FR patients (blue; LT ≤ 312 s) and FR patients (orange; LT > 312 s) are displayed separately

Line = median; ribbon= IQR; thin lines= individuals, $y = \log_{10} \text{value} + \epsilon$

Abbreviations: CRP, C-reactive protein; FR, fibrinolytic resistance; IL-6, interleukin-6; LT, lysis time; s, seconds; vWF, von Willebrand factor.

8.2.3.2. *Coagulation Initiation and Anticoagulant Activity*

Baseline viscoelastic profiles showed mildly prolonged EX-test CT (73 [54–88] s) and high-normal IN-test CT (160 [148–182] s), indicating modest delay in clot initiation. EX-test CT progressively normalized during follow-up, whereas IN-test CT remained stable. (Figure 10)

Thrombin generation was increased at baseline, reflected by elevated thrombin–antithrombin (TAT) complexes (476.8 [272.1–773.3] pg/mL), which persisted with slight increases over time. Antithrombin activity was initially mildly reduced (~80%) and increased during follow-up, consistent with recovery of endogenous anticoagulant pathways. (Figure 10)

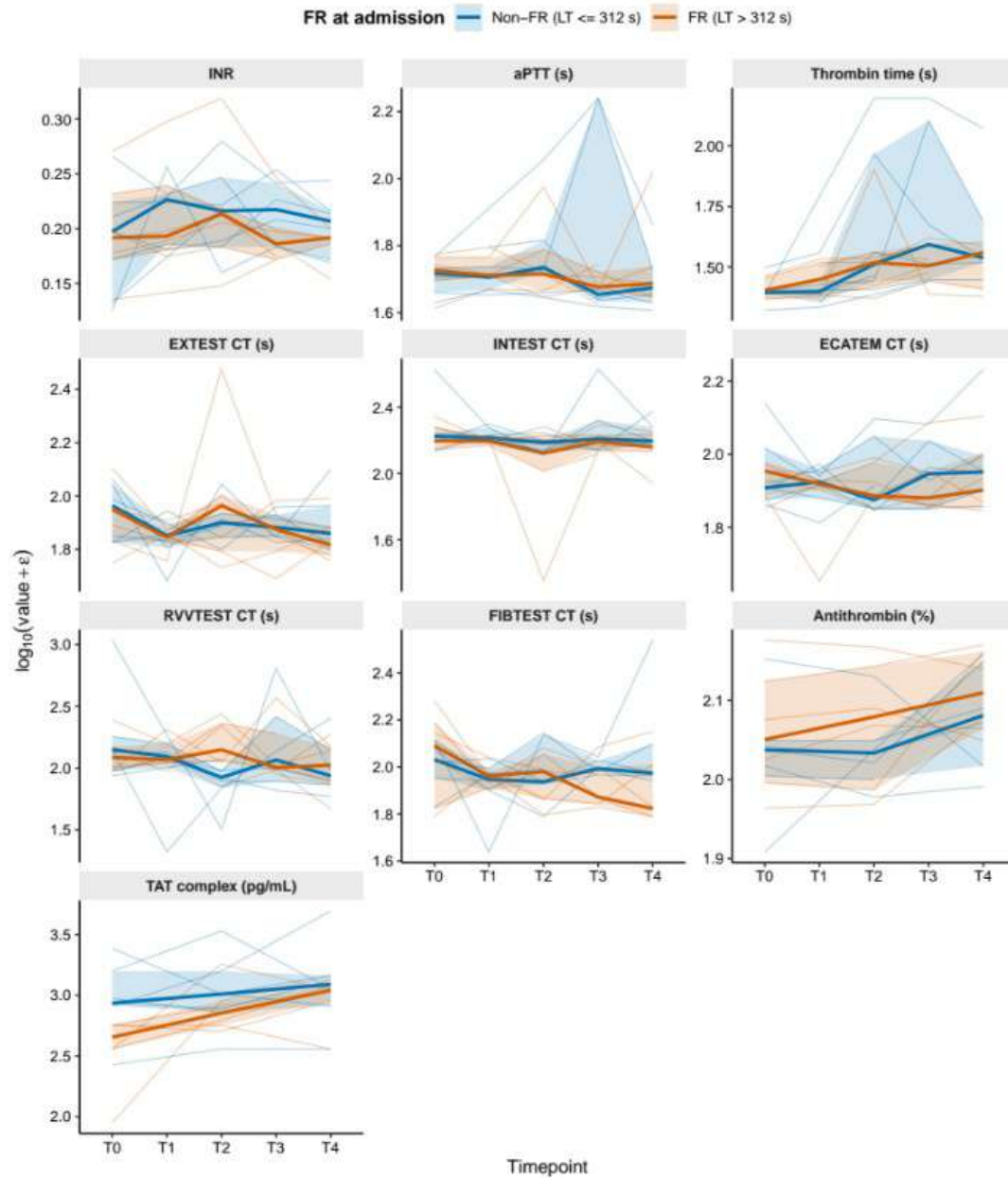


Figure 10: Ribbon plots (log10 scale) display the temporal evolution of coagulation initiation parameters (INR, aPTT, Thrombin time), viscoelastic clotting times (EX-test CT, IN-test CT, ECA-test CT, RVV-test CT, FIB-test CT), and markers of thrombin generation (TAT complex, Antithrombin III) across timepoints T0–T4. Median values are represented by solid lines, shaded ribbons denote the interquartile range (IQR), and individual patient trajectories are shown as thin lines. All biomarkers were transformed using a $\log_{10}(\text{value} + \epsilon)$ function to address right-skewed distributions and zero-inflation. Patients were stratified by fibrinolytic resistance (FR) at admission, defined as TPA-test lysis time > 312 seconds. Non-FR patients (blue) and FR patients (orange) are shown separately. Line = median; ribbon= IQR; thin lines= individuals, $y=\log_{10} \text{value} + \epsilon$

Abbreviations: aPTT, activated partial thromboplastin time; CT, clotting time; ECA-test, ecarin test; EX-test, extrinsic test; FIB-test, fibrinogen test; FR, fibrinolytic resistance; INR, international normalized ratio; IN-test, intrinsic test; LT, lysis time; RVV-test, Russell's viper venom test; s, seconds; TAT complex, thrombin-antithrombin complex.

8.2.3.3. *Clot Propagation and Strength*

Baseline platelet counts were within the lower-normal range (195 [180–218] G/L), while fibrinogen was markedly elevated (5.5 [5.1–6.4] g/L). Platelet counts increased modestly by 48 h (270 [220–312] G/L) and remained stable thereafter, whereas fibrinogen progressively decreased to 2.60 [2.12–2.96] g/L by day 7, paralleling CRP dynamics.

Viscoelastic measurements reflected this pattern, with elevated clot firmness on EX-test and FIB-test MCF (EX-test: 68 [63.5–69.5] mm; FIB-test: 32.5 [29.3–39.0] mm). EX-test MCF remained stable, whereas FIB-test MCF decreased to 22.0 [14.0–24.0] mm by day 7, corresponding to an approximate 30% reduction and reflecting fibrin-driven modulation of clot strength, while platelet-dependent firmness remained preserved (Figure 11).

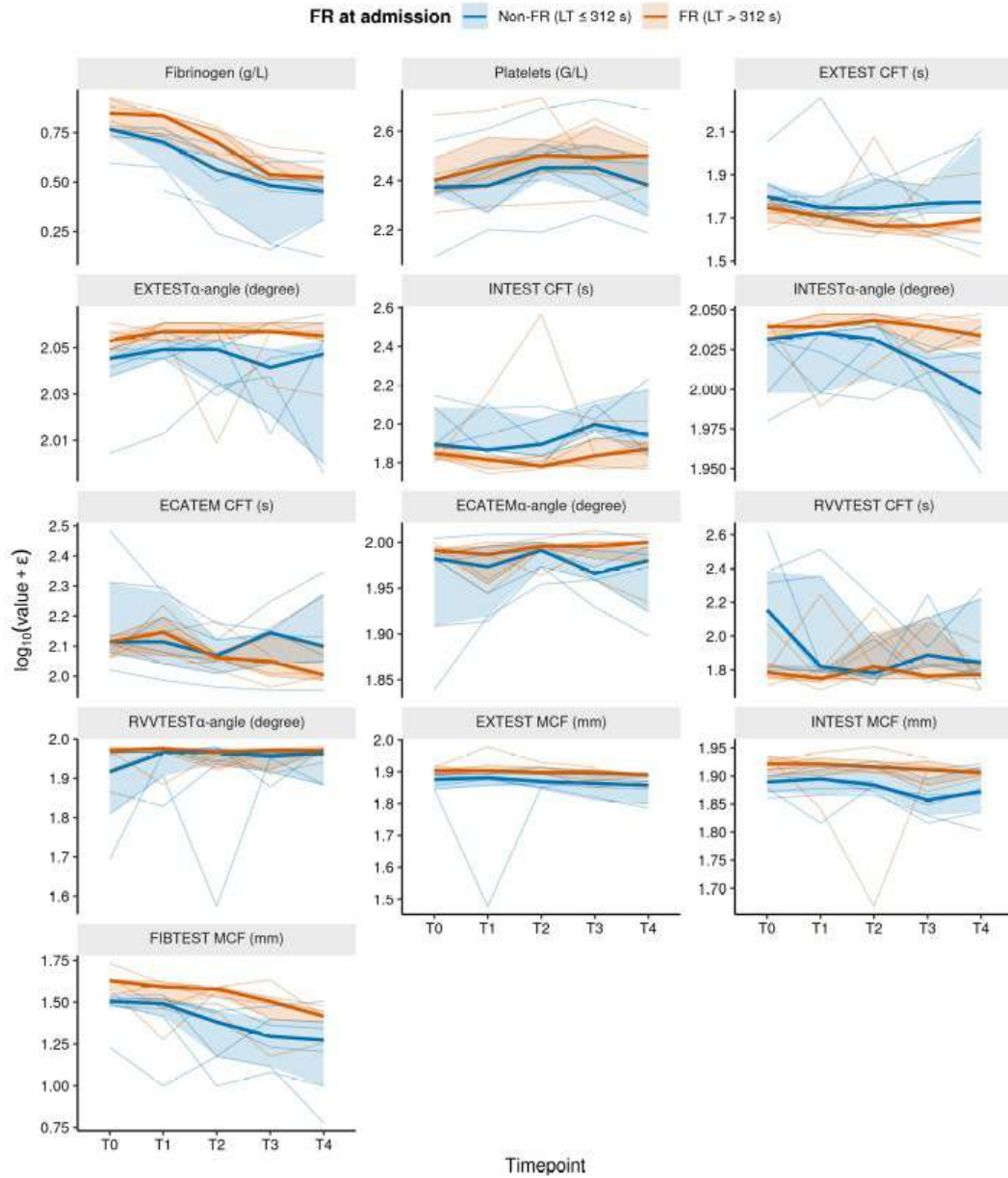


Figure 11: Ribbon plots (log 10 scale) display the temporal trajectories of clot formation and mechanical strength parameters across T0–T4, including fibrinogen concentration, platelet count, clot formation times (CFT) from EX-test, IN-test, ECATEST, and RVV-test, α -angles from EX-test, IN-test, ECATEST, and RVV-test, and maximum clot firmness (MCF) from EX-test, IN-test, and FIB-test. Median values are depicted by solid lines, shaded ribbons represent the interquartile range (IQR), and thin lines correspond to individual patient trajectories. All markers were transformed using a $\log_{10}(\text{value} + \epsilon)$ function to accommodate skewed distributions and zero values. Patients were stratified by fibrinolytic resistance (FR) at admission, defined as TPA-test lysis time > 312 seconds. Non-FR patients (blue) and FR patients (orange) are shown separately. Line = median; ribbon= IQR; thin lines= individuals, $y = \log_{10} \text{value} + \epsilon$

Abbreviations: CFT, clot formation time; ECA-test, ecarin test; EX-test, extrinsic test; FIB-test, fibrinogen test; FR, fibrinolytic resistance; IN-test, intrinsic test; LT, lysis time; MCF, maximum clot firmness; RVV-test, Russell's viper venom test; s, seconds.

8.2.3.4. *Fibrinolytic Activity*

Fibrinolytic parameters demonstrated a dynamic but non-linear trajectory. Plasminogen and α 2-antiplasmin decreased slightly over time (plasminogen: 90.0 [77.0–96.0] to 87.0 [71.5–92.0]%; α 2-antiplasmin: 110.0 [107.0–116.0] to 100.0 [90.8–106.0]%). TAFI decreased early at 48 h with partial recovery by day 7. In contrast, PAI-1 increased progressively (3.42 [2.70–4.90] to 6.29 [5.40–6.83] ng/mL). PAP complexes increased modestly by day 7, while D-dimer peaked around day 5 before a slight decline (Figure 12).

These biochemical changes were mirrored in viscoelastic fibrinolysis assays. EX-test ML decreased from ~5% at baseline to ~2% during follow-up, while CLI-45 remained stable, indicating persistent resistance to spontaneous fibrinolysis. ECA-test LOT showed transient prolongation at 24 h before returning toward baseline by day 7. Under tPA challenge, LT exhibited a biphasic pattern with early prolongation followed by shortening from approximately day 5, whereas TPA-test LOT remained stable. Overall, these findings indicate preserved fibrinolytic capacity with delayed activation dynamics (Figure 13).

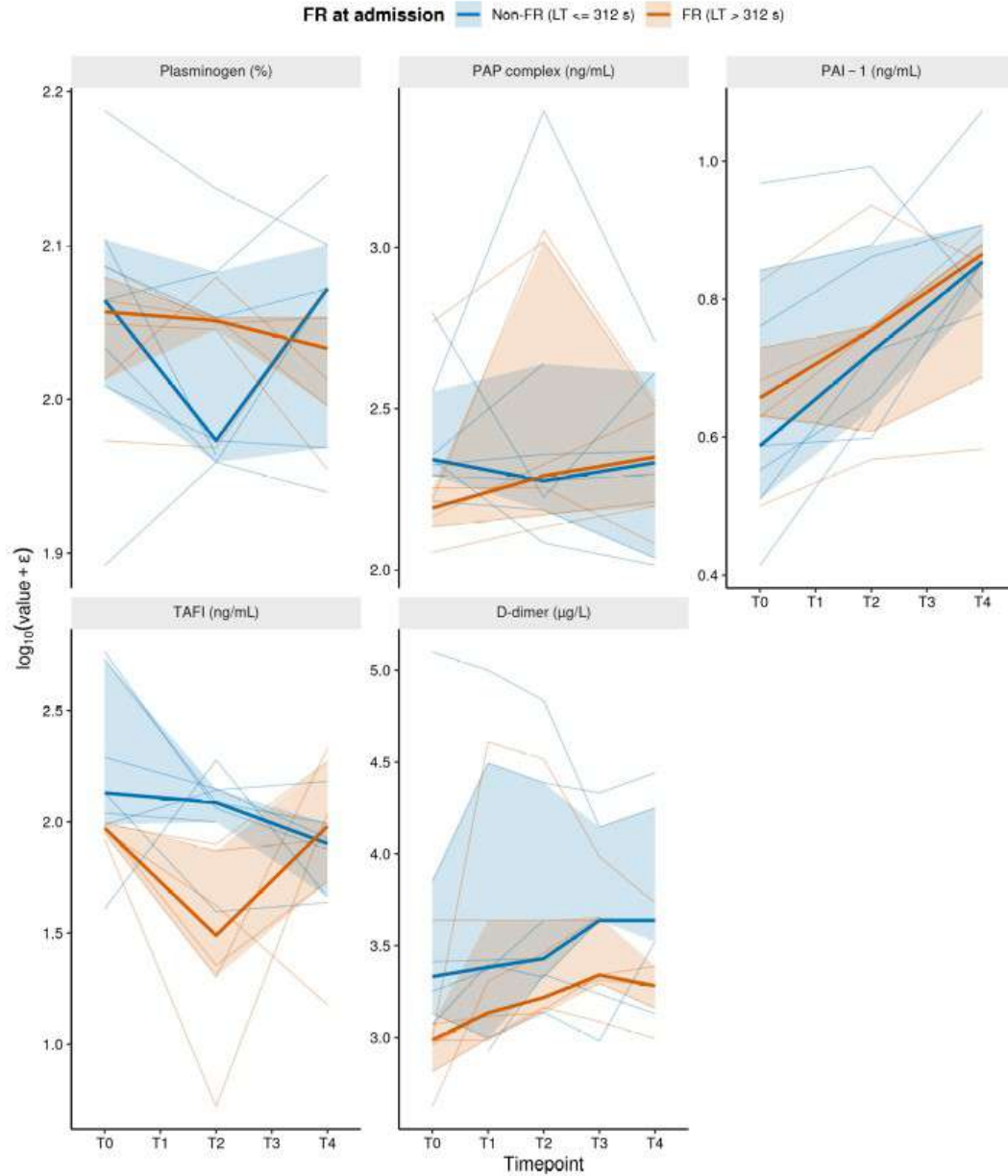


Figure 12: Ribbon plots (log10 scale) illustrate the temporal evolution of plasminogen, plasmin–antiplasmin (PAP) complexes, plasminogen activator inhibitor-1 (PAI-1), thrombin-activatable fibrinolysis inhibitor (TAFI), and D-dimer from T0 to T4. Median trajectories are shown as solid lines, shaded ribbons represent the interquartile range (IQR), and thin lines depict individual patient trajectories. All biomarkers were transformed using $\log_{10}(\text{value} + \epsilon)$ to address skewness and zero-inflation. Patients were stratified by fibrinolytic resistance (FR) at admission, defined as a TPA-test lysis time > 312 seconds. Non-FR patients (blue; LT ≤ 312 s) and FR patients (orange; LT > 312 s) are displayed separately. Line = median; ribbon = IQR; thin lines = individuals, $y = \log_{10} \text{value} + \epsilon$

Abbreviations: FR, fibrinolytic resistance; LT, lysis time; PAI-1, plasminogen activator inhibitor-1; PAP complex, plasmin-alpha2-antiplasmin complex; s, seconds; TAFI, thrombin-activatable fibrinolysis inhibitor.

