Original Study | DOI: https://doi.org/10.17816/KMJ633994

Analysis of plasma hemostasis and the role of microvesicles in the coagulation process in patients with COVID-19

Elena S. Gracheva, Rufina R. Abdullina, Il'shat G. Mustafin, Diana I. Abdulganieva
Kazan State Medical University, Kazan, Russia

ABSTRACT

BACKGROUND: Coagulopathies in COVID-19 are an important aspect in the pathophysiological mechanisms, clinical picture of the disease, and occurrence of delayed complications.

AIM: To study plasma hemostasis using turbidimetry, thromboelastography, and the role of microvesicles in the coagulation process in patients with COVID-19.

MATERIAL AND METHODS: The study used blood samples from patients of the temporary infectious diseases hospital based on the State Autonomous Healthcare Institution "Republican Clinical Hospital of the Ministry of Health of the Republic of Tatarstan" in Kazan (n=213) in the period from June to August 2020. Patients were divided into two groups according to the severity of the disease: the first group — moderate COVID-19 (n=138), the second group — severe COVID-19 (n=75). Patients were treated according to the protocols of the Temporary Methodological Recommendations of the Ministry of Health of the Russian Federation, version 7. The blood of healthy donors (n=20) was used as a control group. Plasma hemostasis was assessed using dynamic turbidimetry (measured lag period — Lag, polymerization rate — V, maximum optical density at a given wavelength — A_{max}) and thromboelastography (determined coagulation activation time — R). Statistical processing of the results was performed using IBM SPSS Statistics 26.0. The groups were compared using the nonparametric Mann–Whitney U-test. Statistical processing of the results following standart normal distribution was performed using the Student's t-test. Differences were considered significant at $p < 0.05$.

RESULTS: Severe COVID-19 was characterized by an increase in the lag period (9.4±0.8 min relative to the control 6.2±1.2 min; $p < 0.0001$), a decrease in the polymerization rate (1.12±0.71 OD units/s relative to the control 3.93±2.3 OD units/s; $p < 0.0001$) and a decrease in the maximum optical density of the clot (0.576±0.17 OD units relative to the control 1.625±0.433 OD units; $p < 0.0001$). In moderate cases, a shortening of the lag period was noted (3.8±1.1 min relative to the control 6.2±1.2 min; $p=0.0004$), the maximum optical density of the clot was lower than the control (1.412±0.351 OD units at 1.625±0.433 OD units, respectively; $p=0.0007$). In patients with moderate disease severity, a 1.6-fold reduction in coagulation activation time was noted relative to the control group. In patients with severe disease, coagulation activation time was increased by 1.5 times relative to the control. After adding microvesicles to the samples, this parameter decreased by 2.12 times in patients with a moderate course of the disease (16.9±1.1 min and 8±0.6 min; $p < 0.0001$), and by 1.44 times in patients with a severe course of the disease (10.8±0.9 min and 7.5±0.5 min; $p < 0.0001$).

CONCLUSION: Moderate COVID-19 is characterized by signs of hypercoagulation, which can lead to the development of thrombotic complications; severe disease is accompanied by hypocoagulation, which contributes to hemorrhagic complications.

Keywords: COVID-19; fibrin clot polymerization; plasma hemostasis; microvesicles.

To cite this article:
Gracheva ES, Abdullina RR, Mustafin IG, Abdulganieva DI. Analysis of plasma hemostasis and the role of microvesicles in the coagulation process in patients with COVID-19. *Kazan Medical Journal.* 2024;105(6):917–925. doi: https://doi.org/10.17816/KMJ633994

Received: 01.07.2024 **Accepted:** 23.08.2024 **Published:** 07.11.2024

DOI: https://doi.org/10.17816/KMJ633994
Оригинальное исследование | УДК 616-092.11: 616.151.5: 578.1

Анализ плазменного гемостаза и роли микровезикул в процессе коагуляции у пациентов с COVID-19

Е.С. Грачева, Р.Р. Абдуллина, И.Г. Мустафин, Д.И. Абдулганиева
Казанский государственный медицинский университет, г. Казань, Россия

АННОТАЦИЯ

Актуальность. Коагулопатии при COVID-19 — важный аспект в патофизиологических механизмах, клинической картине заболевания, возникновении отсроченных осложнений.

Цель. Изучение плазменного гемостаза методом турбидиметрии, тромбоэластографии и роли микровезикул в процессе коагуляции у пациентов с COVID-19.

