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Evaluation of indicators of coagulation hemostasis and endothelial dysfunction in covid-19 convalescents

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Abstract

Purpose: Evaluation of indicators of coagulation hemostasis and humoral markers of endothelial dysfunction in COVID-19 convalescents.

Material and methods: 105 COVID-19 convalescents were examined. Patients were included in the study after suffering COVID-19 after 4-6 months. The average age of the surveyed was 51.8±6.7 years. Women made up - 64 (60.95%), men - 41 (39.05%). Hemostasis indicators were assessed by coagulogram parameters - fibrinogen level, determination of D-dimer by enzyme immunoassay, humoral factors of endothelial dysfunction - endothelin-1, von Willebrand factor, thrombomodulin by enzyme immunoassay.

Results: In the group of patients with elevated fibrinogen levels (more than 400 mg/dl), these values were significantly higher in terms of humoral markers of endothelial dysfunction than in patients with normal fibrinogen levels. Similarly, in patients with elevated levels of D-dimer, these values were significantly higher than in patients with normal levels of this indicator. When studying the levels of fibrinogen and D-dimer in the blood of COVID-19 convalescents, a direct correlation between them was established in relation to endothelin-1, VWF and thrombomodulin. There was a strong correlation between D-dimer and humoral factors endothelin-1 (r=0.88) and VWF (r=0.61), while thrombomodulin had a moderate correlation (r=0.56).

Conclusions: In the blood of COVID-19 convalescents, there was a significant increase in the level of fibrinogen and D-dimer. In COVID-19 convalescents, an increase in the level of markers of endothelial dysfunction was associated with indicators of the clinical course and indicators of fibrinogen and D-dimer.

Keywords: COVID-19 convalescents; Hemostasis indicators; Markers of endothelial dysfunction; **H**ighly sensitive C-reactive protein

1. Introduction

The COVID-19 pandemic poses global challenges for the entire healthcare system of the world, such as early diagnosis, treatment, prevention of complications, rehabilitation, and organization of vaccination [1,2]. According to the World Health Organization (WHO), "a pandemic of infection caused by a new strain of coronavirus - SARS-CoV-2, has led to a rapid increase in the number of cases and high deaths worldwide...". Along with damage to the respiratory organs in COVID-19, it has been established that important prognostic criteria for its course are the age of the patient, the presence of cardiovascular and other concomitant diseases [3,4]. According to multicenter studies, the mortality rate in patients with cardiovascular diseases due to COVID-19 is high, which makes it important to improve the methods for objective

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assessment of clinical and functional processes in them, as well as to develop methods for predicting the development of the disease [5,10]. Studies to assess the clinical course of the disease, complications, clinical and functional features of comorbid conditions in patients with COVID-19 are focused on identifying new aspects and early signs of etiopathogenesis by studying specific biomarkers and molecular genetic factors; scientific research is being carried out to develop a methodology for early detection and prediction of the risk of complications of the disease [6,7,13]. Direct and indirect damage to SARS-CoV-2 endothelial cells in patients with COVID-19 increases the activity of the renin-angiotensin-aldosterone system (RAAS), which increases the risk of worsening the course of the disease and developing an unfavorable prognosis [8,9,11]. In a multicenter cohort study, elevated levels of endothelial dysfunction biomarkers, RAAS hormones, in patients with COVID-19 were associated with an increased risk of death and poor prognosis [10,12].

1.1. Purpose

Evaluation of indicators of coagulation hemostasis and humoral markers of endothelial dysfunction in COVID-19 convalescents.

2. Material and methods

As part of the study, 105 COVID-19 convalescents were examined. Patients were included in the study after suffering COVID-19 after 4-6 months. The average age of the surveyed was 51.8±6.7 years. Women made up - 64 (60.95%), men - 41 (39.05%). The clinical characteristics of patients are presented in tab. 1.1. The survey did not include patients with acute cerebrovascular accident (ACV), past stroke, severe diabetes mellitus and insulin-dependent DM, chronic obstructive pulmonary disease, high-grade arrhythmias, severe liver and kidney diseases. In all COVID-19 convalescents, anamnesis was carefully collected, taking into account cardiovascular risk factors, extracts from the medical history for the treatment of COVID-19 were analyzed, where data were presented on the course of the disease, the degree of lung damage according to MSCT, and the therapy performed. When assessing cardiovascular risks, it was found that aggravated heredity was found in 100 (95.24%) examined patients, obesity - in 40 (38.09%), physical inactivity - 97 (92.38%), dyslipidemia - 72 (68.5%), smoking - 29 (27.61%), AH - 100 (95.2%) examined. In 42 (40%) examined patients, IHD was diagnosed, with FC II in 40 (38.10%) and FC III in two (1.90%). CHF was determined in 68 (64.76%) examined patients. Their distribution according to the 6-MWT data for FC showed that FC I was established in 15 (14.29%), FC II in 33 (31.4%) and FC III in 20 (19.05%). AF was established in one (0.95%) patient.

