Therapeutics and Clinical Risk Management

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Time in Therapeutic Range of Unfractionated Heparin-Based Therapy in Critically Ill Patients with COVID-19 Pneumonia

Tereza Romanová[1,2], Filip Burša[1,2], Peter Sklienka[1,2], Jiří Sagan[3], Michelle Vaňková[1], Denis Buršík[1,2], Markéta Bílená[1,2], Martin Pulcer[4], Michal Burda[5], Jan Máca[1,2,6]

[1]Department of Anesthesiology and Intensive Care Medicine, University Hospital, Ostrava, Czech Republic; [2]Department of Intensive Medicine, Emergency Medicine and Forensic Studies, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic; [3]Department of Infectious Diseases, University Hospital Ostrava, Ostrava, Czech Republic; [4]Institute of Laboratory Medicine, University Hospital Ostrava, Ostrava, Czech Republic; [5]Institute for Research and Applications of Fuzzy Modeling, CE IT4Innovations, University of Ostrava, Ostrava, Czech Republic; [6]Institute of Physiology and Pathophysiology, Faculty of Medicine, University of Ostrava, Ostrava, Czech Republic

Correspondence: Jan Máca, Department of Anesthesiology and Intensive Care Medicine, University Hospital Ostrava, 17. listopadu 1790/5, Ostrava, 708 52, Czech Republic, Tel +420597371111, Email jan.maca@fno.cz

Purpose: Anticoagulation therapy aims to improve the outcome of critically ill patients with severe COVID-19-associated pneumonia. Activated partial thromboplastin time (aPTT) is commonly used to maintain the target therapeutic range of continuous infusion of unfractionated heparin (UFH). The UFH infusion efficacy can be evaluated by determining the time in therapeutic range (TTR) using a modified Rosendaal method. The present study's primary aim was to evaluate TTR based on the aPTT in critically ill patients with severe forms of COVID-19 pneumonia and its influence on survival. The secondary aim was to evaluate the time spent above (TATR) and below the therapeutic range (TBTR).

Patients and Methods: We performed a retrospective analysis of critically ill patients with COVID-19-associated pneumonia. All patients received a continuous infusion of UFH from the 2nd to 8th day since admission to the ICU. TTR, TATR, and TBTR were calculated using the modified Rosendaal method, and survival days were analyzed by regression (censored after 60 days).

Results: Of 103 patients, the median TTR was 49% (IQR 38–63%), TATR 11% (IQR 5–20%), and TBTR 33% (IQR 22–51%). The regression analysis indicated a positive impact of higher TTR and TATR on the number of survival days [β=0.598 (p=0.0367) and β=1.032 (p=0.0208), respectively] and a negative impact of higher TBTR [β=−0.681 (p=0.0033)] on the number of survival days.

Conclusion: Higher TTR and TATR were associated with better survival of critically ill patients with a severe course of COVID-19-associated pneumonia. Higher TBTR was associated with worse survival in these patients.

Keywords: unfractionated heparin, COVID-19, pneumonia, critical care

Introduction

The coronavirus disease 2019 (COVID-19) pandemic highlighted the importance of adequate thromboprophylaxis, especially for severe disease in which the development of microvascular and macrovascular thrombi leads to organ dysfunction and failure. The hypercoagulability state results from complex interactions between the virus, immune system, coagulation, fibrinolytic system, and vascular endothelium.[1,2] This phenomenon, called immunothrombosis, is associated with COVID-19 severity and mortality.[3] Consequently, anticoagulation therapy plays a central role in the treatment of critically ill COVID-19 patients, with either low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH).[4] Heparin may be beneficial for its anticoagulation effect and broad spectrum of anti-inflammatory, immunomodulating, and virostatic properties.[5,6] Reaching and maintaining the desired anticoagulation activity can be challenging for physicians due to altered drug pharmacokinetics in critically ill patients,[7] such as complex interactions with coexisting organ dysfunction, a decrease in subcutaneous absorption, and an increase in the volume of distribution of hydrophilic drugs.[8] Therefore, anticoagulation activity monitoring plays an important role in anticoagulation management. UFH has been frequently used for its rapid onset of the effect, short half-life,