Материал и методы. В исследовании использовали образцы крови пациентов временного инфекционного госпиталя на базе ГАУЗ «РКБ МЗ РТ» г. Казани (n=213) в период с июня по август 2020 г. Пациенты разделены на две группы по степени тяжести заболевания: первая группа — среднетяжёлое течение COVID-19 (n=138), вторая группа — тяжёлое течение COVID-19 (n=75). Лечение пациентов проводили согласно протоколам Временных методических рекомендаций Министерства здравоохранения Российской Федерации, версия 7. В качестве контрольной группы использовали кровь здоровых доноров (n=20). Плазменный гемостаз оценивали методами динамической турбидиметрии (измеряли lag-период — Lag, скорость полимеризации — V, максимальную оптическую плотность при данной длине волны — A_{max}) и тромбоэластографии (определяли время активации коагуляции — R). Статистическую обработку результатов проводили с помощью IBM SPSS Statistics 26.0. Сравнение групп осуществляли с использованием непараметрического U-критерия Манна–Уитни. Статистическую обработку результатов, подчиняющихся закону нормального распределения, выполняли с использованием t-критерия Стьюдента. Достоверными считали различия при $p < 0,05$.

Результаты. Тяжёлое течение COVID-19 отличается удлинением lag-периода (9,4±0,8 мин относительно контроля 6,2±1,2 мин; $p < 0,0001$), снижением скорости полимеризации (1,12±0,71 ед. ОП/с относительно контроля 3,93±2,3 ед. ОП/с; $p < 0,0001$) и уменьшением максимальной оптической плотности сгустка (0,576±0,17 ед. ОП относительно контроля 1,625±0,433 ед. ОП; $p < 0,0001$). При среднетяжёлом течении отметили укорочение lag-периода (3,8±1,1 мин относительно контроля 6,2±1,2 мин; $p=0,0004$), максимальная оптическая плотность сгустка была ниже контроля (1,412±0,351 ед. ОП при 1,625±0,433 ед. ОП соответственно; $p=0,0007$). У пациентов со средней степенью тяжести заболевания отмечено сокращение времени активации коагуляции в 1,6 раза относительно контрольной группы. У пациентов с тяжёлым течением время активации коагуляции увеличено в 1,5 раза относительно контроля. После внесения в пробы микровезикул у пациентов со средним течением данный параметр сократился в 2,12 раза (16,9±1,1 мин и 8±0,6 мин; $p < 0,0001$), а у пациентов с тяжёлым течением в 1,44 раза (10,8±0,9 мин и 7,5±0,5 мин; $p < 0,0001$).

Вывод. Для среднетяжёлого течения COVID-19 характерны признаки гиперкоагуляции, что может привести к возникновению тромботических осложнений; тяжёлое течение заболевания сопровождается гипокоагуляцией, которая способствует геморрагическим осложнениям.

Ключевые слова: COVID-19; полимеризация фибринового сгустка; плазменный гемостаз; микровезикулы.

Как цитировать:
Грачева Е.С., Абдуллина Р.Р., Мустафин И.Г., Абдулганиева Д.И. Анализ плазменного гемостаза и роли микровезикул в процессе коагуляции у пациентов с COVID-19 // Казанский медицинский журнал. 2024. Т. 105, № 6. С. 917–925. doi: https://doi.org/10.17816/KMJ633994

Рукопись получена: 01.07.2024 **Рукопись одобрена:** 23.08.2024 **Опубликована:** 07.11.2024

BACKGROUND

Corona virus disease 2019 (COVID-19) manifests in different forms and varies in the degree of damage and organ and tissue involvement [1]. The World Health Organization lifted the pandemic status of COVID-19 on May 5, 2023. However, currently, COVID-19-associated complications continue to be a relevant issue, and how to prevent them remains unclear. COVID-19 can be virtually asymptomatic to extremely severe, with patients requiring mechanical ventilation [2].

Since the first months of studying the severe acute respiratory syndrome-related coronavirus-2 virus and the disease it causes, the blood coagulation system has been found to be actively involved in their pathogenesis [3]. Thrombotic complications, such as venous and arterial thrombosis, deep vein thrombosis, pulmonary embolism, arterial thromboembolism, microvascular thrombosis, and stroke, are the most common causes of death in COVID-19 patients [4]. Thrombotic complication incidence depends on the severity of the disease, with a higher prevalence in patients in the intensive care unit [5].

The dysfunction of the coagulation system in COVID-19 is crucial in pathophysiological mechanisms, the clinical presentation and, particularly, delayed complications [6]. The risk of thrombotic and hemorrhagic complications should be identified by accurately interpreting standard coagulation parameters and data from additional tests of the hemostasis system, namely, evaluation of fibrin clot formation by turbidimetry and plasma hemostasis by thromboelastography.

These methods provide beneficial information for selecting the appropriate treatment and adequate therapy. A comprehensive evaluation of coagulation disorders in patients with COVID-19 increases the probability of prescribing effective therapy.

The first studies that demonstrated the procoagulant properties of microvesicles (MVs) were published in the 1960–1970s [7, 8].

Published data show a significant impact on development of hypercoagulability in COVID-19, associated with MVs [9–11]. Hamali et al. found significantly increased levels of procoagulant MVs and tissue factor-bearing MVs in COVID-19 patients than in healthy controls ($p < 0.001$). Elevated levels of procoagulant MVs and tissue factor-bearing MVs may play a role in predicting COVID-19 severity and may be a new biomarker for assessing procoagulant activity [12]. MVs secreted by apoptotically stimulated cells from different cell lines (e.g., platelets, leukocytes, macrophages, etc.) have been shown to be reliable markers of vascular injury [13].

