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EFFECT OF CORONAVIRUS DISEASE (COVID-19) ON HEMOSTASIS SYSTEM IN PATIENTS WITH ACUTE CEREBROVASCULAR ACCIDENTS

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ABSTRACT — Acute viral respiratory infections add to the progression risk of the already existing pathologies, including those of cardiovascular origin. Life-threatening complications emerging against SARS-CoV-2 (severe acute respiratory syndrome), which causes COVID-19 (Coronavirus disease 2019), explain the need to study the cardiovascular effects of COVID-19 in order to offer rational medical care to patients belonging to various age categories. This article presents a comprehensive assessment of changes affecting the main hemostasis parameters in patients with acute cerebral circulatory disorders against the SARS-CoV-2 virus infection. There is special attention paid to the pathophysiological features occurring against the infectious process involving the hemostasis system. Our data shows that the differences in the main hemostatic parameters in patients with acute cerebral circulatory disorders occurring against the SARS-CoV-2 virus infection are due to the degree of the body responsiveness and the severity of the comorbid pathology. Reducing the risk of adverse conditions developing in this category of patients is possible in case of responsible monitoring focusing on the main indicators of the cardiovascular system status, as well as provided there are advanced approaches introduced in order to prevent and treat thrombotic/thromboembolic complications.

KEYWORDS — coronavirus, cardiovascular diseases, coronavirus infection 2019, SARS-CoV-2, COVID-19, hemostasis, acute cerebrovascular accident.

INTRODUCTION

Respiratory viral diseases contribute to the progression of pre-existing chronic non-infectious pathology, cardiovascular diseases (CVD) first of all, while the initially existing CVD associated with other concomitant pathology enhance the likelihood of the infection development and progression. The COVID-19 pandemic caused by a new strain of coronavirus SARS-CoV-2, has led to a rapid increase in the number of cases and high mortality globally. Despite the tropism of SARS-CoV-2 to the lungs, COVID-19 contains a high risk of developing multiple organ failure, including due to damage affecting the cardiovascular system [6, 8, 12]. More than half of all patients with SARS CoV-2 feature multimorbidity, the frequency of that increasing up to 72% in severe COVID-19 cases [5,15].

Under the COVID-19 pandemic, patients with CVD are at risk of severe infection, which is due to high metabolic needs experienced by this category of patients, the needs exceeding the available cardiac reserve, thus creating grounds for the previously existing pathology progression. During the acute period of viral infection, there is a release of pro-inflammatory cytokines occurring (cytokine storm). A sharp increase in the interleukin-6 level in the vascular bed promotes the disturbance in the vasoregulatory function of the microcircular bed vascular endothelium, as well as facilitates thrombosis, microcirculation disorders in organs and tissues, acute myocardial damage, arterial hypertension, heart failure, and the development of multisystemic disorders. Given the background of high virus-induced inflammation, the existing atherosclerotic plaques get destabilized. Proinflammatory cytokines produce a systemic procoagulant and thrombogenic effect, whereas the likelihood of developing thrombotic and thromboembolic complications increases sharply [4, 11, 14].

Cerebral vascular pathology is an extremely serious medical and social issue faced by the society nowadays, which can be accounted for by its wide prevalence (1–4 cases per 1,000 population annually), high mortality rate (up to 35% in the acute period of stroke), significant rat of disability and loss of capacity. The WHO claims that stroke, whose rates are very high among cerebrovascular pathologies, is a global epidemic threatening the life and well-being of the world's population. The annual number of brain strokes registered globally exceeds 7 million, while every fourth case is fatal (WHO, 2018).

Research literature contains extensive data concerning hemorheological changes in case of COVID-19, with respective data available regarding an increasing risk of developing acute cerebrovascular accidents (CVA) against the background of COVID-19 [9,13].