Received: 29 April 2024
Accepted: 16 August 2024
Published: 10 September 2024

low cost, and antidote availability. In vitro, UFH has resulted in increased SARS-CoV-2 antiviral activity compared to LMWH.[9] Intermittent measurement of activated partial thromboplastin time (aPTT) is the most used method to monitor the efficacy of continuous UFH infusions. Despite the widespread use of aPTT measurements, no standardized method for assessing the effectiveness of UFH dosing or compliance with titration is currently available. Some authors have evaluated UFH administration by applying the concept of time in therapeutic range (TTR), but its utilization is not common in UFH-based anticoagulation therapy.[10] TTR has been successfully used in warfarin management and for comparing therapy adherence in clinical trials.[11] In these settings, TTR represents the time (as a percentage of total time) in which the international normalized ratio (INR) of prothrombin time (PT) remains in the target range. Longer TTR translates to better clinical outcomes, such as lower incidence of thromboembolic complications and improved therapy safety and survival.[12,13] TTR can be determined using various approaches – simply as a fraction of INR values in the target range, by the cross-section-of-the-files methodology,[14] or by the Rosendaal linear interpolation method.[15] In clinical trials, TTR is typically calculated using the Rosendaal method.[10,11] To the best of our knowledge, there is little evidence for estimating TTR of aPTT monitoring of UFH treatment in critically ill patients and no evidence of its impact on survival, especially in COVID-19 patients. In addition, an isolated TTR value does not consider whether the time out of the therapeutic range of UFH is spent above or below the target range with respective clinical consequences. This additional information and its impact on outcomes have not been reported before. Therefore, we suggest determining time above the therapeutic range (TABR) and time below the therapeutic range (TBTR) using the Rosendaal method as well.

Our study's primary aim was to evaluate continuous UFH infusion efficacy by determining the TTR based on aPTT monitoring in critically ill patients with severe forms of COVID-19 pneumonia and its relationship to survival (censored after 60 days). The secondary aim was to evaluate the role of TATR and TBTR in assessment of UFH efficacy and their impact on survival (censored after 60 days).

Material and Methods

This retrospective study analyzed data from patients with severe COVID-19-associated pneumonia with respiratory failure who were admitted from 1 March 2020 to 31 March 2022 to the Department of Anesthesiology, Resuscitation, and Intensive Care in the tertiary medical facility University Hospital Ostrava. Adult patients positive for SARS-CoV-2 via nasopharyngeal swab polymerase chain reaction (PCR), with acute hypoxemic respiratory failure, and who were administered continuous intravenous infusion of UFH for at least the first 8 days since admission were included in the study. This duration was determined empirically to eliminate short-term UFH administration and to obtain a sufficient number of aPTT values to reliably calculate TTR. According to the clinical protocol, the therapeutic target range of aPTT was 45–60 seconds. The rate of infusion was adjusted based on aPTT levels. Data were extracted from patients' written and electronic records. Mortality data were gathered from the insurance company records. Exclusion criteria were pregnancy and extracorporeal membrane oxygenation (ECMO) support. To obtain aPTT measurements, patients' venous blood was collected into sodium citrate tubes (S-Monovette® Citrate 9NC, 0.106 mol/L, 3.2%, SARDSTEDT AG & Co. KG). The samples were then centrifuged in the central laboratory at 2500 g at 20 °C for 10 minutes and were analyzed within 30 minutes of collection. aPTT was measured using a Sysmex CS-5100 system (Sysmex, Japan) with Pathromtin SL (Siemens AG, Erlangen, Germany). The TTR of UFH therapy was analyzed based on the aPTT measured at 6-hour intervals. TTR was evaluated using a modified Rosendaal method (mod-Rosendaal), which revised the original method to calculate time per hour rather than per day.[10,15] It is a linear interpolation method that includes a straight line between two consecutive measures to interpolate hourly values of aPTT. The time regions of aPTT in the range are based on the interpolated values entering the therapeutic range as shown in Figure 1. TTR is the proportion of time the interpolated aPTT was in the therapeutic range. The TATR and TBTR were estimated using the same method. A censored regression model was used to evaluate the impact of TTR duration on patients' days of survival censored after 60 days. A written informed consent was signed by all patients or legally authorized representatives.

