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Clinical, pathogenetic and prognostic significance of vascular endothelial growth factor content in the blood serum of patients with coronavirus disease (COVID-19) with pneumonia in relation to the parameters of immune inflammation and haemostasis in assessing the risk of death

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

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Aim. To find out the clinical, pathogenetic and prognostic significance of vascular endothelial growth factor in the blood serum of patients with coronavirus disease with pneumonia in relation to the parameters of immune inflammation and haemostasis in assessing the risk of death.

Material and methods. We examined 123 patients with coronavirus disease (COVID-19) with pneumonia. Laboratory examination of patients was carried out in accordance with the Order of the Ministry of Health of Ukraine of 28.03.2020 No. 722. The patients were divided into groups depending on the outcome of the disease: 77 patients who recovered and 46 patients who died. The content of vascular endothelial growth factor (VEGF) (total form) in the blood serum was determined by enzyme-linked immunosorbent assay (Immuno-Biological Laboratories Co., Ltd., Japan). Statistical data processing was performed in the program "Statistica for Windows 13".

Results. It was found that in patients with coronavirus disease with pneumonia, the level of increase in serum VEGF (total form) was associated with the outcome of the disease. When patients were hospitalised on day 9.0 [7.0; 12.0] of illness, a serum VEGF level >32.04 pg/ml (AUC = 0.842, $p < 0.001$) indicated a high risk of adverse outcome, and after 7 days of treatment, with a VEGF level >58.79 pg/ml (AUC = 0.899, $p < 0.001$), there was a high probability of death. The correlation between the development of thrombotic complications and the level of VEGF increase in the blood serum both at the time of hospitalisation (gamma +0.32, $p < 0.05$) and after 7 days of treatment (gamma +0.37, $p < 0.05$) was established. The occurrence of thrombotic complications was more frequent in patients with a fatal outcome than in patients who had recovered (18.4 times, $p = 0.000001$). The level of VEGF in the blood serum >51.91 pg/ml (AUC = 0.680, $p = 0.019$) was prognostic of the risk of developing thrombotic complications only in the dynamics after 7 days of hospital treatment. The clinicopathogenetic role of increased serum VEGF in the progression of the disease in patients with COVID-19 pneumonia was demonstrated by statistically significant correlations.

Conclusions. In patients with COVID-19 pneumonia, VEGF (total form) level elevation in serum is associated with the risk of death. The threshold levels of VEGF, which are informative for prognosing the risk of unfavourable disease course at different follow-up periods, have been established.

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Клініко-патогенетичне та прогностичне значення вмісту VEGF у сироватці крові хворих на коронавірусну хворобу (COVID-19) та пневмонію у взаємозв'язку з параметрами імунного запалення та гемостазу під час оцінювання ризику летального наслідку

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Мета роботи – з'ясувати клініко-патогенетичне та прогностичне значення вмісту VEGF в сироватці крові хворих на коронавірусну хворобу COVID-19 та пневмонію у взаємозв'язку з показниками імунного запалення та гемостазу в оцінюванні ризику летального наслідку.

Матеріали і методи. Обстежили 123 хворих на COVID-19 і пневмонію. Лабораторне обстеження та лікування хворих здійснили згідно з наказом МОЗ України від 28.03.2020 р. № 722. Залежно від наслідків хвороби пацієнтів поділили на групи: 77 осіб, які одужали, та 46 хворих із летальним наслідком. Вміст VEGF (загальна форма) в сироватці крові визначали методом імуноферментного аналізу (Immuno-Biological Laboratories Co., Ltd., Japan). Статистично дані опрацювали за допомогою програми Statistica for Windows 13.

Результати. Встановлено, що у хворих на COVID-19 і пневмонію рівень підвищення VEGF в сироватці крові мав зв'язок із наслідком хвороби. На час госпіталізації хворих на 9,0 [7,0; 12,0] дня хвороби рівень

