Bleeding disorders associated with severity of respiratory failure in COVID-19 patients: a prospective observational study

M.I. Neymark[1,*], A.P. Momot[1,2], M.G. Nikolaeva[1,2,4], A.N. Mamaev[2], S.N. Proskurin[3], P.V. Logvinov[4], A.N. Karkavina[3], D.P. Epp[1,5], V.V. Romanov[6]

[1] *Altai State Medical University, Barnaul, Russia*
[2] *National Medical Research Center for Hematology, Barnaul, Russia*
[3] *City Hospital No. 4 named after N.P. Gull, Barnaul, Russia*
[4] *The diagnostic center of the Altai Krai, Barnaul, Russia*
[5] *Altai Regional Clinical Hospital, Barnaul, Russia*
[6] *OOO "SKR-TEST", Iskitim, Russia*

Abstract

INTRODUCTION: Despite the progress in understanding the pathophysiology of coagulopathy in COVID-19, data about the association and phasing of pathological changes in various parts of the hemostatic system with the development of acute respiratory distress syndrome (ARDS) are insufficient. **OBJECTIVE:** To determine association between the severity of respiratory failure and pathological changes in the hemostatic system in COVID-19 patients. **MATERIALS AND METHODS:** A prospective observational study included 204 patients with a confirmed diagnosis of severe and extremely severe COVID-19. Two groups were identified according to disease outcome: fatal (n = 106) and survived (n = 98) groups. To assess dynamics of the clinical picture of the disease and to study the hemostatic profile, time points were determined: I point — the first day — admission to intensive care unit; II point — 3–5 days, III point — 7–10 days after ICU admission. The respiratory index was calculated to assess the severity of respiratory distress syndrome. Statistical data processing was carried out using the statistical software package MedCalc Version 20.110 (MedCalc Software Ltd, Belgium). **RESULTS:** A 2.15-

Анализ связи нарушений гемостаза со степенью выраженности дыхательной недостаточности у пациентов с COVID-19: проспективное наблюдательное исследование

М.И. Неймарк[1,*], А.П. Момот[1,2], М.Г. Николаева[1,2,4], А.Н. Мамаев[2], С.Н. Проскурин[3], П.В. Логвинов[4], А.Н. Каркавина[3], Д.П. Эпп[1,5], В.В. Романов[6]

[1] *ФГБОУ ВО «Алтайский государственный медицинский университет» Минздрава России, Барнаул, Россия*
[2] *ФГБУ «Национальный медицинский исследовательский центр гематологии», Барнаул, Россия*
[3] *КГБУЗ «Городская больница № 4 им. Н.П. Гулла», Барнаул, Россия*
[4] *КГБУЗ «Консультативно-диагностический центр Алтайского края», Барнаул, Россия*
[5] *КГБУЗ «Краевая клиническая больница», Барнаул, Россия*
[6] *ООО «СКР-ТЕСТ», Искитим, Россия*

Реферат

АКТУАЛЬНОСТЬ: Несмотря на прогресс в понимании патофизиологии коагулопатии при COVID-19, сведений о связи и об этапности патологических сдвигов в различных звеньях системы гемостаза при развитии острого респираторного дистресс-синдрома (ОРДС) недостаточно. **ЦЕЛЬ ИССЛЕДОВАНИЯ:** Определить взаимосвязь тяжести дыхательной недостаточности с патологическими сдвигами в системе гемостаза у больных COVID-19. **МАТЕРИАЛЫ И МЕТОДЫ:** В проспективное наблюдательное исследование включено 204 больных с верифицированным диагнозом COVID-19 тяжелого и крайне тяжелого течения заболевания. В зависимости от исхода заболевания выделено две группы: группа с летальным исходом (n = 106) и группа выживших больных (n = 98). Для динамической оценки клинической картины заболевания и исследования показателей системы гемостаза определены временные точки: точка I — 1-е сутки, поступление в отделение реанимации и интенсивной терапии (ОРИТ); точка II — 3–5-е сутки; точка III — 7–10-е сутки с момента поступления в ОРИТ. Для оценки тяжести респираторного дистресс-синдрома рассчитывался респираторный индекс. Статистическую обработку данных проводили

fold decrease in the respiratory index was determined for fatal outcome in patients with severe and extremely severe COVID-19. The most important hemostatic parameters affecting the severity of respiratory failure are increased Willebrand factor concentration at I point of the study (21% contribution and inverse correlation), increased plasminogen activator inhibitor type 1 (PAI-1) level on 3–5 days (35% contribution and direct correlation), and activation of the coagulative component of hemostasis on 7–10 days (78% contribution and direct correlation). **CONCLUSIONS:** The severity of respiratory failure in patients with a confirmed diagnosis of severe and extremely severe COVID-19 is gradually associated with endotheliopathy (1 day), inhibition of parietal fibrinolysis (3–5 days) and activation of the coagulative component of hemostasis by 7–10 days of ICU stay.

KEYWORDS: COVID-19, hemostatics, fibrinolysis, resuscitation, respiratory insufficiency, respiratory distress syndrome

* *For correspondence*: Mikhail I. Neymark — Dr. Med. Sci., professor, Head of Anesthesiology, Intensive Care and Clinical Pharmacology Department with a course of FVE in Altai State Medical University, Barnaul, Russia; e-mail: mineimark@mail.ru

For citation: Neymark M.I., Momot A.P., Nikolaeva M.G., Mamaev A.N., Proskurin S.N., Logvinov P.V., Karkavina A.N., Epp D.P., Romanov V.V. Bleeding disorders associated with severity of respiratory failure in COVID-19 patients: a prospective observational study. Annals of Critical Care. 2023;2:77–90. https://doi.org/10.21320/1818-474X-2023-2-77-90

Received: 12.09.2022
Accepted: 28.02.2023
Published online: 28.04.2023

с использованием пакета статистического программного обеспечения MedCalc Version 20.110 (MedCalc Software Ltd, Бельгия). **РЕЗУЛЬТАТЫ:** При летальном исходе заболевания COVID-19 тяжелого и крайне тяжелого течения у пациентов определено снижение показателя респираторного индекса в 2,15 раза. Наиболее важные параметры системы гемостаза, влияющие на степень тяжести дыхательной недостаточности: рост концентрации фактора Виллебранда (WF) на точке I исследования (21% вклада и обратная корреляция), увеличение уровня ингибитора активатора плазминогена 1-го типа (PAI-1) на 3–5-е сутки (35% вклада и прямая корреляция) и активация коагуляционного звена гемостаза на 7–10-е сутки (78% вклада и прямая корреляция). ВЫВОДЫ: Степень тяжести дыхательной недостаточности у пациентов с подтвержденным диагнозом COVID-19 тяжелого и крайне тяжелого течения поэтапно связана с эндотелиопатией (1-е сутки), угнетением пристеночного фибринолиза (3–5-е сутки) и активацией коагуляционного звена гемостаза к 7–10-м суткам пребывания в ОРИТ.