The level of significance was set at 0.05. The missing values and outliers were double-checked and imputed or labeled and removed from further statistical processing. Categorical variables were characterized with the absolute count and relative frequency (in %). Fisher's exact test was employed to assess the statistical difference between groups. Numeric variables were described as medians and 25–75% quantiles and statistically tested by two-sample Wilcoxon

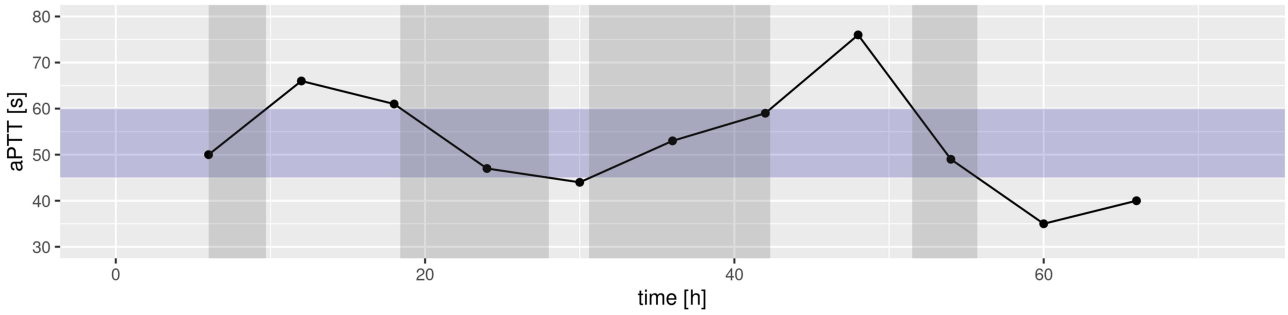

Figure 1 A graphical example of aPTT measurements (black dots) and the application of the modified Rosendaal method to calculate time in therapeutic range (grey). The target therapeutic range (blue).

rank-sum test. All statistical analyses were performed using R statistical software version 4.2.1.[12] Censored regression models were computed using the R package censReg version 0.5–36.

Results

We analyzed data from 419 critically ill patients with COVID-19-associated pneumonia. Based on the inclusion and exclusion criteria, a total of 103 patients were included in the final statistical analysis (Figure 2). The baseline characteristics and comorbidities of the included patients are shown in Table 1. All data were obtained at the time of ICU admission. As generally expected in severe COVID-19, there was a high predominance of males over females. A high proportion of patients were overweight or obese and had a history of arterial hypertension or diabetes mellitus. The median Acute Physiology and Chronic Health Evaluation II (APACHE II) score was 13, which results in a predicted mortality rate of ~15% based on the original validation published by Knaus et al.[16] Outcome characteristics during the ICU stay are presented in Table 2. Acute kidney failure and secondary mycotic infections were the most common complication. Documented bleeding complications were more common than documented venous thromboembolism. Results of a quantitative assessment of anticoagulation therapy are shown in Table 3. The resulting median aPTT was inside the pre-set target range of 45–60 seconds, but the final TTR was only 49%. The aPTT fell below the target range more frequently than it exceeded it. Basic descriptive information on the overall survival and 60-day mortality of patients is provided in Table 4. Results of the regression analysis are presented in Table 5, which shows coefficients and p-values for four censored regression models that differ in the score used as an

