

Quantitative and qualitative changes in blood cells associated with COVID-19

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Abstract

Aim. To establish the relationship of hematological disorders with the pathogenesis, course and outcomes of COVID-19.

Methods. We examined 235 hospitalized patients with moderate and severe forms of acute COVID-19 receiving anticoagulants and immunosuppressive drugs. We studied the full blood cell counts and morphology along with the platelet function by flow cytometry in comparison with the clinical features and synthesis of inflammatory markers. To assess platelet contractility, blood clot contraction (retraction) kinetics was used in combination with scanning electron microscopy of platelets and blood clots.

Results. Hemolytic anemia, neutrophilia and lymphopenia were associated with immature erythrocytes and leukocytes, indicating activation of hematopoiesis. Contraction of blood clots in COVID-19 was impaired, especially in severe and lethal cases, as well as in the presence of comorbidities, including myeloproliferative and coronary heart diseases and acute cerebrovascular disease. In male patients, the changes in clot contraction were more pronounced. Suppression of clot contraction correlated directly with anemia and coagulopathy, including a high D-dimer level, which confirms the pathogenetic significance of blood clot contraction in COVID-19. A decrease in platelet contractility was due to moderate thrombocytopenia in combination with chronic platelet activation and secondary platelet dysfunction. The structure and cellular composition of blood clots depended on the extent of contraction; clots with impaired contraction were porous, had a low content of deformed polyhedral erythrocytes (polyhedrocytes) and an even distribution of fibrin.

Conclusion. Blood cells undergoing both quantitative and qualitative changes are involved in the pathogenesis of COVID-19; the suppressed platelet-driven contraction of intravital blood clots may be a part of the prothrombotic mechanisms.

Keywords: blood cells, blood clotting, contraction of blood clots, COVID-19, platelets.

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Background. Coronavirus disease (COVID-19) usually proceeds as a respiratory pathology of mild or moderate severity, but often, the disease acquires a malignant systemic character with acute respiratory distress syndrome and multiple organ dysfunction, up to death [1]. The proportion of patients with a severe and critical course of infection ranges from 15% to 20% [2], and the average mortality rate for COVID-19 in the general population

ranges from 2% to 3%. But it can reach 15% in the age group over 85 years [3-5].

The recent temporary recommendations of the Ministry of Health of the Russian Federation on the treatment of COVID-19 [2] when selecting schemes for anticoagulant prophylaxis and therapy emphasized monitoring hemostasis indicators. From hematological parameters, the recommendations relate only to the number of platelets, since

thrombocytopenia is a risk factor for bleeding and an indication for reducing or even discontinuing anticoagulant doses. At the same time, moderate and severe cases of COVID-19 are accompanied by significant changes in hematological parameters such as the number of leukocytes and the ratio of platelets and lymphocytes, which reflect systemic inflammation and can have a prognostic value, pre-determining the disease course and outcome [6–9].

Along with changes in white blood, COVID-19 is accompanied by anemia and qualitative composition changes of erythrocytes, reflecting hemolysis and hematopoietic disorders in response to the overproduction of inflammatory mediators [10]. COVID-19 is often accompanied by coagulopathies that are associated with a high frequency of venous and arterial macrothrombosis and microthrombosis [11,12]. In the development of coagulopathy and thrombosis, an important role is played by blood and endothelial cells, which can induce intravascular activation of the hemostasis system and maintain a high thrombotic potential, expressing and secreting procoagulants and forming a matrix for the assembly of enzyme complexes of the coagulation cascade [13]. Blood clotting disorders in combination with systemic vasculitis are becoming the main factors that determine the development of multiple organ failure in patients with severe complications of COVID-19 and a high risk of death [14].

These and other data indicate the importance of quantitative and qualitative changes in blood cells in COVID-19, which determine the most important and diverse pathophysiological processes that determine the clinical picture of the disease and its course. Monitoring hematological parameters and understanding the mechanisms of changes in the cellular composition of the blood in patients with COVID-19 can help determine the prognosis and treatment choice to improve the disease outcome.

Aim. This study aimed to establish the relationship of hematological disorders with the pathogenesis, course, and outcomes of COVID-19.

Materials and methods. *Characterization of clinical material.* The study was conducted on patients with a confirmed diagnosis of COVID-19 who were admitted to a COVID hospital at the City Clinical Hospital No. 16 in Kazan. The study was performed with the written consent of the patients and the permission of the Ethics Committee of the Kazan (Volga Region) Federal University (extract from Protocol No. 27 dated December 28, 2020).

The study involved 235 patients aged 33 to 92 years (mean age 68 years), including 107 (46%) men and 128 (54%) women. Patient inclusion criteria for the study are as follows:

- age over 18 years;
- diagnosis of COVID-19, confirmed by polymerase chain reaction (test for ribonucleic acid of the Severe acute respiratory syndrome coronavirus 2);
- moderate or severe condition [2];
- the presence of infiltrates in the lungs, characteristic of viral pneumonia, according to computed tomography.

Criteria for excluding patients in the study are as follows: age under 18 and the presence of other infections (hepatitis B and C and human immunodeficiency virus infection).

All patients received treatment following the temporary recommendations of the Ministry of Health of the Russian Federation [2], which included etiotropic, pathogenetic (including immunosuppressive and anticoagulant), and symptomatic therapy.

Most patients (90%) received low molecular weight heparins (LMWHs), according to one of the following regimens:

- 1) standard prophylactic dose,
- 2) high prophylactic (intermediate) dose, and
- 3) therapeutic dose [15].

Almost all hospitalized patients were prescribed standard prophylactic doses of LMWH based on body weight [15]. (Patients with severe thrombocytopenia due to concomitant hematologic malignancy did not receive LMWH.) At progressive hypercoagulability, determined by increasing D-dimer and fibrinogen levels, the LMWH doses were increased to high prophylactic (at values for fibrinogen ≤ 6.5 g/L and/or D-dimer ≤ 1500 ng/mL) or therapeutic (fibrinogen > 6.5 g/L and/or D-dimer > 1500 ng/mL).

Blood sampling was conducted on an empty stomach before the next LMWH administration, that is, 24 h after the last subcutaneous injection with a single daily administration or 12 h after the last injection with a double daily administration, due to the persisting residual effect of LMWH [16].