A number of researchers stress that the most important pathophysiological link involved in the chain of stroke development is a disturbance in the vascular-platelet hemostasis system, thrombocytes (TrC) playing a key role in this case. In case of a pathology, there are changes to be observed, which affect morphometric parameters of platelets like the average diameter, the area, the shape factor, the polarization, the specific optical density in three spectral ranges (blue, green, red) and the proportion of blue and red [1, 3]. The available literature sources offer scarce details on TrC morphometry in case of a CVA. This issue requires in-depth consideration, since the information obtained may allow timely diagnosing and assessing the prognosis for stroke course and outcome.

Aim of study:
to study the morphofunctional and geometric parameters of thrombocytes, taking into account the hemostatic parameters of blood plasma in patients with acute cerebrovascular issues in different age groups, in view of the SARS-CoV-2 virus infection background.

MATERIALS AND METHODS

In order to study changes affecting morphofunctional parameters (number, average volume, anisocytosis index, area, average diameter, shape factor, cell polarization) of thrombocytes, which are due to CVAs, 81 patients with a reliably set diagnosis were examined, undergoing inpatient specialized treatment. The CVA diagnosis was verified based on the clinical presentation and magnetic resonance imaging (MRI) data. During the MRI, the nature of the stroke (ischemic or hemorrhagic), the magnitude and prevalence of focal brain changes were identified. The participants, after signing respective voluntary informed consents, were divided into groups based on their age: Group I — 18 patients (aged 35–49; median age — 42±7); Group II — 27 patients (aged 50–60 years; median age – 55±5); Group III — 36 patients (aged 61–75; median age — 68±7). Subject to the aim of the study, the following methods were applied: physical & chemical, conductometric (impedance), mathematical, TrC computer morphometry, coagulation-base. The thrombocyte link indicators were studied based on three features.

The study was carried out on an ADVIA 2120i (Siemens Healthcare Diagnostics) automatic hematology analyzer, with whole blood used as the biological material. Blood sampling was performed in the morning, on an empty stomach. Blood was placed in dry vacuum tubes containing EDTA as an anticoagulant agent. Also, a smear was prepared from the blood for scanning probe microscopy and computer cytomorphometry of thrombocytes. Blood preparations were made by a standard unified method. The study of the hemostasis system was performed on a Sysmex CS-2100i (Sysmex) automatic coagulometer with the following indicators identified: activated partial thromboplastin time (APTT), international normalized ratio (INR), prothrombin index (PTI), prothrombin time (PTT), fibrinogen, antithrombin III, D-dimer. Aggregational examination of thrombocytes is a source offering the most important information for detecting functional disorders of thrombocytes. Aggregation can be primary, which is caused by an inducer, and secondary – due to biologically active compounds released by thrombocytes.

The study was conducted on the BIOLA ALAT-2 220LA (Russia) aggregometer. In our study, we identified the indicators of TrC aggregation with various inducers (ATP, ristocetin, collagen). Morphometric studies of thrombocytes were carried out using the MEKOS-C3 hardware and software complex, as well as the INTEGRA atomic force microscope. The following indicators were identified: the thrombocyte area, the thrombocyte average diameter, the thrombocyte shape factor, the thrombocyte polarization, the blue color fraction, the red color fraction, and the specific optical density (Fig. 1, 2).

The statistical data processing was done with the Microsoft Excel 2013 software as well as employing the package of the SPSS Statistics software (version 22). The critical level of a possible null statistical hypothesis was taken as equal to 0.05.

RESULTS AND DISCUSSION

The study resulted in significant differences revealed in terms of coagulogram and TrC aggregation in the groups. There was a significant decrease in APTT obtained in Groups II (31.6±3.6) ($P<0.02$) and III (30.4±3.6) ($P<0.02$) compared to Group I (40.1±2). PTV is a parameter, which characterizes the activity of the main blood clotting factors (I, II, V, VII, X), whereas its increase may point at the activation of the hemostasis system, which confirms the results obtained here. There was, for instance, a significant increase in the PTV parameter observed in Group III (18.6±2.4) compared to Group I (12.3±0.8) ($P<0.01$) and Group II (13.9±1.1) ($P<0.05$). A change in the entire body of these indicators may be indicative of hypercoagulation that develops in case of a CVA in an older age group within the second maturity period.