VEGF (загальна форма) в сироватці крові >32,04 pg/ml (AUC = 0,842, p < 0,001) свідчив про високий ризик несприятливого наслідку хвороби; через 7 діб лікування, якщо рівень VEGF становив >58,79 pg/ml (AUC = 0,899, p < 0,001), зберігалася висока ймовірність летального наслідку хвороби. Встановлено взаємозв'язок розвитку тромботичних ускладнень із рівнем підвищення VEGF у сироватці крові і на час госпіталізації (гамма +0,32, p < 0,05), і через 7 діб лікування (гамма +0,37, p < 0,05). Розвиток тромботичних ускладнень частіше спостерігали у пацієнтів, які мали летальний наслідок хвороби, порівняно з хворими, котрі одужали (в 18,4 раза, p = 0,000001). Рівень VEGF у сироватці крові >51,91 pg/ml (AUC = 0,680, p = 0,019) мав прогностичну значущість щодо оцінювання ризику розвитку тромботичних ускладнень лише в динаміці через 7 діб лікування в стаціонарі. Клініко-патогенетичну роль підвищення рівня VEGF у сироватці крові в прогресуванні хвороби у пацієнтів із COVID-19 і пневмонією підтверджено статистично значущими кореляціями.

Висновки. У хворих на COVID-19 і пневмонію рівень підвищення VEGF у сироватці крові мав зв'язок із ризиком летального наслідку хвороби. Встановлено межові рівні VEGF (загальна форма), що в різні терміни спостереження є інформативними щодо прогнозування ризику несприятливого перебігу хвороби.

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Today, it is believed that coronavirus disease (COVID-19) should be considered a systemic disease, since in addition to the excessive production of proinflammatory cytokines, several other major pathological phenomena develop due to endothelial cell dysfunction, namely hypercoagulation, micro- and macrothrombosis [1]. One of the explanations for the progression of COVID-19 and the high incidence of thrombotic complications in this disease is the understanding of the formation of the so-called immunothrombosis, the morphological signs of which were detected in 40.8 % of vessels [2]. SARS-CoV-2-associated immunothrombosis is formed as a result of hypercoagulation regulated by inflammatory mediators and accompanied by activation and interaction of platelets, neutrophils and extracellular structures released by activated neutrophils [3]. Today, attention is drawn to the role of activated neutrophils in the formation of immunothrombosis. On the one hand, the release of extracellular structures by activated neutrophils plays a role in the body's defence, and on the other hand, their release is associated with tissue damage, hypercoagulation and thrombosis [4]. It has been proven that extracellular structures released by activated neutrophils directly cause endothelial cell death, activate platelets, recruit procoagulation factors, and stimulate the production of proinflammatory cytokines by immunocompetent cells [4]. Data obtained in clinical practice show that coagulopathy in COVID-19 is caused by endothelial damage, contributes to the severity of the disease and the risk of death [5,6].

In studying the pathogenetic mechanisms of endothelial damage and progression of COVID-19, a number of studies [1,7,8,9,10] have focused on the role of vascular endothelial growth factor (VEGF), which is an important regulator of angiogenesis. It is believed that VEGF plays a key role in regulating the physiological vascular permeability during angiogenesis [11]. To date, the VEGF family is known to consist of eight members: VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F, placental growth factor and endocrine growth factor [1,12]. VEGF A (VEGF-A or VEGF) is a highly conserved secreted signalling protein best known for its role in angiogenesis [13]. Despite a significant number of studies to determine the role of VEGF in the course of COVID-19, their results have some contradictions. Thus, it has been proven that there are elevated levels of VEGF-A and placental growth factor in the blood plasma of patients with COVID-19, with the level of increase having a direct correlation with the severity of the disease [7,8,9]. The researchers found higher levels of VEGF-A in the small intestine tissue of patients with COVID-19 compared to healthy people. At the same time, only VEGF-A was significantly elevated, in contrast to VEGF-B and VEGF-C, whose levels remained unchanged [14].

However, these data also have some contradictions to the results of other researchers. Thus, according to some authors [7,8], in patients with COVID-19, the level of VEGF-B remained almost unchanged, and the level of VEGF-D was even lower compared to healthy individuals and had an inverse correlation with the assessment of multiorgan damage. However, other researchers, on the contrary, demonstrated that the level of VEGF-D elevation is the most important indicator in assessing the severity of the disease, even more informative than D-dimer, interleukin-6 levels, and the absolute number of lymphocytes in the peripheral blood [10].

Thus, the above indicates the need for studies to determine the clinical, pathogenetic and prognostic significance of VEGF in the serum of patients with coronavirus disease (COVID-19) with pneumonia in relation to the parameters of immune inflammation and haemostasis in assessing the probable unfavourable course of the disease.