The general clinical characteristics of the examined patients are presented in Table 1. The control group consisted of 50 healthy donors, of which 23 (46%) were men, and 27 (54%) were women aged from 24 to 87 years (average age 65 years). Thus, the main and control groups were comparable in terms of age and sex.

Obtaining blood and its components. Blood from the antecubital vein was collected in a vacutainer with 3.2% sodium citrate (S-Monovette, Germany), mixing in a ratio of 9:1 by volume. The first citrated blood sample was used to study the contraction of the blood clot. The second blood sample was centrifuged at 200 g for 10 min at

Table 1. Demographic and clinical characteristics of patients with coronavirus disease included in the study

Demographic and clinical characteristics		Number of patients (n = 235), abs. (%)
Sex	Male	107 (46)
	Female	128 (54)
Age, years old	<65	84 (36)
	≥65	151 (64)
Disease severity	Moderate	177 (75)
	Severe	58 (25)
The degree of lung damage (according to computed tomography)	I	47 (20)
	I-II	111 (47)
	III-IV	77 (33)
Disease duration at the moment of examination	<7 days from disease onset	18 (8)
	7–14 days from disease onset	103 (44)
	>14 days from disease onset	114 (49)
Disease outcome	Favorable	217 (92)
	Fatal	18 (8)
Concomitant diseases and risk factors for thrombosis		
Hematological malignancies		7 (3)
Essential hypertension		131 (56)
Diabetes mellitus		56 (24)
Coronary heart disease		44 (18)
Acute myocardial infarction		18 (8)
Acute cerebrovascular accidents		26 (11)
Thrombophlebitis of the vessels of the lower extremities		10 (4)
Smoking		16 (7)
Obesity (body mass index >30 kg/m ²)		78 (33)
Treatment		
Low molecular weight heparin (doses according to [15])	Preventive dose	100 (42)
	High preventive and therapeutic doses	118 (48)
Glucocorticoids (dexamethasone, methylprednisolone, and prednisolone)		221 (94)
Interleukin-6 inhibitors (tocilizumab)		7 (3)
Antiviral drugs (ingavirin and arbidol)		60 (26)
Mucolytics (acetylcysteine and ambroxol)		120 (51)
Hydroxychloroquine (plaquenil)		82 (35)
Artificial pulmonary ventilation		13 (6)

room temperature; platelet-rich plasma was collected and used for flow cytometry and scanning electron microscopy of platelets. A third blood sample was stabilized with K3-EDTA (final concentration 1.6 mg/mL) in S-Monovette vacutainers and used for hematological analyses. Stabilized blood was stored and processed at room temperature and used no later than 4 h after collection.

Study of the contraction of blood clots. Determination of the kinetics and contraction degree (retraction and compression) of a blood clot was conducted

in 215 patients with COVID-19 and 40 healthy donors using an original method based on dynamic registration of the clot size [17]. Before determination, a 12 × 7 × 1 mm two-channel plastic measuring cuvette was rinsed with 4% Triton X-100 solution in 150 mM NaCl to prevent fibrin adhesion to the walls of the cuvette and allow clot compression.

In a plastic test tube, CaCl₂ (final concentration 2 mM) and freshly thawed human thrombin

(final concentration 1 U/mL) were sequentially added to citrated blood to initiate blood coagulation and platelet activation. After that, 80 μ L of the activated sample was quickly transferred into a transparent measuring cuvette and preheated to 37°C in a thermostatically controlled chamber of the Thrombodynamics Registrar (OOO GemaKor, Moscow).

Photo registration of the size of the blood clot was performed automatically every 15 s for 20 min after the addition of thrombin (Fig. 1A). Serial images of the clot were converted into a kinetic curve of contraction (Fig. 1B), from which the following parameters were automatically calculated:

- 1) the final degree of contraction—a value reflecting the degree of clot compression (in percent) relative to its initial size after 20 min of registration;
- 2) lag period—the time during which the clot reaches 95% of its original size;
- 3) the area under the kinetic curve, reflecting the mechanical work of platelets to compress the clot;
- 4) the average contraction rate—the degree of clot compression (in percent) per unit of time.

Hematological and immunochemical analysis. For microscopy, whole blood samples from a fingerstick of 65 patients were mixed with 3% EDTA (Trilon B) solution in a 1:3 ratio. A drop of the mixture was transferred onto a defatted glass slide, and a smear was made, which was dried, fixed in methanol (10 min), and stained according to Romanovsky–Giemsa. In each sample, 10 fields of view were evaluated at $\times 100$ and $\times 400$ magnifications. Blood cell counting in 235 patients and 50 healthy donors was performed on a BC-3600 hematology analyzer (Mindray, China). The interleukin level in the blood serum of 78 patients with COVID-19 who had laboratory signs of inflammation was determined on a StatFax 2100 device using kits from Vector-Best (Russia).

Platelet flow cytometry. The functional state of platelets was assessed by flow cytometry in a randomly selected 20 patients with COVID-19 and 10 healthy donors by the expression of P-selectin (CD62p) and active integrin α IIb β 3 before and after stimulation with a peptide (TRAP-6) that activates the thrombin receptor. For this, a sample of platelet-rich plasma containing 400,000 cells was incubated for 10 min with monoclonal antibodies against CD62p labeled with phycoerythrin (0.045 μ g/mL) and with PAC-1 antibodies against the active form of integrin α IIb β 3 labeled with fluorescein-5-isothiocyanate (1 μ g/mL).