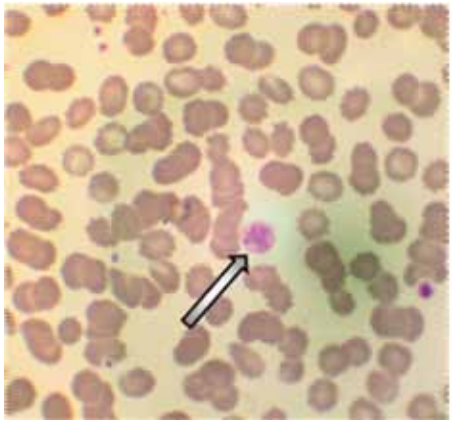


Fig.1. *Thrombocytes of a patient (51 y.o.) with CVA and with the SARS-CoV-2 virus infection in history (MEKOS-C3 hardware and software complex)*
Note: *the arrow points at a giant thrombocyte to be seen on the background of altered erythrocytes.*

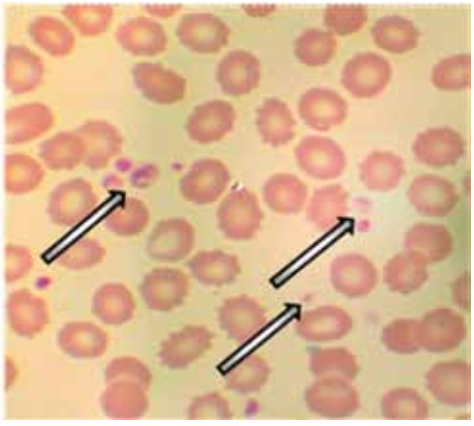


Fig. 2. *Thrombocyte anisocytosis in a patient (58 y.o.) with CVA and with the SARS-CoV-2 virus infection in history (MEKOS-C3 hardware and software complex)*

The prothrombin index (PTI) is a laboratory indicator used to describe the external pathway of blood clotting. When increased, it may point at higher clotting, which is of primary importance in the development of a CVA. Our study revealed a significant increase in the PTI in Group III (121.7±2.4) (P<0.01) compared to Group I (90.2±12.3), with a statistically insignificant PTI increase in Group II (95.4±24.5). There was a significant decrease in the AT-III parameter in Group III (78.3±6.8) (P<0.05) compared to Group I (93.2±3.6). Besides, a statistically insignificant decrease in this indicator in Group II (89.9±11.5) was observed, too. Antithrombin III (AT-III) is a blood clotting inhibitor. A decrease in its level in case of a developing CVA may indicate a decrease in the anticoagulant system activity, which is confirmed by the results of our studies. The study of such a coagulogram indicator as fibrinogen revealed its highly significant increase in Group III (6.1±0.5) if compared to Group I (2.9±0.4) (P<0.001) and Group II (4.6±0.6) (P<0.05), as well as an increase of this indicator in Group II if matched against Group I (P<0.01). Fibrinogen is not only a blood clotting factor, yet also an acute phase protein, which means its increase may point at not only the development of hypercoagulation, but inflammation, too, as well as tissue damage in case of a CVA.

CVAs are characterized by thrombotic conditions associated with the blood containing substances featuring procoagulant properties, D-dimer in particular (Berkovsky, A.V., 2011). In our study, there was a significant increase of D-dimer observed in Group III (9.4± 0.8) compared to Group I (0.25±0.2) (P<0.001) and Group II (4.6±2.6) (P<0.05). There was also a significant increase in this indicator registered in Group II compared to Group I (P<0.05). The obtained data may confirm the presence of a hypercoagulation condition developing in case of a CVA.

