

The State of Microcirculatory Hemodynamics in Patients with Moderate and Severe COVID-19

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Microcirculatory hemodynamic indexes (HI) were assessed in patients with moderate and severe COVID-19. In both groups, a significant increase in the absolute spectral indexes (HI1, HI2, and HI3) and the ratio of low-frequency to high-frequency component (HI1/HI3) was revealed. In the group of severe infection, only the “slow” index (low-frequency HI1) of microcirculatory hemodynamics was significantly lower. The oscillatory indices MAYER1-3 and RESP1-3 were reduced in patients of both groups. The aggravation of the disease course was accompanied by depression of the low-frequency index HI1. Regulatory shifts compensate for disturbances in microcirculatory processes in moderate COVID-19, but severe course was associated with their decompensation.

Key Words: *microcirculation; hemodynamics; hemodynamic indexes; COVID-19*

COVID-19 is associated with high risk of thrombosis due to damaging effect of SARS-CoV-2 on endothelial cells [12], development of the so-called cytokine storm [11], activation of the complement system along the lectin pathway [10], stimulation of angiotensin-converting enzyme 2 [7], and many other factors that enhance the aggregation of blood cells, stimulate expression of tissue factor (TF), and reduce the level of natural anticoagulants, which ultimately leads to a sharp increase in the blood coagulation [3]. At the same time, hemocoagulation and aggregation of blood cells are closely related with changes in hemodynamics in the microvascular bed [1,2]. Cytokine storm accompanied by obvious symptoms of shock (weak peripheral pulse and pallor of the extremities) without obvious hypotension, but with severe metabolic acidosis attest to significant dysfunction of microcirculation in patients with extremely severe COVID-19 [5]. Moreover, analysis of microvascular and endothelial damage is of paramount importance for elucidation of the pathophysiological mechanisms underlying the

clinical course of COVID-19 and for development of new therapies contributing to a decrease in the number of patients who require intensive care. There is no doubt that endothelial damage is the key pathophysiological factor leading to multiple organ failure (MOF) and even death [11].

V. S. Edul, *et al.*, [8] assessed the state of sublingual microcirculation and the time of capillary filling using manual video microscopy in 27 COVID-19 patients with acute respiratory distress syndrome (ARDS), who were on mechanical ventilation. All patients were relatively stable and had normal blood lactate, but the level of D-dimer and capillary filling time were increased, the blood flow heterogeneity index was high, and the proportion of perfused vessels, microvascular flow index, and erythrocyte movement rate were reduced. The fraction of perfused vessels in patients was inversely proportional to the total vascular density. Based on these findings, the authors come to a preliminary conclusion that the decrease in the proportion of perfused vessels and blood flow rate along with high density of vessels can be associated with increased angiogenesis or capillary recruitment caused by hypoxia.

The method of flow-mediated skin fluorescence (FMSF) proposed for evaluation of microcirculation

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allows measuring the shifts in the NADH fluorescence intensity in response to blockage and resumption of blood flow in the forearm [9]. It is assumed that the shifts in microcirculation in COVID-19 are largely due to hypoxia associated with impaired respiratory function and the development of thrombotic microangiopathy.

During the current COVID-19 pandemic, venovenous extracorporeal membrane oxygenation (VV-ECMO) was employed in many patients with severe acute respiratory syndrome caused by the SARS-CoV-2 coronavirus not responding to mechanical ventilation [4]. It was found that in this case, the patients showed an increase in the density and other parameters of microvessels. An inverse correlation between the density of capillaries and the levels of D-dimer has been proven. The use of the VV-ECMO in seriously ill patients slightly improved, but did not normalize the state of microvessels.