Guervilly et al. reported high levels of tissue factor-bearing extracellular MVs in individuals with severe COVID-19 [14]. COVID-19 patients are characterized by the presence of platelet and leukocyte MVs in the extracellular pool. Binding to other blood cells reduces the number of MVs released from endothelial cells. MVs are considered a marker of hemostasis activation, including the risk of thromboembolic complications in COVID-19 patients, with higher sensitivity compared to a well-established D-dimer test. The total number of extracellular MVs ($CD9^+$) in COVID-19 patients at hospital admission was comparable to that in patients with venous thromboembolism and those with post-embolic complications [15].

The impact of MVs on the coagulation system in various diseases indicates that they are actively involved in COVID-19. Therefore, it is critical to evaluate the effects of MVs on the coagulation system in patients with COVID-19.

The current study aimed to evaluate plasma hemostasis in vitro using turbidimetry and thromboelastography and the role of microvesicles in coagulation in patients with COVID-19 of varying severity.

MATERIALS AND METHODS

A retrospective clinical, randomized, case-control study was conducted. According to the type of values received, the study (reported) parameters (endpoints) were quantitative (quantity of MVs, thromboelastographic parameters, and dynamic turbidimetry).

Additionally, 4.5 mL blood samples were extracted from the ulnar vein of 213 patients admitted to the Temporary Infectious Diseases Hospital of the Republican Clinical Hospital of the Ministry of Health of the Republic of Tatarstan, Kazan, between June and August 2020 and were collected in a test tube with sodium citrate. Patients enrolled in this study were diagnosed with COVID-19 (U07.1), confirmed by polymerase chain reaction nasopharyngeal and oropharyngeal analysis. The control group included 20 healthy volunteers. Blood samples were obtained from the Blood Transfusion Department of the Republican Clinical Hospital of the Ministry of Health of the Republic of Tatarstan, Kazan, concurrently with the collection of study material.

The study was approved by the ethics committee of the Kazan State Medical University of the Ministry of Health of the Russian Federation (protocol no. 2, dated February 16, 2021). All patients signed an informed consent to participate in this study.

The patients were divided into two groups according to disease severity:

– Group 1: patients with moderate COVID-19, CT2–3,[1] who received enoxaparin sodium subcutaneously at 0.6 mg twice a day

– Group 2: patients with severe COVID-19, CT4, who received a heparin pump (sodium heparin) at 20,000 U and admitted to the intensive care unit

The mean age of the patients was 63 ± 10.3 years (range: 35–79 years) in group 1, 66.3 ± 12.1 years (range: 32–87 years; $p = 0.008$) in group 2; and 53.5 ± 10.9 years (range: 33–71 years) in the control group. In group 1, 131 (94.9%)

[1] CT, computed tomography.

patients had a history of comorbidities, including 128 (92.7%) with stage I–III hypertension and 99 (71.7%) with type 2 diabetes mellitus. In group 2, 75 (100%) patients had a history of comorbidities, including 74 (98.6%) with stage I–III hypertension and 56 (74.6%) with type 2 diabetes mellitus. All patients in group 2 were on mechanical ventilation, and 66 (88%) died. No significant difference ($p = 0.119$) was noted in sex distribution between the groups compared.

The inclusion criteria were a COVID-19 diagnosis confirmed by polymerase chain reaction and consent of a patient or their representative to participate in the study. The exclusion criteria included refusal by the patient or their representative to participate in the study and unconfirmed COVID-19 diagnosis.

Platelet-free plasma (PFP) was obtained by double centrifugation at 1,500 g for 15 minutes and at 10,000 g for 5 minutes to isolate platelets and purify plasma samples from them. The supernatant was collected in clean Eppendorf tubes for further evaluation.

Fibrin polymerization kinetics was evaluated by dynamic turbidimetry. Furthermore, optical density was measured using an SF-2000 spectrophotometer (OKB Spektr, St. Petersburg, Russia) with Kinetics software. A cell was filled with a mixture of 400 µl PFP and 400 µl 0.025 M $CaCl_2$. Coagulation activation onset was recorded immediately after plasma recalcification (time zero). An increased optical density of the sample caused by the formation of insoluble fibrin fibers from soluble fibrinogen indicated a clot formation. Recording was performed at 340 nm in a 10 mm cell for 60 minutes. Testing was conducted at +24°C.

A lag period (Lag) was determined when evaluating the turbodimetric curve, which corresponds to time of thrombin generation and protofibril formation; the polymerization rate (V) as an increase in optical density in the segment of its increase per unit of time, which characterizes the rate of lateral aggregation and fibrin fiber formation; and the maximum absorbance at a given wavelength (A_{max}), which is determined by the amount of polymerized protein and fibrin fiber thickness.

