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LABORATORY MARKERS OF ENDOTHELIAL DESTRUCTION AND HEMOSTASIS ACTIVATION IN PATIENTS WITH ACUTE CEREBROVASCULAR ACCIDENT AND COVID-19

Olga V. Lyang[1,2✉], Mikhail A. Soldatov[1], Leonid V. Klimov[1], Tatiana V. Kiseleva[1,3], Nataliya A. Marskaya[1], Nikolay A. Shamalov[1]

[1] Federal Center of Brain Research and Neurotechnologies, Moscow, Russia

[2] Patrice Lumumba Peoples' Friendship University of Russia, Moscow, Russia

[3] Research Institute for Healthcare Organization and Medical Management of Moscow Healthcare Department, Moscow, Russia

Introduction. The severity of endothelial destruction in patients with the new COVID-19 new coronavirus infection may be correlated with the risk of developing acute cerebrovascular accident (ACVA).

Objective. To study the role of hemostasis system activation markers and vascular wall damage markers in the development of stroke in patients with the new coronavirus infection.

Materials and methods. The study included 38 patients with the new coronavirus infection and ACVA and 40 patients with the new coronavirus infection without ACVA. All patients were tested for antibodies to β2-glycoprotein, antibodies to cardiolipin, plasminogen activator inhibitor type 1 (PAI-1), α2-antiplasmin, intercellular adhesion molecule type 1 (ICAM-1), von Willebrand factor, and homocysteine.

Results. No statistically significant differences were found between the groups in terms of antiphospholipid antibody levels; however, increased antibodies to β2-glycoprotein relative to the reference interval were more frequent in the group without ACVA. Significant differences in PAI-1 levels were found between the group with ACVA and the comparison group ($p < 0.001$), with the PAI-1 concentration being 1.6 times higher in the comparison group. No significant differences were observed between the groups in terms of α2-antiplasmin, ICAM-1, and von Willebrand factor levels. Significant differences for homocysteine were found between the ACVA group and the comparison group ($p < 0.001$), with the concentration in the comparison group being 1.8 times higher.

Conclusions. The development of acute cerebrovascular accident in patients with lower concentrations of homocysteine and PAI-1 may be explained by weaker compensatory mechanisms aimed at repairing of the vascular wall and harmonization of interaction of hemostasis system links, which eventually led to vascular wall damage.

Keywords: ischemic stroke; hemorrhagic stroke; coronavirus; homocysteine; antiphospholipid antibodies; plasminogen activator inhibitor type 1

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✉ Olga V. Lyang lyang@fccps.ru

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ЛАБОРАТОРНЫЕ МАРКЕРЫ ЭНДОТЕЛИАЛЬНОЙ ДЕСТРУКЦИИ И АКТИВАЦИИ ГЕМОСТАЗА У ПАЦИЕНТОВ С ИНСУЛЬТОМ И COVID-19

О.В. Лянг[1,2✉], М.А. Солдатов[1], Л.В. Климов[1], Т.В. Киселева[1,3], Н.А. Марская[1], Н.А. Шамалов[1]

[1] Федеральный центр мозга и нейротехнологий Федерального медико-биологического агентства, Москва, Россия

[2] Российский университет дружбы народов им. Патриса Лумумбы (РУДН), Москва, Россия

[3] Научно-исследовательский институт организации здравоохранения и медицинского менеджмента Департамента здравоохранения города Москвы, Москва, Россия

Введение. Выраженность эндотелиальной деструкции у пациентов с COVID-19 может быть взаимосвязана с риском развития острого нарушения мозгового кровообращения.

Цель. Изучение роли маркеров активации системы гемостаза и повреждения сосудистой стенки в развитии острого нарушения мозгового кровообращения (ОНМК) у пациентов с новой коронавирусной инфекцией.