Peptide platelet activator TRAP-6 (50 μ M), mimicking the action of thrombin, was added to platelet-rich plasma and incubated for 3 and 10 min at room temperature, after which platelets

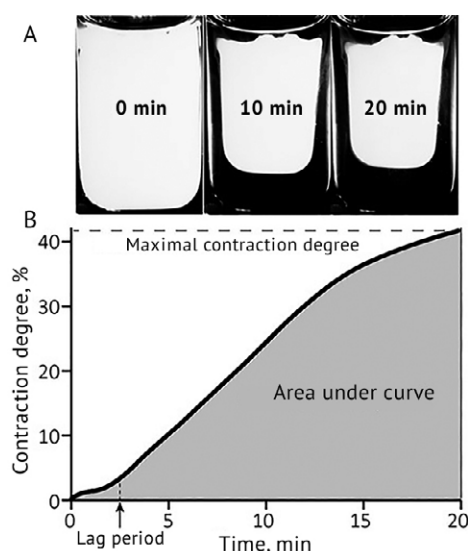


Fig. 1. Optical system for measuring the kinetics of blood clot contraction: (A) examples of images of a clot at different contraction stages; (B) typical kinetic curve of blood clot contraction and its main parameters

were analyzed using a CytoFLEX flow cytometer (Beckman Coulter, USA). Platelet gate was determined based on their size (forward light scatter) and granularity (lateral light scatter); 5000 platelets were counted in each sample. CytExpert 2.4 software (Beckman Coulter, USA) was used for data analysis.

Scanning electron microscopy of platelets. Samples of platelet-rich plasma from the blood of 34 patients with COVID-19 were diluted 10-fold with Tyrode's buffer (4 mM HEPES; 135 mM NaCl; 2.7 mM KCl; 2.4 mM $MgCl_2$; 3.3 mM NaH_2PO_4 ; 5.6 mM D-glucose; 0.3% bovine serum albumin, pH = 7.4), after which platelets were fixed with glutaraldehyde (2%) in physiological saline for 90 min at room temperature, followed by storage at 4°C. The cells were precipitated by centrifugation (500 g, 3 min) on a polycarbonate filter with a pore size of 0.4 μ m and treated with phosphate buffer (3 times, 5 min each) and 30%, 50% (5 min each), 70% (10 min each), 80%, 90%, 95% (5 min each), and 100% ethanol (3 times, 20 min each).

Then, the samples were placed in a 50% solution of hexamethyldisilazane (HMDS) in ethanol (5 min) and 100% HMDS (2 times, 7 min), after which they were air-dried at room temperature for 10–12 h. The dry samples were covered with a gold layer with palladium on Polaron e5100 or Quorum Q150T ES sputters, followed by scanning electron microscopy (Merlin microscope, Carl Zeiss, Germany).

Scanning electron microscopy of blood clots. Clots formed as a result of coagulation and contraction of 80 μ L of whole blood from 24

patients with COVID-19 with normal or reduced contraction were carefully removed from the cuvette of the Thrombodynamics Recorder without mechanical perturbation, washed 2 times in saline, and fixed in 2% glutaraldehyde in saline for 90 min at room temperature. The clots were cut in half and dehydrated, increasing the proportion of ethyl alcohol from 30% to 100%. Then, the alcohol was replaced by the addition of excess HMDS and dried. The samples, oriented with the slice up, were sprayed with a mixture of gold and palladium (Polaron e5100), and the internal content of the clumps was investigated on a Merlin scanning electron microscope.

Statistical analysis of the data obtained was conducted using the GraphPad Prism 8 (GraphPad Software, San Diego, USA) and RStudio software packages. The D'Agostino–Pearson, Kolmogorov–Smirnov, and Shapiro–Wilk tests were used to assess the normality of the sample distribution. Statistical differences for paired comparisons were assessed using the Mann–Whitney nonparametric test. For multivariate analysis, the Kruskal–Wallis test was used, followed by a pairwise comparison using the Dunn test. Rank correlation analysis was performed using Spearman's method. The χ^2 test was used to compare categorical variables. When assessing the reliability, a statistical significance level of 95% ($p < 0.05$) was used.

Results and discussion. *Changes in the hemogram and morphology of blood cells in COVID-19.* Hematological analysis in patients with COVID-19 revealed qualitative and quantitative changes in blood cells of all types.

Among the erythroid cells, the most frequent abnormalities were microanisocytosis (44%), poikilocytosis (30%), and reticulocytosis (6.3%). In combination with Jolly bodies, megaloblasts, normoblasts, and sideroblasts, as well as a decrease in the total erythrocyte number and hemoglobin levels (Table 2), these changes indicate hemolytic anemia and regenerative activation of the bone marrow with incomplete erythropoiesis. This is also evidenced by an increase in the average volume of erythrocytes in combination with hypochromia of erythrocytes (a decrease in the average content and average concentration of hemoglobin in erythrocytes; Table 2). The development of hemolytic anemia not only aggravates hypoxia and ischemia of organs and tissues but also contributes to the progression of hypercoagulability [18]; therefore, timely correction of the anemic syndrome will reduce the risk of thrombotic complications.

Attention is drawn to disorders of granulocytic myelopoiesis, which are manifested by absolute and relative neutrophilia (see Table 2). These changes

indicate systemic inflammation and high inflammatory cytokine levels that stimulate granulocytic myelopoiesis. Leukocytosis was solely due to an increase in the number of neutrophils with an insignificant absolute monocytosis and a normal content of basophils and eosinophils (Table 2). The number of lymphocytes in the blood in the vast majority of patients was reduced; mean lymphopenia, both absolute and relative, was moderate but significant, indicating suppression of adaptive immunity in COVID-19 (Table 2).

Patients with COVID-19 showed a significant decrease in the number of platelets in the blood than the control group, although the median was within the reference values (Table 2). Thrombocytopenia was found in 19% of patients, of which half was moderate ($100\text{--}150 \times 10^9/\text{L}$), and the other half was severe (less than $100 \times 10^9/\text{L}$). Thrombocytosis $>400 \times 10^9/\text{L}$ was registered in 7% of patients. Because of thrombocytopenia, in isolated cases, megakaryocytes were present in blood smears, which indicates compensatory and/or inflammatory activation of megakaryocytopoiesis.

Impaired contraction of blood clots in patients with COVID-19. To assess the functional platelets' state and general thrombotic potential, we developed a test to determine the kinetics of blood clot contraction, which is increasingly used in various types of pathology [17, 19–22]. This test is based on measuring the completeness and rate of contraction of blood clots under the action of activated platelets and depends on the number and quality of platelets and the cellular protein composition of the blood [17]. When comparing the parameters of the kinetics of blood clot contraction in the group of patients and the control group with COVID-19, a pronounced significant inhibition of all stages of the contraction process was found: a decrease in the average degree and rate of contraction and the area under the kinetic curve, and an increase in the lag period (Fig. 2 and Table 3A).