Aim

To find out the clinical, pathogenetic and prognostic significance of vascular endothelial growth factor in the blood serum of patients with coronavirus disease with pneumonia in relation to the parameters of immune inflammation and haemostasis in assessing the risk of death.

Material and methods

The study included 123 patients with coronavirus disease (COVID-19) with pneumonia. In all patients, the diagnosis was etiologically confirmed by the detection of RNA-SARS-CoV-2 by polymerase chain reaction, and the presence of pneumonia was confirmed by imaging methods (CT-scan or chest X-ray radiography). The patients' age ranged from 29 years to 88 years. There were 57 men and 66 women. All patients underwent inpatient treatment in Municipal Non-Profit Enterprise "Regional Infectious

Table 1. Dynamics of VEGF (total form) content in the blood serum of patients with coronavirus disease (COVID-19) with pneumonia based on the consequences of the disease

Parameter, units of measure	Healthy people, n = 20	Patients during hospitalisation who subsequently		Patients in the dynamics after 7 days, who subsequently	
		recovered, n = 77	died, n = 46	recovered, n = 77	died, n = 46
VEGF, Me [Q25; Q75] pg/ml	13.08 [10.50; 14.96]	32.04 [20.07; 51.11][1]	62.82 [39.99; 93.75][1,2]	50.58 [33.84; 62.50][1,3]	72.58 [63.20; 81.19][1,2]

1: the difference is significant compared to healthy people ($p < 0.05$); **2:** compared to patients who subsequently recovered during the relevant follow-up period ($p < 0.05$); **3:** compared to hospitalisation of patients in the corresponding group ($p < 0.05$).

Disease Clinical Hospital" of the Zaporizhzhia Regional Council. Laboratory examination and treatment of patients was carried out in accordance with the Order of the Ministry of Health of Ukraine of 28.03.2020 No. 722 "Organisation of medical care for patients with coronavirus disease (COVID-19)". Patients were included in the study on a random basis and with informed consent.

Depending on the outcome of the disease, patients with coronavirus disease (COVID-19) with pneumonia were divided into groups: 77 patients who recovered and 46 patients who died. A special study included the determination of the content of vascular endothelial growth factor (VEGF) (total form) in the blood serum by enzyme-linked immunosorbent assay according to the instructions offered by the manufacturer (Immuno-Biological Laboratories Co., Ltd., Japan). The immunoassay was conducted at the Training Medical and Laboratory Center of Zaporizhzhia State Medical and Pharmaceutical University (Supervisor – R. O. Shcherbyna, PhD, DSc, Associate Professor). To identify the diagnostic role of VEGF level in predicting the development of a lethal outcome of the disease, its level in the serum of patients at hospitalization and after 7 days of treatment was studied. The control group consisted of 20 healthy individuals.

Statistical processing of the obtained data was performed using the software Statistica for Windows 13 (StatSoft Inc., No. JPZ804I382130ARCN10-J). The normality of the distribution was assessed using the Shapiro–Wilk test. The difference in the distribution of the quantitative traits studied from the normal distribution law led to the use of nonparametric statistical methods. The results of quantitative data are presented in the form of median and interquartile ranges Me [Q25; Q75]. To determine the differences between quantitative features in the independent groups, the Mann–Whitney test was used, in the dependent groups – the Wilcoxon test, and between qualitative features – the χ^2 test. To establish the diagnostic significance of VEGF in predicting the probability of death in COVID-19 with pneumonia, a ROC analysis was performed to determine the threshold level. Spearman's correlation was used to establish relationships between quantitative features, and gamma correlation was used between quantitative and qualitative features, respectively. Differences at $p < 0.05$ were considered significant.

Results

According to the results of the studies, it was found that in patients with COVID-19 pneumonia of both study groups, the level of VEGF (total form) in the blood serum was statistically significantly higher ($p < 0.05$) compared to healthy people during the entire observation period. At the time of hospitalization on day 9.0 [7.0; 12.0] of the disease, in patients who later died, the level of VEGF in the blood serum was 1.9 times higher ($p < 0.05$) than in patients who later recovered. This pattern remained after 7 days of observation, namely, the level of VEGF in the blood serum was 1.4 times higher ($p < 0.05$) in patients with an unfavourable outcome *(Table 1)*.