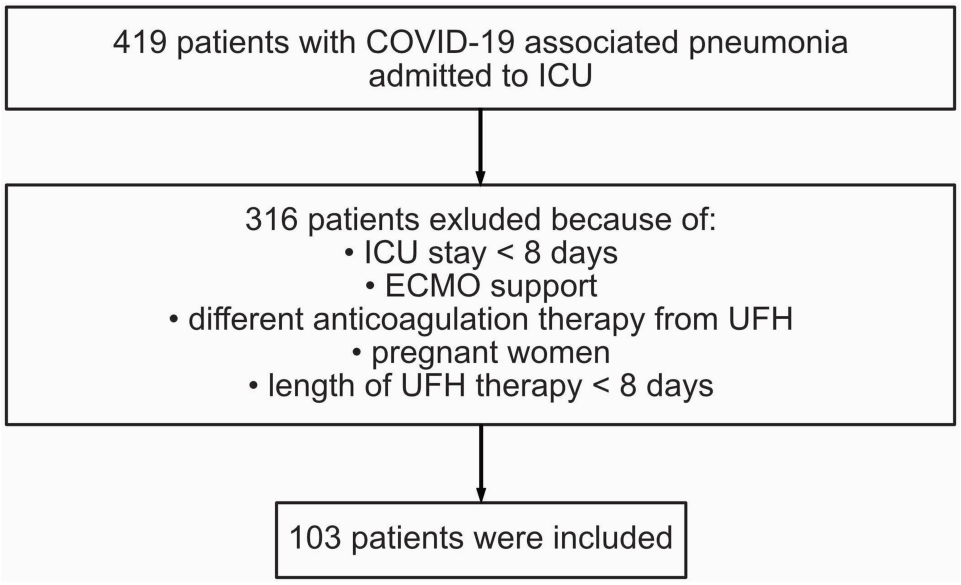

Figure 2 Flowchart of the study.

Table 1 Baseline Characteristics of Included Patients

Parameter	Total (N = 103)
Baseline variables	
Age (years)	59.00 (49.50–66.00)
Sex (male) Sex (female)	73 (70.87) 30 (29.13)
Height (cm)	175.00 (169.00–180.00)
Weight (kg)	97.00 (85.00–118.00)
Body mass index (kg/m^2)	32.41 (27.76–37.04)
APACHE II	13.00 (10.75–17.25)
Time from symptoms to admission (days)	6.50 (3.00–9.00)
Comorbidities	
Diabetes mellitus	25 (24.27)
Hypertension	57 (55.34)
Heart failure	3 (2.91)
Ischemic heart disease	5 (4.85)
Atrial fibrillation or atrial flutter	5 (4.85)
History of stroke	3 (2.91)
History of pulmonary embolism	2 (1.94)
History of deep venous thrombosis	4 (3.88)
Peripheral vascular disease	3 (2.91)
Congenital coagulopathy	3 (2.91)
Bronchial asthma	8 (7.77)
Chronic obstructive pulmonary disease	2 (1.94)
Chronic restrictive pulmonary disease	1 (0.97)
Chronic kidney disease	4 (3.88)
Chronic liver disease	5 (4.85)
Cancer	6 (5.83)
Immunodeficiency	3 (2.91)
Autoimmune disease	6 (5.83)

Note: Values are given as median (range) or n (%).
Abbreviation: APACHE II, Acute Physiology and Chronic Health Evaluation II.

independent variable: APACHE II, TTR, TATR, and TBTR. All models explain the dependent variable survival duration (in days, censored after 60 days). Furthermore, the independent variables age and sex were incorporated in the models as confounders. The effect of increased TTR as a primary outcome of the study significantly affected patients' survival censored after 60 days (p=0.0367). For secondary outcomes, an increased TATR was significantly (p=0.0208) associated with improved censored survival, whereas an increased TBTR significantly (p=0.0033) shortened the censored survival. All

Table 2 Outcome Characteristics During ICU Stay

Parameter	Total (N = 103)
ICU stay duration (days)	17 (12.00–24.00)
Mechanical ventilation (hours)	424.00 (271.75–717.50)
Pronation position	68 (66.02)
Documented bleeding	21 (20.39)
Documented venous thromboembolism	10 (9.71)
Acute kidney failure	46 (44.66)
Acute liver failure	2 (1.94)
Acute heart failure	2 (1.94)
Continual renal replacement therapy	23 (22.33)
Secondary bacterial infection	80 (77.67)
Secondary mycotic infection	40 (38.83)

Note: Values are given as median (IQR) or n (%).
Abbreviation: ICU, Intensive Care Unit.