КЛЮЧЕВЫЕ СЛОВА: COVID-19, гемостаз, фибринолиз, реанимация, дыхательная недостаточность, респираторный индекс

* *Для корреспонденции*: Неймарк Михаил Израилевич — д-р мед. наук, профессор, заведующий кафедрой анестезиологии и реаниматологии с курсом клинической фармакологии с курсом ДПО ФГБОУ ВО «Алтайский государственный медицинский университет» Минздрава России, Барнаул, Россия; e-mail: mineimark@mail.ru

Для цитирования: Неймарк М.И., Момот А.П., Николаева М.Г., Мамаев А.Н., Проскурин С.Н., Логвинов П.В., Каркавина А.Н., Эпп Д.П., Романов В.В. Анализ связи нарушений гемостаза со степенью выраженности дыхательной недостаточности у пациентов с COVID-19: проспективное наблюдательное исследование. Вестник интенсивной терапии им. А.И. Салтанова. 2023;2:77–90. https://doi.org/10.21320/1818-474X-2023-2-77-90

Поступила: 12.09.2022
Принята к печати: 28.02.2023
Дата онлайн-публикации: 28.04.2023

DOI: 10.21320/1818-474X-2023-2-77-90

Introduction

Novel coronavirus disease 2019 (COVID-19), caused by SARS-CoV-2, occurs in various clinical manifestations ranging from mild upper respiratory tract damage to severe pneumonia with significant respiratory failure that requires invasive respiratory support [1–3]. As known, severe course of disease is caused by overproduction of proinflammatory

cytokines (cytokine storm) and systemic hyperinflammatory reaction that significantly predetermines disease course, prognosis and outcome [4, 5]. It is noted that autopsy studies demonstrate extensive inflammatory capillary thrombosis of pulmonary vessels, different from embolic thrombi in pulmonary arteries, that seems to contribute to hypoxemia and respiratory failure progression [6].

There is accumulated evidence that acute respiratory distress syndrome (ARDS) in COVID-19 differs from typical ARDS in that initial hypoxemia is associated with early loss of surfactant, dysregulation of neutrophil extracellular trap formation, alveolar cell damage, and local fibrin deposition [7–9]. In addition to the specific changes in pulmonary vessels, severe COVID-19 is characterized by systemic hypercoagulation represented by arterial and venous thrombotic events, while their contribution to mortality from COVID-19 is unknown. Also, data about the association of pathological changes in the hemostatic system with the development of ARDS are insufficient, and there are few works in this direction [8, 11].

Understanding the pathophysiology of coagulopathy and phasing of the changes in the hemostatic system with the development of ARDS in COVID-19 will allow to identify potential targets for therapeutic intervention, exposure to which can reduce the risk of both intrapulmonary and systemic thrombosis.

Objective: to determine association between the severity of respiratory failure and pathological changes in the hemostatic system in COVID-19 patients.

Materials and methods

A prospective observational two-center study was conducted on the basis of FSBI Federal Center for Traumatology, Orthopedics and Endoprosthetics of the Ministry of Health of the Russian Federation, Barnaul and City Hospital No. 4 named after N.P. Gull, Barnaul. The study period was from November 2020 to January 2022.

Inclusion criteria: Patients with a confirmed diagnosis of COVID-19 (positive laboratory test result for the presence of SARS-CoV-2 RNA using nucleic acid amplification tests (NAAT) or SARS-CoV-2 antigen using immunochromatographic assay); severe and extremely severe course of disease; need for respiratory support in intensive care unit (ICU); lung damage confirmed by computed tomography (CT); age over 18 years.

Exclusion criteria: Refusal of the patient and/or the relatives to participate in the study; age less than 18 years; septicemic condition at ICU admission [12], for women – pregnancy and lactation.

The morbidity as well as the algorithm of diagnosis and treatment were determined according to the temporary guidelines for the prevention, diagnosis and treatment of novel coronavirus infection, version 15 (dated February 22, 2022) [13].

The study included 225 patients at admission to the intensive care unit of a specialized hospital. During the follow-up, 21 patients were excluded from the analysis due to insufficient laboratory and clinical data reflected in the medical history, that did not allow to study the clinical course and outcome of the disease. Among the other 204 patients in the group, 168 patients had severe disease and 36 patients had extremely severe disease at ICU admission. The patients were divided into two groups according to disease outcome: fatal group (n = 106) and survived group (n = 98). The relief of acute respiratory failure (ARF) was achieved in the survived patients in ICU with the use of high-flow oxygenation and non-invasive ventilation that made it possible to transfer the patients to the therapeutic department. In the deceased patient group, 94 patients required noninvasive ventilation at admission, then they were transferred to invasive ventilation during disease progression, and 12 patients initially received respiratory support in the form of invasive ventilation.

The average age of patients was 65 years (minimum age 22 years, maximum age 97 years). The men to women ratio is 91:113. The time from hospital admission to ICU transfer ranged from 0 to 41 days. Median (Me) length of stay in the intensive care unit before the therapeutic department transfer or death was 7.0 (95 % CI for Me — 6.0–7.0, Q1–Q3: 4–10 days).

All patients included in the study had acute respiratory distress syndrome (ARDS) that manifested as tachypnea, a decrease in SpO_2 of less than 90 %, cyanosis of the nasolabial triangle, PaO_2/FiO_2 index <300 mmHg, the results of computed tomography and chest X-ray. 128 patients showed lung damage from 50 % to 75 % that corresponds to CT-3. Lung damage ranged from 75 % to 100 % in 76 patients that corresponds to CT-4. To analyze the severity of respiratory failure, the formulas and criteria presented in the 2012 Berlin definition of ARDS were used (Table 1).

To assess dynamics of the clinical picture of the disease and to study the hemostatic profile, the main time points were determined: I point – the first day, admission to intensive care unit, II point — 3–5 days, III point — 7–10 days of treatment.

In addition to the peripheral blood indicators regulated by the guidelines, 58 different peripheral blood parameters were performed and analyzed within the framework of the study, including the indicators that reflect the functioning of various parts of the hemostatic system and the values related to the immune system and the complement system. The present paper demonstrates an analysis of only the most significant parameters of hemostatic reactions.

Laboratory examination technology

Venous blood samples were collected from the ulnar vein into VACUETTE tubes with sodium citrate buffer

Table 1. Clinical and laboratory characteristics of the severity of acute respiratory distress syndrome [13]

Severity of ARDS	Oxygenation index calculated with the PaO_2/FiO_2 ratio*
Mild	200 mmHg < PaO_2/FiO_2 ≤ 300 mmHg* with PEEP or CPAP ≥ 5 cmH_2O
Moderate	100 mmHg < PaO_2/FiO_2 ≤ 200 mmHg with PEEP ≥ 5 cmH_2O
Severe	PaO_2/FiO_2 ≤ 100 mmHg with PEEP ≥ 5 cmH_2O

in the ratio of 9 : 1 (9NC Coagulation sodium citrate 3.2%, Greiner-Bio-One, Austria). The blood was centrifuged at 1400 g for 15 min at room temperature. Before conducting enzyme immunoassays, plasma was stored at –40 °C in a deep-freeze refrigerator MDF-192 Ultra-low temperature freezer (Sanyo, Japan) during one day up to two months.

The platelet count in the blood was evaluated using the DREW-3 automated hematology analyzer (Drew Scientific, USA). Concentration of von Willebrand factor (vWF) (vWF Ag reagent kit, Siemens Healthcare Diagnostics, Germany), factor II (prothrombin) activity (with the use of factor II deficient plasma and thromboplastin reagent Thromborel, Siemens Healthcare Diagnostics, Germany), factor XIII activity (Berichrom Factor XIII reagent kit), D-dimer concentration (D-dimer Red-700 reagent kit, Helena Bioscience, Great Britain) and α2-antiplasmin (α2-AP) activity (Berichrom α2-Antiplasmin reagent kit, Siemens Healthcare Diagnostics, Germany) were evaluated with Sysmex CA-1500 and Siemens BCS XP automated coagulometers (Sysmex Corporation, Japan).

ADAMTS-13 metalloproteinase activity (TECHNOZYM ADAMTS-13 Activity ELISA test kit), tissue plasminogen activator (t-PA) and plasminogen activator inhibitor type 1 (PAI-1) antigens (t-PA Combi Actibind ELISA test kit and TECHNOZYM® PAI-1 Actibind® ELISA test kit, respectively, Technoclone GmbH, USA) were evaluated with Real-R automated photometer (Vector-Best-Baltika, Russia).