Clinical analysis revealed an association of altered blood clot contraction with the severity and outcome of the disease. In the severe course of the disease, a significant inhibition of the contraction of blood clots was observed in all parameters than in the moderate form (Table 3B).

In addition, in the most severe category of patients on mechanical ventilation, the lag period of contraction was slowed down (median 240 s, interquartile range [IQR] 195; 465) than in those without mechanical ventilation (median 180 s, IQR 150; 210; $p < 0.01$).

In accordance with the revealed dependence on the severity of the disease in the subgroup of patients with a lethal outcome, significant suppres-

Table 2. Hematological parameters in patients with COVID-19 compared with healthy donors

Parameters (reference values)	Patients with COVID-19 ($n = 235$)	Healthy donors ($n = 50$)
Hemoglobin (110–160), g/L	126 (110; 137)**	146 (135; 153)
Hematocrit (37–54), %	39 (34; 43)	41 (36; 43)
Erythrocyte sedimentation rate (2–10), mm/h	32 (18; 45)**	7 (5; 12)
Red blood cells (3.9–4.7), $\times 10^{12}/L$	4,3 (3,8; 4,7)*	4,6 (4,3; 4,9)
Mean cell volume (80–100), fL	89 (86; 93)**	86 (84; 88)
Mean cell hemoglobin (27–34), pg	28,9 (27,6; 29,8)**	31,7 (29,8; 33,7)
Red blood cell distribution width (11–16), %	13,9 (13,4; 15,2)	13,9 (13,4; 14,6)
Mean cell hemoglobin concentration (320–360), g/L	321 (315; 326)**	366 (352; 386)
Platelet (180–360), $\times 10^9/L$	225 (158; 292)*	247 (220; 300)
Mean platelet volume (6.5–12.0), fL	9,7 (9,0; 10,8)**	8,1 (7,5; 8,8)
Plateletcrit (0.108–0.282), %	0,228 (0,179; 0,287)	0,260 (0,200; 0,305)
White blood cells (4–10), $\times 10^9/L$	7,5 (5,2; 11,1)**	5,1 (4,2; 6,2)
Neutrophils (46–72), %	74,0 (64,7; 83,0)**	58,3 (54,6; 64,5)
Neutrophils (2–7), $\times 10^9/L$	5,2 (3,4; 8,3)**	2,9 (2,2; 3,6)
Monocytes (3–11), %	5,2 (4,0; 8,0)	6,0 (5,1; 6,9)
Monocytes (0.1–1.5), $\times 10^9/L$	0,4 (0,2; 0,7)*	0,3 (0,2; 0,4)
Lymphocytes (18–38), %	17 (10; 24)**	35 (29; 39)
Lymphocytes (0.8–4.0), $\times 10^9/L$	1,1 (0,7; 1,7)**	1,7 (1,4; 2,3)
Eosinophils (0.5–5), %	1,0 (0; 3,0)	1,0 (0,8; 1,2)
Eosinophils (0.02–0.30), $\times 10^9/L$	0,10 (0; 0,19)	0,10 (0,07; 0,17)
Basophils (0–1), %	0	0,6 (0,3; 0,7)
Basophils (0–0.07), $\times 10^9/L$	0	0,04 (0,03; 0,05)

Note: Results are presented as median and interquartile range (25th and 75th percentiles). * $p < 0.01$. ** $p < 0.0001$, Mann–Whitney test. COVID-19, coronavirus disease.

sion of contraction was recorded in all parameters than in the subgroup of patients with a favorable outcome (Table 3C). The revealed relationship between blood clot contraction indicators and COVID-19 severity and outcomes indicates the pathogenetic role of impaired contraction and the possible prognostic and diagnostic values of the blood clot contraction kinetic test.

In addition to the underlying disease, the rate and completeness of blood clot contraction depended on concomitant pathology. In particular, in patients with COVID-19 and concomitant hemoblastoses, the degree of contraction and the area under the kinetic curve were significantly reduced, and the lag period was lengthened (Table 3D). Similar changes in the parameters of clot contraction were present because of concomitant cardiovascular diseases, such as ischemic heart disease (Table 3E) and acute cerebrovascular accident history (Table 3F).

These results confirm the previously published data on the inhibition of blood clot contraction in venous thromboembolic complications [19] and

acute ischemic stroke [20]. It should be noted that in male patients with COVID-19, the degree and rate of contraction and the area under the curve are significantly reduced than in female patients (Table 3G), which reflects a more severe course of the disease in men [23,24].

Relationship between the parameters of contraction of blood clots with the cellular and protein composition of the blood in COVID-19. To identify the causes of impaired contraction of blood clots in patients with COVID-19, the contraction parameters were compared with other laboratory test results. It is known that the main causes of impaired contraction of blood clots are thrombocytopenia and platelet dysfunction in combination with pathological changes in blood composition [17]. The dependence of the degree of contraction of blood clots on the number of platelets is clearly shown in Fig. 3. A decrease in the degree, average velocity, and area under the curve of contraction, in comparison with the normal platelet level, occurs even with moderate thrombocytopenia ($100\text{--}150 \times 10^9/L$). However, when the number