Table 3 Parameters Assessing Anticoagulation Therapy Efficacy and Routine Blood Coagulation Parameters at Admission to ICU

Parameter	Total (N = 103)
aPTT (seconds)	48.05 (44.33–50.97)
TTR (%)	49.00 (38.00–63.00)
TATR (%)	11.00 (5.00–20.00)
TBTR (%)	33.00 (22.00–51.00)
Antithrombin (%)	88.00 (78.00–98.38)
D-dimers (mg/l FEU)	2.30 (1.24–4.22)
Fibrinogen (g/l)	4.83 (3.63–5.55)

Note: Values are given as median (IQR) or n (%).
Abbreviations: ICU, Intensive Care Unit; aPTT, activated partial thromboplastin time; TTR, time in therapeutic range; TATR, time above therapeutic range; TBTR, time below therapeutic range; FEU, fibrinogen equivalent units.

Table 4 Survival Characteristics of Included Patients

Survival Characteristics		
Survival days	25 (16.00–34.50)	
60-day mortality	64 (62.14%) survivors	39 (37.86%) died

Note: Values are presented as the median (IQR) or n (%).

Table 5 Regression Models Censored After 60 Days Explaining Survived Days with Age, Sex, and One of the Analyzed Scores

Variable	APACHE II	TTR	TATR	TBTR
(Intercept)	147.22 (<0.0001)	104.55 (0.0006)	132.04 (<0.0001)	165.53 (<0.0001)
Age	−0.97 (0.0388)	−1.32 (0.0030)	−1.54 (0.0009)	−1.44 (0.0011)
Male sex	21.82 (0.0349)	20.66 (0.0351)	22.54 (0.0228)	21.77 (0.0238)
Score	−2.21 (0.0156)	0.60 (0.0367)	1.03 (0.0208)	−0.68 (0.0033)

Note: Data are the regression coefficients (β) and p-values of the coefficient significance test in parentheses.
Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; TTR, time in therapeutic range; TATR, time above therapeutic range; TBTR, time below therapeutic range.

analyzed models were significantly affected by patients age and sex – older patients had shorter survival duration and male sex was associated with improved survival.

Discussion

Our study retrospectively evaluated the efficacy of continuous UFH infusion by determining TTR based on aPTT in critically ill patients with COVID-19-associated pneumonia from day 2 to 8 of their ICU stay. In all 103 patients, the median TTR of 49% was reached, while the median TATR was 11% and the median TBTR 33%. Based on applied regression models, APACHE II, TTR, TATR and TBTR significantly impacted patients' survival. As expected, higher APACHE II score indicates shorter survival. Longer TTR and TATR scores are associated with longer survival period (censored after 60 days). On the other hand, longer TBTR resulted in shorter survival.

The literature assessing TTR in critically ill patients with continuous UFH infusion is sparse. Ting et al[10] estimated the TTR of continuous UFH infusion for the first time. In their cohort, the authors identified an average TTR of 43.7% using the mod-Rosendaal method in non-COVID patients, with similar results after stratification based on ICU and non-ICU admission (41.2% and 43.9%, respectively). In the study, the indication for UFH administration was highly heterogeneous, mostly due to venous thromboembolism, acute coronary syndrome, or stroke prevention in atrial fibrillation. However, the median duration of UFH infusion was shorter (66 hours), and the correlation of TTR with clinical outcomes was not evaluated. As no recommendations are available for optimal TTR, especially in critically ill patients with a possibly variable response to anticoagulants, the relationship between TTR and clinical outcomes is yet to be determined by further studies. However, studies of patients receiving warfarin anticoagulation therapy have shown the best outcomes, with TTR exceeding 70% in an outpatient setting.[13] To further determine the effectiveness of UFH administration, TATR and TBTR can be calculated. To the best of our knowledge, this type of evaluation has never been used before. Longer TBTR, representing a pro-coagulation tendency, could negatively affect the phenomenon of immunothrombosis in COVID-19 patients, with a negative impact on their mortality. Our results suggest that it may be necessary to increase the target range of aPTT to accommodate an increased risk of thrombosis during severe COVID-19, but further studies are necessary to elaborate a recommended range for aPTT.