Материалы и методы. В исследование были включены 38 пациентов с новой коронавирусной инфекцией и ОНМК и 40 пациентов с новой коронавирусной инфекцией без ОНМК. Всем пациентам определяли: антитела к бета-2 гликопротеину, антитела к кардиолипину, ингибитор активатора плазминогена 1-го типа (ИАП-1), α2-антиплазмин, молекулу межклеточной адгезии 1-го типа (ICAM-1), фактор Виллебранда, гомоцистеин.

Результаты. По уровню антифосфолипидных антител не было выявлено статистически значимых различий между группами, однако повышенные относительно референтного интервала антитела к бета-2 гликопротеину чаще встречались в группе без ОНМК. По уровню ИАП-1 были выявлены значимые различия между группой с ОНМК и группой сравнения ($p < 0,001$), в группе сравнения концентрация ИАП-1 была в 1,6 раза выше. По уровню α2-антиплазмина, ICAM-1 и фактора Виллебранда значимых различий между группами выявлено не было. Статистически значимые различия по гомоцистеину были выявлены между группой с ОНМК и группой сравнения ($p < 0,001$), концентрация в группе сравнения была в 1,8 раза выше.

Выводы. Развитие ОНМК у пациентов с более низкими концентрациями гомоцистеина и ИАП-1, вероятно, можно объяснить более слабыми компенсаторными механизмами, направленными на репарацию сосудистой стенки и гармонизацию взаимодействия звеньев системы гемостаза, что в конечном счете приводит к повреждению сосудистой стенки.

Ключевые слова: ишемический инсульт; геморрагический инсульт; коронавирус; гомоцистеин; антифосфолипидные антитела; ингибитор активатора плазминогена

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✉ Лянг Ольга Викторовна lyang@fccps.ru

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INTRODUCTION

The new coronavirus infection (COVID-19) is an acute infectious disease caused by the SARS-CoV-2 strain of coronavirus. Although coronaviruses basically lack neurotropicity, they are capable of affecting the nervous system and disrupting its functions. One of the action mechanisms is respiratory hypoxia accompanying severe coronavirus pneumonia [1]. Another damage mechanism is the cytokine storm, which causes an increase in the pro-inflammatory cytokine level and activation of T-lymphocytes, macrophages, and endothelial cells. Vascular wall permeability increases, the complement system is activated, and blood coagulation properties increase [2]. COVID-19 was shown to develop overexpression of plasminogen activator inhibitor-1 (PAI-1) and thrombin-activated fibrinolysis inhibitor, thus leading to suppression of fibrinolysis and an even greater tendency to hypercoagulation [3].

The role of antiphospholipid antibodies (antibodies to cardiolipin and to β2–glycoprotein) in the pathogenesis of hemostasis system activation was described in previous research. These antibodies activate endothelial cells, monocytes, neutrophils, and platelets, resulting in the transformation of the anticoagulant surface of the endothelium into a procoagulant form [4]. The literature also describes the relationship between the formation of antiphospholipid antibodies as a result of infection and the development of ischemic stroke [5]. According to observations, endotheliopathy is observed in most patients. Markers of endothelial condition, inflammation, and coagulation such as IL-6, TNF-α, von Willebrand Factor (VWF), tissue factor, tissue factor inhibitor, D-dimer, thrombin-antithrombin complex, platelet factor P4, thromboglobulin, P-selectin, and platelet adhesion are significantly increased in mild to moderate COVID-19 [6].

Endothelial wall damage and hemostasis system activation caused by the SARS-COV-2 virus may be the cause of acute vascular events, such as hemorrhagic and ischemic stroke. Moreover, the probability of their development increases in patients with an increased risk of vascular disorders of the brain with upon the onset of the new coronavirus infection [7]. According to the TARGET-VIP hospital registry (Moscow), the incidence of acute cerebrovascular accident (ACVA) in the setting of the coronavirus infection was 0.8%, compared to 2.4% reported by foreign authors [8, 9]. The role of COVID-19 as a risk factor for ischemic brain damage was confirmed by data from the Regional Vascular Center (Botkin Hospital, Moscow). Thus, the number of ischemic stroke cases increased by 2.2–6.1% during the pandemic, while the proportion of hemorrhagic strokes and transient ischemic attacks decreased [10].