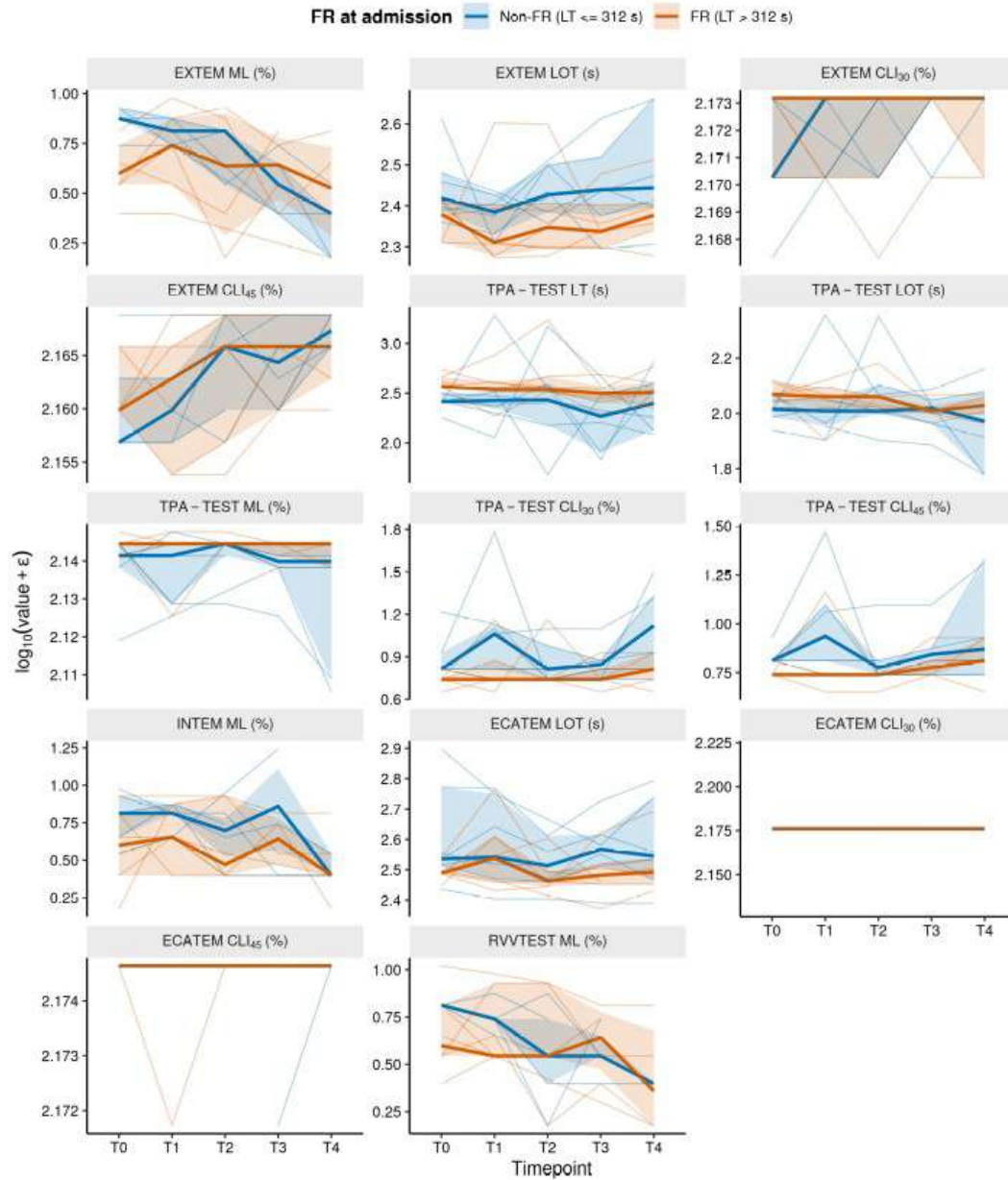


Figure 13: Ribbon plots (log₁₀ scale) depict temporal changes in clot lysis parameters obtained from EXTEM, TPA-test, INTEM, ECATEM, and RVV-test assays from T0 to T4. Parameters include maximum lysis (ML), lysis onset time (LOT), clot lysis at 30 minutes (CLI₃₀), and clot lysis at 45 minutes (CLI₄₅). Median trajectories are shown as solid lines, interquartile ranges (IQRs) as shaded ribbons, and thin lines represent individual patient trajectories. All indices were transformed using log₁₀(value + ε) to mitigate skewness and zero inflation. Patients were stratified by fibrinolytic resistance (FR) at admission, defined as TPA-test lysis time > 312 seconds. Non-FR patients (blue; LT ≤ 312 s) and FR patients (orange; LT > 312 s) are shown separately. Line = median; ribbon = IQR; thin lines = individuals, y = log₁₀ value + ε

Abbreviations: CLI₃₀, clot lysis index at 30 minutes; CLI₄₅, clot lysis index at 45 minutes; CT, clotting time; CFT, clot formation time; ECA-test, ecarin test; EX-test, extrinsic test; FIB-test, fibrinogen test; FR, fibrinolytic resistance; IN-test, intrinsic test; LOT, lysis onset time; LT, lysis time; MCF, maximum clot firmness; ML, maximum lysis; RVV-test, Russell's viper venom test; s, seconds; TPA-test, tissue plasminogen activator test.

9. DISCUSSION

9.1. Summary of Findings

Across both studies, interventions targeting either coagulation (Study I) or upstream inflammation (Study II) produced incomplete modification of the thrombo-inflammatory phenotype in COVID-19.

In **Study I**, higher-dose anticoagulation was not associated with reduced mortality (OR 0.83, 95% CI 0.54–1.28), despite a significant reduction in thrombotic events (OR 0.58, 95% CI 0.44–0.76) which benefit was counteracted by a significant increase in major bleeding (OR 1.64, 95% CI 1.25–2.16). Subgroup analyses showed no mortality benefit in severe (OR 0.84, 95% CI 0.53–1.33) or moderate disease (OR 1.00, 95% CI 0.41–2.33), with a significant reduction in thrombotic events limited to moderate disease (OR 0.48, 95% CI 0.32–0.76) and therapeutic-dose regimens (OR 0.52, 95% CI 0.41–0.66). Disease progression outcomes were neutral for mechanical ventilation, ICU admission, and ARDS, while the exploratory composite endpoint showed a significant reduction (OR 0.46, 95% CI 0.21–0.67).

In **Study II** we conducted an exploratory characterization of longitudinal coagulation, fibrinolysis, and endothelial dynamics following IL-6 receptor blockade in critically ill COVID-19 patients. Despite attenuation of systemic inflammation, persistent endothelial dysfunction and complex, partially dissociated alterations in coagulation and fibrinolysis were observed. IL-6 inhibition was associated with modulation rather than normalization of hemostatic abnormalities, with some ongoing antifibrinolytic activity rather than complete fibrinolytic failure, preserved thrombin generation balance, and sustained endothelial activation. These findings suggest that IL-6 cytokine blockade alone is insufficient to fully restore vascular homeostasis in severe COVID-19.

9.2. Study I - Higher Dose Anticoagulation Cannot Prevent Disease Progression in COVID-19 Patients: A Systematic Review and Meta-Analysis

9.2.1. *Different Dosing Regimens and Disease Severity*

The ACTIV4-B trial was terminated early because of a low number of cardiopulmonary hospitalizations. In outpatients with mild COVID-19, apixaban 2.5 mg twice daily showed no benefit compared with 5 mg twice daily regarding mortality or cardiopulmonary hospitalizations, suggesting that HD anticoagulation is unlikely to provide additional benefit in mild disease (72). Since has been shown that even long-term ambulatory thromboprophylaxis failed to show benefit in ambulatory cases (84).

Similarly, in our analysis of hospitalized COVID-19 patients, HD anticoagulation was not associated with lower mortality compared with SD anticoagulation (OR 0.83 [95% CI 0.54–1.28]), despite reducing thrombotic events (OR 0.58 [95% CI 0.44–0.76]). This benefit was accompanied by an increased risk of major bleeding (OR 1.64 [95% CI 1.25–2.16]), which may have offset any survival advantage.

Several studies reported a higher incidence of pulmonary embolism than deep vein thrombosis in critically ill COVID-19 patients (30,61,63,67,77,85). This observation supports the hypothesis that many pulmonary vascular occlusions represent in situ pulmonary thrombosis driven by hyperinflammation rather than classic embolic disease (63, 86). Severe COVID-19 is associated with hypercoagulation and fibrinolytic resistance, potentially leading to microvascular thrombosis and organ failure that may not be prevented by intensified anticoagulation, while bleeding risk remains increased (87-91).

This concept is supported by multiplatform trials, which demonstrated improved organ support-free days only in non-critically ill patients receiving therapeutic-dose anticoagulation. (29, 61) Consistent with these findings, our analysis found a nonsignificant trend toward reduced intubation rates in predominantly moderately ill patients (OR 0.82 [95% CI 0.63–1.08]), suggesting that therapeutic anticoagulation may be more effective in preventing disease progression than treating established critical illness.

9.2.2. Differences in the Degree of COVID-19-Associated Coagulopathy

COVID-19-associated coagulopathy is characterized by hypercoagulation and impaired fibrinolysis, largely driven by endothelial dysfunction, hyperinflammation, and hypoxia (25, 92, 93). Elevated D-dimer levels are consistently associated with increased mortality and thrombotic complications (94-96). For example, Tang et al. reported significantly higher D-dimer concentrations in non-survivors than survivors (94).

Although D-dimer was used for risk stratification in multiplatform trials, treatment effects did not differ significantly between D-dimer subgroups (29,61). However, these studies included patients regardless of D-dimer levels and generally enrolled individuals with lower median values than some other trials. In contrast, the HEP-COVID trial, which enrolled patients with markedly elevated D-dimer levels, demonstrated reductions in both thromboembolic events and mortality with therapeutic-dose LMWH, without a substantial increase in major bleeding (62). Similarly, the HESACOVID study reported improved gas exchange, greater liberation from mechanical ventilation, and declining D-dimer levels among patients receiving therapeutic anticoagulation (66).

These observations have led to proposals that D-dimer thresholds could help identify patients most likely to benefit from intensified anticoagulation (92, 97-99). Based on previous evidence showing a mean D-dimer difference of 0.97 $\mu\text{g/mL}$ between mild and severe COVID-19, we performed a subgroup analysis using a threshold of 1 $\mu\text{g/mL}$ (100). Although mortality differences did not reach statistical significance, patients with D-dimer levels >1 $\mu\text{g/mL}$ showed a clinically relevant trend toward lower mortality with HD anticoagulation. Furthermore, an exploratory composite outcome including death, pulmonary embolism, and need for mechanical ventilation demonstrated a significant benefit of higher-dose thromboprophylaxis (OR 0.46 [95% CI 0.31–0.67], $p < 0.0001$). These findings suggest that therapeutic-dose anticoagulation may be most beneficial in moderately ill patients with elevated D-dimer levels.

9.3. Study II - Exploratory Analysis of Coagulation and Fibrinolysis Trajectories After IL-6 Antagonist Therapy in COVID-19: A Case Series

This case series provides an in-depth viscoelastic and biochemical characterization of coagulation and fibrinolysis dynamics in critically ill COVID-19 patients treated with tocilizumab. Despite rapid suppression of systemic inflammation, endothelial dysfunction and

hemostatic abnormalities persisted, suggesting that restoration of vascular homeostasis extends beyond IL-6 inhibition alone. These findings should be interpreted cautiously given the small sample size and exploratory design.

At baseline, patients demonstrated marked inflammatory activation accompanied by endothelial-associated hemostatic imbalance. Following immunomodulation, inflammatory markers decreased, whereas biomarkers of endothelial injury continued to rise. This is consistent with evidence that endothelial dysfunction in COVID-19 may persist beyond the acute phase due to ongoing vascular activation, immune-mediated injury, and impaired repair mechanisms (101-103).

Following IL-6 receptor blockade, coagulation parameters suggested partial re-equilibration of the hemostatic system. TAT complexes increased modestly, while antithrombin activity was preserved, indicating controlled thrombin generation without consumption of endogenous anticoagulants. This pattern supports the concept that endothelial injury remains a key driver of coagulation activation despite attenuation of systemic inflammation (104).

Changes in fibrinogen, platelet counts, and viscoelastic parameters suggested a reduced fibrin contribution to clot firmness compensated by preserved platelet-dependent clot strength. Persistent endothelial activation and platelet involvement may also contribute to sustained elevations in PAI-1, maintaining antifibrinolytic pressure and thrombotic risk (105).

Fibrinolytic markers were consistent with persistent antifibrinolytic activity rather than complete fibrinolytic failure. TAFI and plasminogen decreased modestly, D-dimer levels initially increased before declining, and PAP complexes rose slightly, indicating ongoing but limited fibrin turnover. Correspondingly, viscoelastic clot lysis parameters remained largely stable, while TPA-test lysis times showed a delayed but preserved fibrinolytic response. These findings suggest that IL-6 blockade attenuates inflammation and hypercoagulability without fully normalizing fibrinolytic disturbances.

Patients with fibrinolytic resistance at admission displayed lower vWF levels, higher fibrinogen and platelet counts, and viscoelastic features indicative of enhanced clot propagation and mechanical stability. In contrast, those without fibrinolytic resistance exhibited higher D-dimer levels, suggesting more active fibrin turnover. This dichotomy is consistent with the “fibrinolytic shutdown” phenotype described in severe systemic inflammation (106).

Our findings are supported by studies demonstrating that COVID-19 plasma clots exhibit dense fibrin networks with reduced susceptibility to enzymatic degradation, driven by elevated fibrinogen, endothelial injury, neutrophil extracellular traps, and increased thrombin generation (80, 81, 107). Together, these mechanisms promote formation of compact, lysis-resistant clots and contribute to thrombotic risk.

Clinical studies have reported similar observations. Tocilizumab has been associated with improvements in coagulation parameters and respiratory function, transient increases in D-dimer suggesting enhanced fibrin turnover, reduced fibrinogen levels and improved survival in critically ill patients, and modulation of fibrinolytic profiles (108-111). The randomized Coag-ImmCovA trial further demonstrated reduced hypercoagulability and progressive remodeling of fibrin clot structure following tocilizumab treatment (112). These findings collectively support a beneficial but incomplete effect of IL-6 blockade on COVID-19-associated coagulopathy.

9.4. Strengths

Study I is the most up-to-date and comprehensive meta-analysis on the effect of different anticoagulation regimens in COVID-19, including 14 randomized controlled trials and over 6000 patients. It incorporates traditional outcomes (mortality, thrombotic events, and major bleeding) together with additional clinically relevant disease progression endpoints, providing a broader evaluation of anticoagulation effects. We further analysed the interaction between baseline D-dimer levels and all-cause mortality, identifying a selected subgroup of moderately ill patients with elevated D-dimer levels in whom therapeutic anticoagulation may influence disease progression, a finding not previously reported. To enhance robustness, leave-one-out sensitivity analyses were performed for all outcomes with more than five included trials, reducing the influence of individual studies.

Study II provides a novel and detailed temporal characterization of inflammatory, coagulation, endothelial, and fibrinolytic dynamics following IL-6 receptor blockade in critically ill COVID-19 patients. Serial sampling enabled assessment of longitudinal trajectories rather than static measurements. Integration of viscoelastic hemostatic assays with conventional coagulation parameters and circulating biomarkers allowed a comprehensive multimodal evaluation of hemostasis. The study further benefits from simultaneous assessment of endothelial injury, thrombin generation, and fibrinolytic balance, providing a

mechanistic framework for COVID-19-associated coagulopathy in a well-characterized ICU cohort.

9.5. Limitations

Study I is limited by substantial clinical and methodological heterogeneity across included randomized controlled trials, including differences in anticoagulation regimens, dosing strategies, treatment duration, and study populations. Variability in disease severity definitions and ICU admission criteria across countries further limits comparability. Several outcomes were inconsistently reported, restricting pooling for certain endpoints. The low number of studies and low event rates in some trials may have reduced statistical power and increased imprecision. Differences in statistical methods across studies also prevented some preplanned analyses. Finally, inclusion of one non-peer-reviewed study introduces a potential source of bias.