Due to immunological dysregulation, the question remains as to whether aPTT is a suitable method to monitor anticoagulation therapy using UFH in critical patients. The sensitivity of aPTT can be low ("false" or "apparent" heparin resistance) in patients with high concentrations of acute-phase proteins (ie fibrinogen or factor VIII), which renders aPTT monitoring suboptimal in inflammatory illnesses, including severe COVID-19.[17,18] Furthermore, some authors claim that UFH resistance may be present and require a higher dose of UFH.[19] Linear interpolation between aPTT values can be inaccurate in situations with higher doses of UFH in which its anticoagulant effect is nonlinear.[20] Anti-Xa activity testing is generally considered more reliable, but there are issues with standardization and low implementation in routine clinical practice, especially in low to middle-income countries.[17,21]

Our study has several limitations. It was conducted retrospectively in a single center with an institution specific UFH protocol. Changes in the patient´s anticoagulation therapy may have occurred after the study period (8 days of ICU stay),

impacting their mortality. Elevated levels of inflammatory markers could bias measurements of aPTT. The TTR, TATR, and TBTR need to be further examined by larger, preferably prospective, studies with the aim of creating recommendations for evaluating these variables. This also includes their impact on bleeding and thromboembolic complications. Furthermore, there is a potential for TTR, TATR, and TBTR to be used in other groups of critically ill non-COVID-19 patients.

Conclusion

Achieving longer TTR as calculated by the mod-Rosendaal method was associated with better survival of critically ill patients with severe COVID-19-associated pneumonia. This may imply a beneficial effect of a longer duration of adequate anticoagulation. Higher TATR was associated with better survival, whereas higher TBTR was associated with worse survival in these patients.

Abbreviations

APACHE, Acute Physiology and Chronic Health Evaluation; aPTT, activated Partial Thromboplastin Time; BMI, Body Mass Index; CAC, COVID-19 Associated Coagulopathy; ECMO, Extracorporeal Membrane Oxygenation; FNO, University Hospital Ostrava; TTR, Time in therapeutic range; TATR, Time above therapeutic range; TBTR, Time below therapeutic range; ICU, Intensive Care Unit; IQR, Interquartile Range; LMWH, Low Molecular Weight Heparin; PCR, Polymerase Chain Reaction; UFH, Unfractionated Heparin.

Data Sharing Statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

This study was performed in accordance with the Declaration of Helsinki and was approved by an ethics committee under protocol no. 127/2022, University Hospital of Ostrava. All patients signed an informed consent.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This paper was funded by the SGS 06/LF/2022, University of Ostrava, project patronaged by Ministry of Education, Youth and Sports, Czech Republic. Supported by Ministry of Health, Czech Republic - conceptual development of research organization (FNOs/2024).

Disclosure

The authors declare that they have no competing interests in this work.

References

1. Conway EM, Mackman N, Warren RQ, et al. Understanding COVID-19-associated coagulopathy. *Nat Rev Immunol*. 2022;22(10):639–649. doi:10.1038/s41577-022-00762-9
2. Lorini FL, Di Matteo M, Gritti P, et al. Coagulopathy and COVID-19. *Eur Hear J Suppl*. 2021:23(Supplement_E):E95–E98. doi:10.1093/eurheartj/suab100.
3. Xiang G, Hao S, Fu C, et al. The effect of coagulation factors in 2019 novel coronavirus patients. *Medicine*. 2021;100(7):e24537. doi:10.1097/MD.0000000000024537
4. COVID-19 Treatment Guidelines Panel. *Coronavirus Disease 2019 (COVID-19) Treatment Guidelines*. National Institutes of Health; 2024. Available from: https://www.covid19treatmentguidelines.nih.gov/. Accessed September 04, 2024.
5. Thachil J. The versatile heparin in COVID-19. *J Thromb Haemost*. 2020;18(5):1020–1022. doi:10.1111/jth.14821