Thus, the severity of endothelial destruction and the accompanying activation of the hemostasis system towards hypercoagulation in patients with the new coronavirus infection may be correlated with the development of acute cerebrovascular accident. A number of laboratory markers characterizing the state of the endothelium and the hemostatic system in ischemic stroke have been studied. Thus, an extended meta-analysis showed that an increase in the inhibitor of plasminogen activator type 1 (PAI-1), which regulates the intensity of fibrinolysis in plasma, is associated with death, myocardial infarction, or acute cerebrovascular accident [11]. At the same time, it remains unclear whether PAI-1 is just a risk marker or an etiological cause of a vascular event [12]. The α2-antiplasmin acute phase protein, which inhibits the main enzyme of fibrinolysis plasmin, increases damage to the brain and the blood–brain barrier during acute ischemia by activating matrix metalloproteinases. Experiments showed that therapeutic inactivation of α2-antiplasmin reduces microvascular thrombosis, ischemic damage, and cerebral edema [13]. The Inter-Cellular Adhesion Molecule Type 1 (ICAM-1) expressed on the endothelium is significantly increased in stroke patients, reflecting damage to the blood–brain barrier [11]. The endothelial glycoprotein, von Willebrand factor, is involved in platelet adhesion at the vascular wall damage site. It was shown that elevated levels of von Willebrand factor are associated with the development of primary or recurrent stroke, as well as with fatal stroke [14, 15]. Homocysteine, a metabolite of the methionine and cysteine amino acids, is a marker of vascular wall damage and activation of the hemostatic system. This is one of the most studied biomarkers in stroke: hyperhomocysteinemia is associated with the risk of developing cardiovascular diseases, being a predictor of the severity of neurological symptoms in stroke and poor functional recovery [16–18]. Antibodies to β2-glycoprotein and cardiolipin are autoantibodies to phospholipids, which are markers of antiphospholipid syndrome, which is based

on vasculopathy associated with non-inflammatory and/or thrombotic vascular damage. In old age, the prevalence of positive antiphospholipid antibodies can reach 68% [19]. It was shown that these antibodies can participate in the pathogenesis of ischemic stroke, with their levels being positively correlated with the risk of stroke [20, 21].

In this work, we study the role of markers of hemostasis system activation and vascular wall damage in the development of acute cerebrovascular accident in patients with the new coronavirus infection.

MATERIALS AND METHODS

A total of 78 patients participated in the study, including 35 women and 43 men. The patients were divided into two groups: the study group included 38 patients with the new coronavirus infection and ACVA who were admitted to the Federal Center of Brain Research and Neurotechnologies and the Mukhin Hospital in Moscow, of whom four patients developed hemorrhagic stroke (HS) during hospitalization and 34 developed ischemic stroke (IS). Thus, the group of patients with ACVA was divided into two subgroups: patients with IS and patients with HS. The comparison group comprised 40 patients with the new coronavirus infection without ACVA, who were admitted to the Federal Center of Brain Research and Neurotechnologies.

Group 1 (n = 38) — patients with the new coronavirus infection + ACVA;

Group 1.1 (n = 4) — patients with the new coronavirus infection+ ACVA (HS);

Group 1.2 (n = 34) — patients with the new coronavirus infection+ ACVA (IS);

Group 2 (n = 40) — patients with the new coronavirus infection.