Moreover, thromboelastography was used to assess hemostasis using the TEG 5000 thromboelastograph (Haemonetics, USA). Contrary to traditional coagulation tests, thromboelastogram shows kinetics of all stages of thrombus formation, considering contributions of plasma and cellular (platelets, erythrocytes, and leukocytes) components to hemostatic reactions and fibrinolysis [16]. In the current study, this method was employed to assess hemostasis in PFP samples.

The absolute number of MVs was calculated by flow cytometry using FACS Calibur (Becton Dickinson, USA) with modified Iversen technique (2013). PFP was mixed with phosphate buffer (pH = 7.4) at 9 : 1.

The absolute number of MVs per 1 µl was determined by fixed time (60 seconds) light scattering using CellQuest, recording the number of events per time unit (60 seconds), considering the flow rate. Flow cytometry of particle size distribution (FSC) and granularity (SSC) on logarithmic scales allows to locate MV signals in a specific area. Standard synthetic spherical particles with 1, 2, 3, 5, 6, and 10 µm diameters (BD Pharmingen, USA) were used to calibrate the instrument and limit the range of MV counting.

PFP was pre-centrifuged twice with buffered isotonic sodium chloride at 10,000 g for 30 minutes to evaluate the effects of MVs on blood coagulation by thromboelastography. Then, the supernatant was collected, and 200 µL isotonic sodium chloride solution was added to the precipitated MVs to obtain a suspension. Further, 240 µL of the PFP test sample, 20 µL of 0.2 M $CaCl_2$, and 100 µL of MVs were added to the tested samples. The assay was performed in reaction cells containing heparinase to exclude heparin therapy influence on the results. As a control, 340 µL of native patient PFP was used with 20 µL of 0.2 M $CaCl_2$ to activate fibrin formation. Thromboelastograms obtained with and without MVs were compared.

Reaction time (R) (i.e., coagulation activation time) was the thromboelastographic parameter used to examine plasma hemostasis.

OriginLab 2021 (OriginLab Corporation, USA) was used to process and plot the turbidimetric results and IBM SPSS Statistics 26.0 for statistical analysis. The Shapiro–Wilk test was used to test quantitative parameters for normal distribution and the nonparametric Mann–Whitney test to determine significant differences between groups of data that did not conform to the normal distribution law. Student's t-test with calculation of mean and standard deviation was employed for statistical processing of the study results, which conformed to the law of normal distribution.

Tables present data as mean ± standard error of the mean (M ± m), where n is sample size. All presented data were statistically significant ($p < 0.05$).

RESULTS

Fibrin formation parameters obtained by turbidimetry significantly differed between groups. Severe COVID-19 is characterized by significant hypocoagulation and decreased fibrin clot density, indicating a disruption in fibrin formation. Moderate COVID-19 is marked by hypercoagulation and thrombotic complications (Table 1).

We identified 15 (19%) of 75 patients with severe COVID-19 who did not receive heparin therapy (were admitted to the hospital for infectious diseases in a severe condition) (2Hep group). Fibrin generation kinetics data showed that these patients and those on heparin therapy demonstrated hypocoagulation. Figure 1 shows representative kinetic curves for each group.

Thromboelastography results were consistent with our turbidimetry data using PFP. Thromboelastography showed hypercoagulation (decreased coagulation activation time R) in the moderate COVID-19 group and hypocoagulation (increased R) in the severe COVID-19 group. Table 2 shows these results.

Table 1. The influence of the COVID-19 severity on the kinetics of fibrin formation

Parameter	Control, n = 20	Group 1 (moderate course), n = 138	Group 2 (severe course), n = 75
V (fibrin clot polymerization rate), AU/sec	3.93 ± 2.3	5.44 ± 2.47 $p = 0.0013$	1.12 ± 0.71 $p < 0.0001$
Lag period, min	6.2 ± 1.2	3.8 ± 1.1 $p = 0.0004$	9.4 ± 0.8 $p < 0.0001$
A_{max}, AU	1.625 ± 0.433	1.412 ± 0.351 $p = 0.0007$	0.576 ± 0.17 $p < 0.0001$

Note: AU, absorbance unit; A_{max}, maximum absorbance at a given wavelength; *p*, significance of differences compared with the control group.

Table 2. Ratio of coagulation activation time of native sample to coagulation activation time of sample after microvesicle injection

Parameter	Control, n = 20	Group 1 (moderate course) n = 138	Group 2 (severe course) n = 75
R/RMV	0.3 ± 0.06	2.12 ± 0.32 $p < 0.0001$	1.44 ± 0.19 $p < 0.0001$

Note: R, activation time of coagulation of the native sample; RMV, activation time of coagulation after microvesicles were added to the sample; *p*, significance of the differences compared to the control.