The indicators of induced aggregation typically feature a significant increase in aggregation with ristocetin in Groups II (138.1±41.7) (P<0.05) and III (160.7± 26.5) (P<0.001) if compared to Group I (68.1±6.2). As for aggregation with ADP, there no were statistically significant values revealed, yet a tendency towards its increase detected in Groups II (79.8±13.4) and III (87±9.7) compared to Group I (71.2±10.1). The aggregation with collagen index revealed a significant increase in Group III (96.1±8) (P<0.05) and a statistically insignificant increase in Group II (80.2±13.5) compared to Group I (72.5±10.6). Therefore, there are changes in the hemostasis system indicators, both in case of CVAs with no concomitant pathology, and in case of CVAs on the background of smoking and SARS-CoV-2. A decrease in APTT, AT-III, an increase in PTI, PTV, fibrinogen, D-dimer, for instance, may point at the development of hypercoagulation with anticoagulation system inhibition. Changes in these indicators point at a strain in the hemostasis system in case of the pathology in question, which also is confirmed by an increase in TrC induced aggregation.

To complete the objectives set within the study, we analyzed the quantitative and morphometric indicators of TrC. In order to identify various changes in the thrombocyte link of hemostasis, the examination implied a statistical analysis of the obtained data. Based on the data, there were some quantitative and

morphometric differences of TrC identified in the groups (Fig. 3).

The study showed that the TrC number featured a statistically significant increase in Group II (361.4±26.6) ($p<0.01$), with a decrease in Group III (190.0±30.0) ($P>0.001$) if compared to Groups I and II. Unlike the TrC numbers, the PDW and MPV indicators revealed no statistically significant, reliable differences, yet a tendency towards an increase in these indicators was identified in Groups II (PDW 42.3±3.6; MPV 10.2±0.7) and III (PDW 43±6.6; MPV 10.6±0.6). It suggests that these changes in thrombocytes are associated with their activation through a CVA development, namely, an increase in the MPV parameter was noted, which points at the appearance of younger forms of thrombocytes. An increase in the PDW parameter indicates an increase in the TrC anisocytosis. A decrease in the TrC number in case of a CVA against SARS-CoV-2 in different age groups may indicate the severity of the disease course. Since SARS-CoV affects the lung tissue, which may be the site of TrC release from mature megakaryocytes, disturbances in the capillary pulmonary blood flow may be associated with higher consumption and reduced production of thrombocytes (Fig. 4).

The TrC average diameter in Group III (4.5±0.6) ($p<0.02$) was significantly above that in Groups I (2.6±0.6) and II (3.2±0.23) ($p<0.02$). There was a significant increase noted in the TrC area in Group III (7.8±0.5) ($p<0.02$) if compared to Groups I (4.8±0.27) and II (5.3±0.31) ($p<0.02$). The shape factor describes the degree of the cell edge indentation, which is due to the development of pseudopodia, occurring both with in case of CVAs without concomitant pathologies, and with CVAs on the background of COVID-19. The study revealed a significant increase in the TrC form factor in Group III (18.3±1.8) ($p<0.05$) compared to Groups I (13.9 ±2.39) and II (14.9±0.81). There were no statistically significant differences detected in the TrC polarization index, however, a tendency towards its increase was determined in Group III (0.24±0.02) compared to Groups I (0.2±0.07) and II (0.22±0.02). Changes in these TrC geometric parameters are indicative of a hyperactivated thrombocyte link in case of a CVA.

3D scanning probe microscopy and scanning electron microscopy was used to make images of activated thrombocytes in patients with acute cerebrovascular accident on the background of SARS-CoV-2 (Fig. 5–7).

We have revealed that in case of a CVA on the background of the SARS-CoV-2 virus infection in history, there are changes affecting the hemostasis main parameters, as well as quantitative and morphometric

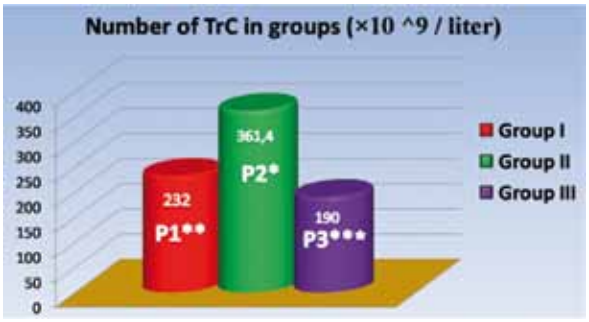


Fig.3. *Number of TrC in groups: Group I (age — 35–49); Group II (age — 50–60); Group III (age — 61–75)*
Note 1: *P1 — reliability of differences between indicators in Groups I and II; P2 — reliability of differences between indicators in Groups I and III; P3 — reliability of differences between indicators in Groups II and III.*
Note 2: ** — P<0.05, ** — P<0.01, *** — P<0.001.*