The average age of patients with HI was 70.9 ± 8.0 years; patients with IS — 72.0 ± 7.3 years; the comparison group — 58.2 ± 16.7 years. The criteria for inclusion of patients in the study were age over 18 years, detection of SARS-CoV2 RNA by PCR, clinical signs of pneumonia (body temperature above 38.5°C, respiratory rate above 22 per min, shortness of breath during physical exertion, saturation less than 95% in the air), pneumonia signs in the chest organs confirmed by computed tomography. All participants signed a voluntary informed consent to participate in the study.

Arterial hypertension (64%), chronic heart failure (39%), chronic kidney disease (25%), and diabetes mellitus (21%) prevailed among the concomitant diseases in the general sample. The stroke severity at admission according to the National Institutes of Health Stroke Scale (NIHSS) in the study group was 10.4 ± 5.7 points (13.8 ± 7.6 points in patients with HS; 9.9 ± 5.3 points in patients with IS). The distribution of pathogenetic variants of ischemic stroke was as follows: atherothrombotic — 5 patients (14.7%), cardioembolic — 11 patients (32.4%), lacunar — 2 patients (5.9%), unspecified — 16 patients (47.1%). At the beginning of hospitalization, in 52.6% of patients, the lung damage severity was established at the CT-1 level (77.5% in the comparison group; 29.4% in patients with IS; there were no patients with HS and CT-1). At the same time, grade 1 respiratory failure was detected in 70.5% of patients: in 85.0% of patients from the comparison group; in 58.8% of patients with IS, in 25.0% of patients with HS. The average hospital stay of patients in the study group was 11.0 ± 4.7 days. Hospital mortality was 5.2% (2 patients), caused by ischemic stroke; the immediate cause of death was cerebral edema. Upon discharge, the median on the Rehabilitation Routing Scale (RRS) was 4.0 (Q 3.0–4.3) points, on the modified Rankin scale — 3.0 (Q 3.0–3.3) points. The neurological symptoms severity according to NIHSS was 8.9 ± 7.2 points. The groups were representative in terms of concomitant diseases and severity of coronavirus infection.

Taking into account their biological role, antibodies to β2-glycoprotein, antibodies to cardiolipin, plasminogen activator inhibitor type 1 (PAI-1), α2-antiplasmin, intercellular adhesion molecule type 1 (ICAM-1), von Willebrand factor, homocysteine were selected for the study.

Venous blood in a volume of 5 mL was collected during the first hospital day in tubes with coagulation activator and separation gel (VACUTEST KIMA, Italy). About 30 min after blood collection, the tubes were centrifuged for 15 min at 1500 g on an ELMI CM-6MT centrifuge; the blood serum was aliquoted and frozen at –70 °C. Further, total antibodies to β2-glycoprotein (Euroimmun, Germany), total antibodies to cardiolipin (Euroimmun, Germany), type 1 plasminogen activator inhibitor (PAI-1) (ABclonal, China), a2-antiplasmin (ABclonal, China) were determined by enzyme immunoassay (ELISA) in serum; intercellular adhesion molecule type 1 (ICAM-1) (ABclonal, China), von Willebrand factor (VWF) (ABclonal, China), homocysteine (ABclonal, China) were determined on an Infinite F50 enzyme microplate automatic immunoassay analyzer using a Shellab GI2-2 laboratory incubator, a Titramax 101 platform vibrating shaker and a HydroFlex microplate flushing analyzer.

For each set of the reagents, a calibration curve was constructed based on measuring the optical density of standard solutions. The conversion of optical density into concentration in serum samples was carried out using the software installed in the Infinite F50 analyzer, taking the calibration data into account.

Statistical data processing was carried out using the SPSS 25.0, Microsoft Excel 2016 software packages. Descriptive statistics of continuous quantitative data after analysis of the normality of distribution are presented as the mean (M) and standard deviation for a normal distribution, or as the median (Md) and values of 25% of the lower and 75% of the upper quartiles using the sign Q [25–75%] for an abnormal distribution. The normal distribution was assumed to have a criterion for distinguishing the Kolmogorov–Smirnov type from a theoretically normal distribution of more than 0.05. Analytical statistics were performed using the Student's t-test for quantitative data with a normal distribution or the Wilcoxon, Mann–Whitney rank/sign sum test for quantitative data with a distribution other than normal. The probability value of $p < 0.05$ demonstrated statistical significance.