Table 3. Absolute number of microvesicles in platelet-free plasma samples from patients with COVID-19 of varying severity, obtained by flow cytometry

Group	Absolute number of MVs per 1 mL
Control, n = 20	14.4 ± 1.9 × 106
Group 1 (moderate course of COVID-19), n = 138	12.2 ± 1.6 × 106, p = 0.3967
Group 2 (severe course of COVID-19), n = 75	3.7 ± 0.3 × 106, p < 0.0001

Note: p, significance of differences compared to control.

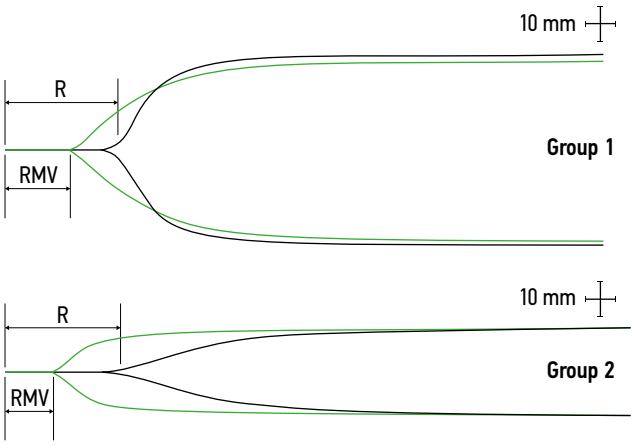

Fig. 2. Thromboelastograms demonstrating the effect of microvesicles on coagulation parameters, "typical" for each of the study groups (group 1 and group 2); R is the coagulation activation time of the native sample; RMV is the coagulation activation time after adding microvesicles to the sample

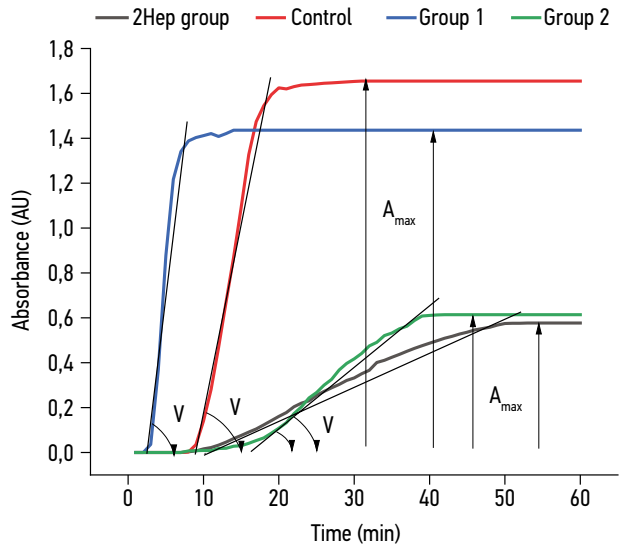

Fig. 1. Registration of fibrin polymerization kinetics by dynamic turbidimetry. Determined parameters: lag period (Lag) — time before the density increase; V — increase in optical density during its increase per unit time; A_{max} — maximum optical density at a given wavelength; control — group of healthy donors; group 1 — moderate course; group 2 — severe course, patients of the intensive care unit; group 2Hep — patients with severe course who did not receive heparin therapy

Data confirming the role of MVs in fibrin clot formation by reducing coagulation activation time were obtained from evaluating the effect of MVs on plasma hemostasis. This parameter decreased by 2.1 times (16.9 ± 1.1 min and 8.0 ± 0.6 min; $p < 0.0001$) in patients with moderate COVID-19 and by 1.44 times (10.8 ± 0.9 min and 7.5 ± 0.5 min; $p < 0.0001$) in those with severe COVID-19 after MVs were added to the samples (Figure 2). In the control group, no significant effect of MVs was observed on R coagulation activation time ($p = 0.0917$).

Notably, the higher the R value (coagulation activation time), the more significant the influence of MVs (e.g., for native PFP, R = 39.3 minutes; after addition of washed MVs to the test sample, R = 13.7 minutes). However, if a patient was hypercoagulated, MVs less significantly shortened the fibrin activation time (for the patient's native PFP, R = 9.4 minutes; after addition of MVs to the test sample, R = 8.6 minutes).

Flow cytometry was used to calculate the absolute number of MVs in the PFP samples; Table 3 presents the results.

DISCUSSION

Study of the kinetics of fibrin formation using turbidimetry and thromboelastography in COVID-19 patients showed specific characteristics in the pathogenetic processes of coagulopathy development depending on severity. Fibrin formation indicated hypocoagulation in patients with severe COVID-19 and hypercoagulation in those with moderate COVID-19. Furthermore, hypocoagulation was reported in patients with severe COVID-19 in the heparin and no-anticoagulant groups.

Several studies revealed the critical role of MVs in blood coagulation. The procoagulant role of MVs and their involvement in fibrin clot formation have been reported in our studies [17].

In the present study, relevant results were obtained on a significant and reliable decrease in the content of MVs in severe COVID-19 patients with hypocoagulation. A model experiment to investigate the effect of MVs on plasma hemostasis demonstrated a significant change in the kinetics of fibrin clot formation when PFP with a high MV content was added to the test plasma of severe COVID-19 patients. This exhibits the procoagulant role of MVs, and the decrease in their peripheral blood content may be related to their active participation in coagulation processes.