Fluoroscan Ascent plate fluorimeter (ThermoFisher SCIENTIFIC, Finland) equipped with a dispenser with Thrombinoscope 3.0.0.26 software was used to perform TGT. Coagulation of the studied blood plasma was carried out in the presence of 5.0 pmol of tissue factor and 4 μmol of phospholipids (PPP-Reagent 5 pM, Thrombin Calibrator, FluCa-Kit, CAT Thrombinoscope BV, the Netherlands). Thrombin generation was recorded by measuring a fluorogenic substrate (Z-Gly-Gly-Arg-AMC) signal. The following indicators were taken into account: PTC (Peak thrombin - peak thrombin concentration, nmol/L) — the maximum thrombin concentration per unit time, and ETP (endogenous thrombin potential, nmol/min) — an indicator estimating the area under the thrombin generation curve that counts on the features of inactivation of this enzyme. All sample tests were carried out in two parallel versions, the average was automatically taken into account.

The tactics of intensive care, resuscitation, manipulation and transportation of patients with severe and extremely severe COVID-19 were carried out according to the Guidelines of the All-Russian public organization "Federation of Anesthesiologists and Reanimatologists" [14].

Also, it should be noted that before peripheral blood sampling for analysis at the first point, the patients did not receive anticoagulation. Further, anticoagulation is represented by: unfractionated heparin in 57.4% (n = 117) of cases and low molecular weight heparin (LMWH: sodium enoxiparin and calcium nadroparin in equal proportions) in 42.6% (n = 87) of cases. Anticoagulants were prescribed in a weight-adjusted therapeutic dose. At the second and third study points, blood sampling was carried out in the morning, at least 6 hours after the last intake of anticoagulants. The selected time range is due to the pharmacokinetics of the drugs: the duration of action of UFH is 4–6 hours [15], the half-life of LMWH is 5–6 hours [16].

Statistical analysis

Statistical data processing was carried out using the statistical software package MedCalc Version 20.110 (a product of MedCalc Software Ltd, Belgium). The Kolmogorov-Smirnov test was used to check the variation series for normality of distribution. Nonparametric methods were used for statistical while the results of the analyzed parameters of hemostatic tests and respiratory index values did not correspond to the normal distribution. The data of laboratory and instrumental indicators are presented in the form of median (Me), 95% confidence interval (95% CI) and interquartile range [Q1–Q3]. The degree of correlation between individual parameters of the hemostatic system and the data of the oxygenation index was determined with the Spearman correlation coefficient. Multiple regression analysis with the Kolmogorov-Smirnov test was used to determine association between respiratory failure and general disorders in the hemostatic system.

The study was approved by the local Ethics Committee of Altai State Medical University, Protocol No. 9 dated October 23, 2020.

Results

The results of a dynamic study of the indicators of various parts of the hemostatic system are presented in Figures 1, 2, 3 and 4.

When coagulative hemostasis was studied (Fig. 1 and 2), no statistical differences were found in comparing the fibrinogen concentration ratio at all study points in the comparison groups: p = 0.6004, p = 0.4707 and p = 0.6127, respectively. At the same time, the activity of the precursor of thrombin, factor II (prothrombin), in the deceased group was statistically significantly lower compared to the survived patients. Decreased prothrombin activity in blood plasma was specific to both groups by 7–10 days of ICU stay, but significantly lower values were characteristic of the group of deceased patients (89 % *vs.* 126 %, $p = 0.0095$).

When the activity of fibrin stabilizing factor (F XIII) was analyzed, the medians of this indicator were found to be

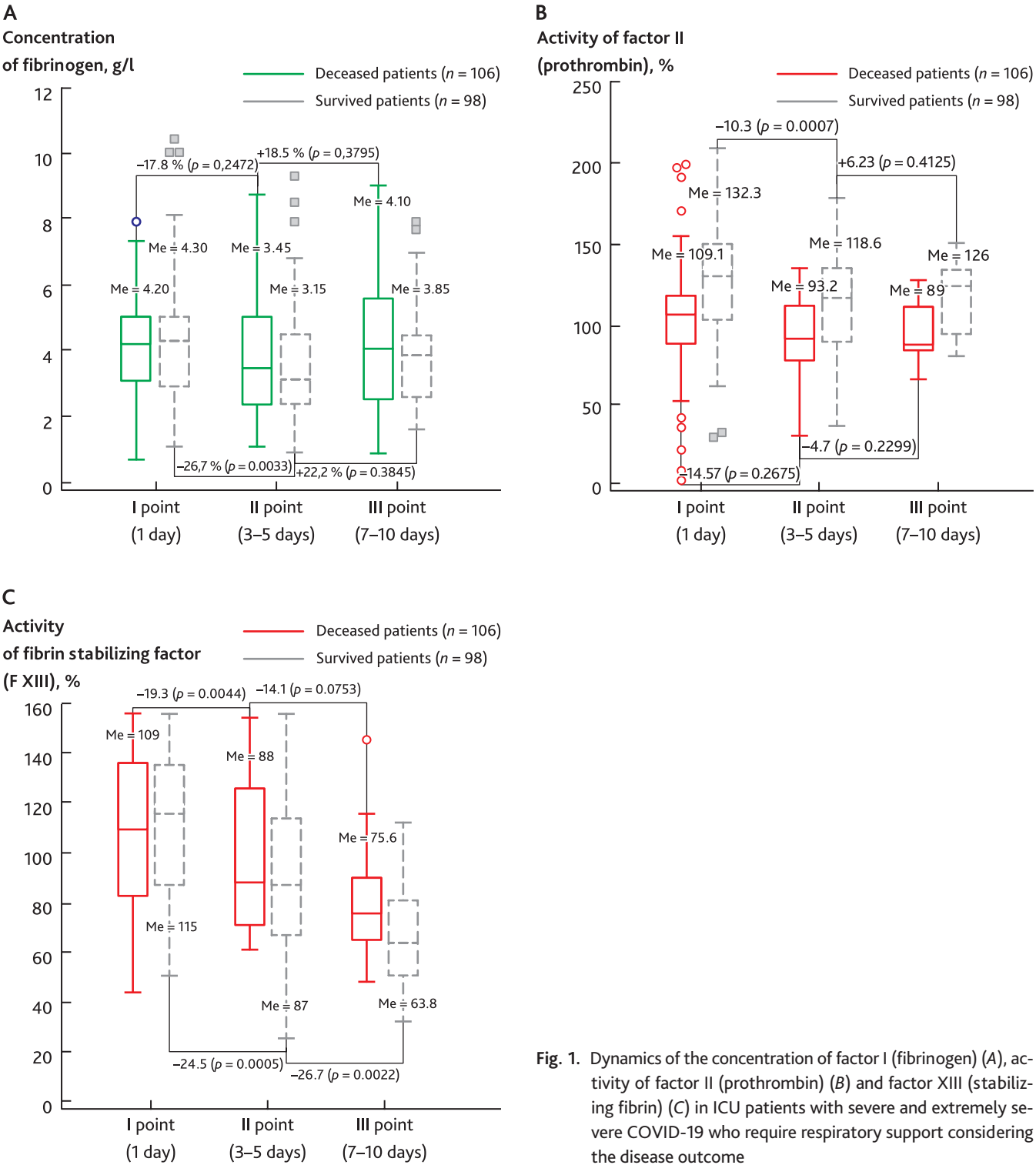

Fig. 1. Dynamics of the concentration of factor I (fibrinogen) (*A*), activity of factor II (prothrombin) (*B*) and factor XIII (stabilizing fibrin) (*C*) in ICU patients with severe and extremely severe COVID-19 who require respiratory support considering the disease outcome