Study II is limited by its observational pilot design, small sample size, and absence of a control group, precluding causal inference and limiting generalizability. Patient selection was pragmatic, introducing potential selection bias. The study was conducted during the late pandemic phase, which may limit extrapolation to earlier waves or unvaccinated populations due to changes in immunity and treatment standards. Concomitant therapies, including corticosteroids and anticoagulation, represent unavoidable confounders inherent to standard ICU care. Therefore, results should be interpreted as exploratory and hypothesis-generating.

10. CONCLUSIONS

Study I does not support routine use of therapeutic-dose thromboprophylaxis in all hospitalized COVID-19 patients, as no effect on mortality was observed and the reduction in thrombotic events was counterbalanced by an increased risk of bleeding. However, a non-significant tendency suggests a potential benefit of higher-dose anticoagulation on disease progression, particularly in non-ICU patients with markedly elevated D-dimer levels receiving UFH or LMWH.

Study II characterizes in a hypothesis-generating approach the parallel temporal trajectories in inflammatory, endothelial, and coagulation parameters following IL-6 receptor antagonism. While inflammatory markers decreased over time, endothelial activation and hemostatic imbalance persisted in several patients. These exploratory observations highlight the complexity of COVID-19-associated coagulopathy and support further investigation into mechanisms beyond cytokine-driven inflammation.

11. IMPLICATIONS FOR PRACTICE

Based on the results of **Study I**, widespread use of higher-dose anticoagulation in COVID-19 cannot be recommended, as any potential reduction in thrombotic events appears to be offset by an increased risk of bleeding, without a mortality benefit. Standard-dose thromboprophylaxis remains the default, particularly in critically ill patients, in whom no benefit was observed. Escalation could be considered only in ward-level patients with elevated D-dimer ($>1 \mu\text{g/mL}$), weighed against individual bleeding risk.

Study II was underpowered to inform practice; it does, however, generate the hypothesis that IL-6 receptor blockade does not attenuate the endothelial disruption that sustains the hemostatic imbalance.

12. IMPLICATIONS FOR RESEARCH

With the recession of the pandemic, the relevant questions extend from COVID-19 itself toward the broader response to pandemics. On the methodological side, future research should concentrate on the adequacy of endpoints and on patient selection rather than on further undifferentiated dosing comparisons. All-cause mortality is a distal, multifactorial outcome that may fail to register a genuine correction of coagulopathy, while composite outcomes, though more sensitive, require cautious interpretation, since an apparent benefit may be driven by their least severe or most frequent component and, as in **Study I**, may derive from a post hoc rather than a pre-specified analysis.

The principal direction, however, concerns the interaction between immunomodulatory therapy and haemostasis, which remains insufficiently characterized. In the hypothesis-generating **Study II**, hemostatic trajectories following IL-6 blockade were explored to better understand its impact on coagulation and fibrinolysis. These observations indicate that endothelial dysfunction can persist despite attenuation of systemic inflammation, suggesting that the associated hemostatic imbalance may be sustained in a possibly non-overt form even as the clinical course improves. Future studies should therefore clarify how inflammatory and hemostatic pathways interact and how targeting one influences the other, rather than assume that control of inflammation entails correction of coagulopathy. In this context, the persistently activated endothelium warrants further investigation and may represent a therapeutic target in its own right.

13. FUTURE PERSPECTIVES

The endothelium represents the most compelling direction for future work. Both studies converge upon it: endothelial activation is what persists once inflammation has been attenuated, and it is the point at which anticoagulation and immunomodulation alike prove insufficient. Understanding how immunomodulation influences haemostasis ultimately returns to this common mechanism, which links the inflammatory and coagulation systems. Crucially, the resulting thromboinflammatory imbalance is not specific to COVID-19; the same endothelial disturbance characterizes sepsis and other critical illnesses, so endothelial-directed strategies may prove relevant well beyond the setting in which they were first observed.

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Systematic Review

Higher Dose Anticoagulation Cannot Prevent Disease Progression in COVID-19 Patients: A Systematic Review and Meta-Analysis

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Abstract: Implementation of higher dose (HD) thromboprophylaxis has been considered in patients infected with coronavirus disease 2019 (COVID-19). Our aim was to compare HD to standard dose (SD) thromboprophylaxis in COVID-19 patients. The protocol is registered on PROSPERO (CRD42021284808). We searched for randomised controlled studies (CENTRAL, Embase, Medline and medRxiv) that compared HD to SD anticoagulation in COVID-19 and analysed outcomes such as mortality, thrombotic events, bleedings, and disease progression. The statistical analyses were made using the random effects model. Fourteen articles were included (6253 patients). HD compared with SD showed no difference in mortality (OR 0.83 [95% CI 0.54–1.28]). The use of HD was associated with a decreased risk of thrombosis (OR 0.58 [95% CI 0.44–0.76]), although with an increased risk of major bleeding (OR 1.64 [95% CI 1.25–2.16]). The cohort with D-dimer < 1 mg/mL showed no effect (OR 1.19 [95% CI 0.67–2.11]), but in the case of D-dimer > 1 mg/mL, a tendency of lower risk in the HD group was observed (OR 0.56 [95% CI 0.31–1.00]). The need for intubation in moderately ill patients showed a nonsignificant lower likelihood in the HD group (OR 0.82 [95% CI 0.63–1.08]). We cannot advocate for HD in all COVID-19 patients, although it shows some nonsignificant benefits on disease progression in those with elevated D-dimer who do not need ICU admission.

Keywords: COVID-19; anticoagulation; thromboinflammation; disease progression

1. Introduction

In December 2019, the news reported the emergence of atypical pneumonia in Wuhan, China, for the first time [1]. To date, there have been more than 6 million deaths attributed to this new virus, called severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) [2].

The emergence of the SARS-CoV2 virus behind coronavirus disease 2019 (COVID-19) underlined the importance of thromboinflammatory processes after an increased number of thrombotic complications was reported and autopsies described microthrombi and fibrin deposits among their findings [3–6]. Reports have shown that coagulation parameters, namely D-dimer levels, correlate with the outcomes of COVID-19 patients [7,8].

These findings encouraged clinicians to suggest the implementation of higher dose thromboprophylaxis in the case of COVID-19 patients, typically with low molecular weight (LMWH) and unfractionated heparin (UFH) [9,10]. Besides their anticoagulant benefits, they show antiinflammatory properties and might improve clinical outcomes [11]. Thus,

questions arose concerning the optimal dose of anticoagulation in COVID-19. The administration of intermediate or therapeutic dose anticoagulation has resulted in somewhat controversial findings based on observational studies [12–18].

Thus, there was an urgent call for randomized controlled trials (RCTs) on this topic. The HEP-COVID [19] trial showed significant benefits of survival in the case of moderately ill patients, but not in those admitted to ICU when they were administered therapeutic dose anticoagulation compared with usual-care thromboprophylaxis. The multiplatform trials by REMAP-cap, ATTAC, and ACTIV-4b investigators found that therapeutic dose thromboprophylaxis was associated with an increased number of organ-support-free days in moderately ill patients, which was further confirmed by the results of the RAPID trial [20,21]. Other trials with similar patient populations failed to show any significant advantage of the higher dose thromboprophylaxis in COVID-19 patients [22–25].

As new findings are published, there is a need for a newly updated synthesis that could shed new light on the topic and determine more precisely the patient population who would and those who would not benefit from higher dose anticoagulation.

The aim of this systematic review is to assess whether higher dose (HD) compared with standard dose (SD) versions of thromboprophylaxis have different effects on clinical outcomes without jeopardizing the safety of COVID-19 patients.

2. Materials and Methods

2.1. Research Question

We performed a meta-analysis in accordance with the Cochrane Handbook for Systematic Reviews of Interventions [26]. This protocol was registered in PROSPERO, the International Database of Prospectively Registered Systematic Reviews, with the identification number CRD42021284808 (<https://www.crd.york.ac.uk/prospero> (accessed on 30 July 2022)) [27].

To address our research question, we included RCTs that defined their population as adults with clinically or laboratory-confirmed COVID-19 infection and that compared higher dose to standard dose thromboprophylaxis. We defined our primary outcomes as organ support-free days (defined as days without respiratory, inotrope/vasopressor support); length of hospital stay (in days); mortality (ICU mortality, in-hospital mortality, and 30-day mortality); safety outcomes such as the incidence of thrombotic events (number of arterial and venous thrombotic events); bleeding event rate (number of major bleeding events, clinically relevant non-major bleeding, and minor bleeding as per International Society on Thrombosis and Haemostasis [ISTH]); and requirement for transfusion (packed red blood cell, platelet, fresh frozen plasma, cryoprecipitate, and prothrombin complex concentrate). Our predefined secondary outcomes were as follows: change in $\text{PaO}_2/\text{FiO}_2$ ratio from baseline to 7 and 14 days (in mmHg); duration of supplemental O_2 therapy (days); duration of invasive mechanical ventilation (days); duration of vasopressor/inotrope support (days); duration of renal replacement therapy (days); other adverse events (e.g., heparin-induced thrombocytopenia); progression of disease (number of patients who needed intensive care unit (ICU) admission); and variation in markers of inflammation and coagulation (e.g., D-dimer).

Because of the quick pandemic response, and thus rapidly changing guidelines and shifts in the interest in research, we adapted our analysis to assess available data. We included the number of patients that required intubation instead of the duration of mechanical ventilation, as well as progression to acute respiratory distress syndrome (ARDS).

Additionally, we included an exploratory composite outcome in our posthoc analysis that consisted of death, pulmonary embolism (PE), and the need for invasive mechanical ventilation to assess the severity of pulmonary involvement.

2.2. Search Strategy, Selection Process, and Data Extraction

We conducted our systematic search on 18 October 2021 and performed an updated search on 23 May 2022 in MEDLINE, Embase, and CENTRAL using the following

searchkeys: “(covid* OR SARS-CoV* OR ncov OR novel coronavirus OR COVID-19 OR coronavirus) AND (thrombosis prevention OR thromboprophylaxis OR thromboembolism prophylaxis OR anticoag* OR anticoagulation OR heparin OR UFH OR LMWH OR low molecular weight heparin OR dalteparin OR tinzaparin OR enoxaparin OR clexane OR fondaparinux OR argatroban OR bivalirudin OR rivaroxaban OR apixaban OR dabigatran OR pradaxa OR edoxaban OR betrixaban)”. In addition to the databases mentioned in the protocol registered in PROSPERO, we searched an archive (medRxiv) to include the latest, although not peer-reviewed, articles published from 1 January to 23 May 2022.

Records were screened based on title, abstract, and full-text by two independent review authors (E.H.K. and C.T.), using a reference manager software. Cohen’s kappa was calculated after each step of the selection process to measure inter-rater reliability. An independent third investigator (Z.M.) resolved the disagreements.

We extracted data in a standardized data extraction sheet. In addition to the abovementioned outcomes, we retrieved the following data from the eligible articles: title, first author, year of publication, countries, study design, eligibility criteria, anticoagulant regimen, patient demographics, and interventions. Two independent review authors (E.H.K. and B.Y.L.) extracted data using the standardized data collection form, and a third independent reviewer (F.D.) resolved the disagreements.

2.3. Subgroup Analyses

We planned to perform subgroup analyses to reduce the heterogeneity of the pooled data according to the severity of the disease, different dosing regimens, and baseline coagulation disorders assessed by the D-dimer level.

In order to evaluate the effect of HD compared with SD in cohorts with different disease severity, we defined them according to the level of care they needed. Thus, a “severe disease cohort” was described as patients who needed ICU level care and a “moderate disease cohort” as those who did not require organ-support and thus admission to the ICU.

We defined SD as low dose, preventive thromboprophylaxis. In the HD group, we defined intermediate and therapeutic dose anticoagulation according to the guidelines of the American Society of Hematology 2021 [28]. Thus, we considered the therapeutic dose the equivalent dose of enoxaparin 1.5 mg/kg once daily or 1 mg/kg twice daily for patients with CrCl > 30 mL/min and BMI < 40 kg/m², and unfractionated heparin to target aPTT in the therapeutic range as per local guidelines or anti-Xa activity 0.3–0.7 IU/mL. The intermediate dose was defined as the equivalent of enoxaparin 0.5 mg/kg once daily or 40 mg (4000 U) twice daily for patients with CrCl > 30 mL/min and BMI < 40 kg/m².

The value of 1 mg/mL D-dimer level was chosen as a cut-off point as early reports showed that, above this value, at baseline, there is an 18-fold increased risk of mortality [8,29–32]. As most of the studies reported median baseline levels, we distinguished the two cohorts: studies that enrolled patients who were admitted to hospital in more than 50% of cases with D-dimer levels > 1 mg/mL and those with baseline D-dimer < 1 mg/mL.

2.4. Risk of Bias and Evidence Level

The risk of bias assessment was performed by two independent review authors (E.H.K. and C.T.) following the recommendations of the Cochrane Collaboration [33]. Disagreements were resolved by a third review author (F.D.). RoB2 (risk of bias assessment) tool [34] was used to assess the transparency of the included randomised controlled studies and GRADE-Pro [35] to grade the quality of evidence. Publication bias was assessed by visual inspection of the funnel plots.

2.5. Statistical Analysis

The statistical analyses were carried out by R (R Core Team 2021, v 4.1.1, R Foundation for Statistical Computing, Vienna, Austria) [36], using the meta (Schwarzer 2022, v5.2.0) [37] and dmetar (Cuijpers, Furukawa, and Ebert 2022, v0.0.9000) [38] packages for calculations and plots.

To assess the effect measure, we used the odds ratio (OR) with 95% confidence intervals (95% CI) for dichotomous outcomes. For this calculation, we extracted the total number of patients in each group and those with the event of interest from each study [39–41].

The random effects model was used for meta-analyses. The Hartung–Knapp adjustment was used in the case where the study number for the given outcome was over five [42,43]. For the pooled results, the exact Mantel–Haenszel method (without continuity correction) was applied to handle zero cell counts [44,45]. To estimate τ^2 , we used the Paule–Mandel method [46], and the Q profile method was used for calculating the confidence interval of τ^2 [47]. Means of the Cochrane Q test and the I^2 values were used for the assessment of statistical heterogeneity, where $p < 0.1$ was considered as statistically significant [48].

The publication bias was evaluated by a funnel plot of the logarithm of effect size and comparison with the standard error for each trial.

Outlier and influence analyses were carried out following the recommendations of Harrer et al. (2021) and Viechtbauer and Cheung (2010) [47,49].

3. Results

3.1. Systematic Search and Selection

Our systematic search resulted in 17,114 eligible studies (8749 from the search on 18 October 2021 and 8365 from 23 May 2022). After the selection process, 14 articles were included in the meta-analysis [19–25,50–56] and 15 [57] articles in the systematic review. Figure 1 shows the PRISMA 2020 Flow diagram of the updated search, and the one for the initial search can be found in Supplementary material (Figure S1) [58].

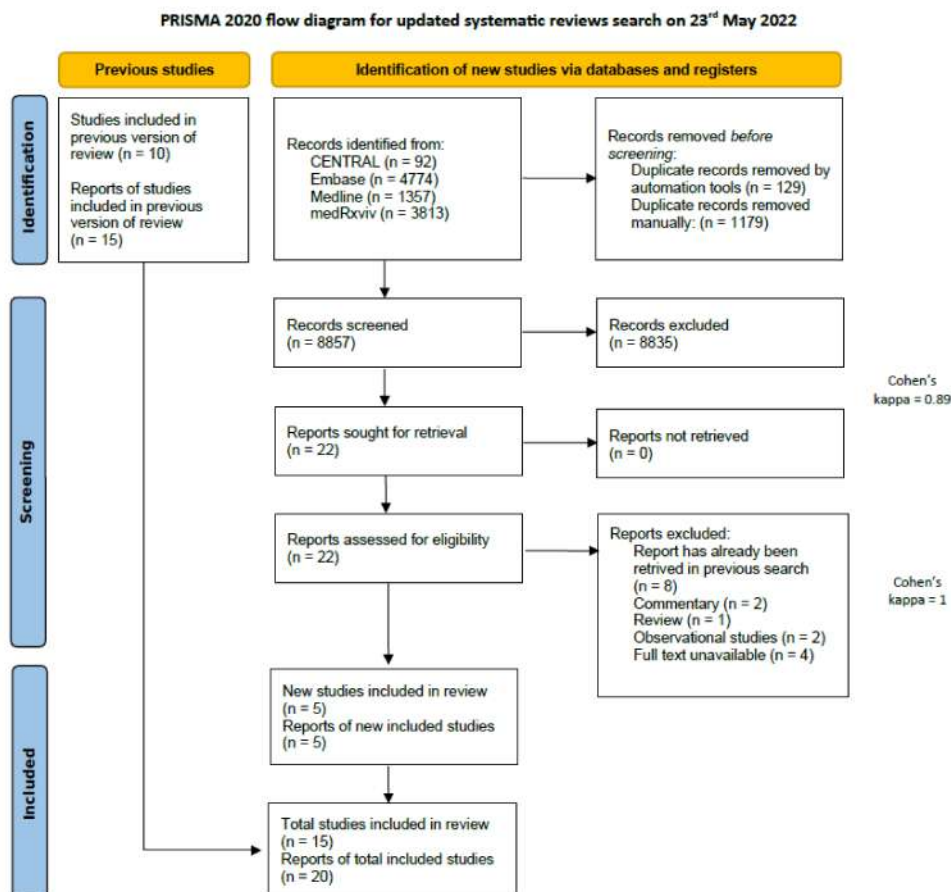


Figure 1. PRISMA 2020 flow diagram of the updated search.