The following laboratory studies were carried out complete blood count, coagulogram, enzyme immunoassay for determination of D-dimer (JSC Vector-Best, Russia), highly sensitive C-reactive protein (CRP) (Demeditec Diagnostics, Germany), endothelin-1 (Elabscience, USA), von Willebrand factor (Elabscience, USA), thrombomodulin (Elabscience, USA).

The data obtained during the study were subjected to statistical processing on a Pentium-IV personal computer using the Microsoft Office Excel-2019 software package, including the use of built-in statistical processing functions. Methods of variational parametric and nonparametric statistics were used with the calculation of the arithmetic mean of the studied indicator (M), standard deviation (SD), relative values (frequency, %), the statistical significance of the measurements obtained when comparing the average values was determined by Student's t test (t) with the calculation of the error probability (R). Comparison of three or more independent groups was carried out by one-way analysis of ANOVA variations. Significance level $P < 0.05$ was taken as statistically significant changes. To identify the most reliable indicators, the presence of a correlation dependence between them was determined. At the same time, the relationship was designated as having a strong relationship at $r = 0.6$-1, moderate - at $r = 0.3$-0.6, weak - at $r < 0.3$.

3. Results and discussion

In the blood of COVID-19 convalescents, there was a significant increase in the level of CRP, fibrinogen and D-dimer. Highly sensitive CRP amounted to 6.03±0.88 mg/ml, fibrinogen - 398.71±8.14 mg/dl, D-dimer - 214.55±4.73 ng/ml, while in patients with IHD the level of highly sensitive CRP increased by 2 times amounting to 12.8±0.70 mg/l ($p < 0.01$), the level of fibrinogen and D-dimer increased by 13.4% and 31%, amounting to 503.6±9.6 mg/dl and 248.72±8.03 mg/ml (Table 1). It was noted that the level of CRP ($p < 0.05$), highly sensitive CRP ($p < 0.001$), fibrinogen ($p < 0.01$) and D-dimers ($p < 0.01$) in the blood of COVID-19 convalescents with IHD and CHF were significantly higher than in the general group of patients. At the same time, the level of these indicators in patients with CHF was: CRP was 25.6±4.17 mg/l ($p < 0.001$); fibrinogen - 568.6±10.3 mg/dl ($p < 0.01$) and there was a 2-fold increase in the amount of D-dimer amounting to 13.4±0.42 ($p < 0.01$).

Table 1 Indicators of inflammation and coagulation hemostasis in COVID-19 convalescents

№	Indicators	Total (n=105)	AH (n=100)	IHD (n=42)	CHF (n=68)
1	A high-sensitivity C-reactive protein (hs-CRP) (mg/ml)	6.03±0.88	10.80±0.28	12.8±0.70*	13.4±0.42*
2	Fibrinogen (mg/dl)	398.71±8.14	498.1±8.33	503.6±9.6*	568.6±10.3*
3	D-dimer (ng/ml)	214.55±4.73	221.6±4.8	248.72±8.03*	289.8±6.02*

Note: *p<0.05; ** - p<0.001 differences are significant in comparison with the indicators of the general group

The study of indicators of inflammation and coagulation hemostasis in COVID-19 convalescents, depending on the occurrence of cardiovascular risk factors in one person, showed that the level of high sensitivity CRP in persons with 3 and 4 risk factors increased almost 2 times, amounting to 13.2 ± 0.3 and 14 .7±1.5 mg/l (p<0.01) versus 7.9±0.75 in individuals with 1 risk factor. The same dynamics was observed in relation to the indicators of fibrinogen and D-dimer, the level of fibrinogen and D-dimer significantly increased by 13.4% and 31% in patients with 3 risk factors and by 55.7% and 34.1% in patients with 4 risk factors (Table 2). In comorbid patients included in the ACTIV SARS-CoV-2 registry and other studies, in the presence of cardiovascular risk factors, higher laboratory indicators of inflammation and D dimer were observed, which were associated with an increase in readmissions and mortality in the post hospital period [10,11].