A prolonged coagulation activation time in patients with severe COVID-19 should be considered a marker of disseminated vascular coagulation syndrome (consumption phase) and, as a consequence, possible hemorrhagic complications. Moderate COVID-19 is characterized by Lag shortening, indicating the presence of hypercoagulation.

Moreover, according to thromboelastographic parameters, these patients manifested increased coagulation activity as evidenced by a decreased activation time. Without appropriate anticoagulant therapy, this condition may lead to thrombosis (venous thromboembolism or pulmonary embolism).

CONCLUSION

1. Dynamic turbidimetry and thromboelastography are used as objective and demonstrative methods to assess plasma hemostasis in patients with COVID-19. They allow a more detailed evaluation of changes in the patient's coagulation system and timely adjustment of treatment strategy.

2. The plasma hemostasis system of patients with COVID-19 shifts to hypo- and hypercoagulation depending on disease severity.

3. The absolute number of microvesicles in peripheral blood is significantly lower in patients with severe disease than in controls and those with moderate disease.

ADDITIONAL INFORMATION

Authors' contribution. E.S.G. — conceptualization, formal analysis, writing — review and editing, investigation; R.R.A. — methodology, validation, writing — original draft; I.G.M — writing — review and editing, final approval of the article; D.I.A. — resources, supervision.
Funding source. The study had no sponsorship support.
Competing interests. The authors declare no conflict of interest regarding the presented article.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Е.С.Г — концептуализация, анализ, редактирование рукописи, исследование; Р.Р.А — методология, валидация, создание черновика; И.Г.М. — редактирование рукописи, окончательное утверждение статьи; Д.И.А. — ресурсы, общее руководство.
Источник финансирования. Исследование не имело спонсорской поддержки.
Конфликт интересов. Авторы заявляют об отсутствии конфликта интересов по представленной статье.

REFERENCES

1. Baklaushev VP, Kulemsin SB, Gorchakov AA, Lesnyak VN, Yusubalieva GM, Sotnikova AG. COVID-19. Etiology, pathogenesis, diagnosis and treatment. *Journal of Clinical Practice*. 2020;11(1):7–20. (In Russ.) doi: 10.17816/clinpract26339

2. Wang D, Hu B, Hu C. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020;323(11):1061–1069. doi: 10.1001/jama.2020.1585

3. Roitman EV, Vavilova TV, Markin SM, Kravtsov PF, Mazayshvili KV. The realities of the anticoagulant therapy using in COVID-19. *Tromboz, Gemostaz i Reologiya*. 2021;(1):18–25. (In Russ.) doi: 10.25555/THR.2021.1.0957

4. Kolesnikov VV. Roitman EV, Leonov AA. Anticoagulant treatment for severe COVID-19 with high bleeding risk. *Tromboz, Gemostaz i Reologiya*. 2022;(1):59–68. (In Russ.) doi: 10.25555/THR.2022.1.1010