RESULTS AND DISCUSSION

The assessment of distribution normality of laboratory marker values showed that most of the markers did not have a normal distribution across a set of factors. As a result,

nonparametric analysis methods were used. Limitations on the choice of statistical methods were also associated with the limited number of patients in the HS subgroup. It should be noted that the objectives of the study did not include calculation of reference intervals, which reagent manufacturers propose to calculate for each laboratory independently through their own sampling. The results of laboratory measurements are shown in the Table.

No statistically significant differences were observed in the level of antiphospholipid antibodies between the groups. At the same time, antibodies to β2-glycoprotein were completely absent (not detected by ELISA) in 16 patients (42%) and antibodies to cardiolipin in 34 patients (89%). In the comparison group, 12 (30%) patients had no antibodies to β2-glycoprotein and 32 (80%) patients had no antibodies to cardiolipin. In 10 (26%) patients from the ACVA group and in 15 (38%) patients from the comparison group, the concentration of antibodies to β2-glycoprotein was higher than the reference range specified in the kit instructions (10 RU/mL), i.e., increased concentrations were more often observed in the comparison group. Elevated concentrations of antibodies to cardiolipin relative to the reference range (more than 10 RU/mL) were much less common: in 1 (2.6%) patient from the ACVA group and in 1 (2.5%) patient from the comparison group. β2-glycoprotein is a serum cofactor, having anticoagulant activity in vivo. Antibodies to β2-glycoprotein, in addition to directly suppressing activity, induce the expression of E-selectin on the membrane of endothelial cells and the secretion of proinflammatory cytokines and prostaglandin E2, which can lead to endothelial damage and activation of the hemostasis system towards hypercoagulation.[1] A single measured increase in the concentration of antiphospholipid antibodies in some patients does not yet indicate the development of antiphospholipid syndrome, although supporting the data of other authors that the appearance of antiphospholipid antibodies is associated with the viral infection caused by SARS-CoV-2 [22].

According to the PAI-1 level, statistically significant differences were found between the group with ACVA and the comparison group ($p < 0.001$), as well as between the group with IS and the comparison group ($p < 0.001$). In the comparison group, the concentration of PAI-1was 1.6 times higher than in patients with ACVA. The lower levels of PAI-1 in the ACVA group are likely to be due to the activation of fibrinolysis processes, which are triggered after a thrombotic vascular event, and suppression of blood antifibrinolytic activity.

No statistically significant differences were observed between the groups in terms of α2-antiplasmin, ICAM-1, and von Willebrand factor. At the same time, attention should be drawn to the tendency towards a higher level of the ICAM-1 endothelial molecule in patients with ACVA, which may be caused by damage to the blood–brain barrier due to stroke.

The results of measuring homocysteine in the studied groups turned out to be unexpected. Since the units of measurement from the kit instructions — ng/mL — were used to determine the concentration of homocysteine, and the kit itself was intended strictly for scientific use, we did not compare the level of homocysteine in the studied groups with the generally accepted threshold level[2] of 11.4 μmol/L.The calculation of our own reference interval was not part of the objectives of our study. Statistically significant differences were found between the group with ACVA and the comparison group ($p < 0.001$), the group with IS and the comparison group ($p < 0.001$), as well as between the group with HS and the comparison group ($p < 0.05$). The concentration in the comparison group was 1.8 times higher than in the group with ACVA, although the concentration of homocysteine tends to increase with age

Table. Laboratory parameters measured in patients of the main group and the comparison group