Considering the established pattern of changes in the content of VEGF (total form) in the serum of patients with COVID-19 pneumonia in the dynamics depending on the outcome of the disease, we performed a ROC analysis at different follow-up periods to establish the diagnostic significance in assessing the probability of the risk of death. According to the results of the ROC analysis, it was found that at the time of hospitalisation, the threshold level of VEGF in the blood serum was >32.04 pg/ml (AUC = 0.842, $p < 0.001$). That is, exceeding this level indicated a high probability of death in the future (sensitivity – 100 %, specificity – 54.17 %) *(Fig. 1A)*. The analysis of the diagnostic relevance of this indicator after 7 days of treatment showed that at its level >58.79 pg/ml (AUC = 0.899, $p < 0.001$), patients with COVID-19 pneumonia had a high probability of death (sensitivity – 92.86 %, specificity – 72.00 %) *(Fig. 1B)*.

An analysis of the incidence of thrombotic complications in patients with coronavirus disease (COVID-19) with pneumonia showed that one in ten patients (9.8 %) developed thrombotic complications, namely ischemic stroke, myocardial infarction and pulmonary embolism. One patient had a combination of ischemic stroke and myocardial infarction. The incidence of thrombotic complications was clearly associated with an unfavourable outcome of COVID-19. Thus, the development of thrombotic complications was statistically significantly more common in patients with a fatal course of the disease compared to patients who had recovered (18.4 times, $\chi^2 = 16.73$, $p = 0.000001$). In addition, myocardial infarction ($\chi^2 = 8.72$, $p = 0.003$) and pulmonary embolism ($\chi^2 = 6.63$, $p = 0.01$) were more common in the unfavourable course of COVID-19 than in patients who recovered *(Table 2)*.

The GAMMA correlation analysis allowed us to establish the relationship between the development of thrombotic complications and the level of VEGF increase in the blood serum, both at the time of hospitalisation (gamma +0.32, $p < 0.05$), and after 7 days of treatment (gamma +0.37, $p < 0.05$), which confirmed the clinical and pathogenetic relationship of changes in the level of VEGF (total form) in the blood serum of patients with COVID-19 with pneumonia in the formation of thrombotic complications.

We considered the established patterns of changes in VEGF content in the blood of patients with COVID-19 pneumonia in the dynamics in relation to the progression of thrombotic com-

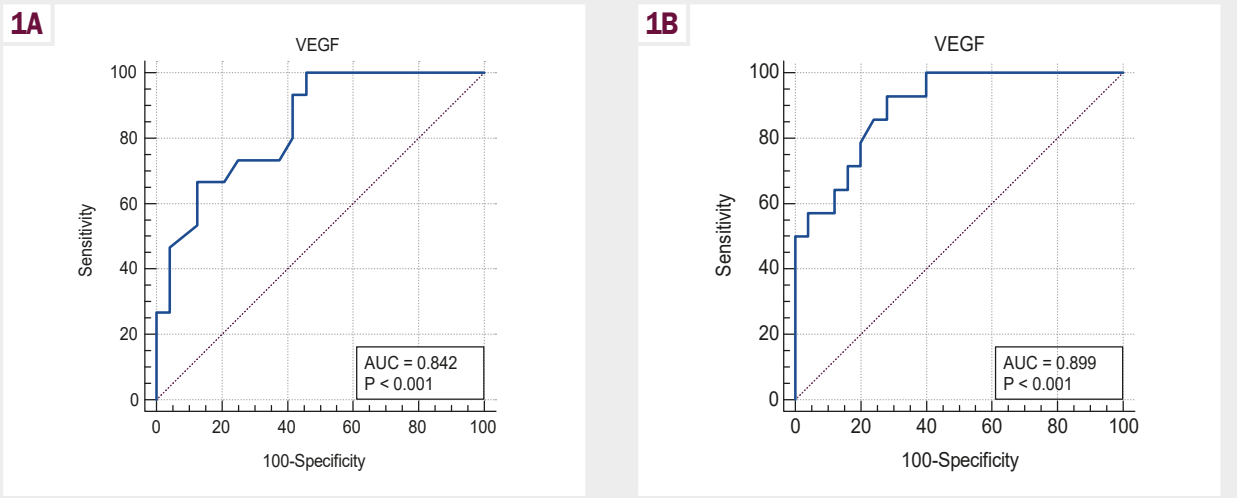

Fig. 1. Diagnostic significance of VEGF (total form) in the serum of patients with COVID-19 pneumonia at hospitalisation **(A)** and after 7 days of treatment **(B)** in assessing the probability of death.