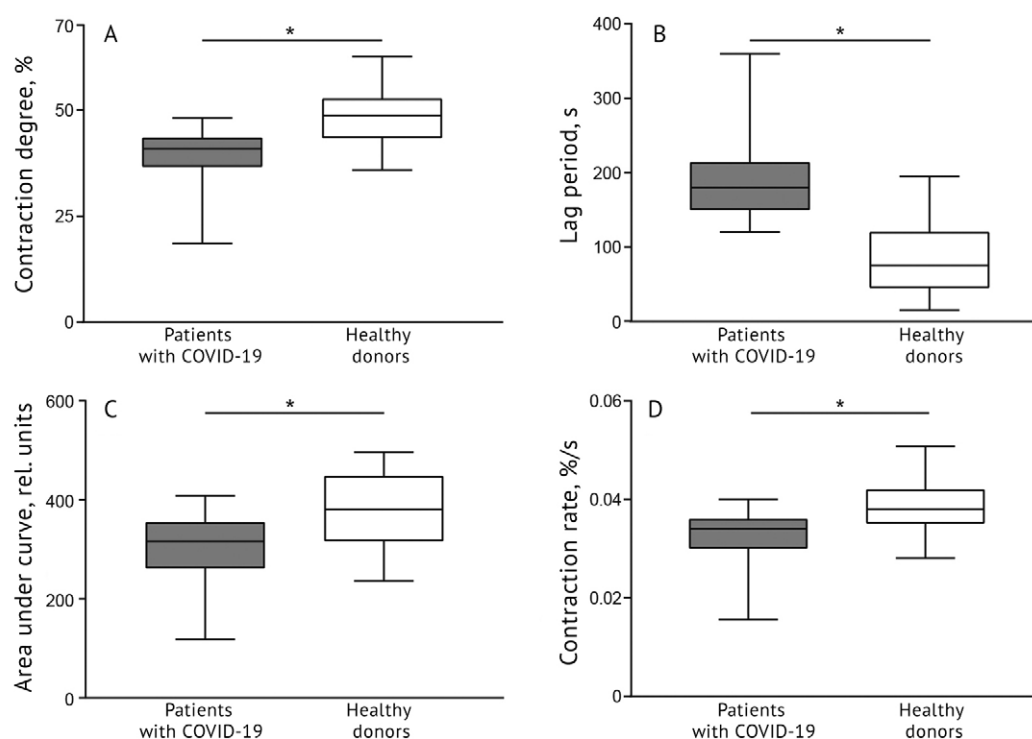


Fig. 2. Contraction parameters of blood clots of patients with coronavirus disease (COVID-19; $n = 215$) and healthy donors ($n = 40$): (A) degree of contraction; (B) lag period; (C) the area under the curve; (D) average speed. Results are presented as median and interquartile range (25th and 75th percentiles). * $p < 0.001$, Mann–Whitney test.

of platelets falls to $50 \times 10^9/L$ and below, there is progressive deceleration and almost complete suppression of contraction. These data confirm the key role of platelet contractile function in the contraction of blood clots and the important pathogenetic role of thrombocytopenia and possibly thrombocytopathy in COVID-19.

Correlation analysis of indicators of blood clot contraction and hemocoagulation tests revealed weak or moderate but significant relationships. The degree of contraction positively correlated with the number of platelets ($r = 0.25$, $p < 0.001$) and also negatively correlated with the mean volume of erythrocytes ($r = -0.24$, $p < 0.05$) and mean content of hemoglobin ($r = -0.29$, $p < 0.01$). Platelet count and thrombocrit were positively correlated with the area under the kinetic curve ($r = 0.31$, $p < 0.001$ and $r = 0.22$, $p < 0.05$, respectively) and contraction rate ($r = 0.25$, $p < 0.001$ and $r = 0.24$, $p < 0.05$, respectively). The lag period was inversely related to the number of platelets ($r = -0.26$, $p < 0.001$) and positively correlated with prothrombin time ($r = 0.35$, $p < 0.01$). The area under the kinetic curve negatively correlated with prothrombin time ($r = -0.33$, $p < 0.01$). The average contraction rate correlated negatively with the average erythrocyte volume ($r = -0.22$, $p < 0.05$) and the average hemoglobin content ($r = -0.25$, $p < 0.01$). In addition to correla-

tions, the lag periods were 203 s (IQR 165; 251) and 180 s (IQR 150; 210) at hemoglobin levels <110 and >110 g/L, respectively ($p < 0.01$).

The relationships between these different parameters indicate that the reasons for the change in the kinetics of contraction of blood clots include, but are not limited to, the number and functional state of platelets. The association of impaired blood clot contraction with the average erythrocyte volume and hemoglobin content in erythrocytes suggests that changes in “red” blood should also be considered when assessing the risk of thrombotic complications and thromboprophylaxis in patients with COVID-19.

It is important to note that the degree of contraction of blood clots was associated with the D-dimer level in the blood: with inhibited contraction (degree of contraction $< 41\%$), the proportion of patients with an increased D-dimer level (>1000 ng/mL) was significantly higher than in the subgroup patients with normal contraction ($\geq 41\%$; $p = 0.047$, χ^2 test). This result confirms the association of impaired contraction of blood clots with intravascular fibrin deposition since the lysis of which becomes a source of D-dimer.

These patterns indicate the pathogenetic relationship of impaired contraction of blood clots with changes in the cellular and protein compositions of

Table 3. Parameters of blood clot contraction in patients with COVID-19

Comparison groups and subgroups	Параметры контракции			
	Contraction degree, %	Lag period, s	Area under curve, rel. units	Mean rate, %/s $\times 10^{-3}$
A. Patients with COVID-19 and healthy donors				
Patients ($n = 215$)	41 (37; 43)***	180 (150; 213)***	315 (261; 354)***	34 (31; 36)***
Donors ($n = 40$)	48 (43; 52)	75 (45; 120)	380 (316; 447)	38 (35; 42)
B. Dependence on the severity of the disease				
Moderate form ($n = 149$)	41 (38; 44)*	180 (150; 195)**	322 (280; 358)**	34 (32; 36)*
Severe form ($n = 51$)	39 (33; 43)	210 (165; 255)	298 (220; 337)	32 (27; 35)
C. Dependence on the disease outcome				
Favorable outcome ($n = 184$)	41 (38; 44)	180 (150; 210)	317 (271; 357)	34 (31; 36)
Fatal outcome ($n = 16$)	35 (27; 42)*	255 (195; 349)***	238 (156; 320)**	30 (22; 35)**
D. Dependence on the presence of concomitant hematological malignancies				
Hematological malignancies present ($n = 8$)	28 (17; 42)*	218 (150; 251)	200 (117; 304)**	23 (14; 34)
No hematological malignancies ($n = 207$)	41 (37; 44)	180 (150; 210)	316 (270; 357)	34 (31; 36)
E. Dependence on the presence of concomitant CHD				
CHD present ($n = 41$)	39 (33; 41)**	195 (165; 240)*	298 (227; 335)*	32 (29; 35)*
No CHD ($n = 159$)	41 (38; 44)	180 (150; 210)	324 (276; 361)	34 (32; 36)
F. Dependence on the presence of concomitant ACA				
ACA present ($n = 23$)	36 (27; 43)*	225 (165; 285)**	272 (152; 318)*	30 (22; 36)*
No ACA ($n = 162$)	41 (38; 44)	180 (150; 210)	323 (279; 358)	34 (31; 36)
G. Dependence on sex				
Female ($n = 115$)	42 (39; 45)***	180 (150; 225)	323 (291; 371)**	34 (32; 37)***
Male ($n = 100$)	39 (35; 42)	180 (150; 210)	306 (253; 341)	33 (29; 35)

Results are presented as median and interquartile range (25th and 75th percentiles). * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$, Mann-Whitney test. COVID-19, coronavirus disease; CHD, coronary heart disease; ACA, acute cerebrovascular accidents.

the blood, which confirms the informative value of the contraction kinetic test in COVID-19.