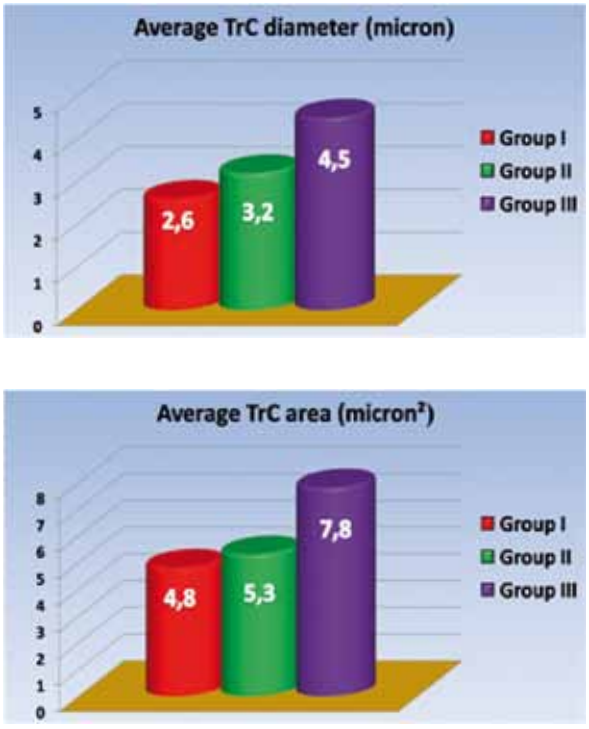


Fig.4. *Average TrC diameter and area in studied groups*

indicators of thrombocytes, while the dynamics of changes in the indicators is multidirectional. As far as the hemostasis indicators are concerned, the following was observed: a shortened APTT, an increase in the PTV, PTI, fibrinogen, D-dimer, induced aggregation with various inducers (ADP, ristocetin, collagen), while the severity of the changes was the biggest in Group III. These changes indicate the development of