6. Gozzo L, Viale P, Longo L, Vitale DC, Drago F. The Potential Role of Heparin in Patients With COVID-19: beyond the Anticoagulant Effect. A Review. *Front Pharmacol*. 2020;11:1307. doi:10.3389/fphar.2020.01307
7. Morales Castro D, Dresser L, Granton J, Fan E. Pharmacokinetic Alterations Associated with Critical Illness. *Clin Pharmacokinet*. 2023;62 (2):209–220. doi:10.1007/s40262-023-01213-x
8. Zufferey PJ, Dupont A, Lanoiselée J, et al. Pharmacokinetics of enoxaparin in COVID-19 critically ill patients. *Thromb Res*. 2021;205:120–127. doi:10.1016/j.thromres.2021.07.010
9. Tree JA, Turnbull JE, Buttigieg KR, et al. Unfractionated heparin inhibits live wild type SARS-CoV-2 cell infectivity at therapeutically relevant concentrations. *Br J Pharmacol*. 2021;178(3):626–635. doi:10.1111/bph.15304
10. Ting C, Sylvester KW, Schurr JW. Time in the Therapeutic Range for Assessing Anticoagulation Quality in Patients Receiving Continuous Unfractionated Heparin. *Clin Appl Thromb*. 2018;24(9_suppl):178S–181S. doi:10.1177/1076029618798944
11. Reiffel J. Time in the Therapeutic Range (TTR): an Overly Simplified Conundrum. *J Innov Card Rhythm Manag*. 2017;8(3):2643–2646. doi:10.19102/icrm.2017.080302
12. R Core Team. *R: A Language and Environment for Statistical computing*. 2013.
13. Gallagher A, Setakis E, Plumb J, Clemens A, van Staa TP. Risks of stroke and mortality associated with suboptimal anticoagulation in atrial fibrillation patients. *Thromb Haemost*. 2011;106(11):968–977. doi:10.1160/TH11-05-0353
14. Loeliger EA. Laboratory Control, Optimal Therapeutic Ranges and Therapeutic Quality Control in Oral Anticoagulation. *Acta Haematol*. 1985;74 (3):125–131. doi:10.1159/000206187
15. Rosendaal FR, Cannegieter SC, van der Meer FJ, Briët E, A method to determine the optimal intensity of oral anticoagulant therapy. *Thromb Haemost*. 1993;69(3):236–239.:.
16. Knaus WA, Draper EA, Wagner DP, Zimmerman JE, APACHE II: a severity of disease classification system. *Crit Care Med*. 1985;13(10):818–829.
17. Cauchie P, Piagnerelli M. What Do We Know about Thromboprophylaxis and Its Monitoring in Critically Ill Patients? *Biomedicines*. 2021;9 (8):864. doi:10.3390/biomedicines9080864
18. van Roessel S, Middeldorp S, Cheung YW, Zwinderman AH, de Pont ACJM. Accuracy of aPTT monitoring in critically ill patients treated with unfractionated heparin. *Neth J Med*. 2014;72(6):305–310.
19. Uprichard J, Manning RA, Laffan MA. Monitoring heparin anticoagulation in the acute phase response. *Br J Haematol*. 2010;149(4):613–619. doi:10.1111/j.1365-2141.2010.08129.x
20. Hirsh J, Warkentin TE, Shaughnessy SG, et al. Heparin and Low-Molecular-Weight Heparin Mechanisms of Action, Pharmacokinetics, Dosing, Monitoring, Efficacy, and Safety. *Chest*. 2001;119(1):64S–94S. doi:10.1378/chest.119.1_suppl.64S
21. Toulon P, Smahi M, De Pooter N. APTT therapeutic range for monitoring unfractionated heparin therapy. Significant impact of the anti-Xa reagent used for correlation. *J Thromb Haemost*. 2021;19(8):2002–2006. doi:10.1111/jth.15264