The structure and composition of blood clots in patients with COVID-19. According to scanning electron microscopy of blood clots, the main structural elements are fibrin fibers and erythrocytes. Since the clots undergo compression (contraction or retraction), erythrocytes, depending on the degree of their compression deformation, are conventionally divided into four types as follows [25]:

- 1) biconcave (original and undeformed);
- 2) partially deformed, but close to the original ("intermediate biconcave");
- 3) more deformed, acquiring the characteristics of polyhedra ("intermediate polyhedral");
- 4) maximally deformed to the stage of polyhedra, or polyhedra (polyhedrocytes).

The formation of polyhedrocytes is due to their compression under the action of the force generated by the fibrin-platelet complex. A common phy-

siological consequence of the compact packing of deformed red blood cells is that it makes the clot dense and impermeable, including for pathogens and fibrinolytic enzymes.

The structure and composition of blood clots in patients with COVID-19, despite the similarity of the qualitative composition, varied greatly depending on the ability of the clots to contract. Structural differences are most clearly seen when comparing clots with the lowest (13%) and highest (49%) degrees of contraction (Fig. 4).

In the first case (Fig. 4A), the clot is characterized by higher porosity, higher fibrin content, and an almost complete absence of deformed cells (polyhedrocytes and intermediate polyhedral cells close to them) while maintaining the fraction of biconcave erythrocytes. The pathophysiological consequences of the porosity of blood clots and thrombi may be associated with their reduced hemostatic properties due to increased permeability

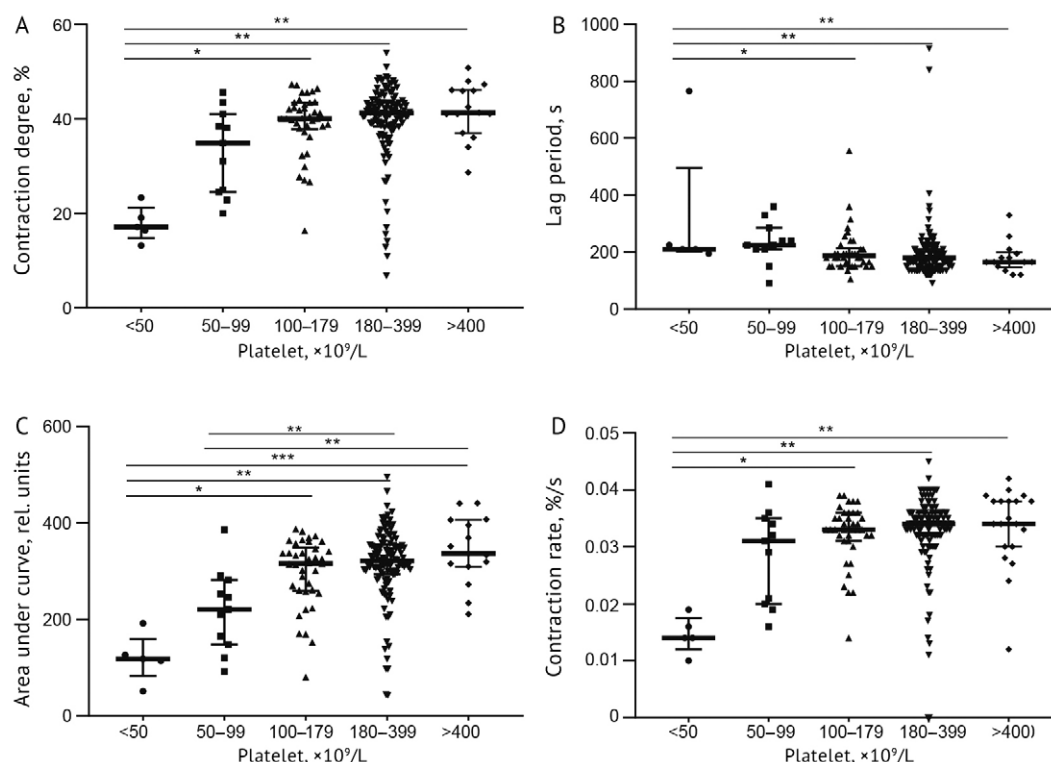


Fig. 3. Dependence of the contraction parameters of blood clots in patients with coronavirus disease on the number of platelets: (A) degree of contraction; (B) lag period; (C) the area under the curve; (D) average speed. Results are presented as median and interquartile range (25th and 75th percentiles). * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$. Kruskal–Wallis method followed by Dunn’s test was used for paired comparisons.

to blood and its components. In other words, such clots cannot effectively prevent hemorrhagic diathesis.

At a high degree of compression (Fig. 4B), the clot becomes dense, without intercellular pores, with a high content of polyhedrocytes in the complete absence of biconcave erythrocytes. It is important to note that complete contraction of the clot leads to a redistribution of fibrin, which predominates at the edge of the clot and is almost completely absent in the central part, as previously described [26].

These differences acquire the character of a general pattern when comparing the quantitative composition of clots from the blood of patients with COVID-19 with a normal ($\geq 41\%$) and reduced ($< 41\%$) degree of contraction (Table 4). With full contraction, erythrocytes with signs of compression deformity predominated, fibrin accumulated on the periphery of the clot, and porosity was reduced. Conversely, with impaired clot contraction, the proportion of deformed (multifaceted) erythrocytes was low, the porosity was relatively high, and fibrin was present in all parts of the clot without a tendency to redistribute it to the periphery.