3.2. Study Characteristics

The included open-label studies were conducted between April 2020 and September 2021 in 15 countries [19–25,50–56]. In total, they enrolled 6253 adult patients with an average age above 50 years in the cohorts. Seven studies included patients with severe disease [50–56], seven with moderate disease at presentation, and one with mild disease [19–25,57]. The eligibility criteria of trials included higher than the upper limit of normal D-dimer levels or DIC score ≥ 3 in ten of the included studies [19,21,22,24,25,50–54,56]. Four studies compared intermediate dose thromboprophylaxis to standard dose [23,52,55]; among them, one was a three-arm study that included therapeutic anticoagulation as well [25]. The rest of the studies compared therapeutic dose to usual care thromboprophylaxis, mostly using enoxaparin [19–23,50,51,53,54,56]. One trial compared high dose Rivaroxaban to standard of care LMWH [22]. In a three-arm intervention study, pnyk et al. compared therapeutic UFH with therapeutic and standard dose LMWH [54]. One trial assessed the efficacy of different dosing regimens of bempiparin, while another study used tinzaparin for the same aim [24,25].

The characteristics of each included study can be found in Table 1. The detailed inclusion and exclusion criteria of included trials with baseline D-dimer levels can be found in Supplementary material (Table S1).

3.3. All-Cause Mortality

To evaluate the effectiveness of HD compared with SD anticoagulation, we extracted data about all-cause mortality reported between 21 and 30 days in 12 of the included RCTs [19,21–25,50,52–56]. This primary outcome occurred in 232 out of 1525 patients assigned to the HD group and 235 out of 1405 patients assigned to the SD group, which indicated no significant difference (OR 0.83, 95% CI [0.54–1.28], $p = 0.3748$; $I^2 = 41\%$, $p = 0.06$).

3.3.1. Different Dosing Regimens in Moderate and Severe Disease

There was little or no effect between the SD and the HD groups when we assessed the severe disease and moderate disease cohort separately (OR 0.84 [95% CI 0.53–1.33] and OR 1.00 [95% CI 0.41–2.33], respectively) (Figure 2A).

We found no effect of HD compared with SD in the case of intermediate dose anticoagulation used as HD with OR 1.08 [95% CI 0.44–2.65]. In the case of therapeutic anticoagulation administered in the HD group, the pooled effect was 0.72 OR [95% CI 0.40–1.31], showing little or no effect. The overall pooled effect of the two cohorts was an OR of 0.85 [95% CI 0.55–1.31, $p = 0.4237$] (Figure 2B).

Table 1. Characteristics of included studies.

Study	Design	Enrollment Period	Country	Sample Size	Patients Admitted to	Intervention Arms	Follow-Up Period (days)
HESACOVID 2020 [50]	Open-label, single center RCT	From April 2020 to July 2020	Brazil	20	100% ICU	Therapeutic: enoxaparin 1 mg/kg twice daily or UFH adjusted to aPTT 1.5–2 Standard: enoxaparin 40 mg once daily or UFH 5000 three times a day ^a	28
Goligher et al., 2021 [51]	Open-label, adaptive, multiplatform RCT	From 21 April 2020 to 19 December 2020	United States, Canada, the United Kingdom, Brazil, Mexico, Nepal	1103	100% ICU	Therapeutic: enoxaparin 1.5 mg/kg once daily, UFH titrated to have 1.5–2.5 aPTT, dalteparin 200 UI once daily Tinzaparin 175 UI/kg once daily Standard: enoxaparin 40 mg once daily, dalteparine 5000 UI once, tinzaparine 4500 UI, fondaparinux 2.5 mg, UFH 5000 UI/ 8–12 h ^b	21
Lawler et al., 2021 [20]	Open-label, adaptive, multiplatform RCT	From 21 April 2020 to 22 January 2021	United States, Canada, the United Kingdom, Brazil, Mexico, Nepal	2219	100% ward	Therapeutic: enoxaparin 1.5 mg/kg once daily, UFH titrated to have 1.5–2.5 aPTT, dalteparin 200 UI once daily OR 100 UI twice daily tinzaparin 175 UI/kg once daily Standard: enoxaparin 40 mg once daily, dalteparine 5000 UI once, tinzaparine 4500 UI, fondaparinux 2.5 mg UFH 5000 UI/ 8–12 h ^b	21
Perepu et al., 2021 [52]	Open-label, multicenter RCT	From 26 April 2020 to 6 January 2021	USA	173	38% ward 62% ICU	Intermediate: enoxaparin 1 mg/kg SC daily Standard: enoxaparin 40 mg daily ^b	30
X-COVID 2021 [23]	Open-label, multicentre RCT	From 30 April 2020 to 25 April 2021	Italy	183	100% ward	Intermediate: enoxaparin 40 mg twice daily Standard: enoxaparin 40 mg once daily	30
COVID-HEP 2022 [53]	Open-label, multicentre RCT	From April 2020 to June 2021	Switzerland	159	45% ward 26% intermediate care 28% ICU	Therapeutic: enoxaparin 1 mg/kg twice daily or UFH with anti-Xa titration Standard: enoxaparin 40 mg once daily or UFH 5000 IU twice daily at ward enoxaparin 40 mg twice daily or UFH 5000 three times daily ^c	30
HEP-COVID 2020 [19]	Single-blinded, multicentre RCT	From 8 May 2020 to 14 May 2021	USA	253	67% ward 32% ICU	Therapeutic: enoxaparin 1 mg/kg twice daily Standard dose: enoxaparin 30 or 40 mg once or twice daily ^d	30

Table 1. Cont.

Study	Design	Enrollment Period	Country	Sample Size	Patients Admitted to	Intervention Arms	Follow-Up Period (days)
RAPID 2021 [21]	Open-label, adaptive, multicentre RCT	from 29 May 2020 to 12 April 2021	Brazil, Canada, Ireland, Saudi Arabia, United Arab Emirates, United States of America	465	100% ward	Therapeutic: enoxaparin 1.5 mg/kg once daily, dalteparin 200 UI/kg once daily, tinzaparin 175 IU/kg once daily, or UFH titrated according to aPPT Standard: 40 mg enoxaparin once daily, dalteparin 5000 UI daily, tinzaparin 4500 UI daily, UFH 5000 UI two or three times a day ^b	28
ACTION 2021 [22]	Open-label, pragmatic, multicentre RCT	From 24 June 2020 to 26 February 2021	Brazil	615	93% ward 6% ICU	Therapeutic: Rivaroxaban 20 mg once daily, enoxaparin 1 mg/kg twice daily, UFH titrated until anti-Xa 0.3–0.7 Standard: local prophylactic guidelines	30
Oliynyk et al., 2021 [54]	Open-label RCT	From July 2020 to 1 March 2021	Ukraine	126	100% ICU	Therapeutic: group 1: enoxaparin of 100 antiXa IU/kg twice daily group 2: UFH titrated to aPTT 40–70 s Standard: enoxaparin of 50 antiXa IU/kg once daily	28
INSPIRATION 2021 [55]	Open-label, multicenter RCT	From 29 July 2020 to 19 November 2020	Iran	562	100% ICU	Intermediate: enoxaparin 1 mg/kg daily Standard: enoxaparin 40 mg daily	30
Rashidi et al., 2021 [56]	Open-label, pilot RCT	From September 2020 to April 2021	Iran	10	100% ICU	Therapeutic dose: UFH 5000 IU every 8 h Standard: UFH titrated until aPTT 5–70	30
BEMICOP 2022 [24]	Open-label, multicenter, RCT	From October 2020 to May 2021	Spain	65	100% ward	Therapeutic: bemiparin 115 UI/kg daily Standard: bemiparin 3500 UI daily	30
PROTHROMCOVID 2022 [25]	Open-label, multicenter RCT	From 1 February 2021 to 30 September 2021	Spain	300	100% ward	Therapeutic: tinzaparin 175 UI/kg Intermediate: tinzaparin 100 UI/kg Standard: tinzaparin 4500 UI daily	30

Abbreviations used in this table: RCT: randomised controlled trial, BMI: body mass index, CrCl: creatinine clearance, UFH: unfractionated heparine, UI: international units. Explanations: ^a adjusted to age, BMI, and CrCl; ^b adjusted to BMI; ^c adjusted to patient's weight; ^d adjusted for CrCl.

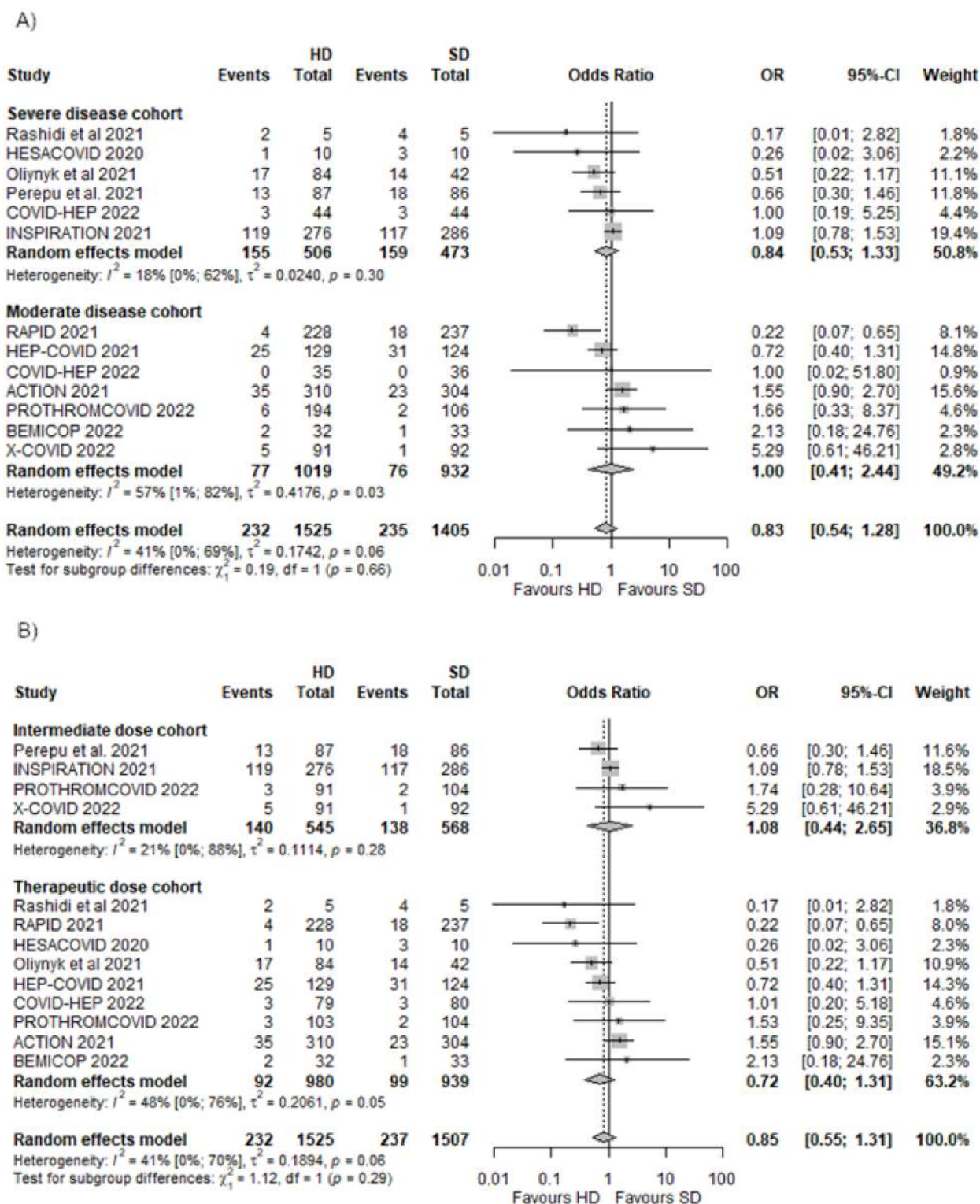


Figure 2. All-cause mortality in different dosing regimens in moderate and severe disease cohorts [19,21–25,50,52–56]. (A) All-cause mortality in severe and moderate disease cohorts; (B) all-cause mortality in cohorts where intermediate or therapeutic dose was used in the HD group.

3.3.2. Different D-Dimer Levels in the Included Trials

The cohort with D-dimer levels <1 mg/mL [22–24,52,54] showed no significant difference between the HD and the SD groups (OR 1.19 [95% CI 0.67–2.11]). Although nonsignificant, there is a tendency towards a decreased number of events in the HD group (OR 0.56 [95% CI 0.31–1.00]) in the case of the cohort with D-dimer >1 mg/mL [19,21,25,50,53,54,56]. The overall effect in the cohorts was an OR of 0.83 [95% CI 0.51–1.32, $p = 0.3914$] (Figure 3).

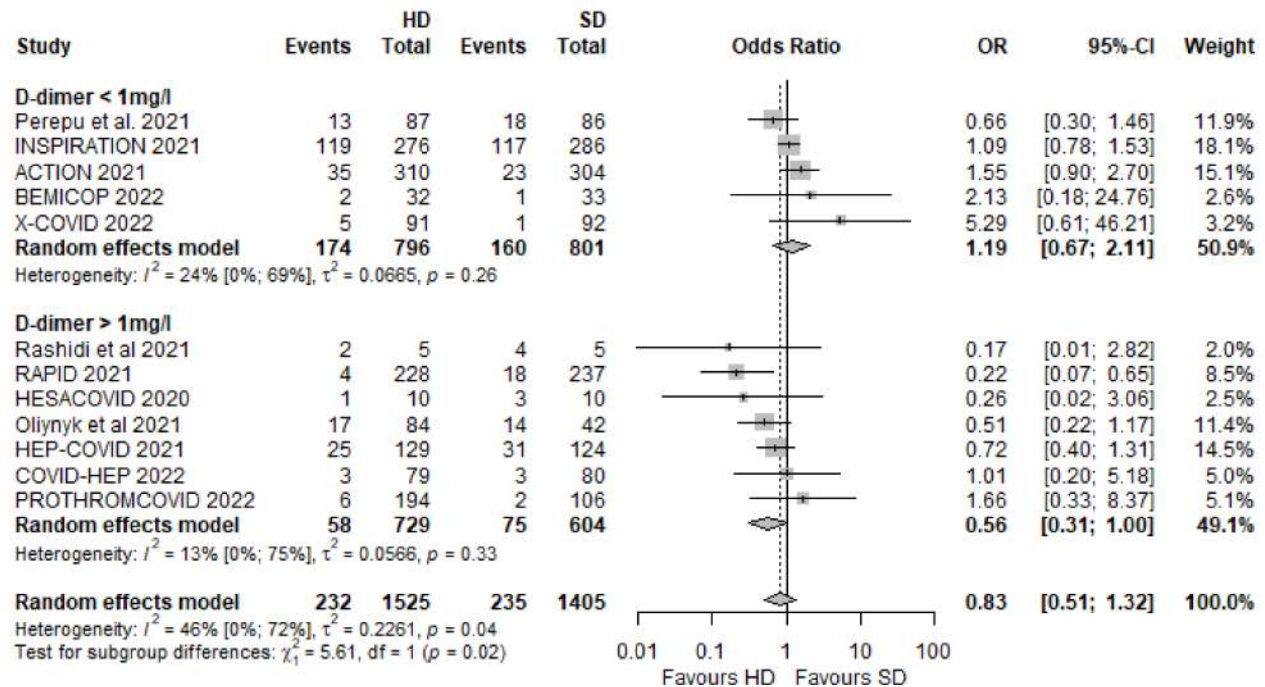


Figure 3. All-cause mortality in cohorts with different baseline D-dimer levels [19,21–25,50,52–56].