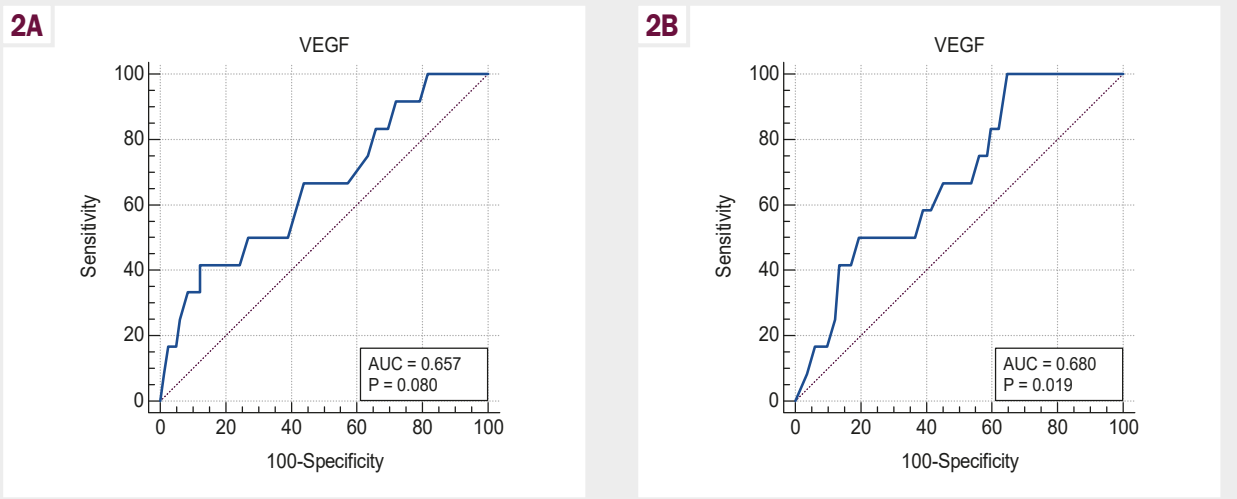

Fig. 2. Diagnostic significance of VEGF (total form) in the serum of patients with COVID-19 pneumonia at hospitalisation **(A)** and after 7 days of treatment **(B)** in assessing the risk of developing thrombotic complications.

plications. To establish the diagnostic significance in assessing the likelihood of their development, we have performed a ROC analysis at different follow-up periods. It was found that at the moment of hospitalisation, the level of VEGF (total form) in the blood serum was not prognostic of the risk of developing thrombotic complications (AUC = 0.657, p = 0.08) *(Fig. 2A)*. However, in the dynamics of 7 days of treatment, the information value of this indicator became statistically significant. With serum VEGF level >51.91 pg/ml (AUC = 0.680, p = 0.019), patients with COVID-19 pneumonia were at risk of developing thrombotic complications (sensitivity – 100 %, specificity – 35.39 %) (*Fig. 2B*).

Comparative analysis of acute inflammatory response and haemostasis in patients with COVID-19 pneumonia depending on the course of the disease indicated that at the time of hospitalization on 9.0 day [7.0; 12.0] of illness in patients who later died, acute inflammatory changes and signs of hypercoagulability were more pronounced than in patients who later recovered, which was confirmed by a higher ($p < 0.05$) level of C-reactive protein (CRP), the ratio of absolute neutrophils to absolute lymphocytes (N/L) and the content of D-dimer in the blood *(Table 3)*.

In the dynamics of observation after 7 days in patients who later recovered, there was a decrease in serum CRP levels (3.6 times, $p < 0.05$), an increase in lymphocytes ($p < 0.05$), a decrease in N/L ($p < 0.05$) in combination with a decrease in fibrinogen content (1.4 times, $p < 0.05$) and an increase in platelet count ($p < 0.05$). In contrast to patients who recovered, in patients with COVID-19 pneumonia who subsequently died, in the dynamics of observation after 7 days there was an increase in leukocytosis (2.6 times, $p < 0.01$), an increase in the absolute number of neutrophils (2.7 times, $p < 0.01$), a decrease in the relative (2.5 times, $p < 0.01$) number of lymphocytes and, accordingly, an increase in the N/L ratio (2.6 times, $p < 0.01$), which was combined with a sharp increase in the content of D-dimer (6.8 times, $p < 0.05$) *(Table 3)*.