Many diseases, especially in the elderly, are risk factors for the development of severe complications and even deaths in COVID-19. These primarily include damage to the cardiovascular system, including hypertension, as well as diabetes, lung disease and obesity. It was hypothesized [13] that vascular endothelial cells are a common link between SARS-CoV-2 invasion and pre-existing cardiometabolic diseases. Cytokine storm is induced by rapid viral replication and excessive release of proinflammatory cytokines, including IL-1 β , IFN-1, and IL-6 and leads to apoptosis of endothelial cells. In addition, proinflammatory cytokines increase vascular inflammation and expression of leukocyte adhesion molecules to the vascular endothelium. This leads to activation of the endothelium accompanied by the procoagulant and proadhesive phenotype typical of endothelial dysfunction, which ultimately causes serious changes in microcirculation and, therefore, in tissue perfusion. At the same time, specific mechanisms associated with the violation of microcirculatory processes and hemodynamics in COVID-19 remain poorly studied.

Our aim was to study fine mechanisms of microcirculatory hemodynamics in patients with moderate and severe course of COVID-19.

MATERIALS AND METHODS

The study was carried out in accordance with the principles of Declaration of Helsinki of World Medical Association (Ethical Principles for Medical Research Involving Human Subject, 2008) and Order No. 200n of the Ministry of Health of the Russian Federation (On Approval of the Rules of Good Laboratory Practice; April 1, 2016). Written informed consent was obtained from all patients.

The study involved COVID-19 patients in moderate condition ($n=31$; mean age 58.4 ± 6.7 years) treated in the infectious diseases department and seriously ill patients (age 59.4 ± 9.2 years) receiving treatment in the intensive care unit of the 1st City Clinical Hospital of Chita, repurposed for the treatment of patients with COVID-19. The control group consisted of 69 subjects (age 62.5 ± 9.6 years). To evaluate the direct contribution of coronavirus infection disease into the dynamics of changes, we selected the control group taking into account the complications typical of seriously ill COVID-19 patients. The proportion of concomitant diseases (type 2 diabetes mellitus, hypertension, arthritis and arthrosis, and metabolic syndrome) was identical in both the control and the studied groups of patients.

Standard therapy was carried out in accordance with the current version of the Temporary Guidelines of the Ministry of Health of the Russian Federation "Prevention, Diagnosis, and Treatment of the New Coronavirus Infection COVID-19". Antiviral therapy included hydroxychloroquine, lopinavir/ritonavir. In addition, two-component antibiotic therapy was carried out. The patients received low-molecular-weight heparins (LMWH) in a dose of 1 mg/kg 2 times a day or continuous intravenous infusion of unfractionated heparin (UFH) with a starting rate of 1000 U/h with subsequent correction of the rate of administration based on the blood coagulation parameters. Respiratory therapy included inhalations of humidified oxygen, non-invasive mechanical ventilation, and invasive mechanical ventilation. Symptomatic therapy consisted of correction of glycemia, nutritional support, and maintenance of water and electrolyte balance.

The state of blood flow was studied using an mDLS sensor (Dynamic Light Scattering) and using an original algorithmic approach. For this purpose, a technique has been developed for spectral decomposition of the signal into frequency components associated with hemodynamic sources of different shear rates of blood layers [6].

For interpretation of multifrequency analysis, we used a hemodynamic index (HI) determined as the intensity of oscillation of the reflected laser radiation in a frequency band corresponding to the volume blood flow at a certain shear rate. The low-frequency index (HI1) is determined by the slow interlayer interaction, the high-frequency region (HI3) characterizes the fast shear processes of the layers. HI2 occupies an intermediate position (precapillary and capillary blood flow). The relative indices RHI1, RHI2, and RHI3 denote the normalized (relative) contribution of each component to the total dynamic processes: $RHI1=HI1/(HI1+HI2+HI3)$, $RHI2=HI2/(HI1+HI2+HI3)$, and $RHI3=HI3/(HI1+HI2+HI3)$. To assess the tendencies of blood flow redistribution between the fast and slow

processes, the indices of the difference (HI1-HI3) and the ratio and (HI1/HI3) were introduced. For each HI (HI1, HI2, and HI3), an additional measure of slow fluctuations in blood flow, the oscillatory hemodynamic index (OHI), is used. The following OHI were determined: 0.005-0.05 Hz, blood movement associated with the endothelium (NEUR); 0.05-0.15 Hz, blood movement determined by the muscular layer of the vessels (MAYER); 0.15-0.6 Hz, blood movement determined by the respiratory cycle (RESP), and 0.6-3 Hz, pulse tremors (PULSE). The method is implemented using a dynamic light scattering (DLS) sensor from Elfi-Tech and measures signals initiated by cutaneous blood flow.