5. Shatohin YuV, Snezhko IV, Ryabikina EV. Violation of hemostasis in coronavirus infection. *South Russian Journal of Therapeutic Practice*. 2021;2(2):6–15. (In Russ.) doi: 10.21886/2712-8156-2021-2-2-6-15
6. Yokota S, Kuroiwa E, Nishioka K. Novel coronavirus disease (COVID-19) and cytokine storms. For more effective treatments from the viewpoints of an inflammatory pathophysiology perspective. *Infectious diseases: news, opinions, training*. 2020;9(4):13–25. doi: 10.33029/2305-3496-2020-9-4-13-25
7. Wolf P. The nature and significance of platelet products in human plasma. *Br J Haematol.* 1967;13(3):269–288. doi: 10.1111/j.1365-2141.1967.tb08741.x
8. Zubairov DM, Zubairova LD. *Mikrovezikuly v krovi: funktsii i ikh rol' v tromboobrazovanii.* (Microvesicles in the blood: functions and their role in thrombus formation.) Moscow: GEOTAR-MED; 2009. 167 p. (In Russ.)
9. Panigada M, Bottino N, Tagliabue P, Grasselli G, Novembrino C, Chantarangkul V, Pesenti A, Peyvandi F, Tripodi A. Hypercoagulability of COVID-19 patients in intensive care unit: A report of thromboelastography findings and other parameters of hemostasis. *J Thromb Haemost.* 2020;18(7):1738–1742. doi: 10.1111/jth.14850
10. Hippensteel JA, Burnham EL, Jolley SE. Prevalence of venous thromboembolism in critically ill patients with COVID-19. *Br J Haematol*. 2020;190(3):e134–e137. doi: 10.1111/bjh.16908
11. Khismatullin RR, Ivaeva RA, Abdullayeva S, Shakirova AZ, Khuzin FF, Kiassov AP, Litvinov RI. Pathological manifestations of inflammatory microthrombosis in COVID-19. *Kazan Medical Journal*. 2022;103(4):575–586 (In Russ.) doi: 10.17816/KMJ2022-575
12. Hamali HA, Saboor M, Dobie G, Madkhali AM, Akhter MS, Hakamy A, Al-Mekhlafi HM, Jackson DE, Matari YH, Mobarki AA. Procoagulant microvesicles in COVID-19 patients: Possible modulators of inflammation and prothrombotic tendency. *Infect Drug Resist*. 2022;15:2359–2368. doi: 10.2147/IDR.S355395
13. Nieri D, Neri T, Petrini S, Vagaggini B, Paggiaro P, Celi A. Cell-derived microparticles and the lung. *Eur Respir Rev*. 2016;25(141):266–277. doi: 10.1183/16000617.0009-2016
14. Guervilly C, Bonifay A, Burtey S, Sabatier F, Cauchois R, Abdili E, Arnaud L, Lano G, Pietri L, Robert T, Velier M, Papazian L, Albanese J, Kaplanski G, Dignat-George F, Lacroix R. Dissemination of extreme levels of extracellular vesicles: tissue factor activity in patients with severe COVID-19. *Blood Adv*. 2021;5(3):628–634. doi: 10.1182/bloodadvances.2020003308
15. Sirotkina OV, Ermakov AI, Zhilenkova YI, Zolotova EA, Kudlay DA, Vavilova TV, Gaykovaya LB. Dynamics of microvesicle formation in blood in patients with COVID-19 at different stages of the disease. *Preventive and clinical medicine.* 2021;81(4):68–74 (In Russ.) doi: 10.47843/2074-9120_2021_4_68
16. Yarets YI. *Tromboehlastografiya: osnovnye pokazateli, interpretatsiya rezul'tatov.* (Thromboelastography: main indicators, interpretation of results.) Gomel: State Institution "RNPC RMiEH"; 2018. 26 p. (In Russ.)
17. Nabiullina RM, Mustafin IG, Ataullakhanov FI, Litvinov RI, Zubairova L.D. Thrombin-mediated effects of blood microparticles on formation, structure, and stability of fibrin clots. *IM Sechenov Russian Journal of Physiology*. 2015;101(7):812–821. (In Russ.) EDN: UABUTT

СПИСОК ЛИТЕРАТУРЫ

1. Баклаушев В.П., Кулемзин С.В., Горчаков А.А., и др. COVID-19. Этиология, патогенез, диагностика и лечение // Клиническая практика. 2020. Т. 11, № 1. С. 7–20. doi: 10.17816/clinpract26339
2. Wang D., Hu B., Hu C., et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China // JAMA. 2020. Vol. 323, N. 11. P. 1061–1069. doi: 10.1001/jama.2020.1585
3. Ройтман Е.В., Вавилова Т.В., Маркин С.М., и др. Реалии применения антикоагулянтной терапии при COVID-19 // Тромбоз, гемостаз и реология. 2021. № 1. С. 18–25. doi: 10.25555/THR.2021.1.0957
4. Колесников В.В., Ройтман Е.В., Леонов А.А. Антикоагулянтная терапия у пациентов с тяжёлым течением COVID-19 и высоким риском кровотечения // Тромбоз, гемостаз и реология. 2022. № 1. С. 59–68. doi: 10.25555/THR.2022.1.1010
5. Шатохин Ю.В., Снежко И.В., Рябикина Е.В. Нарушение гемостаза при коронавирусной инфекции // Южно-Российский журнал терапевтической практики. 2021. Т. 2, № 2. С. 6–15. doi: 10.21886/2712-8156-2021-2-2-6-15
6. Йокота Ш., Куройва Е., Нишиока К. Новая коронавирусная болезнь (COVID-19) и «цитокиновый шторм». Перспективы эффективного лечения с точки зрения патофизиологии воспалительного процесса // Инфекционные болезни: новости, мнения, обучение. 2020. Т. 9, № 4. С. 13–25. doi: 10.33029/2305-3496-2020-9-4-13-25
7. Wolf P. The nature and significance of platelet products in human plasma // Br J Haematol. 1967. Vol. 13, N. 3. P. 269–288. doi: 10.1111/j.1365-2141.1967.tb08741.x
8. Зубаиров Д.М., Зубаирова Л.Д. Микровезикулы в крови: функции и их роль в тромбообразовании. Москва: ГЭОТАР-Медиа, 2009. 167 с.
9. Panigada M., Bottino N., Tagliabue P., et al. Hypercoagulability of COVID-19 patients in intensive care unit: A report of thromboelastography findings and other parameters of hemostasis // J Thromb Haemost. 2020. Vol. 18, N. 7. P. 1738–1742. doi: 10.1111/jth.14850
10. Hippensteel J.A., Burnham E.L., Jolley S.E. Prevalence of venous thromboembolism in critically ill patients with COVID-19 // Br J Haematol. 2020. Vol. 190, N. 3. P. 134–137. doi: 10.1111/bjh.16908
11. Хисматуллин Р.Р., Иваева Р.А., Абдуллаева Ш., и др. Патоморфологические проявления воспалительного микротромбоза при COVID-19 // Казанский медицинский журнал. 2022. Т. 103, №4. С. 575–586. doi: 10.17816/KMJ2022-575
12. Hamali H.A., Saboor M., Dobie G., et al. Procoagulant microvesicles in COVID-19 patients: Possible modulators of inflammation and prothrombotic tendency // Infect Drug Resist. 2022. Vol. 15. P. 2359–2368. doi: 10.2147/IDR.S355395
13. Nieri D., Neri T., Petrini S., et al. Cell-derived microparticles and the lung // Eur Respir Rev. 2016. Vol. 141, N. 25. P. 266–277. doi: 10.1183/16000617.0009-2016
14. Guervilly C., Bonifay A., Burtey S., et al. Dissemination of ex-