Comparison of acute inflammatory response and haemostasis in patients with COVID-19 pneumonia depending on the

Table 2. Comparison of the frequency and list of thrombotic complications in patients with coronavirus disease (COVID-19) with pneumonia depending on the outcome of the disease

Thrombotic complications, abs. (%)	COVID-19 patients with pneumonia, n = 123	COVID-19 patients with pneumonia	
		recovered, n = 77	died, n = 46
The presence of thrombotic complications, including:	12 (9.8)	1 (1.3)	11 (23.9)*
– ischemic stroke	4 (3.3)	1 (1.3)	3 (6.5)
– myocardial infarction	5 (4.1)	–	5 (10.9)*
– pulmonary embolism	4 (3.3)	–	4 (8.7)*
– combination of thrombotic complications	1 (0.8)	–	1 (9.1)

*: the difference is significant compared to patients who recovered during the corresponding follow-up period ($p < 0.05$).

Table 3. Dynamics of the main indicators of acute inflammatory response and haemostasis in patients with COVID-19 pneumonia depending on the course of the disease, Me [Q25; Q75]

Indicator, units of measure	Patients during hospitalisation who subsequently		Patients in the dynamics after 7 days, who subsequently	
	recovered, n = 77	died, n = 46	recovered, n = 77	died, n = 46
Indicators of acute inflammatory reaction				
CRP mg/L	53.45 [34.90; 114.10]	131.40 [67.4; 180.0][1]	15.0 [5.00; 27.5][2]	84.75 [25.95; 161.50][1]
Leukocytes, $\times 10^9$/L	6.5 [4.8; 9.0]	6.9 [4.6; 9.6]	8.0 [6.0; 9.2]	18.1 [12.1; 28.2][1,2]
Neutrophils, $\times 10^9$/L	5.46 [3.67; 7.74]	6.25 [3.96; 8.73]	6.01 [4.19; 7.56]	16.96 [11.76; 25.73][1,2]
Lymphocytes, %	12.0 [9.0; 22.0]	10.0 [7.0; 16.0]	19.0 [14.0; 26.0][2]	4.0 [3.0; 7.0][1,2]
Lymphocytes, $\times 10^9$/L	0.9 [0.6; 1.2]	0.8 [0.5; 1.0]	1.3 [1.1; 1.8][2]	0.8 [0.6; 1.1][1]
N/L	6.44 [3.30; 10.00]	8.15 [4.98; 12.96][1]	4.40 [2.56; 6.40][2]	21.33 [11.83; 32.22][1,2]
Haemostatic parameters				
Prothrombin index, %	110.0 [98.4; 124.0]	107.1 [94.8; 123.6]	104.1 [92.4; 119.1]	90.0 [69.0; 101.7][1,2]
International normalized ratio	0.94 [0.87; 1.04]	0.93 [0.83; 1.03]	0.96 [0.89; 1.05]	1.07 [0.99; 1.26][1,2]
Fibrinogen, g/L	5.2 [3.9; 5.9]	5.6 [4.3; 6.3]	3.8 [2.9; 4,5][2]	5.0 [3.8; 6.1][1]
D-dimer, μg/ml	0.9 [0.5; 1.8]	1.3 [0.9; 2.3][1]	1.3 [0.6; 2.3]	8.8 [3.7; 21.4][1,2]
Platelets, $\times 10^9$/L	220.0 [181.0; 287.0]	204.0 [158.0; 251.0]	283.0 [224.0; 332.0][2]	219.5 [148.0; 303.0][1]

1: the difference is significant compared to patients who subsequently recovered during the relevant follow-up period ($p < 0.01$); **2:** compared to hospitalisation of patients in the corresponding group ($p < 0.05$).

course of the disease after 7 days of treatment showed a statistically significant greater severity of these changes in patients with an unfavourable outcome. Namely, in patients who later died, in contrast to patients who later recovered, the CRP content, leucocytosis, absolute neutrophils count, N/L ratio, D-dimer level (6.8 times, $p < 0.05$) were statistically significantly higher ($p < 0.05$) and the relative and absolute lymphocyte counts were lower ($p < 0.05$) *(Table 3)*.