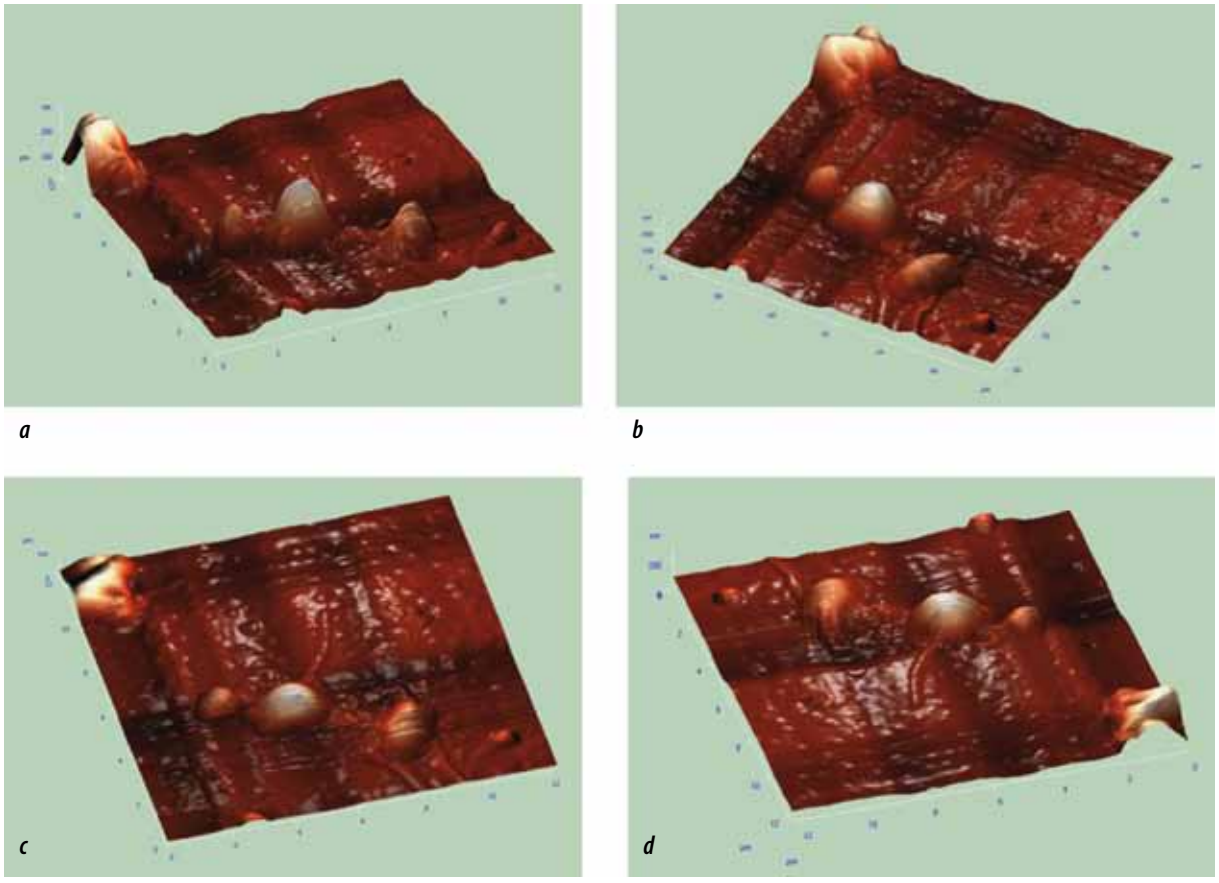


Fig.5 *(a–d). Activated thrombocyte — 3D projection (size 12 × 12 μm). Blood preparation; patient 51 y.o. with a CVA on the background of the SARS-CoV-2 virus infection in history*

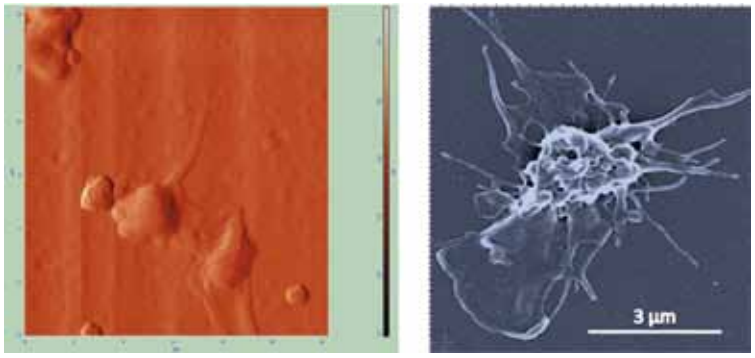
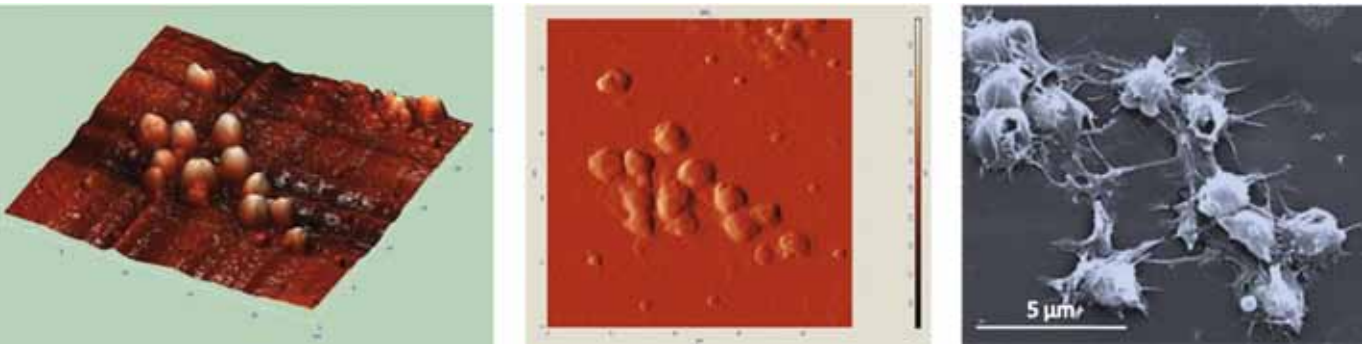