treme levels of extracellular vesicles: tissue factor activity in patients with severe COVID-19 // Blood Adv. 2021. Vol. 5, N. 3. P. 628–634. doi: 10.1182/bloodadvances.2020003308

15. Сироткина О.В., Ермаков А.И., Жиленкова Ю.И., и др. Динамика образования микровезикул клеток крови у больных COVID-19 на разных стадиях заболевания // Профилактическая и клиническая медицина. 2021. Т. 81, № 4. С. 68–74. doi: 10.47843/2074-9120_2021_4_68

16. Ярец Ю.И. Тромбоэластография: основные показатели, интерпретация результатов. Гомель: ГУ «РНПЦ РМиЭЧ», 2018. 26 с.

17. Набиуллина Р.М., Мустафин И.Г., Атауллаханов Ф.И., и др. Опосредованное тромбином влияние микровезикул крови на кинетику образования, структуру и свойства фибринового сгустка // Российский физиологический журнал им. И.М. Сеченова. 2015. Т. 101, № 7. С. 812–821. EDN: UABUTT

AUTHORS' INFO

***Elena S. Gracheva**, Postgrad. Stud., Assist.,
Depart. of Biochemistry and Clinical Laboratory Diagnostics,
Kazan State Medical University, Kazan, Russia;
ORCID: 0000-0002-8543-6529;
eLibrary SPIN: 3290-5459;
e-mail: gracheva020688@mail.ru

Rufina R. Abdullina, Assist., Depart. of Biochemistry and Clinical Laboratory Diagnostics, Kazan State Medical University, Kazan, Russia;
ORCID: 0009-0000-5975-4390;
eLibrary SPIN: 7099-8375;
e-mail: rufina.abdullina.r@mail.ru

Il'shat G. Mustafin, MD, D. Sci. (Med.), Prof., Head of Depart., Depart. of Biochemistry and Clinical Laboratory Diagnostics, Kazan State Medical University, Kazan, Russia;
ORCID: 0000-0001-9683-3012;
eLibrary SPIN: 1588-6988;
e-mail: ilshat.mustafin@kazangmu.ru

Diana I. Abdulganieva, MD, D. Sci. (Med.), Prof., Head of Depart., Depart. of Hospital Therapy, Kazan State Medical University, Kazan, Russia;
ORCID: 0000-0001-7069-2725;
eLibrary SPIN: 6676-4270;
e-mail: diana_s@mail.ru

* Corresponding author / Автор, ответственный за переписку

ОБ АВТОРАХ

***Грачева Елена Сергеевна**, асп., асс., каф. биохимии и клинической лабораторной диагностики, ФГБОУ ВО Казанский ГМУ Минздрава России, г. Казань, Россия;
ORCID: 0000-0002-8543-6529;
eLibrary SPIN: 3290-5459;
e-mail: gracheva020688@mail.ru

Абдуллина Руфина Рустамовна, асс., каф. биохимии и клинической лабораторной диагностики, ФГБОУ ВО Казанский ГМУ Минздрава России, г. Казань, Россия;
ORCID: 0009-0000-5975-4390;
eLibrary SPIN: 7099-8375;
e-mail: rufina.abdullina.r@mail.ru

Мустафин Ильшат Ганиевич, д-р мед. наук, проф., зав. каф., каф. биохимии и клинической лабораторной диагностики, ФГБОУ ВО Казанский ГМУ Минздрава России, г. Казань, Россия;
ORCID: 0000-0001-9683-3012;
eLibrary SPIN: 1588-6988;
e-mail: ilshat.mustafin@kazangmu.ru

Абдулганиева Диана Ильдаровна, д-р мед. наук, проф., зав. каф., каф. госпитальной терапии, ФГБОУ ВО Казанский ГМУ Минздрава России, г. Казань, Россия;
ORCID: 0000-0001-7069-2725;
eLibrary SPIN: 6676-4270;
e-mail: diana_s@mail.ru