Spearman's correlation analysis allowed us to establish the relationship between the level of increase in serum VEGF (total form) with indicators of acute inflammatory response and haemostatic parameters both at the time of hospitalisation and in the dynamics in patients with COVID-19 pneumonia. The level of increased serum VEGF (total form) was associated with a decrease in oxygen saturation ($r = -0.24$, $p < 0.05$) at the moment of hospitalization on day 9.0 [7.0; 12.0] of the disease. This indicated the role of this growth factor in the progression of lung damage in patients with COVID-19 pneumonia. VEGF (total form) level in the blood serum had correlations with the level of CRP ($r = +0.31$, $p < 0.05$), relative ($r = -0.29$, $p < 0.05$) and absolute ($r = -0.37$, $p < 0.05$) blood lymphocyte count at the time of hospitalisation. When assessing the correlations in the dynamics after 7 days of treatment, it was noted that there were correlations between the level of increase in VEGF in the blood serum and indicators of acute inflammatory response, namely, CRP level ($r = +0.35$, $p < 0.05$), total leukocyte count ($r = +0, 47$, $p < 0.05$), absolute number of neutrophils ($r = +0.50$, $p < 0.05$), relative ($r = -0.53$, $p < 0.05$) and absolute ($r = -0.29$, $p < 0.05$) number of blood lymphocytes, N/L ratio ($r = -0.50$, $p < 0.05$), as well as with haemostatic parameters, namely fibrinogen ($r = +0.24$,

p < 0.05) and D-dimer (r = +0.54, p < 0.05). The established correlations allowed us to confirm the clinical and pathogenetic role of increased levels of VEGF (total form) in the blood serum in the progression of the disease in patients with coronavirus disease (COVID-19) with pneumonia.

Discussion

According to the results of our study, we found a high level of VEGF (total form) in the blood serum of patients with COVID-19 with pneumonia during the entire observation period compared with healthy people (p < 0.05). At the same time, the level of increase in VEGF depended on the subsequent outcome of the disease, namely, during hospitalisation in patients who later died, the level of VEGF in the blood was 1.9 times higher (p < 0.05) than in patients who later recovered. This pattern persisted after 7 days of observation.

Our data are consistent with the results of other studies [15,16], which also found that VEGF can be considered one of the key factors involved in the progression of COVID-19, and its sharp increase correlates with the course of the disease. In the current literature, there is even a certain explanation for the significant increase in the level of VEGF-A in the blood of patients with COVID-19. It is assumed that the S-protein of SARS-CoV-2 has the ability to bind to neuropilin-1, which is usually a co-receptor for VEGF-A. That is, since S-protein is able to antagonise the interaction of VEGF-A with neuropilin-1, this can lead to disruption of physiological pathways involved in angiogenesis. As a result of the above mechanism, the content of freely circulating forms of VEGF-A in the blood may increase, which can then bind to other receptors, which in turn can cause diffuse microvascular damage [17,18,19,20]. The authors believe that an excess of VEGF-A can also lead to remodelling of the interstitial spaces of the myocardium, which probably explains the development of cardiac arrhythmias in a number of patients with COVID-19 [21].

In our study, we assessed the diagnostic value of VEGF (total form) level elevation in predicting the risk of adverse outcome. Namely, using ROC analysis, we calculated the threshold VEGF levels in the blood at different periods of observation, the excess of which indicates a high risk of fatal outcome. We had calculated threshold VEGF (total form) level in the blood was >32.04 pg/ml (AUC = 0.842, p < 0.001) at the moment of hospitalisation on day 9.0 [7.0; 12.0] of the disease. In the literature, we found a study [9], in which the authors determined the prognostic value of another member of the VEGF family, namely placental growth factor in the blood plasma of patients with COVID-19. The researchers proved that the level of placental growth factor increase had a direct correlation with the severity of the disease and also calculated the threshold level of placental growth factor, which was 30 pg/ml, exceeding which was determined to be the best predictor of in-hospital mortality in patients with COVID-19 [9].

According to the results of our study, we have established a correlation between the development of thrombotic complications and the increase in the level of VEGF (total form) in the blood, both at the moment of hospitalisation (gamma +0.32, p < 0.05), and after 7 days of our treatment (gamma +0.37, p < 0.05), which confirmed the clinical and pathogenetic relationship of changes in the VEGF level in the blood of COVID-19 patients with pneumonia in the formation of thrombotic complications. The immune mechanism of procoagulant changes and, consequently, the formation of thrombotic complications is supported not only by their higher frequency of development in the unfavourable course of the disease (18.4 times, p = 0.000001) compared with patients who recovered, but also by statistically significantly higher rates of both acute inflammatory response and haemostatic parameters during the specified observation period. The clinical and pathogenetic role of VEGF in the progression of COVID-19 with pneumonia is proved by the established statistically significant correlations with oxygen saturation, acute inflammatory parameters and haemostatic parameters.