Statistical data processing was performed using version 4.0.3 of the specialized language for statistical analysis R. To describe quantitative features, medians (Me) and percentiles (25%-75%) were determined. To compare quantitative indicators, the nonparametric Wilcoxon's test was used. The null hypothesis was rejected at a significance level of 0.05.

RESULTS

In patients with moderate COVID-19, a significant increase in all hemodynamic indices HI1, HI2, and HI3 was observed (Table 1), which undoubtedly indicate a significant increase in shear processes in microcirculation. At the same time, the most significant changes are observed in the hemodynamic indices HI1 and HI2, which characterize the parietal (endothelial) and intermediate (between axial and endothelial, HI2) blood flow. HI3 index characterizing central blood flow was less involved in this process. Similar changes in hemodynamics were observed in patients with severe COVID-19.

There is no doubt that the increase in all shear rates in the microcirculation system of patients with COVID-19 of moderate severity is a compensatory re-

action to inflammation of the endothelium, thrombotic angiopathy, enhanced aggregation of blood cells, and increased blood viscosity, and, most likely, is a result of increased cardiac activity. It is possible that the increase in all major hemodynamic indices is due to a sharp decrease in the number of functioning small vessels due to their massive thrombosis. An insignificant tendency towards a decrease in the hemodynamic index HI1 in patients with severe, in comparison with moderate course of the disease, can be regarded as a failure of compensatory mechanisms aimed at the maintenance of blood flow in affected organs. This assumption is confirmed by an increase in the HI1/HI3 ratio, which is most pronounced in COVID-19 patients with moderate disease severity.

These data suggest that the contribution of the low-frequency component relative to other components of microcirculation increases, *i.e.* the effect of high-frequency components — cardiac and respiratory oscillations on the microcirculation did not significantly change in patients. However, the contribution of low-frequency fluctuations (endothelial, myogenic, and neurogenic components) increased, *i.e.* the incretory function of the endothelium was activated, the ratio of vasoconstrictor and vasodilatation factors (endothelins, nitrogen oxide, *etc.*) changed. On the other hand, increasing activity of the low-frequency components indicates disorders in tissue metabolism and attempts to optimize tissue perfusion and metabolism via endothelial, myogenic and neurogenic mechanisms.

Of particular interest, in our opinion, are changes in oscillatory indices that characterize the regulatory processes caused by impulses from receptors of various tissues, organs and organ systems in COVID-19 of different severity (Table 2).

In patients with moderate and severe course of the disease, we revealed equal decrease in Mayer 1-3 and Resp1-3 in comparison with controls. The

TABLE 1. Hemodynamic Indices in Patients with COVID-19 (Me (25%-75%); arb. units)

Parameter	Control	Moderate COVID-19	Severe COVID-19
HR	69 (62-81.2)	77 (71.2-84.8)*	78.5 (61-87.8)
HI1	134 (123-150)	1420 (1280-1540)***	1250 (1120-1440)***
HI2	337 (282-377)	1510 (1160-1850)***	1600 (1340-1850)***
HI3	161 (131-187)	656 (450-893)***	871 (491-1170)***
HI1/HI3	0.785 (0.689-0.886)	2.08 (1.61-2.64)***	1.55 (1.12-2.37)***
RHI1	0.211 (0.195-0.228)	0.403 (0.337-0.439)***	0.349 (0.318-0.43)***
RHI2	0.521 (0.508-0.539)	0.409 (0.38-0.44)***	0.414 (0.391-0.427)***
RHI3	0.266 (0.242-0.287)	0.186 (0.172-0.227)***	0.223 (0.18-0.272)**

Note. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ in comparison with the control (Mann—Whitney test with correction for multiple comparison).