3.4. Any Thrombotic Events

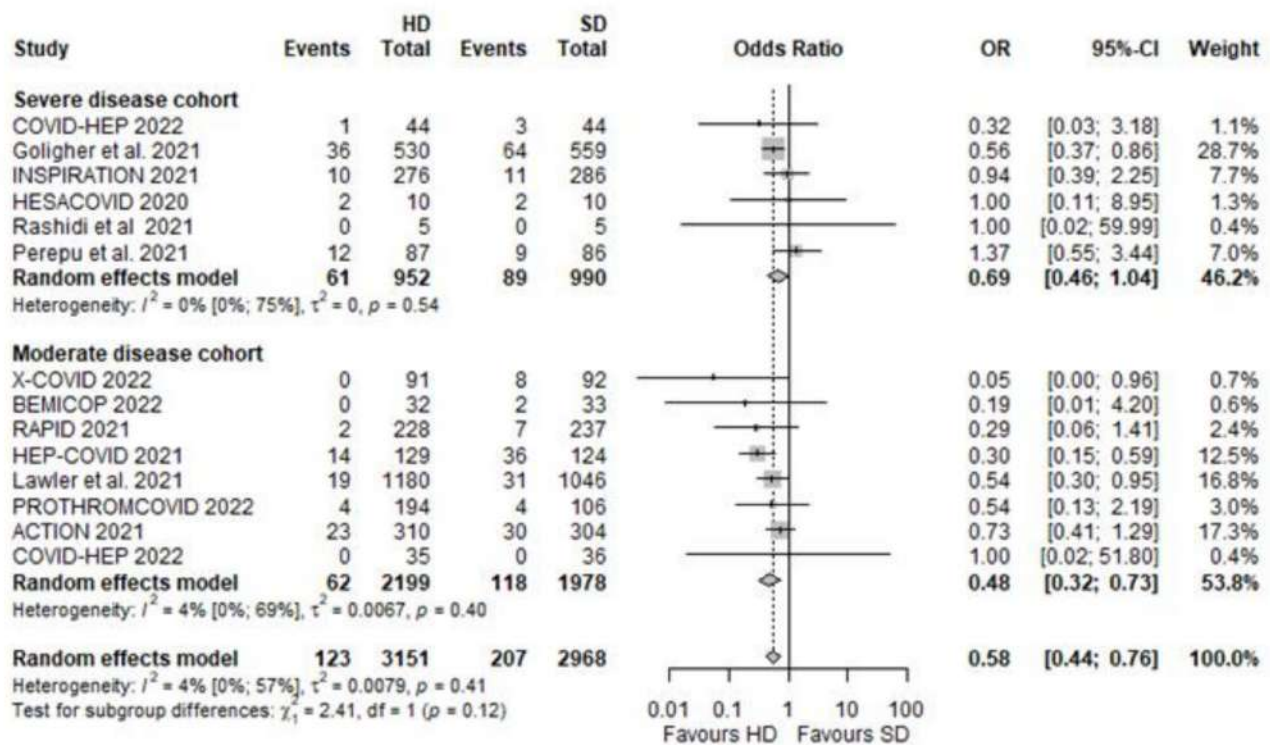
We included 13 RCTs [19–25,50–53,55,56] to assess the net effect regarding any thrombotic events, which consisted of any venous or arterial thrombotic events. This outcome covered a total of 6119 patients, out of which 123 patients in the HD group and 207 patients in the SD group suffered an adverse outcome. The overall effect size was an OR of 0.58 [95% CI 0.44–0.76, $p = 0.0000$], which indicates a significant difference favouring HD (Figure 4A).

Different Dosing Regimens in Moderate and Severe Disease

In the severe disease cohort, there was a numerical nonsignificant association of reducing the odds of thrombotic events in the HD group (OR 0.69 [95% CI 0.46–1.04]). This tendency favouring HD is associated with a significant decrease in thrombotic complications in the case of the moderate disease cohort with an OR of 0.48 [95% CI 0.32–0.76] (Figure 4A).

In the intermediate dose cohort, there was an association of a decreased number of thrombotic events in the HD group, but this did not reach statistical significance (OR 0.71 [95% CI 0.13–4.00]). Nevertheless, there was a significant association of reduction of thrombotic complications when patients were anticoagulated with therapeutic doses (OR 0.52 [95% CI 0.41–0.66]) (Figure 4B).

A)



B)

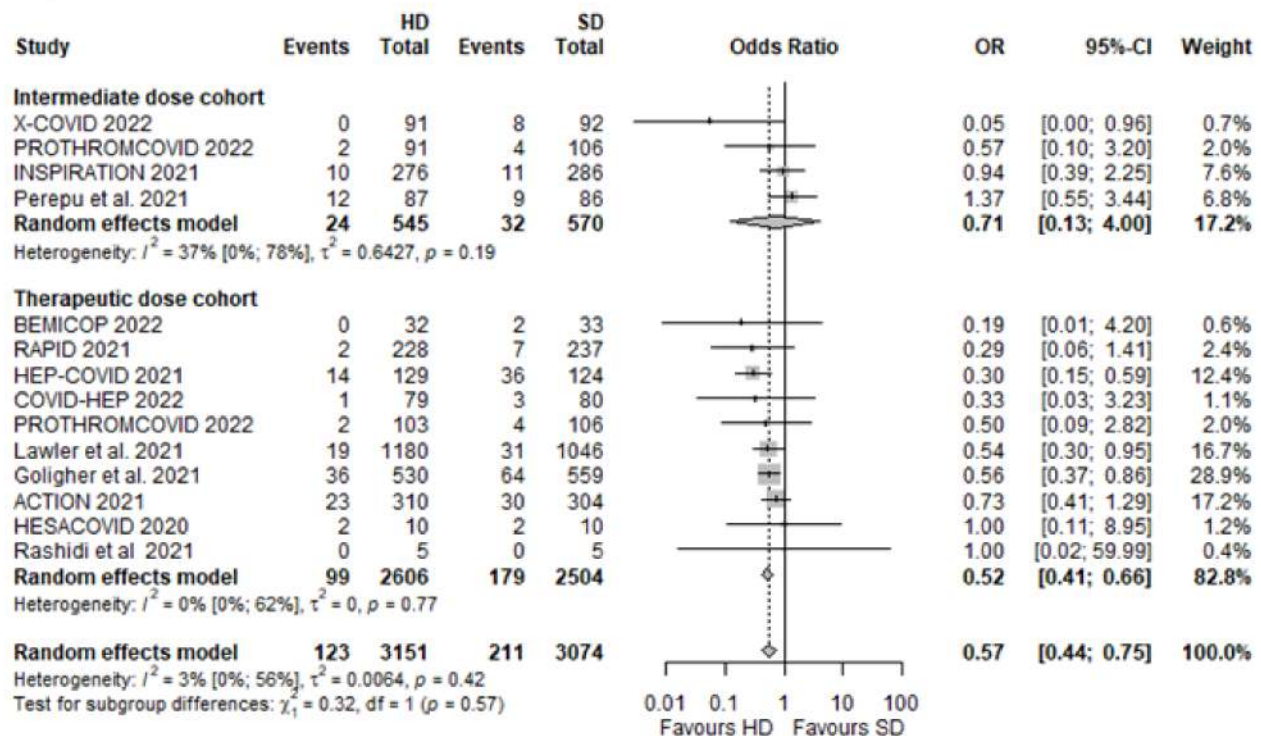


Figure 4. Any thrombotic events in different dosing regimens in moderate and severe disease cohorts [19–25,51–56]. (A) Any thrombotic events in severe and moderate disease cohorts; (B) any thrombotic events in cohorts where an intermediate or therapeutic dose was used in the HD group.

3.5. Major Bleedings

We included 14 RCTs covering 6250 patients [19–25,50–56]. We found a significant association of increased risk of bleedings in the case of the HD group compared with the SD group (OR 1.64 [95% CI 1.25–2.16], $p < 0.0017$) (Figure 5).

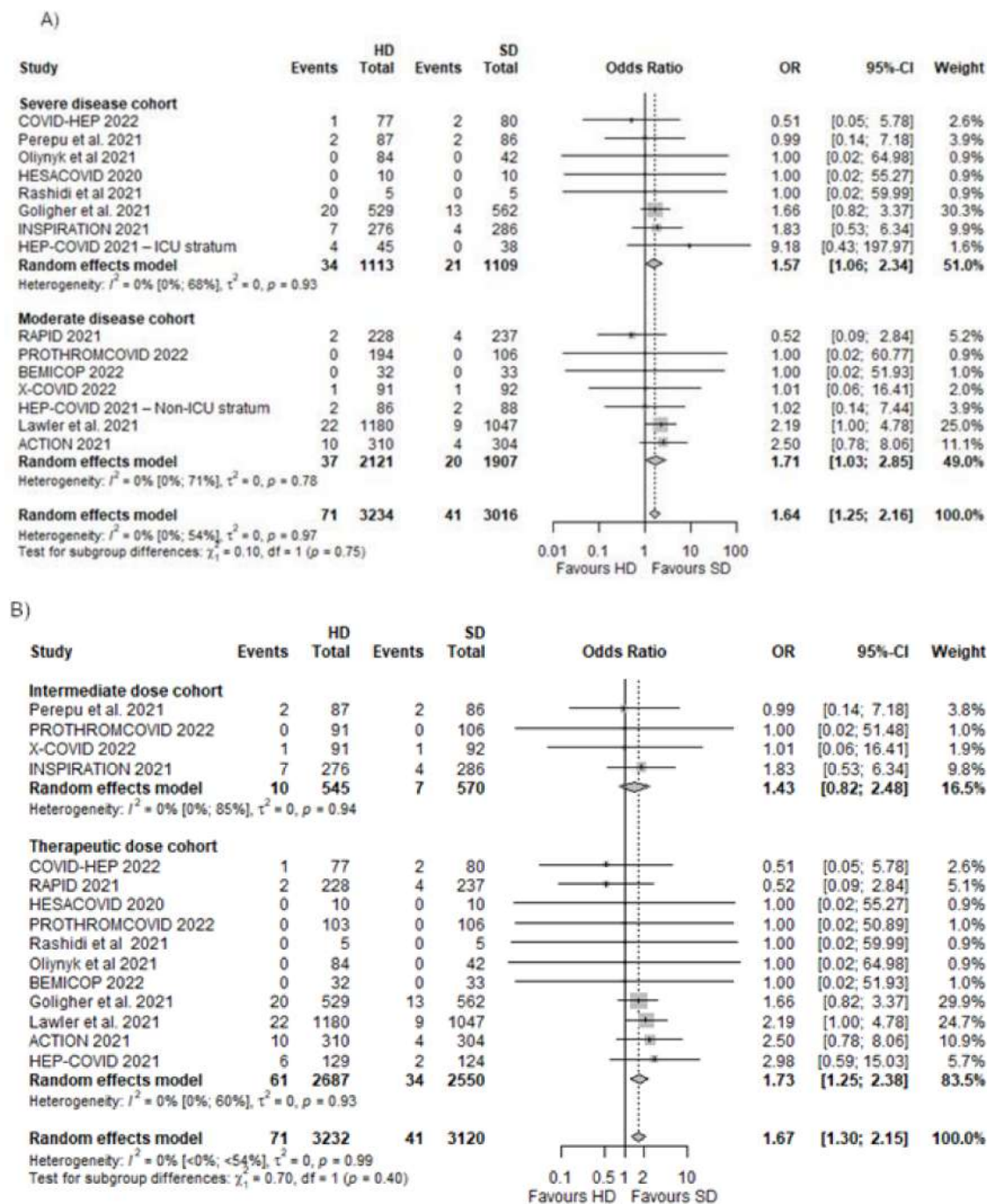


Figure 5. Major bleedings in different dosing regimens in moderate and severe disease cohorts [20–25,50–56]. (A) Major bleeding events in severe and moderate disease cohorts; (B) major bleeding events in cohorts where intermediate or therapeutic dose was used in the HD group.

Different Dosing Regimens in Moderate and Severe Disease

We found similar results favouring SD in severe and moderate disease cohorts (OR 1.57 [95% CI 1.06–2.34] and OR 1.71 [95% CI 1.03–2.85], respectively), both associated with an increased likelihood of the occurrence of bleedings (Figure 5A).

The association with increased major bleeding events did not reach the level of significance in the cohort where intermediate doses were administered in the HD group (OR 1.43 [95% CI 0.82–2.48]), although there was a significant association of increased likelihood of bleeding in the cohort with therapeutic anticoagulation used as HD (OR 1.73 [95% CI 1.25–2.15] (Figure 5B).

3.6. Progression of Disease

In order to examine the effect of different anticoagulant doses on disease progression, we used the following outcomes: the need for invasive mechanical ventilation, the need for ICU admission, progression to ARDS and an exploratory composite outcome of death, pulmonary embolism, and the need for invasive mechanical ventilation.

3.6.1. Need for Invasive Mechanical Ventilation

Data from six RCTs [19–21,23,25,54] were included in this analysis covering 3548 patients. There was no significant difference between the HD and SD groups regarding the number of patients who needed intubation, although a tendency towards a lower likelihood of occurrence of this event could be seen (OR 0.82 [95% CI 0.63–1.08], $p = 0.1214$) (Figure 6).

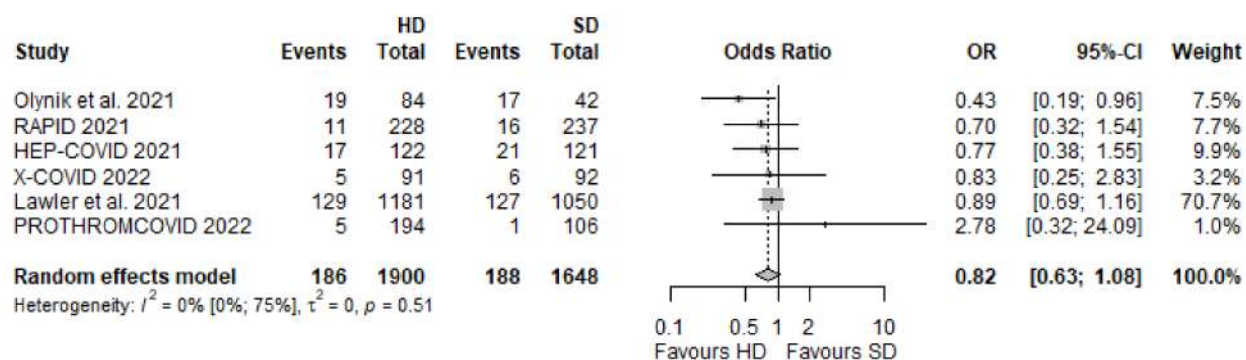


Figure 6. Need for invasive mechanical ventilation [19–21,23,25,54].

3.6.2. Need for ICU Admission

Data from four RCTs were used in this analysis [21,24,25,53]. The need for ICU admission showed no significant difference between the HD and SD groups (OR 0.98 [95% CI 0.65–1.45, $p = 0.9047$] (Figure S2 in Supplementary Material).

3.6.3. Progression to ARDS

Three RCTs reported this outcome [19,23,25]. Progression to ARDS occurred in 16 cases out of 412 in the higher dose cohort and 10 out of 319 in the standard dose cohort. The overall effect was 1.22 OR [95% CI 0.35–4.24, $p = 0.7563$], which did not reach the significance level (Figure S3 in Supplementary Material).

3.6.4. Death, Pulmonary Embolism, and Need for Invasive Mechanical Ventilation

In the exploratory composite outcome, we pooled data from four RCTs [19,21,23,54]. When the numbers of death, pulmonary embolism, and intubations are taken together, there is a significant difference between the HD and SD groups, because, in the HD group, the event was less likely to occur (OR 0.46 [95% CI 0.21–0.67], $p < 0.0001$) (Figure S4 in Supplementary Material).

3.7. Assessment of Quality of Evidence

The risk of bias was assessed using the RoB2 tool [32,33]. The overall risk of bias was low in three of the RCTs [21,23,53]. One trial had a high risk because of missing outcome data [24]. There were some concerns in the case of the overall risk of other trials (Figure S5 in Supplementary Material).

The GRADE assessment resulted in “moderate” certainty owing to imprecision for all-cause mortality and major bleedings, while any thrombotic events were downgraded as “low” owing to the risk of bias and imprecision. The need for invasive mechanical ventilation, the need for ICU admission, and the composite outcome were qualified as “very low” certainty owing to serious risk of bias, indirectness, and imprecision. Progression to ARDS also resulted in “very low” certainty owing to the serious risk of bias, indirectness, very serious inconsistency, and imprecision (Figure 7).

Certainty assessment							No. of patients		Effect		Certainty
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	HD group	SD group	Relative (95% CI)	Absolute (95% CI)	
All-cause mortality (follow-up: 21-30 days)											
12	randomised trials	not serious ^a	not serious	not serious	serious ^b	none	232/1525 (15.2%)	235/1405 (16.7%)	OR 0.83 (0.54 to 1.28)	24 fewer per 1,000 (from 69 fewer to 37 more)	⊕⊕⊕○ Moderate
Any thrombotic events (follow-up: 21-30 days)											
13	randomised trials	serious ^a	not serious	not serious	serious ^c	none	123/3151 (3.9%)	207/2968 (7.0%)	OR 0.58 (0.44 to 0.76)	28 fewer per 1,000 (from 38 fewer to 16 fewer)	⊕⊕○○ Low
Major bleeding											
13	randomised trials	not serious ^a	not serious	not serious	serious ^c	none	71/3234 (2.2%)	41/3016 (1.4%)	OR 1.69 (1.17 to 2.44)	9 more per 1,000 (from 2 more to 19 more)	⊕⊕⊕○ Moderate
Need for invasive mechanical ventilation											
6	randomised trials	serious ^a	not serious	serious ^d	serious ^e	none	186/1900 (9.8%)	188/1648 (11.4%)	OR 0.82 (0.63 to 1.08)	19 fewer per 1,000 (from 39 fewer to 8 more)	⊕○○○ Very low
ICU admission											
4	randomised trials	serious ^a	not serious	serious ^d	serious ^b	none	61/489 (12.5%)	57/412 (13.8%)	OR 0.98 (0.65 to 1.45)	2 fewer per 1,000 (from 44 fewer to 51 more)	⊕○○○ Very low
Progression to ARDS											
3	randomised trials	serious ^a	very serious ^a	serious ^d	very serious ^b	none	16/412 (3.9%)	10/319 (3.1%)	OR 1.22 (0.35 to 4.24)	7 more per 1,000 (from 20 fewer to 89 more)	⊕○○○ Very low
Composite outcome of death, pulmonary embolism and need for invasive mechanical ventilation											
4	randomised trials	serious ^a	not serious	serious ^d	serious ^c	none	108/532 (20.3%)	145/495 (29.3%)	OR 0.46 (0.31 to 0.67)	133 fewer per 1,000 (from 179 fewer to 76 fewer)	⊕○○○ Very low

CI: confidence interval; OR: odds ratio

Figure 7. GRADE assessment of the included studies. ^a The included studies are open-label studies, some of them with blinded adjudication. All-cause mortality and major bleeding are objective outcomes are thus less influenced by a lack of allocation concealment. ^b Some studies included a small sample size and low number of events. The 95% confidence interval is wide and crosses the line of no effect. ^c Some of the studies included a small sample size and low number of events. ^d There were some differences in outcome measures. Need for invasive mechanical ventilation, ICU admission, progression to ARDS, and the composite outcome included a subjective clinical decision—this and the open-label design could have influenced the measurement of the outcomes. ^e There were wide differences in point estimates across studies.