Considering that a decrease in the degree of contraction relative to the norm was found in 53%

of the examined patients with COVID-19 and that this violation was statistically significantly combined with a more severe course and an unfavorable outcome of the disease (Table 3), the morphological data obtained can explain this pathogenetic relationship if we extrapolate them to intravital microthrombi and macrothrombi.

First, the compaction of the intercellular spaces and the compactness and compression of the thrombus due to contraction reduce the occlusion of the vessel and improve local blood flow. Conversely, in violation of contraction, thrombotic obstruction of the vessels is more pronounced, which aggravates ischemia [20].

Second, contracted clots and thrombi with a high density of fibrin fibers and an increased local concentration of fibrinolytic are lysed from the inside faster in contrast to loose, poorly contracted clots, which have a low sensitivity to internal fibrinolysis and, therefore, last longer [27].

Third, the degree of contraction determines the mechanical stability of thrombi, that is, the tendency to embolization. Thus, thrombi with a lower degree of contraction are potentially more embologic [19], which may explain the high incidence of pulmonary embolism and cardioembolic ischemic strokes in COVID-19 [14,28,29].

Table 4. Comparative characteristics of the structure and composition of blood clots in patients with COVID-19 with reduced (<41%) and normal (≥41%) degrees of contraction

Studied structures	Contraction < 41% (<i>n</i> = 17)	Contraction ≥ 41% (<i>n</i> = 7)
Volume fraction of the clot occupied by each type of structural elements		
Red blood cells	70% (60; 80)**	90% (85; 94)
Fibrin	15% (10; 20)**	5% (5; 10)
Pores	15% (10; 20)**	5% (3; 10)
The relative number of erythrocytes with an ascending degree of compression		
Biconcave erythrocytes (original, no compression)	10% (10; 25)***	0
Intermediate biconcave erythrocytes	35% (30; 45)**	10% (3; 20)
Intermediate polyhedral erythrocytes	30% (20; 40)	20% (10; 30)
Erythrocytes in the form of a polyhedron (polyhedrocytes)	15% (10; 20)***	70% (40; 80)

Note: Results are presented as median and interquartile range (25th and 75th percentiles). * $p < 0.05$. ** $p < 0.01$. *** $p < 0.001$, Mann–Whitney test.

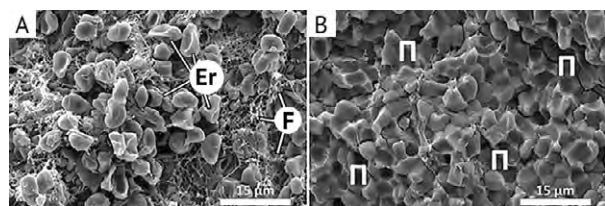


Fig. 4. Representative micrographs of blood clots in patients with coronavirus disease with (A) low (13%) and (B) high (49%) degrees of contraction, obtained using scanning electron microscopy: (A) clot with high porosity, high fibrin content (F) and fraction preservation of biconcave erythrocytes (Er); (B) a dense clot, without intercellular pores, with a high content of polyhedral erythrocytes—polyhedrocytes (II).

Thus, the structure and composition of blood clots form the structural basis for the pathophysiological consequences of impaired contraction of blood clots and blood clots in patients with COVID-19.

Changes in the functional state of platelets in COVID-19. Relative thrombocytopenia was combined with moderate changes in the functional state of platelets, determined by the expression of activation markers (P-selectin and active integrin $\alpha\text{IIb}\beta 3$), secretion of soluble sP-selectin, and morphological features (scanning electron microscopy).

According to flow cytometry data, an average of twofold background (“spontaneous”) platelet activation was found in the ability to express P-selectin on the surface of the plasma membrane in the blood of patients with COVID-19 (median 2.3%, IQR 1.2; 3.0) compared with healthy donors (median 1.1%, IQR 0.6; 1.8; $p < 0.05$). After a 3-min incubation with an activator (TRAP-6), the median proportion of P-selectin-expressing platelets in patients with COVID-19 was 85% (IQR 77; 87), 12% higher than in healthy donors (median 73%,

IQR 65; 81; $p < 0.05$). However, this significant difference leveled out by 10 min after activation.

At the same time, after 10 min of incubating platelets with TRAP-6 in patients with COVID-19, there was a slight but significant decrease in the expression level of active integrin $\alpha\text{IIb}\beta 3$: the proportions of activated platelets were 91% (IQR 89; 94) in patients with COVID-19 and 95% (IQR 93; 96) in healthy donors ($p < 0.01$).

These multidirectional changes indicate a not very pronounced but reliable activation of circulating platelets with simultaneous signs of dysfunction, although they did not reach the degree of exhaustion and refractoriness.

The expression of P-selectin on the outer membrane is accompanied by its secretion; therefore, an increased soluble sP-selectin level in the blood also serves as a biochemical marker of platelet activation [30]. Patients with COVID-19 were characterized by a significant increase in the concentration of sP-selectin (median 81 ng/mL, IQR 55; 104) relative to healthy donors (median 55 ng/mL, IQR 31; 70; $p < 0.001$). It is important to emphasize that the sP-selectin level is directly correlated with the inflammatory cell contents in the blood: neutrophils ($r = 0.53$; $p < 0.0001$), lymphocytes ($r = 0.29$; $p = 0.014$), and monocytes ($r = 0.25$; $p = 0.045$).

In addition, a high content of sP-selectin (median: 82.5 ng/mL; IQR 64.6; 111.6) was combined with hyperproduction of the inflammatory cytokine interleukin-8 (>35 pg/mL), whereas at a lower concentration (<35 pg/mL), the content of sP-selectin in the blood was significantly lower (median 58.2; IQR 50.2; 87.5; $p = 0.029$). A related but important observation confirming the importance of inflammatory cytokines in the development of respiratory syndrome in COVID-19: in patients with interleu-

kin-8 levels >35 pg/mL, the blood oxygen saturation level (median: 94%; IQR 92; 96) was significantly lower than in patients with interleukin-8 levels < 35 pg/mL (median: 96%; IQR 93; 97; $p = 0.038$).