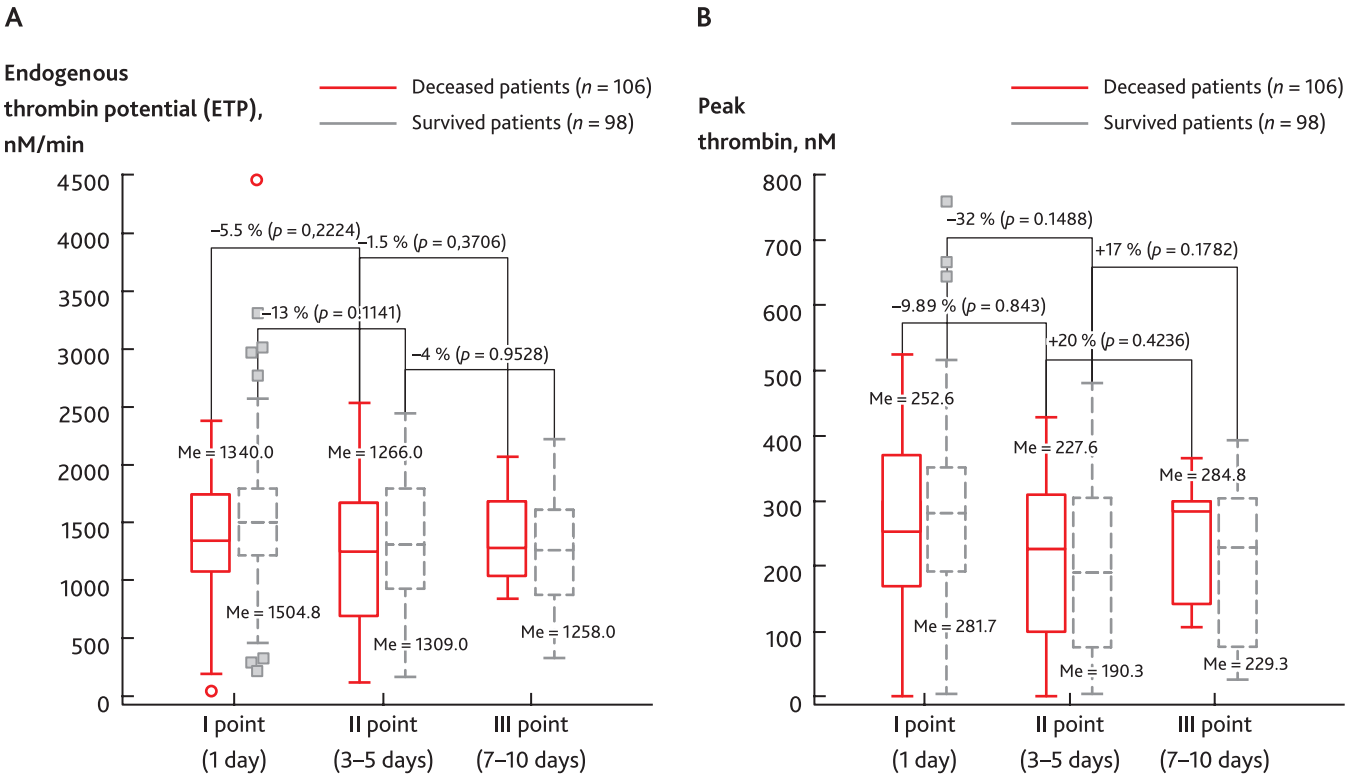

Fig. 2. Dynamics of indicators of endogenous thrombin potential (ETP) (*A*) and peak thrombin (*B*) in ICU patients with severe and extremely severe COVID-19 who require respiratory support considering the disease outcome

comparable at the first and second study points ($p = 0.5240$ and $p = 0.4630$, respectively). However, its activity was significantly higher ($p = 0.0043$) in the group of deceased patients than in that of survived patients on days 7–10.

Analysis of the thrombin formation dynamics based on the values of the thrombin generation test indicates that thrombin generation (according to the Peak thrombin indicator) is generally higher in the group of deceased patients than in that of deceased patients, but there was no statistically significant difference.

A significantly higher concentration of von Willebrand factor ($p < 0.0001$) and definitely lower activity of ADAMTS-13 metalloproteinase ($p = 0.0037$) were determined in the fatal group on the very first day of ICU stay (Fig. 3). Both comparison groups are characterized by an increased von Willebrand factor level, with a significant difference in the indicator values at the second ($p = 0.0001$) and third ($p = 0.0242$) study points. At the same time, the concentration of ADAMTS-13 metalloproteinase remained stable in the survived group by 7–10 days of ICU stay ($p = 0.0530$), and it decreased slightly by 19 % in the fatal group ($p = 0.2788$).

Platelet count analysis showed that their number had no statistically significant differences in the comparison groups (survived and deceased patients) on the first day of the study — at ICU admission ($p = 0.3254$). The platelet count decreased in both groups by 3–5 days of following up, without reaching statistical significance ($p = 0.3304$). On 7–10 days in the group of deceased patients, the platelet count decreased by 53.2 % in respect of the first study point ($p = 0.0001$), with a significant difference in the same indicator in the survived group ($p = 0.0251$).

The study of dynamics of the indicators reflecting the fibrinolysis system function (Fig. 4) allowed to establish that multidirectional reactions were noted in the comparison groups, given its regulation complexity. According to the findings, significantly higher t-PA concentrations (1.6 times median) and significantly lower α2-antiplasmin activity were determined in the fatal group at the first study point that may indicate fibrinolysis activation in the systemic circulation. Later, however, by 7–10 days of ICU stay, the t-PA concentration significantly decreased (by 16 %; $p = 0.0482$) in patients with a fatal outcome with an increased concentration of its inhibitor (PAI-1) by 29 % ($p = 0.0610$). In contrast, the survived group was characterized by significantly increased t-PA concentration ($p = 0.0028$) with identical PAI-1 values on the first and 7–10 days of ICU stay ($p = 0.1064$). It should be noted that the activity of α2-antiplasmin, the main plasmin inhibitor, was comparable by study points in the comparison groups.

The concentration of D-dimer, one of the probable fibrin degradation products, was determined to be significantly higher in the fatal group at the first study point ($p = 0.0011$), while the values of this indicator in 2 and 3 study points were comparable to each other