4. Discussion

The COVID-19 pandemic has shocked the world and highlighted the importance of up-to-date knowledge. New treatments and old repurposed ones were demanded to curb the numbers of recently infected and dead.

In this meta-analysis, we analysed 14 RCTs [19–25,50–56], including a total number of 6253 patients, comparing the effects of HD with those of SD anticoagulation on mortality, any thrombotic events, major bleedings, and the progression of the disease in COVID-19 patients.

4.1. Different Dosing Regimens and Disease Severity

The ACTIV4-B trial [57] reported a low number of cardiopulmonary hospitalizations, thus it was halted. However, according to their findings, the use of apixaban 2.5 mg twice daily compared with apixaban 5 mg twice daily showed no benefit in the case of outpatients with a mild disease, as the authors found no effect on mortality or cardiopulmonary hospitalizations. Therefore, they concluded that the use of HD compared with SD seems futile in the case of a mild disease.

According to our results, the HD group compared with the SD group was not associated with lower mortality in all hospitalised COVID-19 patients (OR 0.83 [95% CI 0.54–1.28]). Although it was associated with a decreased risk of thrombotic events (OR 0.58 [95% CI 0.44–0.76]), it was parallelly associated with an increased chance of major bleeding (OR 1.64 [95% CI 1.25–2.16]). Therefore, we cannot exclude that the positive effects on thrombotic events were counteracted by the increased risk of major bleedings, thus resulting in no significant effect on mortality. Moreover, mortality in COVID-19 might have other causes that cannot be influenced by anticoagulation. On the other hand, owing to the short follow-up period of most of the included studies, we cannot assess the long-term benefits of anticoagulation in the case of these patients [59].

When we analysed the moderate and severe disease cohorts separately, we did not find significant differences in mortality (OR 1.00 [95% CI 0.41–2.44] and OR 0.84 [95% CI 0.53–1.33], respectively) in the HD group compared with the SD group.

In the moderate disease cohort, the effect of therapeutic dose anticoagulation compared with SD on thrombotic events was associated with a significant decrease in the incidence of any thrombotic events (OR 0.52 [95% CI 0.41–0.66]) and a nonsignificant tendency of decreasing all-cause mortality (OR 0.72 [95% CI 0.40–1.31]) compared with SD. Regarding the intermediate dose cohort as the HD group, we did not find a statistically significant effect regarding mortality (OR 1.08 [95% CI 0.44–2.65]) or thrombotic events (OR 0.71 [95% CI 0.13–4.00]).

The therapeutic dose anticoagulation was associated with a significant decrease in thrombotic events in the moderate disease cohort, but in a parallel way, it was associated with an increased risk of major bleedings (OR 1.73 [95% CI 0.25–2.38]). HD anticoagulation also significantly increased the risk of major bleeding in the severe disease cohort (OR 1.57 [95% CI 1.06–2.34]), but without a statistically significant benefit regarding the prevention of thrombotic events (OR 0.69 [95% CI 0.46–1.04]).

We have to take into consideration that some of the larger studies in severe disease cohorts used intermediate doses in the HD group [51,54], but only a minority of patients were administered intermediate doses in the moderate disease cohort [23,25].

It is important to note that a higher incidence of PE than deep vein thrombosis (DVT) was found in critically ill COVID-19 patients compared with non-COVID-19 patients, as reported by several studies [20–23,51,60]. These authors hypothesized that, in many instances, pulmonary artery occlusions in these patients could be interpreted as a manifestation of local pulmonary thrombi due to hyperinflammation rather than a consequence of an embolic event per se [23]. Pulmonary hyperinflammation in severe COVID-19 patients may be associated with hemostatic disorders such as hypercoagulation with fibrinolysis resistance [61–65], which can lead to organ failure due to thrombi in small vessels that theoretically cannot be prevented or treated with HD anticoagulation, although it could still be associated with an increased risk of bleeding events.

This assumption has been supported by recent multiplatform trials [20,51], which reported a significant increase in organ support-free days only in non-critically ill COVID-19 patients receiving a therapeutic dose of anticoagulation (OR 1.27 [95% CI 1.03–1.57]). Similar results were reported in the RAPID trial [21]. Unfortunately, outcomes of organ support-free days and ventilator-free days were only reported in three of the eligible RCTs [20,21,51], with different adjustment of statistical calculations; hence, the data were ineligible to be pooled together in the current meta-analysis (Supplementary Material Table S2). Therefore, we decided to analyse data based on disease progression such as the need for invasive

mechanical ventilation, progression to ARDS, and need for ICU admission. According to our result, therapeutic dose anticoagulation in a cohort with mostly moderately ill patients showed a slight tendency towards a reduction in the intubation rate (OR 0.82 [95% CI 0.63–1.08]). Although the result is statistically nonsignificant, it suggests that HD anticoagulation might be suitable as a preventive measure regarding disease progression in selected moderately ill patients, rather than a treatment option in the severe disease cohort with already settled respiratory failure.

Nevertheless, the severity of the coagulation disorder in COVID-19 patients could also differ and might influence the effectiveness of anticoagulation therapy.

4.2. Differences in the Degree of COVID-19-Associated Coagulopathy

The mechanism responsible for COVID-19-associated coagulopathy has not been fully elucidated, but it seems that hypercoagulation with impaired fibrinolysis induced by endothelial dysfunction due to hyperinflammation and hypoxia has a pivotal role in critically ill patients [66–68].

Hypercoagulation may be indicated by increased D-dimer levels, which are associated with an increased mortality and risk of complications in COVID-19 [8,29–31]. For instance, Tang et al. [7] found significantly higher D-dimer levels in non-survivors compared with those who survived (2.12 µg/mL [IQR 0.77–5.27] and 0.61 µg/mL [IQR 0.35–1.29], respectively, $p < 0.001$). Other studies had similar findings with significantly higher levels of D-dimer in patients with complications with PE [67–69].

In the multiplatform trials [20,51], D-dimer levels were used for risk stratification, but this did not result in significant variation in treatment effects between HD and SD anticoagulation. In these trials, patients were enrolled independently of their D-dimer levels and the median D-dimer levels were lower compared with those in some other studies [19,21,25,50,53,54,56]. In the HEP-COVID trial on patients with more considerably elevated D-dimer levels, therapeutic-dose LMWH reduced not only the risk of thromboembolism, but also mortality, compared with low or intermediate-dose LMWH (absolute risk reduction, 13.2%) without increasing the risk of major bleeding (absolute incremental risk, 3.0%) [19]. In the pilot HESACOVID study, the use of therapeutic enoxaparin improved gas exchange over time and resulted in a higher ratio of successful liberation from mechanical ventilation in the case of severe COVID-19 patient with very high D-dimer levels (median: 4176 [1986–6365] µg/L); furthermore, D-dimer levels showed a significant decrease in the HD group (4176 [95% CI 1986–6365] µg/L vs. 1469 µg/L [95% CI 1034–1904] µg/L, $p = 0.009$), and a significant increase in the SD group (3408 µg/L [95% CI 1283–5532] µg/L vs. [95% CI 2291–7465]), $p = 0.004$) [50].

Therefore, D-dimer thresholds have been proposed to identify high-risk patients and guide decisions regarding anticoagulation on whether a therapeutic or prophylactic dose should be applied [69–72]. In a recently published meta-analysis, the weighted mean difference of D-dimer was 0.97 µg/mL (95% CI 0.65–1.29) between mild and severe groups of patients hospitalized with COVID-19; therefore, we performed a subgroup analysis of included RCTs with D-dimer levels below and above 1 µg/mL [31]. We found that, although none of the cohorts were associated with statistically significant effects, there was a clinically relevant tendency of decreased likelihood of death in the HD group in the cohort admitted to hospital with a D-dimer level > 1 µg/mL. In order to investigate whether HD anticoagulation prevented thrombotic events and the progression of disease with elevated D-dimer levels in a cohort consisting mostly of moderately ill patients, we performed an exploratory composite outcome that included death, the incidence of pulmonary emboli, and the need for mechanical ventilation. We found statistically significant results favouring higher dose thromboprophylaxis (OR 0.46 [95% CI 0.31–0.67], $p < 0.0001$). On the basis of our results, one may assume that therapeutic dose anticoagulation could be beneficial in moderately ill patients with elevated D-dimer levels.

4.3. Strengths and Limitations

To date, this is the most up-to-date and comprehensive meta-analysis on the effect of different anticoagulant regimens on COVID-19 infection. We included traditional outcomes that assess the effect of different anticoagulation doses on thrombotic events and bleeding, but included additional clinical outcomes that point towards the course of the disease by comparing higher and standard dose thromboprophylaxis. Furthermore, we analysed the interplay of baseline D-dimer levels on the effect of anticoagulation on all-cause mortality. We defined a selected subpopulation of patients, the moderately ill with elevated D-dimer levels, in which the progression of the disease may be prevented by therapeutic anticoagulation. This had not been reported before. We also tried to minimize the effects of confounding factors present in different trials by conducting leave-one-out analyses on all outcomes that had more than five trials included (Supplementary Material Figures S6–S13).

Our study has several limitations. First, data extraction resulted in some compromise as Lawler et al. published survival without intubation through 28 days as the number of patients per total number, but we chose to calculate the number of patients who were intubated or died and pooled together with the number of patients who needed intubation, as this was an outcome to evaluate the progression of disease [20]. In addition, in the case of composite outcomes, we cannot exclude that there are overlaps in the patient population. Second, the low number of studies and the clinical heterogeneity of the population make it difficult to address the question of thromboprophylaxis, as the difference in anticoagulant regimens, dose, duration, and the trial population may work as confounding factors. Third, there are differences in ICU admission among countries. Fourth, some trials enrolled a low number of participants, thus a low event rate was reported, which may have influenced our analysis [50,56]. Besides, some preplanned analyses could not be performed because of the differences in statistical adjustments between the trials. Finally, we included an article that was not peer-reviewed [25].

5. Conclusions and Implications for Practice and Research

Our analysis including recently published RCTs [25,53,54,56] confirmed the main finding of previous meta-analyses [73–79] and endorsed the current guidelines (Table S3 in Supplementary Material) [80–83]. On the basis of our results, we cannot advocate for the routine use of therapeutic dose thromboprophylaxis in all COVID-19 patients either. Nevertheless, there is a tendency that favours HD regarding disease progression, specifically therapeutic dose anticoagulation with UFH or LMWH, in patients with considerably elevated D-dimer levels who did not need ICU level care at hospital admission.

Further results of ongoing studies are needed to define more precisely at what time it is appropriate to start higher dose thromboprophylaxis and in which selected patients it should be applied. However, there is a great risk of the populations of these trials being notably heterogeneous, as diverse variants of the SARS-CoV2 virus have emerged, new therapeutic options were implemented, and rates of immunizations varied between different countries and periods. Thus, what seemed beneficial in the earlier wave of the pandemic with considerable thrombotic complications reported might have less importance later, as these might serve as confounding factors that influenced the presentation of the disease.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/biomedicines10092194/s1>, **Figure S1:** PRISMA 2020 flow diagram of the initial search, **Table S1:** Detailed inclusion and exclusion criteria of included trials with baseline D-dimer levels, **Figure S2:** Need for ICU admission, **Figure S3:** Progression to ARDS, **Figure S4:** Composite outcome of death, pulmonary embolism and need for invasive mechanical ventilation, **Figure S5:** Risk of bias assessment 2 in the included trials, **Table S2:** Other clinical outcomes, **Table S3:** Latest recommendations of guidelines on anticoagulation in patients infected with COVID-19, **Figure S6:** All-cause mortality in cohorts with different disease severity, **Figure S7:** All-cause mortality in cohorts with different anticoagulant doses, **Figure S8:** All-cause mortality in cohorts with different baseline d-dimer levels, **Figure S9:** Any thrombotic events in cohorts with

different disease severity, **Figure S10**: Any thrombotic events in cohorts with different anticoagulant doses, **Figure S11**: Major bleeding events in cohorts with different disease severity, **Figure S12**: Major bleeding events in cohorts with different anticoagulant doses, **Figure S13**: The need for invasive mechanical ventilation.

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Article

Exploratory Analysis of Coagulation and Fibrinolysis Trajectories After IL-6 Antagonist Therapy in COVID-19: A Case Series

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Abstract

Background/Objectives: Severe COVID-19 is marked by IL-6-driven inflammation, endothelial injury, and dysregulated coagulation. Although IL-6 antagonists improve clinical outcomes, their effects on the temporal evolution of coagulation and fibrinolysis remain insufficiently defined. This study characterizes inflammatory, endothelial, coagulation, and fibrinolytic trajectories following IL-6 receptor blockade in critically ill COVID-19 patients. **Methods:** In this prospective, exploratory multicenter case series (ClinicalTrials.gov NCT05218369), 15 ICU patients with PCR- or antigen-confirmed COVID-19 received tocilizumab per protocol. Serial sampling at five timepoints (T0–T4) included routine laboratories, comprehensive viscoelastic hemostatic assays (ClotPro[®]), and ELISA-based endothelial and fibrinolytic biomarkers. Analyses were primarily descriptive, emphasizing temporal patterns through boxplots; paired Wilcoxon tests with FDR correction contextualized within-patient changes. **Results:** Patients exhibited marked inflammation, hyperfibrinogenemia, endothelial activation, and delayed fibrinolysis at baseline. IL-6 blockade induced rapid suppression of CRP and PCT, progressive declines in fibrinogen, and modest platelet increases. In contrast, vWF antigen and activity further increased, indicating persistent endothelial dysfunction. Viscoelastic testing showed preserved thrombin generation and sustained high clot firmness, while biochemical markers (rising PAI-1, modest PAP increase, and progressively increasing D-dimer) and VHA indices suggested ongoing antifibrinolytic activity despite resolution of systemic inflammation. **Conclusions:** IL-6 antagonism was associated with rapid attenuation of systemic inflammation but was not accompanied by normalization of endothelial activation or fibrinolytic resistance. The observed hemostatic profile was consistent with attenuation of inflammation-associated



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coagulation features, while endothelial and prothrombotic alterations appeared to persist during follow-up, warranting further investigation in larger controlled studies.

Keywords: coagulation; fibrinolysis; COVID-19; thromboinflammation; interleukin-6 antagonist; endothelial dysfunction; viscoelastic hemostatic assay

1. Introduction

The COVID-19 pandemic unveiled the critical crosstalk between inflammation and coagulation, with cytokine-driven immune activation contributing to a markedly prothrombotic state [1]. The inflammatory and coagulation systems have evolved in close interdependence, a relationship evident throughout phylogeny [2]. Inflammatory stimuli and endothelial injury create a pro-coagulant environment, commonly referred to as thromboinflammation, which not only promotes clot formation but also aids in pathogen containment and immune regulation [3].

Cytokines such as interleukin-6 (IL-6) play a central role in this crosstalk. IL-6 enhances coagulation through multiple mechanisms: upregulating the hepatic synthesis of fibrinogen, increasing expression of factor VIII and tissue factor, and downregulating natural anticoagulants such as antithrombin and protein S. Additionally, IL-6 promotes antifibrinolytic activity by stimulating endothelial cells to produce plasminogen activator inhibitor-1 (PAI-1) via trans-signaling pathways. The cumulative effect of elevated IL-6 is a shift in hemostatic balance toward a procoagulant, hypofibrinolytic state [4].

The abovementioned phenomena are not confined to a single disease. In chronic inflammatory disorders such as rheumatoid arthritis and systemic juvenile idiopathic arthritis, persistently elevated IL-6 contributes to a hypercoagulable state, mirroring the hemostatic disturbances seen in acute inflammation, albeit typically less pronounced [5,6]. Clinical experience and mechanistic studies support the notion that IL-6 is a key driver of inflammation-induced coagulation abnormalities and that IL-6 blockade may reduce thrombotic risk [7].

The prothrombotic effects of IL-6 have also been implicated in systemic inflammatory response (SIRS), where elevated IL-6 levels correlate with increased tissue factor and PAI-1 expression, potentially contributing to disseminated intravascular coagulation (DIC) and multiorgan failure. Interrupting the IL-6/sIL-6R pathway may, therefore, attenuate coagulopathy and improve outcomes [8].