Scanning electron microscopy of platelets isolated from the blood of 34 randomly selected COVID-19 patients also revealed morphological signs of moderate spontaneous platelet activation in all patients examined. Platelet activation manifested itself in the form of a loss of the disc-shaped body of the platelet, a decrease in the diameter of the platelet body, and the formation of multiple filopodia (Fig. 5A). Despite the presence of activated platelets, most of the cells retained their discoid shape in the absence or presence of one or two short filopodia, which is typical of resting nonactivated platelets (Fig. 5B).

These results indicate that platelets are involved in the pathogenesis of COVID-19. This is manifested in both quantitative (relative thrombocytopenia) and qualitative (expression and secretion of P-selectin and decreased expression of active integrin $\alpha\text{IIb}\beta 3$ with morphological signs of cell activation) changes.

Platelet dysfunction in combination with impaired contraction of blood clots and hypercoagulation according to routine coagulogram data, which develop due to prophylactic and therapeutic doses of LMWH, indicates persistent intravascular activation of blood coagulation in patients with COVID-19 and requires the search for additional methods for its correction. We can talk about both the earlier appointment, along with anticoagulants and glucocorticoids, which have an indirect antithrombotic effect, and antiplatelet agent use, especially in patients with cardiovascular diseases and arterial thrombosis in history. The presence of a correlation between platelet activation and inflammatory changes (neutrophilia, monocytosis, and cytokine hyperproduction) allows us to consider inflammation as a risk factor for thrombotic complications and serves as an additional basis for immunosuppressive therapy.

Conclusions. We studied the hemogram and morphology of blood cells, as well as the functional state of platelets in the acute period of COVID-19 in comparison with the clinical picture and laboratory parameters of inflammation due to anticoagulant and immunosuppressive therapy. In most of the examined patients, along with signs of anemia, neutrophilia, and lymphopenia, immature forms of erythrocytes and leukocytes are present in the blood, which indicates regenerative and/or inflammatory activation of hematopoiesis.

For an integral assessment of the number and quality of platelets, we used the test of the kinetics

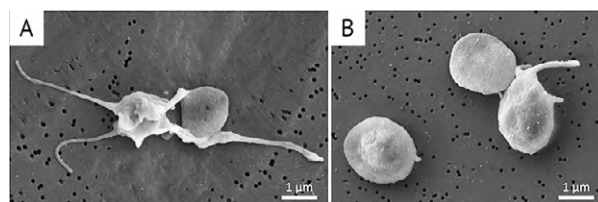


Fig. 5. Representative scanning electron micrographs of partially activated (A) and resting (B) platelets from the blood of patients with coronavirus disease.

of contraction (retraction) of blood clots, and the inhibition of which, according to the literature, is combined with a predisposition to thrombosis. In patients with COVID-19, compared with the control group, a pronounced inhibition of the contraction of blood clots was found, which directly correlates with the severity of the disease and likelihood of death. A decrease in the rate and completeness of contraction of blood clots is aggravated by the presence of concomitant diseases, including hematologic malignancy, ischemic heart disease, and acute cerebrovascular accidents.

In male patients with COVID-19, the parameters of contraction are significantly reduced than in female patients, indicating a higher risk of thrombotic complications in men. The degree of clot contraction directly correlates with the number of platelets in the blood and laboratory signs of anemia and coagulopathy, including the D-dimer level, which confirms the pathogenetic significance of contraction and informative value of the clot contraction kinetic test in COVID-19.

The impaired contraction of blood clots in patients with COVID-19 is based on a decrease in the total contractile potential of platelets against a background of moderate thrombocytopenia in combination with chronic activation and secondary dysfunction of platelets, detected by increased secretion of P-selectin, decreased expression of active integrin $\alpha\text{IIb}\beta 3$, and morphological signs of cell activation.

According to the data of scanning electron microscopy of blood clots, their structure and cellular composition depend on the completeness of contraction. With the incomplete contraction of clots, which was detected in 53% of the examined patients, clots are characterized by high porosity, low content of deformed polyhedral erythrocytes (polyhedrocytes), and uniform distribution of fibrin, whereas with full contraction, clot porosity is reduced, polyhedrocytes prevail, and fibrin accumulates at the clot periphery. These data reveal the structural mechanisms of the pathogenetic relationship of impaired contraction of blood clots with a high risk of thrombotic complications in COVID-19.

The results indicate that all blood cells are involved in the pathogenesis of COVID-19, which undergo both quantitative and qualitative changes. The predisposition to thrombotic complications in COVID-19 can be associated with the formation of uncompressed and noncompact blood clots, which increases thrombotic obstruction of blood vessels, decreases sensitivity to fibrinolysis, and enhances the embolism of intravital blood clots and thrombi.

The totality of the data obtained confirms the important pathogenetic role of blood cells in COVID-19 and substantiates the information content of laboratory monitoring of hematological parameters, including the kinetics of contraction of blood clots. It is important to note that, despite the use of anticoagulants, most of the examined patients with COVID-19 retain signs of activation of both the cellular and plasma components of the hemostatic system, which means that the risk of thrombotic complications remains.

Insufficient effect of heparin prophylaxis and heparin therapy contributes to persistent disorders of microcirculation, which predetermines a long course of the disease and can cause a transition to a more severe form, up to multiple organ failure and an unfavorable outcome. The reason for the ineffective anticoagulant and anti-inflammatory therapy in COVID-19 may be an underestimation of the cellular mechanisms of activation of the hemostasis system, especially in patients with concomitant cardiovascular diseases and complicated thrombotic history.

Understanding the important role of hematological disorders and laboratory monitoring can help determine the severity, prognosis, and choice of personalized drug treatment for patients with COVID-19 to improve the disease course and outcome.

CONCLUSIONS

1. Blood cells are involved in the pathogenesis of COVID-19, which undergo both quantitative and qualitative changes.

2. One of the prothrombotic mechanisms may be a violation of the contraction of blood clots.

3. The results obtained confirm the important pathogenetic role of blood cells in COVID-19 and substantiate the information content of laboratory monitoring of hematological parameters, including the kinetics of contraction of blood clots.

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S.I.S., N.G.E., A.D.P., R.R.Kh., and I.A.A.—writing the article.

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Conflict of interest. The authors declare that they have no conflicts of interest regarding the presented article.

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