TABLE 2. Oscillatory Indices in Patients with COVID-19 (Me (25%-75%); arb. units)

Parameter	Control	Moderate COVID-19	Severe COVID-19
MAYER1	0.031 (0.021-0.049)	0.019 (0.014-0.027)***	0.022 (0.016-0.031)*
MAYER2	0.040 (0.021-0.057)	0.024 (0.012-0.036)**	0.018 (0.010-0.030)***
MAYER3	0.024 (0.012-0.041)	0.015 (0.009-0.026)*	0.014 (0.008-0.025)**
RESP1	0.128 (0.114-0.143)	0.102 (0.090-0.106)****	0.099 (0.094-0.105)****
RESP2	0.153 (0.137-0.170)	0.097 (0.081-0.122)****	0.100 (0.084-0.113)****
RESP3	0.142 (0.122-0.151)	0.071 (0.059-0.101)****	0.082 (0.067-0.105)****
PULSE1	0.739 (0.710-0.766)	0.815 (0.752-0.836)****	0.812 (0.780-0.834)****
PULSE2	0.683 (0.653-0.712)	0.757 (0.701-0.788)****	0.758 (0.701-0.807)****
PULSE3	0.710 (0.68-0.753)	0.791 (0.701-0.843)*	0.734 (0.673-0.819)****

Note. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ in comparison with the control.

absence of these changes can be due to not only endothelial dysfunction, but also inflammatory changes in the endothelium leading to the deposition of thrombotic masses (thrombotic microangiopathy) accompanied by impaired activity of the receptor apparatus. In both moderate and severe COVID-19, we observed a clear-cut tendency to a decrease in the amplitudes of oscillations primarily associated with MAYER 2 and to a lesser extent with MAYER 3. Changes in oscillatory indices determined by MAYER 1 and 2 did not differ in patients with moderate and severe course of the disease. The decrease in oscillations determined by the muscular layer of blood vessels that affect microcirculation, from our point of view, is due to damage to the vascular system and failure of regulatory mechanisms.

At the same time, in both moderate and severe COVID-19, the oscillations associated with PULSE1 and PULSE2 slightly increase, which is due to an increased load on the heart. However, we found no significant differences in the oscillatory indices PULSE1 and PULSE2.

Finally, we found a significant decrease (by more than 1.5 times) in RESP1, RESP2, and RESP3, which was undoubtedly associated with damage to the lung tissue (pneumonia) and the development of fibrosis.

In addition, changes in respiratory oscillation can be associated with respiratory insufficiency, shortness of breath, and the effect of the “chest pump” on preload. In patients with a severe course of the disease against the background of mechanical ventilation, the inspiratory pressure in the thorax increases which significantly changes the state of hemodynamics. Deviations of blood flow fluctuations in the pulse range, obviously, reflect compensatory changes in central hemodynamics in response to decompensated respiratory failure.

These findings indicate that the inflammatory changes in blood vessels and the heart, micro- and

macrothrombosis, and the development of pulmonary fibrosis in both moderate and severe forms of COVID-19 induce changes in hemodynamic functions aimed at adaptation of the organism to new conditions. In the moderate form, these shifts more or less compensate for disturbances in microcirculatory processes, but in a severe form, they are insufficient, which ultimately leads to multiple organ failure and can be fatal. However, conclusions on this issue can only be drawn if we carefully analyze the hemodynamic state in critically ill COVID-19 patients who ended in a favorable or fatal outcome, which was beyond the scope of our study.