While tocilizumab improves outcomes in severe COVID-19, its influence on the temporal interplay between inflammation, coagulation, and fibrinolysis remains unclear. Given that severe COVID-19 is characterized by both cytokine storm and coagulopathy, we hypothesize that immunomodulation with IL-6 antagonists could mitigate hemostatic disturbances without the need for intensified anticoagulation beyond standard of care [9]. This study characterizes hemostatic alterations in critically ill COVID-19 patients undergoing IL-6 receptor blockade. We assessed longitudinal changes in coagulation and fibrinolytic parameters before and after treatment using ClotPro[®] viscoelastic assays. We further explored associations between coagulation abnormalities, endothelial dysfunction, and systemic inflammation in this observational setting.

2. Materials and Methods

2.1. Study Design and Setting

We conducted a prospective, exploratory, multicenter case series involving three Hungarian intensive care units (ICUs). This study complied with the Declaration of Helsinki

and was approved by the Hungarian Medical Research Council (1405-3/2022/EÜG). The protocol has been published previously and was registered on ClinicalTrials.gov (NCT05218369) [10].

2.2. Eligibility

Patients were enrolled without active recruitment based on the treating physician's decision to initiate IL-6 receptor blockade according to local institutional protocols.

Inclusion criteria comprised adults with polymerase chain reaction (PCR) or antigen-confirmed SARS-CoV-2 infection and acute respiratory failure requiring immunomodulation. Typical indications included invasive or non-invasive ventilation, or high-flow nasal oxygen therapy ($\text{FiO}_2 > 0.4$, flow > 30 L/min), in the presence of systemic inflammation defined by C-reactive protein (CRP) > 75 mg/L.

Exclusion criteria included prior administration of immunomodulatory agents, chronic immunosuppression, suspected active bacterial infection, platelet count $< 50 \times 10^9$ /L, recent fibrinolytic therapy or blood product administration, pregnancy, inability to obtain informed consent from the patient or legally authorized representative, and a delay exceeding 120 h between ICU admission and IL-6 antagonist administration.

2.3. Data Collection

Prospective data included demographics, comorbidities, clinical status, therapies, blood cultures, routine labs, and viscoelastic hemostatic assays (VHA). Sites entered data into standardized eCRFs in a centralized database; patients were de-identified with unique study IDs.

Blood was sampled prior to tocilizumab administration (T0) and subsequently at 24 h (T1), 48 h (T2), 120 h (T3), and 168 h (T4). Viscoelastic hemostatic assays (VHA; ClotPro[®], Haemonetics Corporation, Boston, MA, USA) included extrinsic pathway (EX), intrinsic pathway (IN), fibrinogen (FIB), tissue plasminogen activator-enhanced (TPA), Russell viper venom (RVV), and ecarin clotting (ECA) assays. Parameters recorded reflected clot initiation (clotting time, CT), clot propagation and mechanical strength (clot formation time, CFT; maximum clot firmness, MCF), and fibrinolytic activity (maximum lysis, ML; clot lysis index at 30 and 45 min, CLI-30/45; lysis onset time, LOT; and lysis time, LT). Interpretation of the ClotPro assays and derived parameters is summarized in Supplementary Table S4.

Routine laboratory tests, including complete blood count (CBC), C-reactive protein (CRP), procalcitonin (PCT), ferritin, international normalized ratio (INR), activated partial thromboplastin time (aPTT), thrombin time (TT), fibrinogen, and D-dimer, were performed daily. Additional plasma samples were stored at -80 °C for centralized enzyme-linked immunosorbent assay (ELISA) measurements of inflammatory, endothelial, and fibrinolytic biomarkers, including interleukin-6 (IL-6), syndecan-1, von Willebrand factor (vWF) antigen and activity, antithrombin, plasminogen (PLG), τ 2-antiplasmin, thrombin-activatable fibrinolysis inhibitor (TAFI), thrombin-antithrombin complex (TAT), plasmin-antiplasmin complex (PAP), plasminogen activator inhibitor-1 (PAI-1), and tissue plasminogen activator (tPA). Biomarkers constrained by logistics were assayed at T0, T2, and T4.

2.4. Outcomes

The primary outcome was VHA-derived fibrinolysis dynamics: LT and LOT (TPA- and ECA-tests) and ML and CLI-45 (EX-test), measured at T0 and T1–T4. Secondary outcomes included trajectories of plasma biomarkers of coagulation system (PLG, PAP, TAFI, PAI-1, τ 2-antiplasmin, and tPA; T0/T2/T4), inflammatory markers (procalcitonin, CRP, ferritin, and lactate dehydrogenase [LDH]), endothelial markers (syndecan-1 and vWF antigen/activity), classical coagulation tests, fibrinogen, antithrombin, TAT, and platelet count, and their relationships with VHA parameters.

2.5. Statistical Analysis

All statistical analyses were performed using R statistical software (R version 4.5.1, R Core Team, 2021) [10]. Given the exploratory nature of this study and the small sample size, the primary focus was on describing trends and patterns rather than establishing causal relationships. Descriptive statistics were used to summarize categorical variables as counts and percentages, while continuous variables were expressed as mean $\pm$ SD or median with IQR, depending on normality. No imputation, winsorization, or outlier deletion was applied and analyses used available cases.

Paired comparisons across timepoints were performed using the Wilcoxon signed-rank test, applying the Hodges–Lehmann median difference and 95% confidence intervals with false discovery rate adjustment.

For each biomarker and timepoint, box-and-jitter plots were generated to display the distribution of values with individual patient measurements superimposed. Survivors were plotted as grey circles and non-survivors as red triangles. Several biomarkers exhibited pronounced right-skewed distributions; therefore, to improve visual interpretability without modifying the underlying data, predefined upper display limits were applied for selected variables. Values exceeding these limits were displayed at the axis boundary and indicated by asterisks, explicitly denoting out-of-range observations while preserving all data points. No observations were removed or altered for statistical analyses.

For fibrinolysis-related visualizations, patients were additionally stratified by fibrinolytic resistance status using the TPA-test lysis time (LT) threshold of 312 s at admission, as proposed by Coupland et al. [11].

To illustrate temporal evolution, longitudinal “spaghetti” plots were also constructed, showing individual patient trajectories with median trend lines and interquartile-range ribbons.

Due to the study design, loss to follow-up was not applicable. Statistical significance was set at $p < 0.05$.

3. Results

Between January 2022 and January 2023, 15 critically ill COVID-19 patients receiving IL-6 receptor blockade were included across three Hungarian ICUs. Enrollment concluded earlier than planned as national case numbers declined; results are therefore presented as an exploratory case series.

All included patients received immunomodulation therapy and were followed for the full study period. Reasons for exclusion included cases when tocilizumab was finally not administered due to physician’s decision although the patient fit inclusion criteria or the patient presented a co-infection. The baseline characteristics of the study population can be found in Table 1.

Table 1. General characteristics of the study population at admission.

Characteristic	Value
Study population	15
Male	12/15 (80%)
Age	68.3 (8.6); 69.0 [64.0, 74.0]
BMI	32.2 (6.2); 30.0 [27.2, 35.4]
Hypertension	15/15 (100.0%)
Ischemic heart disease	6/15 (40.0%)
Atrial fibrillation	4/15 (26.6%)
Diabetes Type II	7/14 (50.0%)

Table 1. Cont.

Characteristic	Value
Chronic Respiratory Disease	2/15 (13.3%)
Autoimmune disease	2/15 (13.3%)
Malignant Disease	1/15 (6.7%)
Renal disease	1/15 (6.7%)
Neurological or psychiatric disease	3/15 (20.0%)
Obesity	7/15 (46.7%)
APACHE II	11.2 (3.4); 12.0 [8.2, 13.0]
Clinical frailty scale	3.5 (1.2); 3.0 [3.0, 4.0]
Severe organ failure at admission	7/15 (46.7%)
Vasopressor support	4/15 (26.7%)
Currently on oxygen or ventilated	15/15 (100.0%)
Type of respiratory support	
High-Flow Nasal Cannula	4/15 (26.7%)
Invasive Mechanical Ventilation	6/15 (40.0%)
Non-Invasive Ventilation	3/15 (20.0%)
Non-rebreather mask	2/15 (13.3%)
Prone position	7/15 (46.7%)
Neuromuscular relaxant	2/15 (13.3%)
PaO ₂	74.4 (21.2); 69.2 [60.4, 80.2]
FiO ₂	0.9 (0.2); 1.0 [0.8, 1.0]
PaO ₂ /FiO ₂ (Horowitz index)	91 (40); 74 [65, 114]
PaCO ₂ (mmHg)	39.4 (6.4); 36.6 [35.1, 43.9]
Ferritin (ug/L)	1351.2 (1930.1); 673.0 [602.8, 1105.2]
CRP (mg/L)	183.7 (58.6); 180.9 [150.8, 211.0]
LDH (U/L)	841.4 (396.1); 759.5 [531.2, 1176.0]
Leucocytes (G/L)	10 (5); 8 [7, 13]
Hemoglobin (g/L)	127 (20); [120, 111, 146]
Hematocrit (%)	37 (5); 36 [33, 42]
Thrombocytes (G/L)	209 (81); 195 [180, 218]
ASAT/GOT (U/L)	76.3 (45.6); 62.0 [42.0, 109.5]
ALAT/GPT (U/L)	47.7 (33.1); 31.0 [26.5, 64.5]
APTT (s)	39.0 (6.5); 40.4 [36.0, 44.7]
Thrombin time (s)	18.8 (2.9); 18.1 [17.4, 21.6]
Fibrinogen (g/L)	5.6 (1.5); 5.5 [5.1, 6.4]
D dimer (ug/L)	10,674.9 (33,130.4); 1125.5 [837.5, 2347.5]
Tocilizumab dose (mg)	704.0 (98.9); 720.0 [600.0, 800.0]
Corticosteroids	15/15 (100.0%)
Antiviral medication (acyclovir, remdesivir, or both)	14/15 (93.3%)

Table 1. Cont.

Characteristic	Value
Blood products or derivatives	0/15 (0.0%)
Immunoglobulin	0/15 (0.0%)
Antiplatelet agents	8/15 (53.3%)
Aspirin	8/15 (53.3%)
Clopidogrel	2/15 (13%)
Anticoagulants	13/15 (86.7%)
DOAC	1/13 (7.7%)
LMWH	12/13 (92.3%)
Daily dose of LMWH (IU)	11,500.0 (5125.7); 12,000.0 [8000.0, 13,000.0]
Admission source to ICU	Emergency department: 12 (80%); Hospital ward: 3 (20%)
Patient outcome at the end of the study period (7 days)	
Deceased	3/15 (20.0%)
Remained in ICU	8/15 (53.3%)
Discharged from ICU and transferred to ward	4/15 (26.7%)

Continuous: Mean (SD); Median [IQR]; Binary: n/N, (%).

3.1. Primary Outcomes

Paired Wilcoxon analyses revealed nominally significant changes in four parameters: ECA-test LOT (T0 vs. T2) and EX-test CLI-45 (T0 vs. T2, T3, and T4) ($p < 0.05$; Table S2). However, none of these remained statistically significant after false discovery rate adjustment. Consequently, interpretation focused on biological trends across timepoints rather than isolated p -values.

3.2. Inflammation and Endothelium

At admission, IL-6 (49.6 [16.2–79.2] pg/mL) and CRP (180.9 [150.8–211.0] mg/L) were markedly elevated. Following IL-6 antagonist therapy, CRP declined rapidly, with a >90% reduction within five days. Circulating IL-6 increased transiently at 48 h before declining during follow-up.

Procalcitonin showed a gradual decline after a minor early increase, while ferritin rose during the first 24 h and then stabilized (826 µg/L at 168 h), suggesting slower resolution of macrophage activation. Leukocyte counts increased follow-up, while LDH declined but remained above normal.

vWF activity (351.7 [341.8–438.0] %) and antigen (449.3 [321.6–595.3] %) were markedly elevated, indicating endothelial injury, and rose further to 483.9% and 630.0% at 168 h, respectively. Syndecan-1 was generally low or undetectable, with sporadic peaks reflecting discrete glycocalyx shedding (Figure 1).

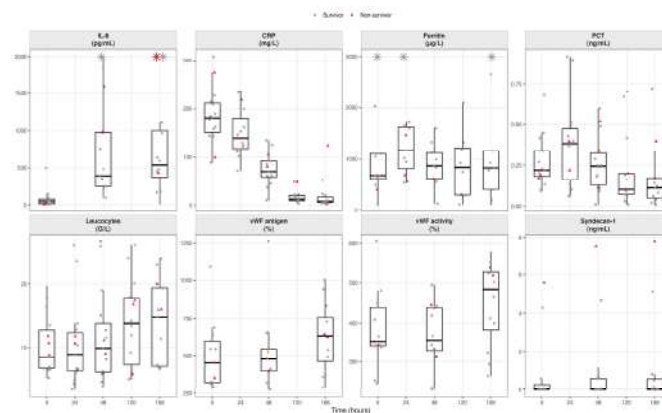


Figure 1. Endothelium and inflammation: upstream drivers of hemostatic shift. Boxplots show inflammatory (IL-6, CRP, ferritin, procalcitonin, and leukocytes) and endothelial injury markers (vWF antigen/activity and syndecan-1). Boxes represent median and interquartile range; whiskers indicate $1.5 \times \text{IQR}$. Individual patient values are overlaid (grey circles = survivors; red triangles = non-survivors). Data are shown across timepoints (T0–T4). Values exceeding prespecified display ranges (IL-6 > 2000 pg/mL; CRP > 300 mg/L; and ferritin > 3000 µg/L) are indicated by star symbols at the upper axis boundary.

3.3. Coagulation Initiation and Anticoagulant Activity

Baseline viscoelastic measurements showed mildly prolonged EX-test clotting time (CT; 73 [54–88] s) and IN-test CT near the upper reference range (160 [148–182] s), indicating a modest delay in clot initiation. During follow-up, EX-test CT progressively shortened toward normal values, whereas IN-test CT remained largely unchanged, reflecting preserved extrinsic and intrinsic pathway activation despite ongoing anticoagulation.

Markers of thrombin generation were elevated at admission, with increased thrombin–antithrombin (TAT) complexes (476.8 [272.1–773.3] pg/mL) that persisted with a slight increase during follow-up. Antithrombin was mildly reduced (~80%) initially and increased over time, consistent with recovery of endogenous anticoagulant activity as inflammation resolved (Figure 2).

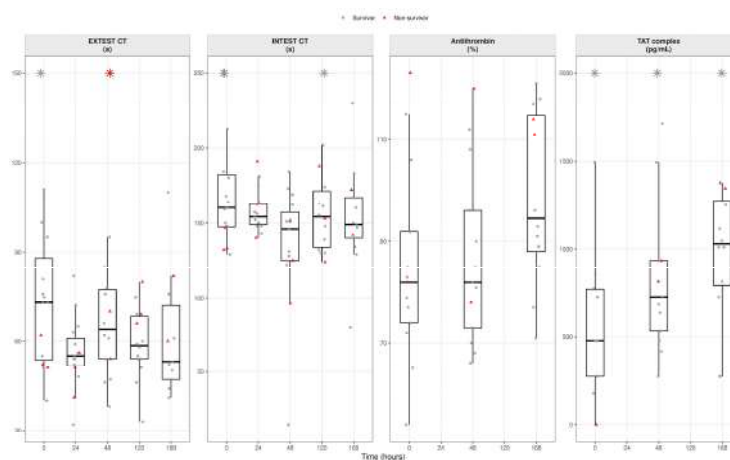


Figure 2. Coagulation initiation and thrombin generation. Boxplots show viscoelastic markers of coagulation initiation (EX-test and IN-test clotting time) and biochemical markers of thrombin regulation (antithrombin activity and thrombin–antithrombin complex). Boxes represent the median and interquartile range; whiskers indicate $1.5 \times \text{IQR}$. Individual patient values are overlaid (grey circles = survivors; red triangles = non-survivors). Data are shown across timepoints (T0–T4). Values exceeding prespecified display ranges (EX-test CT > 150 s; IN-test CT > 250 s; and TAT complex > 2000 pg/mL) are indicated by star symbols at the upper axis boundary.

3.4. Clot Propagation and Strength

At baseline, platelet counts were within the lower-normal range (195 [180–218] G/L), while fibrinogen concentrations were markedly elevated (5.5 [5.1–6.4] g/L). Platelet counts increased modestly during follow-up (270 [220–312] G/L at 48 h) and remained stable thereafter. Fibrinogen declined steadily to 2.60 [2.12–2.96] g/L by day 7, paralleling the CRP decrease and signaling resolution of acute-phase synthesis.

Baseline viscoelastic measurements were consistent with this profile, showing elevated clot firmness on both EX-test and FIB-test maximum clot firmness (MCF) (EX-test: 68 [63.5–69.5] mm; FIB-test: 32.5 [29.3–39.0] mm), indicating formation of strong, fibrin-rich clots. During follow-up, EX-test MCF remained largely preserved, whereas FIB-test MCF declined to 22.0 [14.0–24.0] mm by day 7, representing an approximate 30% reduction, paralleling the decrease in fibrinogen concentration. These findings indicate a gradual re-equilibration of clot composition driven predominantly by changes in the fibrin component, while platelet-dependent clot firmness remained stable (Figure 3).