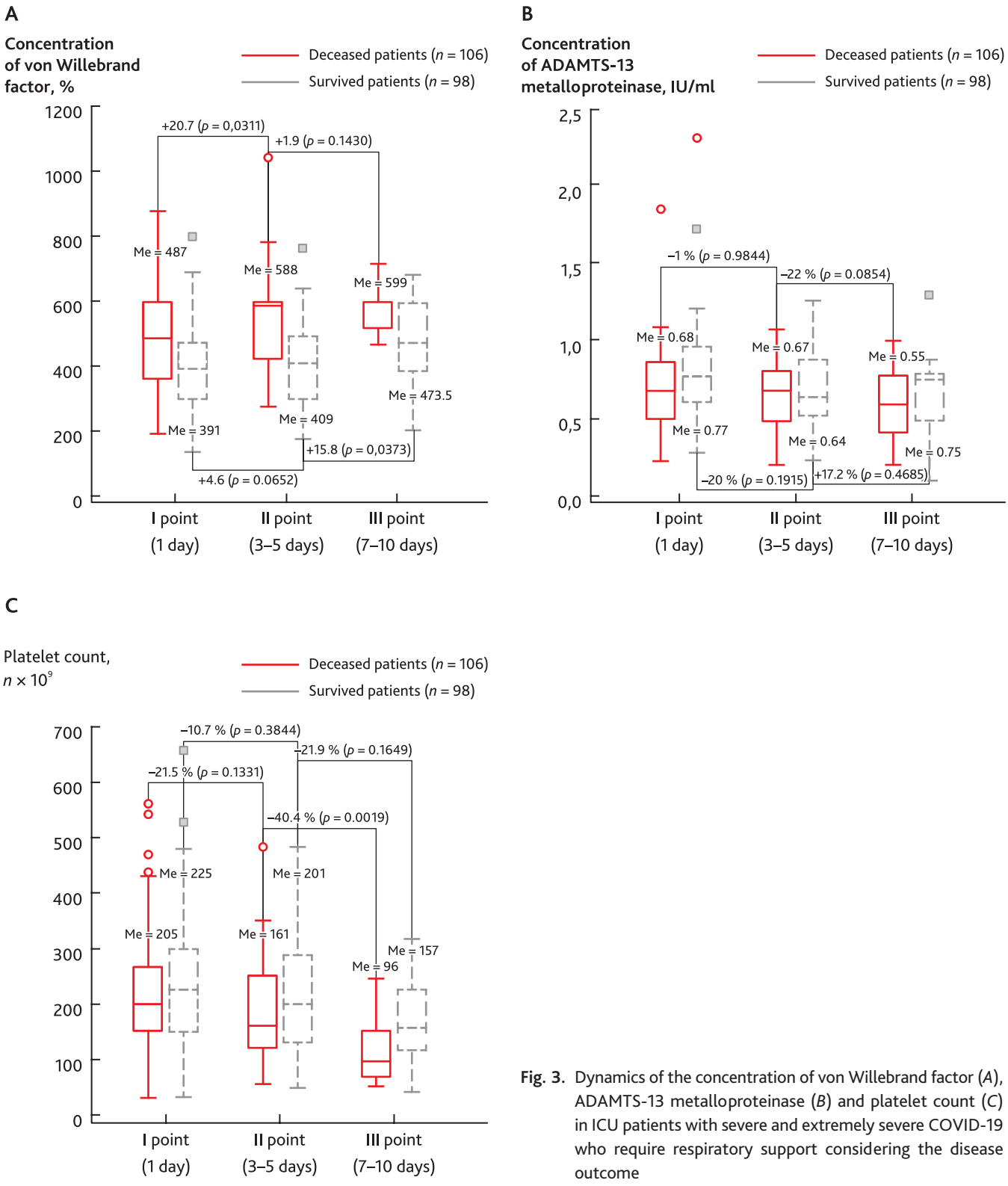

Fig. 3. Dynamics of the concentration of von Willebrand factor (*A*), ADAMTS-13 metalloproteinase (*B*) and platelet count (*C*) in ICU patients with severe and extremely severe COVID-19 who require respiratory support considering the disease outcome

(p = 0.3030 and p = 0.4179, respectively). Along with this, by 7–10 days of ICU stay, a dynamic increase in D-dimer concentration was revealed by 26.6 % in the group of deceased patients (p = 0.4822) and by 83.9 % in that of survived patients (p = 0.2412).

Summing up the above, we can conclude that severe and extremely severe COVID-19 is associated with dysfunction in all parts of the hemostatic system that is characterized by endothelial cell activation, hypercoagulation shift and fibrinolysis inhibition.

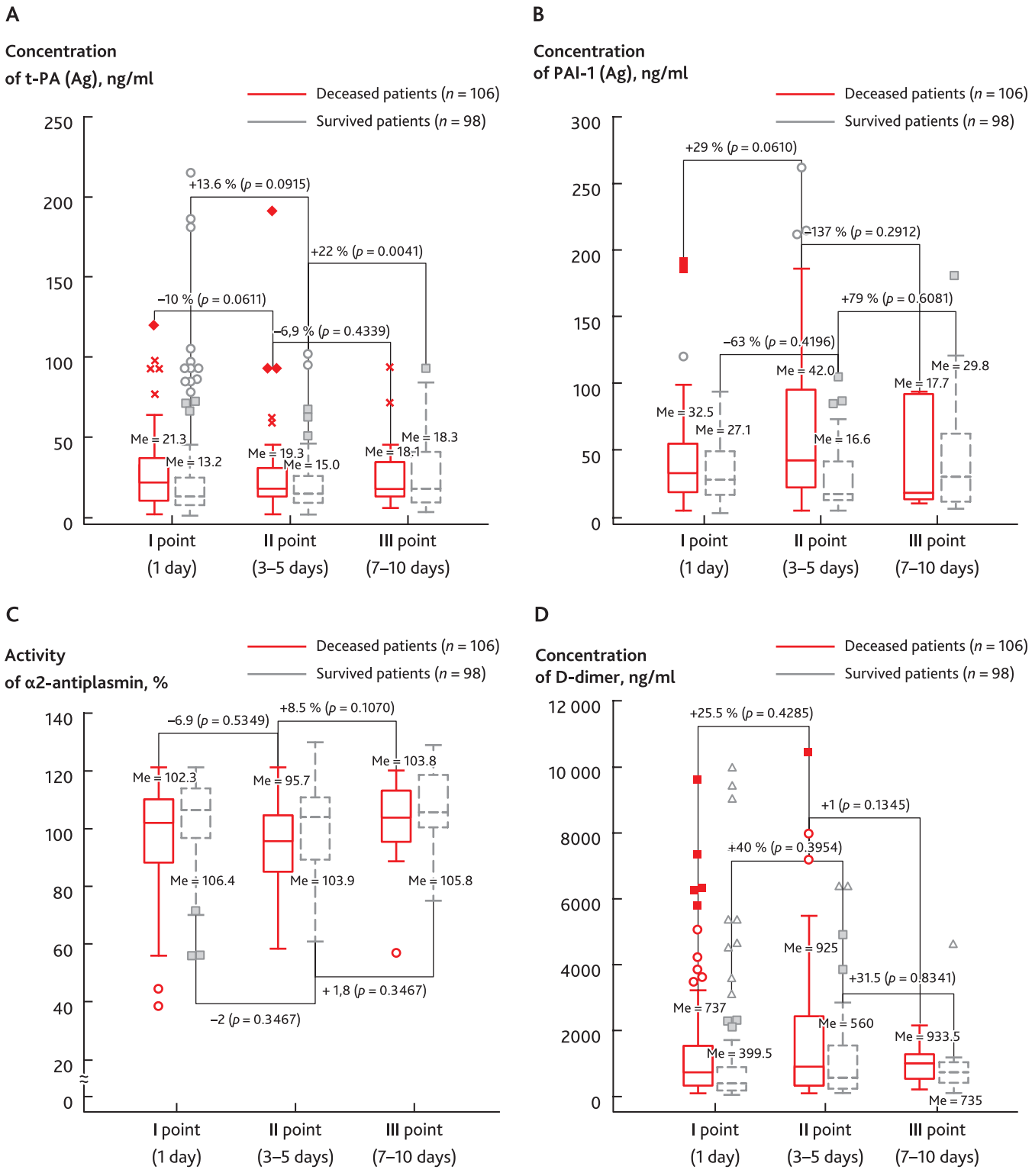

Fig. 4. Dynamics of the concentration of t-PA (*A*), PAI-1 (*B*), activity of α2-antiplasmin (*C*) and D-dimer (*D*) in ICU patients with severe and extremely severe COVID-19 who require respiratory support considering the disease outcome

When the respiratory index value was assessed, it was determined that patients with a fatal outcome had its significant decrease compared to the survived group. Thus, by 7–10 days in the survived group, the oxygenation index was statistically significantly higher by 2.15 times ($p = 0.0001$) than in the fatal group (Fig. 5).