Fig. 6. *Activated thrombocyte: a — plane projection (size 12 × 12 μm); b — scanning electron microscopy (increase ×17 500). Blood preparation; patient 51 y.o. with a CVA on the background of the SARS-CoV-2 virus infection in history*

hypercoagulation, and a decrease in the anticoagulant system function in case of a CVA. Blood coagulopathic potential occurs through substances entering the bloodstream, where the substances in question gave procoagulant properties, while the sources of these substances are to be found in the decomposition foci of the brain tissue.

It is important to note that the SARS-CoV-2 virus infection is followed with more profound changes in hemostasis. The obtained results concerning the hemostasis status in the patients are consistent with published research data on the pathophysiological mechanisms behind the development of cardiovascular complications in case of COVID-19: a direct damaging effect of the SARS-CoV-2 virus on pericytes, cardiomyocytes and fibroblasts; an indirect effect that the SARS-CoV-2 virus has on the myocardium under a cytokine storm; a direct damaging effect



A B C

Fig. 7. *A — Thrombocyte conglomerates (aggregates), 3D projection (size 25 × 25 µm); B — plane projection (size 25 × 25 µm); C – scanning electron microscopy (increase ×10 000). Blood preparation; patient 51 y.o. with a CVA on the background of the SARS-CoV-2 virus infection in history*

of the SARS-CoV-2 virus on the vascular endothelium, resulting in its dysfunction; hypercoagulation due to endothelial dysfunction, increased thrombocyte activity and decreased plasminogen production; pronounced hypoxemia, which leads to increased anaerobic processes, intracellular acidosis and oxidative stress; imbalance between myocardial oxygen demand and its delivery on the background of virus-induced inflammation, hypoxia, oxidative stress, endothelial damage and hypercoagulation; activation of the sympathetic system with stress-induced release of catecholamines into the blood; electrolyte imbalance, which facilitates the development of tachyarrhythmia [2, 7, 10].

CONCLUSION

1. Diagnostically significant changes in the hemostasis system in patients with CVAs against the background of the SARS-CoV-2 virus infection include increased blood levels of D-dimer, increased prothrombin time, thrombin and activated partial thromboplastin time (APTT). The early stages of the disease feature an increase in the fibrinogen concentration; however, as the disorders progress, the blood levels of fibrinogen and antithrombin decrease. Thrombocytopenia is also associated with the severity and the prognosis of the disease, yet is rarely significant.

2. Out of the examined hemostatic parameters, D-dimer is the most interesting as a marker of severity and unfavorable prognosis in case of a CVA against the background of the SARS-CoV-2 virus infection, since its definition is widely available and standardized. Prothrombin time is of prognostic value, too, however, during hospitalization, its changes in patients with unfavorable prognosis are not as obvious as in case of D-dimer, and will not typically go much beyond the upper normal limit.

3. Patients with a CVA on the background of the SARS-CoV-2 virus infection, when hospitalized, are recommended to have the blood level of D-dimer, prothrombin time, fibrinogen concentration, identified, as well as to take a detailed general blood test, including such as to detect the level of thrombocytes, followed by regular monitoring of these indicators. These hemostasis indicators, if exceeding the normal values significantly, serve signs of the disease taking a worse turn with the development of serious consumption coagulopathy, where intensification of COVID-19 treatment and/ or the introduction of blood components may be required.

4. The prothrombin ratio and prothrombin time are not recommended to be replaced with the international normalized ratio, since it does not detect relatively small changes that may occur in case of COVID-19.

5. The difference in the main morphometric, hemostatic parameters is a distinctive feature of patients with CVAs on the background of the SARS-CoV-2 virus infection, whereas the variability of these parameters reflects the degree of the body responsiveness, the severity of the disease, as well as it allows making forecast regarding the outcome.

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