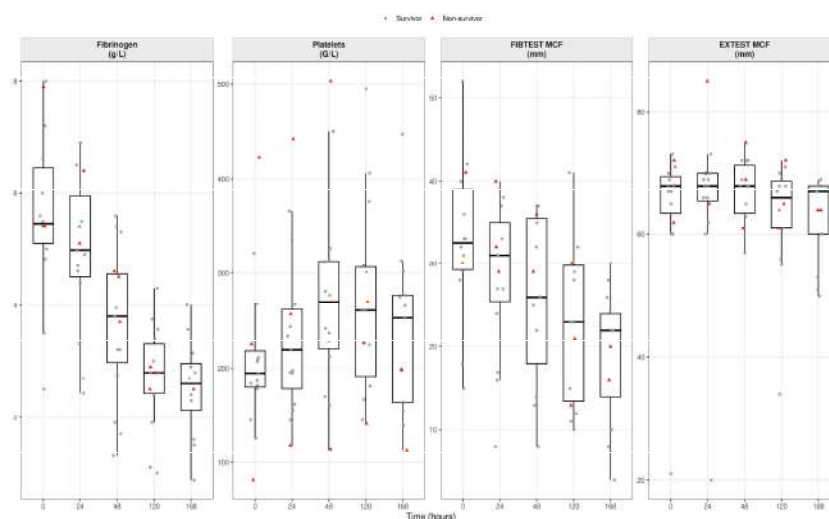


Figure 3. Clot propagation and strength. Boxplots show substrates of clot formation (fibrinogen concentration and platelet count) and viscoelastic measures of clot strength (FIB-TEST and EX-test maximum clot firmness). Boxes represent the median and interquartile range; whiskers indicate $1.5 \times$ IQR. Individual patient values are overlaid (grey circles = survivors; red triangles = non-survivors). Data are shown across timepoints (T0–T4).

3.5. Fibrinolytic Activity

The fibrinolytic profile showed dynamic but non-linear evolution over the observation period. Plasminogen and τ_2 -antiplasmin decreased modestly from baseline to day 7 (plasminogen: 90.0 [77.0–96.0] to 87.0 [71.5–92.0]%; τ_2 -antiplasmin: 110.0 [107.0–116.0] to 100.0 [90.8–106.0]%). TAFI declined early at 48 h and partially recovered by day 7. In contrast, plasminogen activator inhibitor-1 (PAI-1) increased steadily throughout follow-up (3.42 [2.70–4.90] to 6.29 [5.40–6.83] ng/mL). Plasmin–antiplasmin (PAP) complexes increased modestly toward day 7, while D-dimer concentrations rose markedly, peaking around day 5 before slightly declining by day 7.

These biochemical patterns were reflected in viscoelastic findings. On the EX-test, maximum lysis (ML) decreased from approximately 5% at baseline to around 2% during follow-up, while the clot lysis index at 45 min (CLI-45) remained relatively stable, indicating persistent resistance to spontaneous clot breakdown. ECA-test lysis onset time (LOT) showed a transient prolongation at 24 h before returning toward baseline values by day 7. Under tissue plasminogen activator challenge, lysis time (LT) exhibited a biphasic

pattern, with early prolongation followed by shortening from approximately day 5 onward, whereas TPA-test LOT remained stable. Together, these findings indicate preserved overall fibrinolytic capacity with delayed activation (Figure 4).

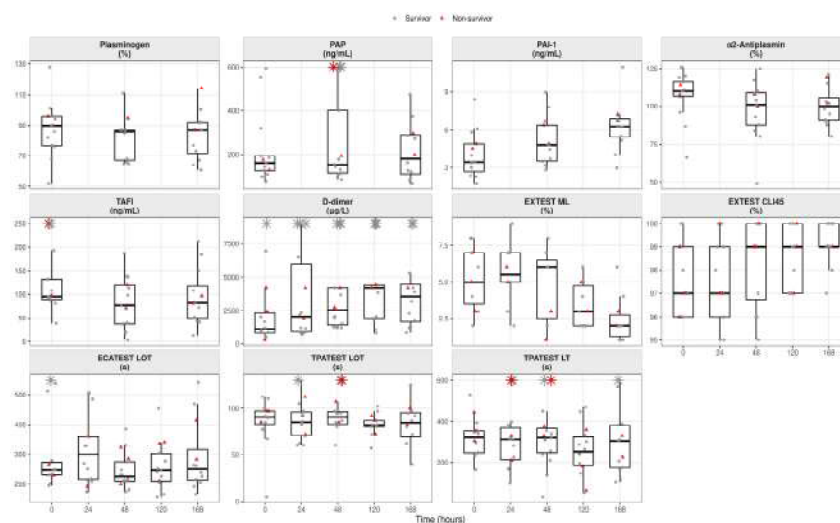


Figure 4. Fibrinolysis-related biomarkers and viscoelastic parameters. Boxplots depict fibrinolytic parameters derived from viscoelastic hemostatic assays, including EX-test maximum lysis (ML), EX-test clot lysis index at 45 min (CL45), ecarin clotting assay lysis onset time (ECA LOT), and tissue plasminogen activator (TPA)-challenge lysis onset (TPA LOT) and lysis time (TPA LT). Data are shown across timepoints (T0–T4). Values exceeding prespecified display ranges (PAP > 300 ng/mL, TAFI > 250 ng/mL, D-dimer > 7500 μ g/L, ECA-test > 500 s, TPA-test LOT > 150 s, and TPA-test LT > 600 s) are indicated by star symbols at the upper axis boundary.

Inter-individual variability and within-patient trends were illustrated using longitudinal ribbon plots with median overlays, with additional stratification by fibrinolytic resistance status at admission (Supplementary Figures S1–S11).

4. Discussion

This case series provides an in-depth viscoelastic and biochemical characterization of coagulation and fibrinolysis dynamics in critically ill COVID-19 patients treated with tocilizumab. Despite rapid suppression of systemic inflammation, our findings reveal a dissociation between cytokine blockade and persistent endothelial dysfunction and hemostatic imbalance, suggesting that restoration of vascular homeostasis may extend beyond IL-6 inhibition alone. These exploratory results should be interpreted with caution due to the limited sample size and hypothesis-generating design.

At baseline, patients showed inflammatory activation with endothelial-associated hemostatic imbalance; following immunomodulation, systemic inflammation subsided, whereas markers of endothelial injury continued to rise.

Physiologically, the endothelium maintains vascular homeostasis through anticoagulant, anti-inflammatory, and antioxidant pathways; however, in COVID-19 it rapidly transitions to a dysfunctional, proinflammatory, and prothrombotic phenotype. Excessive IL-6 signaling disrupts endothelial integrity, increases permeability, and promotes oxidative stress and vasoconstriction, leading to structural injury and impaired repair. Accelerated endothelial detachment and impaired regeneration may therefore contribute to sustained vascular injury and delayed recovery [12]. Recent studies have shown that endothelial dysfunction can persist well beyond the acute phase, with ongoing endothelial

activation, immune-mediated injury, and sustained vascular stiffness months after recovery, underscoring the long-term vascular sequelae of COVID-19 [13,14].

Following IL-6 receptor blockade, coagulation parameters indicated partial re-equilibration of the hemostatic system. Thrombin generation markers (TAT complexes) rose modestly, while antithrombin activity increased, consistent with ongoing but controlled thrombin formation without consumption of endogenous anticoagulants. This pattern is consistent with attenuation of IL-6-driven inflammation, with endothelial injury remaining a predominant driver of thrombin generation. Consequently, antithrombin is preserved unlike in bacterial sepsis, where intense and sustained thrombin production leads to marked depletion. This profile appears characteristic of COVID-19-associated coagulopathy, with IL-6 blockade amplifying the distinction rather than causing it [15].

The kinetics of platelets and fibrinogen, together with corresponding viscoelastic patterns, indicated a reduction in the fibrin contribution to clot firmness that was offset by preserved platelet-dependent clot strength. This profile is compatible with persistent endothelial activation and platelet involvement, which may also contribute to elevated PAI-1 levels, thereby reinforcing antifibrinolytic pressure and maintaining thrombotic risk [16].

With respect to fibrinolytic balance, the observed patterns were consistent with persistent antifibrinolytic activity rather than complete fibrinolytic failure. TAFI and plasminogen declined modestly, while D-dimer concentrations increased during the first five days before slightly decreasing by day seven, indicating ongoing fibrin turnover. A modest rise in plasmin–antiplasmin (PAP) complexes further suggests limited but continuous plasmin generation.

From a viscoelastic perspective, it is important to recognize that ML and related whole-blood indices should not be interpreted as isolated markers of plasmin-mediated fibrinolysis. Tissue-factor-triggered assays contain cellular components, and post-MCF decreases in clot firmness represent a composite of enzymatic fibrinolysis and cell-mediated clot contraction. Platelet-driven contraction markedly alters clot architecture, permeability, and mechanical stability, thereby modifying the apparent “lysis” behavior recorded by viscoelastic devices. Mechanistic studies have demonstrated that clot contraction can mimic reduced fibrinolysis or, conversely, mask ongoing plasmin activity [17,18]. Consequently, low ML values or “shutdown-like” patterns in cellular assays should be interpreted with caution, as they may arise in part from altered clot-contraction dynamics rather than true suppression of fibrinolysis.

Consistent with these considerations, viscoelastic parameters showed overall stability of clot lysis indices and a non-linear evolution of TPA-test lysis time, characterized by delayed but preserved fibrinolytic capacity. IL-6 blockade was associated with attenuation of inflammation and hypercoagulability without reactivation of pathological fibrinolysis, indicating modulation rather than normalization of the hemostatic imbalance.

Interestingly, patients with fibrinolytic resistance at admission exhibited a distinct hemostatic profile during follow-up, with slightly lower vWF levels, higher fibrinogen and platelet counts, and viscoelastic features consistent with enhanced clot propagation and mechanical stability. In contrast, patients without fibrinolytic resistance showed higher D-dimer levels, suggesting more active fibrin turnover. This dichotomy aligns with the “fibrinolytic shutdown” phenotype described in severe systemic inflammation, where dense fibrin architecture and antifibrinolytic pressure coexist with low circulating fibrin degradation products [19].

Our observations are consistent with microscopic studies demonstrating that plasma clots from COVID-19 patients exhibit increased fibrin fiber density, reduced pore size, and occasionally thicker fibers, rendering clots less susceptible to enzymatic lysis. Dense

fibrin networks impede diffusion of plasminogen and fibrinolytic enzymes, while elevated fibrinogen accelerates polymerization and fiber packing. In parallel, endothelial injury, neutrophil extracellular traps, and high thrombin flux promote extensive cross-linking and tighter fibrin alignment. Together, these processes generate compact, lysis-resistant clots that persist within a pro-inflammatory and pro-coagulant milieu, amplifying thrombotic risk in COVID-19 [20–23].

Several clinical studies corroborate our findings. Di Nisio et al. reported rapid improvements in coagulation parameters following a single subcutaneous dose of tocilizumab in hospitalized COVID-19 patients, with reductions in D-dimer and fibrinogen, rising platelet counts, and shortened PT/aPTT concurrent with improved respiratory function [24]. Conversely, Chan et al. observed transient D-dimer surges after tocilizumab, particularly in patients not receiving full-dose anticoagulation, suggesting transient unmasking of latent fibrinolytic activity and release of clot-bound degradation products [25]. More recently, Aljuhani et al. demonstrated in a large propensity-matched ICU cohort that early tocilizumab use was associated with lower fibrinogen trajectories and improved survival without excess thrombosis, suggesting an overall hemostatic benefit [26]. Similarly, Nagy et al. found divergent fibrinolytic responses, with tocilizumab-treated patients showing shorter tPA-ROTEST lysis times compared with controls and increasing PAI-1 levels over time, whereas control and dexamethasone groups showed the opposite pattern [27]. The randomized Coag-ImmCovA trial further showed that, by day 29, tocilizumab reduced hypercoagulability (prolonged CFT and reduced MCF) and decreased fibrinogen compared with anakinra or standard care. Structural analysis revealed denser fibrin networks early after treatment, followed by remodeling with larger pores and thicker fibers, indicating gradual reorganization of the clot matrix [28].

Strengths and Limitations

The novelty of this study lies in the detailed temporal characterization of inflammatory, coagulation, endothelial, and fibrinolytic dynamics following IL-6 receptor blockade.

Nevertheless, the findings presented here must be interpreted cautiously, given several inherent limitations. Primarily, this investigation was an observational pilot study involving a small number of critically ill COVID-19 patients at a late pandemic stage, when widespread vaccination and prior viral exposures may have modified patient phenotypes and immune responses. Thus, extrapolation of our results to earlier pandemic waves or unvaccinated populations warrants caution. Patient selection was pragmatic and may introduce selection bias. Additionally, the absence of a control group and reliance on before-and-after comparisons further limit the robustness and causal interpretability of our results. Consequently, our findings should be viewed as hypothesis-generating rather than definitive evidence. Finally, concomitant therapies, including corticosteroids and anticoagulation, represent unavoidable confounders inherent to standard-of-care management in severe COVID-19.

5. Conclusions

Taken together, our findings describe parallel temporal patterns in inflammatory, endothelial, and coagulation parameters following IL-6 antagonism. While inflammatory markers declined, indices of endothelial activation and hemostatic imbalance persisted in several patients. These exploratory observations highlight the complexity of COVID-19-associated coagulopathy and support further investigation into mechanisms beyond cytokine-mediated inflammation.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/biomedicines14010254/s1>; Figure S1: Temporal evolution of endothelial and inflammatory biomarkers (boxplots); Figure S2: Temporal evolution of coagulation initiation and thrombin generation parameters (boxplots); Figure S3: Temporal evolution of clot propagation and mechanical clot strength parameters (boxplots); Figure S4: Temporal evolution of fibrinolytic and thrombin-regulatory biomarkers (boxplots); Figure S5: Temporal evolution of viscoelastic fibrinolysis parameters (boxplots); Figure S6: Longitudinal ribbon plots (log₁₀ scale) of endothelial and inflammatory biomarkers; Figure S7: Longitudinal ribbon plots (log₁₀ scale) of coagulation initiation and thrombin generation parameters; Figure S8: Longitudinal ribbon plots (log₁₀ scale) of clot propagation and mechanical strength parameters; Figure S9: Longitudinal ribbon plots (log₁₀ scale) of fibrinolytic and thrombin-regulatory biomarkers (excluding tPA); Figure S10: Longitudinal ribbon plots (log₁₀ scale) of viscoelastic fibrinolysis indices; Figure S11: Complete viscoelastic hemostatic assay panel, including FIBTEST, stratified by survival status; Table S1: Detailed general characteristics of the study population; Table S2: Paired Wilcoxon signed-rank analyses with Hodges–Lehmann estimators and Benjamini–Hochberg false discovery rate correction for primary and secondary viscoelastic fibrinolysis outcomes; Table S3: Trajectories of inflammation and hemostasis parameters across time points (T0–T4); Table S4: ClotPro viscoelastic assays and parameters: physiological basis and interpretive framework; Table S5: Normal values on ClotPro as indexed by manufacturer; Table S6: STROBE Statement—checklist of items that should be included in reports of observational studies.

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Abbreviations

The following abbreviations are used in this manuscript:

aPTT	Activated Partial Thromboplastin Time
CFT	Clot Formation Time
CLI-30/45	Clot Lysis Index at 30 or 45 min
COVID-19	Coronavirus Disease 2019
CRP	C-reactive protein
CT	Clotting Time
D-dimer	Fibrin degradation product
ECA-test	Ecarin Clotting Assay
eCRF	Electronic Case Report Form
ELISA	Enzyme-Linked Immunosorbent Assay
EX-test	Extrinsic Pathway Test
FDR	False Discovery Rate
FIBTEST	Fibrinogen Test
FiO ₂	Fraction of Inspired Oxygen
FR	Fibrinolytic Resistance
G/L	Giga per Liter
HFNO	High-Flow Nasal Oxygen
ICU	Intensive Care Unit
IL-6	Interleukin-6
INR	International Normalized Ratio
IN-test	Intrinsic Pathway Test
LOT	Lysis Onset Time
LT	Lysis Time
MCF	Maximum Clot Firmness
ML	Maximum Lysis
NIV	Non-invasive ventilation
PaCO ₂	Partial Pressure of Carbon Dioxide
PaO ₂	Partial Pressure of Oxygen in Arterial Blood
PAI-1	Plasminogen Activator Inhibitor-1
PAP	Plasmin–Antiplasmin Complex
PCT	Procalcitonin
PCR	Polymerase Chain Reaction
PLG	Plasminogen
RVVTEST	Russell Viper Venom Test
sIL-6R	Soluble Interleukin-6 Receptor
SIRS	Systemic Inflammatory Response Syndrome
TAT	Thrombin–Antithrombin Complex
TAFI	Thrombin-Activatable Fibrinolysis Inhibitor
Tpa	Tissue Plasminogen Activator
TPA-test	tPA-Enhanced Lysis Test
TT	Thrombin Time
VHA	Viscoelastic Hemostatic Assay
vWF	von Willebrand Factor
vWF:Act	von Willebrand Factor Activity
vWF:Ag	von Willebrand Factor Antigen

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