Considering that the main objective of our study was to determine the association between the severity of respi-

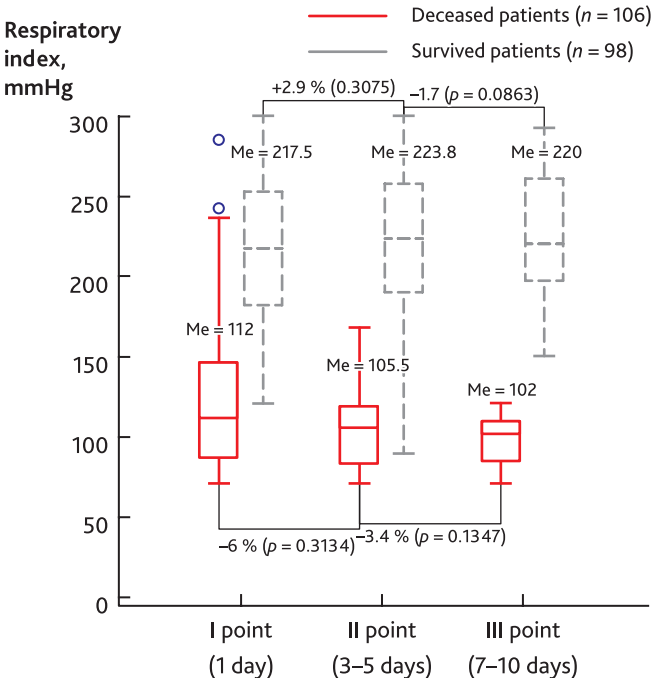

Fig. 5. Dynamics of the respiratory index in ICU patients with severe and extremely severe COVID-19 who require respiratory support considering the disease outcome

ratory failure and pathological changes in the hemostatic system, a correlation analysis of the respiratory index value and the analyzed parameters of the hemostatic system was carried out at the selected study points to identify the direction and connection strength (Table 2).

According to the findings, a reliable positive correlation of the activity of factor II, ADAMTS 13 metalloproteinase, α2-antiplasmin and a reliable negative correlation of the concentration of factor vWF, PAI-1 and D-dimer with the respiratory index at various study points were identified. At the same time, connection strength for all the parameters was weak, that does not make it possible to identify the dominant dysfunction in the hemostatic system that determines the severity of respiratory failure.

To better predict the possible association of the analyzed parameters of the hemostatic system with the respiratory index value at the study points, a multiple regression analysis was performed considering the multicollinearity of factors and their backward selection in the model (Table 3).

According to the findings, the contribution of specific factors of the hemostatic system to the formation of the severity of respiratory failure at each study point was different. On the first day of ICU stay, the concentration of von Willebrand factor explains 21% of the variability of the respiratory index value, while an inverse correlation of average strength is observed. When the patients were examined on 3–5 days, only the concentration of plasminogen activator inhibitor type 1 (PAI-1) significantly contributed to the respiratory index value variation determining it by 35% with an average correlation between the variables. The other factors of the hemostatic system lose their significance in the formation of the severity of respiratory failure at the first two study points. Activation of the coagulative hemostasis component on 7–10 days of following up is the determining factor of the critical values of the respiratory index that determines it by 78% with a strong correlation of the analyzed parameters.

Table 2. The results of the correlation analysis of the respiratory index and the hemostatic system indicators at the study points

Hemostatic system parameters	I point		II point		III point	
	Spearman's coefficient	*p*	Spearman's coefficient	*p*	Spearman's coefficient	*p*
Activity of factor II, %	0.2110	0.0040	0.261	0.0120	0.3690	0.0268
Activity of factor XIII, %	0.0490	0.5562	–0.0783	0.4984	–0.2550	0.1597
Peak thrombin, nmol/L	0.0308	0.7054	0.0233	0.8357	–0.1960	0.3172
Endogenous thrombin potential (ETP)	0.0948	0.2517	0.0330	0.7744	–0.1070	0.6121
Platelet count ×10⁹, %	0.0353	0.6252	0.0730	0.4728	0.3140	0.0561
Concentration of factor vWF, %	–0.2220	0.0019	–0.278	0.0043	0.3880	0.0232
Activity of ADAMTS 13, ME/ml	0.2430	0.0013	-0.0033	0.9741	0.0800	0.6670
Concentration of tPA (Ag), ng/ml	–0.2120	0.0024	–0.0688	0.4858	0.1160	0.5013
Concentration of PAI-1 (Ag), ng/ml	–0.1910	0.0327	–0.3880	0.0013	–0.180	0.3883
Activity of α2-antiplasmin, %	0.1470	0.0502	0.1340	0.2287	0.1120	0.5291
Concentration of D-dimer, ng/ml	–0.1420	0.0470	–0.1840	0.1370	–0.2270	0.3351

Table 3. The results of the multiple regression analysis of the dependence of the respiratory index at different study points on the activity/concentration of specific parameters of the hemostatic system

Study point	Independent variable	Ratio	*t*	*p*	R^2	*R*
1 day	Constant	260.9	—	—	0.2097	0.4316
	Concentration of factor vWF, %	–0.1892	–3.236	0.0017		
3–5 days	Constant	219.3	—	—	0.3524	0.6024
	Concentration of PAI-1 (Ag), ng/ml	–0.6498	–3.582	0.0010		
7–10 days	Constant	492.3	—		0.7813	0.8756
	Activity of factor II, %	1.3980	2.463	0.0263		
	Peak thrombin, nmol/l	–0.3375	–3.304	0.0048		
	Activity of factor XIII, %	–1.6773	–3.809	0.0017		

Note: *p* — ratio significance level; *R* — multiple correlation coefficient; *R2* — determination coefficient; *t* — empirical value of t-test for checking the statistical significance of the specific ratio.

Thus, the severity of respiratory failure in patients with a confirmed diagnosis of severe and extremely severe COVID-19 is gradually associated with endothelial dysfunction (activation) (1 day), inhibition of parietal fibrinolysis (3–5 days) and activation of the coagulative hemostasis component by 7–10 days of ICU stay.

Discussion

Pulmonary intravascular coagulation plays a major role in the pathogenesis of acute respiratory failure in COVID-19 [17]. As known, microthromboembolization of pulmonary vessels in COVID-19 patients causes a progressive decrease in lung perfusion, hypoxic vasoconstriction and increasing hypoxemia [18, 19]. At the same time, its severity can be objectively evaluated by the respiratory index, an integral indicator, that characterizes the severity of respiratory distress syndrome [20–22].

In the present work, it was found that the respiratory index was 2.15 times lower in the fatal group than in the survived group. Hence, it was of interest to identify the association of phasing and severity of disorders in the hemostatic system that determine the possible microthrombosis in the pulmonary vessels with the severity of respiratory failure.

As known, one of the main causes of thrombosis in COVID-19 is vascular endothelial damage caused by viral invasion [19] resulting in that endothelial cells increase the expression of proinflammatory cytokines and von Willebrand factor, and decreased fibrinolytic activity of mast cells is recorded [23]. Our study is consistent with the earlier studies according to a number of the indicators confirming the dysfunction of the hemostatic system. Thus, it was determined that platelet count in the blood significantly decreased in both outcome comparison groups, however, thrombocytopenia was much more significant in deceased patients on 7–10 days, the median was 97×10^9 versus 157×10^9/l in the group of survived patients ($p = 0.0251$). According to E.L. Bulanova et al., the presence of thrombocytopenia increases the risk of death in COVID-19 infection by 5.5 times (95 % CI 2.979–10.031) [24].