REFERENCES

1. Kuznik BI, Smolyakov YuN, Guseva ES, Davydov SO, Fine IV. Effect of moderate physical exercises on the relationship of variability of the heart rhythm with the level of blood pressure and hemodynamic functions in women with essential hypertension. *Patol. Fiziol. Eksp. Ter.* 2020;64(3):64-73. doi: 10.25557/0031-2991.2020.03.64-73. Russian.
2. Smolyakov YN, Kuznik BI, Romanyuk SV. Adaptive response of microcirculatory hemodynamics indexes on the state of general dehydration. *Zabaikal. Med. Vestn.* 2019;(2):83-89. Russian.
3. Barnes BJ, Adrover JM, Baxter-Stoltzfus A, Borczuk A, Cools-Lartigue J, Crawford JM, Daßler-Plenker J, Guerci P, Huynh C, Knight JS, Loda M, Looney MR, McAllister F, Rayes R, Renaud S, Rousseau S, Salvatore S, Schwartz RE, Spicer JD, Yost CC, Weber A, Zuo Y, Egeblad M. Targeting potential drivers of COVID-19: Neutrophil extracellular traps. *J. Exp. Med.* 2020;217(6):e20200652. doi: 10.1084/jem.20200652
4. Carsetti A, Damiani E, Casarotta E, Scorcella C, Domizi R, Montomoli J, Gasparri F, Gabbanelli V, Pantanetti S, Carozza R, Adrario E, Donati A. Sublingual microcirculation in patients with SARS-CoV-2 undergoing veno-venous extracorporeal membrane oxygenation. *Microvasc. Res.* 2020; 132:104064. doi: 10.1016/j.mvr.2020.104064

5. Colantuoni A, Martini R, Caprari P, Ballestri M, Capecchi PL, Gnasso A, Lo Presti R, Marcoccia A, Rossi M, Caimi G. COVID-19 sepsis and microcirculation dysfunction. *Front. Physiol.* 2020;11:747. doi: 10.3389/fphys.2020.00747
 6. Fine I, Kuznik B, Kaminsky A, Shenkman L. A non-invasive method for the assessment of hemostasis in vivo by using dynamic light scattering. *Laser Physics.* 2012;22(2):469-475. doi: 10.1134/S1054660X12020090
 7. Henry BM, Vikse J, Benoit S, Favalaro EJ, Lippi G. Hyperinflammation and derangement of renin-angiotensin-aldosterone system in COVID-19: a novel hypothesis for clinically suspected hypercoagulopathy and microvascular immunothrombosis. *Clin. Chim. Acta.* 2020;507:167-173. doi: 10.1016/j.cca.2020.04.027
 8. Kanoore Edul VS, Caminos Eguillor JF, Ferrara G, Estensoro E, Siles DSP, Cesio CE, Dubin A. Microcirculation alterations in severe COVID-19 pneumonia. *J. Crit. Care.* 2021;61:73-75. doi: 10.1016/j.jcrc.2020.10.002
 9. Katarzynska J, Borkowska A, Los A, Marcinek A, Cypryk K, Gebicki J. Flow-mediated skin fluorescence (FMSF) technique for studying vascular complications in type 2 diabetes. *J. Diabetes Sci. Technol.* 2020;14(3):693-694. doi: 10.1177/1932296819895544
 10. Keragala CB, Draxler DF, McQuilten ZK, Medcalf RL. Haemostasis and innate immunity — a complementary relationship: a review of the intricate relationship between coagulation and complement pathways. *Br. J. Haematol.* 2018;180(6):782-798. doi: 10.1111/bjh.15062
 11. Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, Pan P, Wang W, Hu D, Liu X, Zhang Q, Wu J. Coronavirus infections and immune responses. *J. Med. Virol.* 2020;92(4):424-432. doi: 10.1002/jmv.25685
 12. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J. Thromb. Haemost.* 2020;18(4):844-847. doi: 10.1111/jth.14768
 13. Tibirica E, De Lorenzo A. Importance of the evaluation of systemic microvascular flow and reactivity in critically ill patients with coronavirus disease 2019 — COVID-19. *Microvasc. Res.* 2020;131:104028. doi: 10.1016/j.mvr.2020.104028
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