Laboratory parameter	Patients with Acute Cerebrovascular Accident (ACVA)			Comparison group *n* = 40
	Total patients of the ACVA group (HS+IS) *n* = 38	Patients with IS *n* = 34	Patients with HS *n* = 4	
Antibodies to β2-glycoprotein, RU/mL	5.0 [0.0–11.0]	6.8 [0.0–11.5]	0.0 [0.0–9.0]	7.2 [0.0–14.5]
Antibodies to cardiolipin, IU/mL	0.0 [0.0–0.0]	0.0 [0.0–0.0]	0.0 [0.0–0.0]	0.0 [0.0–0.0]
PAI-1, pg/mL	332* [157–456]	318* [152–456]	362 [321–528]	543 [354–773]
α2-antiplasmin, pg/mL	43 [19–90]	49 [19–90]	26 [21–44]	40 [27–81]
ICAM-1, pg/mL	45 [9–45]	37 [9–45]	45 [15–45]	15 [5–45]
von Willebrand factor, ng/mL	91 [110–138]	114 [91–140]	95 [87–122]	116 [77–131]
Homocysteine, ng/mL	22* [16–31]	22* [16–32]	24♦ [16–31]	39 [33–45]

Table prepared by the authors using their own data

Note: Data are presented as the median (Md) values of the lower and upper quartiles of *Q* [25%–75%]; statistical significance of differences with the comparison group: * $p < 0.001$; ♦ $p < 0.05$.

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due to a decrease in renal excretory function, and the age of patients with ACVA was higher than in the comparison group. We expected to obtain the opposite results, taking into account the damaging effect of homocysteine on endothelial cells, its suppressive effect on the natural anticoagulant antithrombin III, direct neurotoxic effect on brain neurons [23] and, consequently, the creation of prerequisites for the development of vascular events. However, the results obtained forced us to take a fresh look at the pathogenesis of ACVA in coronavirus infection.

Thus, the results obtained confirm the data that the new coronavirus infection can induce the formation of antiphospholipid autoantibodies, which were detected in more than half of the studied patients. Such patients should be further monitored and examined to exclude the development of antiphospholipid syndrome and thrombotic complications. Suppression of the antifibrinolytic link in patients with ACVA, manifested by a decrease in the concentration of PAI-1, may be a consequence of a compensatory increase in fibrinolysis in response to a thrombotic event that led to a stroke. Higher levels of homocysteine in patients with coronavirus infection without ACVA probably indicate an initially higher protective potential of the endothelial wall and the hemostatic system, which reduces the risk of developing ACVA in such patients. However, this assumption requires further elucidation.

CONCLUSION

The development of ACVA in patients with lower concentrations of homocysteine and PAI-1 may probably be explained by weaker compensatory mechanisms aimed at repairing the vascular wall and harmonizing the interaction of hemostasis system links, which ultimately leads to damage to the vascular wall. A more accurate understanding of the molecular mechanisms of stroke in coronavirus infection will be possible provided an extended panel of laboratory biomarkers and an increased patient cohort. Studying the mechanism of formation of antiphospholipid antibodies in the setting of the new coronavirus infection is another undoubtedly urgent task in the light of preventing thrombotic complications.

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AUTHORS

Olga V. Lyang, Dr. Sci. (Med.)
https://orcid.org/0000-0002-1023-5490
lyang@fccps.ru

Mikhail A. Soldatov
https://orcid.org/0000-0002-5294-5706
soldatov1477@gmail.com

Leonid V. Klimov, Cand. Sci. (Med.)
https://orcid.org/0000-0003-1314-3388
dr.klimov@mail.ru

Tatiana V. Kiseleva
https://orcid.org/0000-0003-4913-351X
tatiana-kis17@yandex.ru

Nataliya A. Marskaya
https://orcid.org/0000-0002-0789-4823
marskaya@fccps.ru

Nikolay A. Shamalov, Dr. Sci. (Med.)
https://orcid.org/0000-0001-6250-0762
shamalov@fccps.ru