According to the study results, the decreased respiratory index value on the first day was due to the increased concentration of von Willebrand factor. At the same time, significantly higher concentration ratios were found in the group of deceased patients on 3–5 and 7–10 days of following up compared with the group of survived patients. Significantly lower activity of ADAMTS-13 metalloproteinase ($p = 0.0037$) in the group of deceased patients was noted only at the first study point. Probably, the discrepancy of its stable activity at 2 and 3 study point is more clinically significant against the background of an increasing concentration of von Willebrand factor, that can result in the increased processes of microthrombosis specific for thrombotic microangiopathy (TMA). Consideration of coagulopathy in COVID-19 as a variant of TMA, as known, supposes a paradigm shift in therapy, for example, plasma exchange [25].

In the conducted work, it was found that severe COVID-19 is characterized by multidirectional fibrinolytic reactions depending on the duration of ICU stay, and, therefore, on the duration of respiratory support. Thus, on the first day of respiratory failure, activation of parietal fibrinolysis (increased concentration of t-PA) was established, followed by its inhibition on 3–5 days of treatment (increased concentration of PAI-1), that is especially marked

in patients with a fatal outcome. It was found that it was the excessive concentration of PAI-1 that determined the severity of respiratory distress syndrome at the second study point.

A similar conclusion was made by the researchers in South Carolina. Decreased concentration of PAI-1 was associated with severe hypoxemia, fibrinolysis inhibition in the microcirculation system and subsequent micro- and macrovascular thrombosis in severe COVID-19 [11].

The revealed pattern suggests that the basis for the correction of bleeding disorders in patients with severe and extremely severe COVID-19 should be not anticoagulant, but fibrinolytic therapy, that is confirmed by a few studies considering intravenous administration of fibrinolytics [6, 26, 27].

Now there is convincing evidence that hypercoagulation in COVID-19 is secondary to elevated levels of tissue factor, von Willebrand factor, fibrinogen, a decrease in ADAMTS-13 concentration with platelet activation and fibrinolysis inhibition [6, 28], that is confirmed by the findings of the present work.

On the one hand, we identified facts of common knowledge when we analyzed the parameters of the hemostatic system at 3 study point. The severity of respiratory failure, namely, critical values of the respiratory index by the 7–10 days of ICU stay are associated with activation of the coagulative component of hemostasis, that is confirmed by an increase in peak thrombin by 12.7 % in thrombin generation test and by statistically significantly higher activity of factor XIII in the group of deceased patients (p = 0.0043). On the other hand, prothrombin activity value has a negative correlation with the respiratory index. In our opinion, the nature of dynamics of FII and FXIII activity values from the moment of ICU admission to disease outcome suggests intensive consumption of the indicated factors for thrombosis, that warrants further studies.

It should be noted that there are quite few works characterizing decreased activity of factor XII. At the same time, the researchers' results are comparable to our findings. For example, in the work of Austrian researchers, it is reported that the concentration of fibrin-stabilizing factor is one of the important predictors of poor outcome in COVID-19 [29].

Thus, it can be assumed that the severity of respiratory failure in patients with severe and extremely severe COVID-19 correlates with pathological changes in the hemostatic system. At the same time, there is a certain phasing of the detected hemostatic disorders that indicates primary acute endothelial damage followed by inhibition of parietal fibrinolysis and the development of secondary hypercoagulation by 7–10 days of respiratory support in the ICU. The revealed regularities of phasing of pathological changes in the hemostatic system that correlate and determine the severity of respiratory failure are a prerequisite for revising therapeutic approaches to the treatment of patients with severe and extremely severe COVID-19.

Conclusions

The severity of respiratory failure in patients with severe and extremely severe COVID-19 is associated with pathological changes in all parts of the hemostatic system.

In patients with severe and extremely severe COVID-19 receiving respiratory support, the phasing of hemostatic changes is determined that correlates with the respiratory index value and consists in endothelial dysfunction followed by inhibition of parietal fibrinolysis and secondary hypercoagulation.

The hypercoagulation shift determines the respiratory index value by 78 % with a strong correlation by 7–10 days against the background of concomitant respiratory support in patients with severe and extremely severe COVID-19 (R = 0.8756). At the same time, increased thrombin generation is accompanied by a statistically significant decrease in the activity of factors II and XIII.

Disclosure. The authors declare that they have no competing interests.

Author contribution. All authors according to the ICMJE criteria participated in the development of the concept of the article, obtaining and analyzing factual data, writing and editing the text of the article, checking and approving the text of the article.

Ethics approval. This study was approved by the local Ethical Committee of Altai State Medical University (reference number: 9-23.10.2020).

Funding source. This study was not supported by any external sources of funding.

Data Availability Statement. The data that support the findings of this study are openly available in repository Mendeley Data at https://data.mendeley.com/datasets/2z3dmg4y5f

Author's ORCID:

Neymark M.I. — 0000-0001-9135-6392
Momot A.P. — 0000-0002-8413-5484
Nikolaeva M.G. — 0000-0001-9459-5698
Mamaev A.N. — 0000-0002-3313-7295
Proskurin S.N. — 0000-0003-3216-4029
Logvinov P.V. — 0000-0002-4359-2769
Karkavina A.N. — 0000-0002-9037-8781
Epp D.P. — 0000-0002-2261-6442
Romanov V.V. — 0000-0003-1953-2536

References

[1] *Allegra A., Innao V., Allegra A.G., et al*. Coagulopathy and thromboembolic events in patients with SARS-CoV-2 infection: Pathogenesis and management strategies. Ann Hematol. 2020; 99: 1953–65. DOI: 10.1007/s00277-020-04182-4

[2] *Armstrong R.A., Kane A.D., Cook T.M.* Outcomes from intensive care in patients with COVID-19: a systematic review and meta-analysis of observational studies. Anaesthesia. 2020; 75: 1340–9. DOI: 10.1111/anae.15201

[3] *Dolhnikoff M., Duarte-Neto A.N., de Almeida Monteiro R.A., et al*. Pathological evidence of pulmonary thrombotic phenomena in severe COVID-19. J Thromb Haemost. 2020; 18 (6): 1517–9. DOI: 10.1111/jth.14844

[4] *Бобкова С.С., Жуков А.А., Проценко Д.Н. и др*. Критический анализ концепции «цитокиновой бури» у пациентов с новой коронавирусной инфекцией COVID-19. Обзор литературы. Вестник интенсивной терапии им. А.И. Салтанова. 2021; 1: 57–68. DOI : 10.21320/1818-474X-2021-1-57-68 [*Bobkova S.S., Zhukov A.A., Protsenko D.N., et al*. Critical appraisal of the "cytokine storm" concept in new coronavirus disease COVID-19. Review. Annals of Critical Care. 2021; 1: 57–68. DOI: 10.21320/1818-474X-2021-1-57-68 (In Russ)]

[5] *Bi X., Su Z., Yan H., et al*. Prediction of severe illness due to COVID-19 based on an analysis of initial fibrinogen to albumin ratio and platelet count. Platelets. 2020; 31(5): 674–9. DOI: 10.1080/09537104.2020.1760230

[6] *Chowdary P*. COVID-19 coagulopathy — what should we treat? Exp Physiol. 2022; 107(7): 749–58. DOI: 10.1113/EP089404

[7] *Бицадзе В.О., Слуханчук Е.В., Хизроева Д.Х. и др*. Внеклеточные ловушки нейтрофилов (NETs) в патогенезе тромбоза и тромбовоспалительных заболеваний. Вестник Российской академии медицинских наук. 2021; 76(1): 75–85. DOI: 10.15690/vramn1395 [*Bitsadze V.O., Slukhanchuk E.V., Khizroeva J.H., et al*. Extracellular neutrophil traps (NETs) in the pathogenesis of thrombosis and thromboinflammation. Annals of the Russian academy of medical sciences. 2021; 76(1): 75–85. DOI: 10.15690/vramn1395 (In Russ)]