Currently, coagulation disorders and thrombotic complications are perceived as the dominant manifestations of endothelial dysfunction in severe COVID-19 [22]. It is known that one of the main functions of the endothelium at rest is to prevent unnecessary thrombosis, while procoagulant effects can be caused by activated endothelial cells due to virus infection [23]. Already at the beginning of the COVID-19 pandemic, there were numerous reports of coagulation disorders in patients with SARS-CoV-2 infection. At the same time, alveolar capillary microthrombi were recorded 9 times more often in patients with COVID-19 than in patients with influenza [24]. According to researchers [2], 57 % of patients with COVID-19 had pulmonary microthrombi, and in 15 % of autopsies of those who died as a result of COVID-19, thrombosis of medium and large pulmonary vessels was detected.

Even though activation of the VEGF pathway is not exclusive to COVID-19, a comparison of VEGF expression in SARS-CoV-2 infection and other respiratory viral infections of other etiologies has revealed different trends. Thus, in a study [25], when assessing immune changes in patients with moderate and severe COVID-19 course and pandemic influenza A(H1N1), a higher expression of VEGF in the serum of patients with COVID-19 was found. Similar results were obtained by other researchers [26], who demonstrated that children hospitalised for COVID-19, respiratory syncytial infection, influenza, and adenovirus infection had elevated levels of various cytokines, but only VEGF was significantly associated with COVID-19.

One of the latest findings in the progression of COVID-19 is pathological angiogenesis [1]. In the formation of clinically significant forms of coronavirus disease, there is an overexpression of various proangiogenic factors, such as VEGF, a fibroblast growth factor, in both plasma and lung biopsy of patients with COVID-19 [1]. It is believed that hypoxia and ischemia stimulate the production of growth factors that promote angiogenesis, with VEGF being one of the key angiogenic factors that contributes to the formation of new blood vessels based on existing ones [27]. It has been proven that age and gender do not have a significant effect on the level of VEGF, while factors leading to hypoxia or endothelial damage stimulate an increase in its concentration [28].

Based on an understanding of the role of VEGF in the development of acute respiratory distress syndrome in SARS-CoV-2 infection, a clinical trial of monoclonal recombinant antibodies targeting VEGF (bevacizumab) was even launched during the pandemic, targeting VEGF (bevacizumab) in the treatment of severe lung damage in patients with critical course of COVID-19 [29].

Conclusions

1. In patients with COVID-19 pneumonia, the level of serum VEGF (total form) elevation is associated with the outcome of the disease. At hospitalisation on day 9.0 [7.0; 12.0] of illness, a serum VEGF level >32.04 pg/ml (AUC = 0.842, p < 0.001) indicates a high risk of adverse outcome, and after 7 days of treatment, with a VEGF level >58.79 pg/ml (AUC = 0.899, p < 0.001), there is a high probability of death.

2. There is a correlation between the development of thrombotic complications and the level of increase in VEGF (total form) in the blood serum both at the time of hospitalisation (gamma +0.32, p < 0.05) and after 7 days of treatment (gamma +0.37, p < 0.05). The development of thrombotic complications is more common in patients with a fatal outcome than in patients who recovered (18.4 times, p = 0.000001). The level of VEGF in the blood serum >51.91 pg/ml (AUC = 0.680, p = 0.019) has prognostic significance for assessing the risk of developing thrombotic complications only in the dynamics after 7 days of hospital treatment.

3. The clinicopathogenetic role of increased serum VEGF (total form) in the progression of the disease in patients with COVID-19 pneumonia is demonstrated by statistically significant correlations (p < 0.05) of VEGF with oxygen saturation in the air at the moment of hospitalisation, as well as with the main acute inflammatory parameters and haemostasis parameters both at the time of hospitalisation and in the dynamics of treatment.

Prospects for further research. In our opinion, the prospects for further research in this area are to develop medical methods of correction of endothelial dysfunction in patients with coronavirus disease (COVID-19) with pneumonia.

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