Table 2 Indicators of inflammation and coagulation hemostasis in COVID-19 convalescents depending on the occurrence of risk factors

№	Indicators	1 risk factor (n=5)	2 risk factors (n=12)	3 risk factors (n=45)	4 or more risk factors (n=42)
1	High sensitivity CRP (mg/ml)	7.9±0.75	10.5±0.61	13.2±0.3	14.7±1.5
2	Fibrinogen (mg/dl)	383.5±13	503.6±9.6	545.6±45.9**	568.6±10.3**
3	D-dimer (ng/ml)	210±14.5	224.1±12.58	243±7.78*	274.6±7.47*

Note: p<0.05; ** - p<0.001 differences are significant in comparison with the indicators of persons with 1 risk factor

Endothelin-1, one of the biomarkers of endothelial dysfunction, was 90.61±2.36 pg/ml in COVID-19 convalescents, and 80.76±16.23 in individuals with 1 RF; with 2 FR - 88.87±3.68 pg/ml, with 3 FR - 89.07±3.34 pg/ml and 4 FR - 94.3 ±7.49 pg/ml, which was 29.3 % higher compared to persons with 1 RF, respectively. Analysis of this parameter depending on the presence of CVD showed that in patients with AH this indicator was 91.57±2.45, in patients with IHD - 94.21±4.39 and CHF - 99.5±3.31 pg/ml (p<0.01) (Table 3).

Table 3 Indicators of endothelial function in COVID-19 convalescents

№	Indicator	Total n=105	AH n=100	IHD n =42	CHF n =68
1	Endothelin I (pg/ml)	90.61±2.36	91.57±2.45	94.21±4.39	99.5±3.31*
2	VWF (%)	128.67±1.76	129.2±1.83	134.43±2.51	139.1±2.17
3	Thrombomodulin (pg/ml)	1388.5±18.2	1393±18.6	1394.07±23.9	1436±21.7*

The VWF index in persons who had undergone COVID-19 was 128.67 ± 1.76%, in persons with 1 risk factor this indicator was 117.06 ± 10.8%; with 2 RF - 124.1±2.56%, with 3 RF - 129.9±3.66% (p<0.05) and 4 RF - 134±2.67% (p<0.01), which was 23.5% higher compared to persons of 1 RF, respectively. Analysis of this parameter depending on

the presence of CVD showed that in patients with AH this indicator was 129.2±1.83%, in patients with IHD - 134.43±2.51% and CHF - 139.1±2.17% (p<0.05).

The index of thrombomodulin in persons who had undergone COVID-19 was 1388.5±18.2 pg/ml, in persons with 1 RF this indicator was 1351±69.79; with 2 RF - 1365±38.1 pg/ml, with 3 RF - 1410±26.14 (p<0.05) and 4 RF - 1425±25.5 (p<0.01), which was 19 .6% higher compared to persons of 1 RF, respectively. Analysis of this parameter depending on the presence of CVD showed that in patients with AH this indicator was 1393±18.6 pg/ml, in patients with IHD - 1394.07±23.9 and CHF - 1436±21.7 pg/ml (p <0.05).

4. Conclusion

The study of the consequences of the transferred COVID-19, as well as the tactics of actions in the post-COVID period, is of particular interest and has many controversial and unresolved issues. By definition, post-COVID syndrome includes signs and symptoms that develop during or after COVID-19 and last >12 weeks that cannot be explained by another cause. In the blood of COVID-19 convalescents, there was a significant increase in the level of coagulation hemostasis and inflammation, characterized by a significant increase in fibrinogen and D-dimer, a highly sensitive CRP. The results obtained indicate the feasibility using the proposed laboratory tests to determine the level of indicators of coagulation hemostasis and humoral markers of endothelial dysfunction in the blood of patients with the risk of a complicated course of coronary heart disease in concomitant COVID-19 patients. In IHD patients, the level of highly sensitive CRP increased by 2 times, amounting to 12.8±0.70 mg/l (p<0.01), the level of fibrinogen and D-dimer increased by 13.4% and 31%, amounting to 503.6± 9.6 mg/dl and 248.72±8.03 mg/ml. In COVID-19 recombinants, an increase in the level of endothelial dysfunction parameters was associated with the clinical course of the disease and coagulation hemostasis and inflammation.

Thus, the combined use of three markers of endothelial dysfunction - endothelin-1, von Willebrand factor and thrombomodulin - opens up new prospects in the study of patients with coronary pathology in COVID-19 convalescents.

Disclosure of conflict of interest

The authors declare that no conflict of interest.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

Contribution of the authors

The concept and design of the study - Kamilova U.K.; collection of material - Zakirova G.A.; material processing and laboratory tests - Utemuradov B.B.; statistical data processing - Zakirova G.A., Utemuradov B.B.; writing the text - Kamilova U.K., Zakirova G.A.; editing - Kamilova U.K.

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