[8] *Gando S., Wada T*. Pathomechanisms Underlying Hypoxemia in Two COVID-19-Associated Acute Respiratory Distress Syndrome Phenotypes: Insights From Thrombosis and Hemostasis. Shock. 2022; 57(1): 1–6. DOI: 10.1097/SHK.0000000000001825

[9] *Zhu Y., Chen X., Liu X*. NETosis and Neutrophil Extracellular Traps in COVID-19: Immunothrombosis and Beyond. Front Immunol. 2022; 13: 838011. DOI: 10.3389/fimmu.2022.838011

[10] *Kwee R.M., Adams H.J.A., Kwee T.C.* Pulmonary embolism in patients with COVID-19 and value of D-dimer assessment: a meta-analysis. Eur Radiol. 2021; 31(11): 8168–86. DOI: 10.1007/s00330-021-08003-8

[11] *Corey K.M., Olson L.B., Naqvi I.A., et al*. Suppression of Fibrinolysis and Hypercoagulability, Severity of Hypoxemia, and Mortality in COVID-19 Patients: A Retrospective Cohort Study. Anesthesiology. 2022; 137(1): 67–78. DOI: 10.1097/ALN.0000000000004239

[12] *Evans L., Rhodes A., Alhazzani W., et al*. Surviving sepsis Campaign: International guidelines for management of sepsis and septic shock 2021. Intensive Care Med. 2021; 47(11): 1181–247. DOI: 10.1007/s00134-021-06506-y

[13] Временные методические рекомендации профилактика, диагностика и лечение новой коронавирусной инфекции (COVID-19). Версия 15 (22.02.2022) [Internet] [updated 2022 november 16; cited 2022 november 16]. Available from: https://static-0.minzdrav.gov.ru/system/attachments/attaches/000/059/392/original/BMP_COVID-19_V15.pdf [Vremennye metodicheskie rekomendacii profilaktika, diagnostika i lechenie novoj koronavirusnoj infekcii (COVID-19). Versiya 15 (22.02.2022) [Internet] [updated 2022 november 16; cited 2022 november 16]. Available from: https://static-0.minzdrav.gov.ru/system/attachments/attaches/000/059/392/original/BMP_COVID-19_V15.pdf (In Russ)]

[14] *Заболотских И.Б., Киров М.Ю., Лебединский К.М. и др*. Анестезиолого-реанимационное обеспечение пациентов с новой коронавирусной инфекцией COVID-19. Методические рекомендации Общероссийской общественной организации «Федерация анестезиологов и реаниматологов». Вестник интенсивной терапии им. А.И. Салтанова. 2022; 1:5–140. DOI: 10.21320/1818-474X-2022-1-5-140 [*Zabolotskikh I.B., Kirov M.Yu., Lebedinskii K.M., et al*. Anesthesia and intensive care for patients with COVID-19. Russian Federation of anesthesiologists and reanimatologists guidelines. Annals of Critical Care. 2022; 1: 5–140. DOI: 10.21320/1818-474X-2022-1-5-140 (In Russ)]

[15] *Hirsh J., Anand S.S., Halperin J.L., et al*. Guide to anticoagulant therapy: heparin. A statement for health-care professionals from the American Heart Association. Arterioscler Thromb Vasc Biol. 2001; 21(7): E9–9. DOI: 10.1161/hq0701.093520

[16] *Garcia D.A., Baglin T.P., Weitz J.I., et al*. Parenteral anticoagulants: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest. 2012; 141(2 Suppl): e24S–e43S. DOI: 10.1378/chest.11-2291

[17] *Colling M.E., Kanthi Y*. COVID-19-associated coagulopathy: An exploration of mechanisms. Vasc Med. 2020; 25(5): 471–8. DOI: 10.1177/1358863X20932640

[18] *Ahmed S., Zimba O., Gasparyan A.Y.* Thrombosis in Coronavirus disease 2019 (COVID-19) through the prism of Virchow's triad. Clin Rheumatol. 2020; 1–15. DOI: 10.1007/s10067-020-05275-1

[19] *Mancini I., Baronciani L., Artoni A., et al*. The ADAMTS13-von Willebrand factor axis in COVID-19 patients. J Thromb Haemost. 2021; 19(2): 513–21. DOI: 10.1111/jth.15191

[20] *Wu C., Chen X., Cai Y., et al*. Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. JAMA Intern Med. 2020; 180(7): 934–43. DOI: 10.1001/jamainternmed.2020.0994

[21] *Puah S.H.* ATS and APSR Joint Webinar: Global perspectives on COVID-19 [Internet]. [updated 2020 March 27]. Available from: https://www.apsresp.org/archive/2020-covid-19-webinar.html

[22] *Xu Z., Shi L., Wang Y., et al*. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med. 2020; 8: 420–2. DOI: 10.1016/S2213-2600(20)30076-X

[23] *Suzuki Y., Yasui H., Brzoska T., et al*. Surface-retained tPA is essential for effective fibrinolysis on vascular endothelial cells. Blood. 2011; 118: 3182–5. DOI: 10.1182/blood-2011-05-353912

[24] *Буланова Е.Л., Работинский С.Е., Дегтярев П.А. и др.* Тромбоцитопении в ОРИТ до и во время пандемии новой коронавирусной инфекции COVID-19: ретроспективное сравнительное когортное исследование. Вестник интенсивной терапии им. А.И. Салтанова. 2022; 4: 66–73. DOI: 10.21320/1818-474X-2022-4-66-73 [*Bulanova E.L., Rabotinsky S.E., Degtyarev P.A., et al*. Thrombocytopenia in the ICU before and during the pandemic of the new coronavirus infection COVID-19: a comparative retrospective cohort study. Annals of Critical Care. 2022; 4: 66–73. DOI: 10.21320/1818-474X-2022-4-66-73 (In Russ)]

[25] *Doevelaar A.A.N., Bachmann M., Hölzer B., et al*. von Willebrand Factor Multimer Formation Contributes to Immunothrombosis in Coronavirus Disease 2019. Crit Care Med. 2021; 49(5): e512–e520. DOI: 10.1097/CCM.0000000000004918

[26] *Barrett C.D., Moore H.B., Moore E.E., et al*. Fibrinolytic therapy for refractory COVID-19 acute respiratory distress syndrome: Scientific rationale and review. Res Pract Thromb Haemost. 2020; 4 (04): 524–31. DOI: 10.1002/rth2.12357

[27] *Zając P., Kaziród-Wolski K., Oleś I., et al*. Role of Fibrinolysis in the Management of Patients with COVID-19 and Thromboembolic Complications: A Review. J Cardiovasc Dev Dis. 2022; 9(10): 356. DOI: 10.3390/jcdd9100356

[28] *Sugimoto M.A., Perucci L.O., Tavares L.P., et al*. Current Drug Targets (CDT) special issue on Covid-19 and Coagulopathy Fibrinolysis in COVID-19: impact on clot lysis and modulation of inflammation. Curr Drug Targets. 2022. Oct 11. DOI: 10.2174/1389450123666221011102250

[29] *Ercan H., Schrottmaier W.C., Pirabe A., et al*. Platelet Phenotype Analysis of COVID-19 Patients Reveals Progressive Changes in the Activation of Integrin αIIbβ3, F13A1, the SARS-CoV-2 Target EIF4A1 and Annexin A5. Front Cardiovasc Med. 2021; 8: 779073. DOI: 10.3389/fcvm.2021.779073