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Pathogenetic and prognostic significance of inflammation and altered ADAMTS-13/vWF axis in patients with severe COVID-19

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Abstract

Introduction. Currently, endothelial dysfunction caused by inflammation and immunothrombosisis considered as one of the crucial mechanisms in developing the SARS-CoV-2 virus-mediated coronavirus disease 2019 (COVID-19). A mass endothelial damage followed by release of untypical large quantity of von Willebrand factor (vWF) multimers and subsequent consumption of metalloproteinase ADAMTS-13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) is described during severe COVID-19. The activation of innate immune cells including neutrophils results in formation of neutrophil extracellular traps (NETs) and myeloperoxidase (MPO) release that, in turn, contributes to spread of inflammation and microvascular thrombosis.

Aim: to evaluate a pathogenetic role and predictive significance for serum markers of inflammation, endothelial dysfunction and hemostatis activation such as vWF, ADAMTS-13 and MPO for in-hospital mortality in severe COVID-19 patients requiring mechanical lung ventilation.

2022 • Vol. 16 • № 3
Obstetrics, Gynecology and Reproduction

Materials and Methods. There was performed a single-center observational study with 129 severe COVID-19 patients on mechanical lung ventilation at the intensive care unit, by assessing serum in all subjects vWF, ADAMTS-13 as well as in 79 patients MPO level along with other potential predictors for in-hospital mortality.

Results. A multivariate analysis revealed that increased serum level for vWF antigen (vWF:Ag) and MPO antigen (MPO:Ag) were significantly and independently related to high mortality probability: vWF:Ag (IU/ml) – adjusted odds ratio (OR) = 3.360; 95 % confidence interval (95 % CI) = 1.562–7.228 (p = 0.0019); MPO:Ag (ng/ml) – adjusted OR = 1.062; 95 % CI = 1.024–1.101 (p = 0.0011). Such data allowed to obtained a simplified mortality score for categorizing patients as those having a higher or lower score compared with the median score level: a high score was associated with lower cumulative survival rate (p < 0.0001), with 50 % of the cases linked to lethal outcome on day 13 post-hospital admission.

Conclusion. Severe COVID-19 patients requiring mechanical lung ventilation were found to have elevated level of serum MPO activity and vWF correlating with poor survival.

Keywords: COVID-19, ADAMTS-13, von Willebrand factor, vWF, myeloperoxydase, MPO, survival

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Патогенетическое и прогностическое значение воспаления и нарушений в оси ADAMTS-13/vWF у больных тяжелой формой COVID-19

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2022 • Том 16 • № 3
Акушерство, Гинекология и Репродукция

Резюме

Введение. В настоящее время эндотелиальная дисфункция, вызванная воспалением и иммунотромбозом, рассматривается в качестве одного из ключевых механизмов COVID-19. При тяжелом течении COVID-19 описано массивное повреждение эндотелия с высвобождением большого количества мультимеров фактора фон Виллебранда (англ. von Willebrand factor, vWF) и последующим потреблением металлопротеиназы ADAMTS-13 (англ. a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13). Активация клеток врожденного иммунитета, в том числе нейтрофилов, приводит к образованию внеклеточных ловушек нейтрофилов (англ. neutrophil extracellular traps, NETs) и высвобождению миелопероксидазы (МПО), что в свою очередь способствует распространению процессов воспаления и тромбоза в микрососудистом русле.

Цель: изучение патогенетической роли и прогностической ценности циркулирующих в крови маркеров воспаления, дисфункции эндотелия и активации системы гемостаза, в частности, vWF, ADAMTS-13 и МПО в отношении внутрибольничной смертности у пациентов с тяжелой формой COVID-19, нуждающихся в искусственной вентиляции легких (ИВЛ).

Материалы и методы. Проведено одноцентровое ретроспективное наблюдательное исследование с участием 129 пациентов с тяжелым течением COVID-19, находившихся в отделении интенсивной терапии на ИВЛ. У всех пациентов определяли содержание vWF, ADAMTS-13 и у 79 – концентрацию МПО в сыворотке крови, а также другие показатели как потенциальные предикторы внутрибольничной смертности.

Результаты. Путем проведения многофакторного анализа было показано, что увеличение концентрации таких маркеров, как антиген vWF (vWF:Ag, МЕ/мл) и МПО человека (МПО:Аг, нг/мл) достоверно и независимо связаны с высокой вероятностью смертности: vWF:Ag – скорректированное отношение шансов (ОШ) = 3,360; 95 % доверительный интервал (95 % ДИ) = 1,562–7,228 (p = 0,0019); МПО:Аг – скорректированное ОШ = 1,062; 95 % ДИ = 1,024–1,101 (p = 0,0011). На основании этих результатов был получен упрощенный показатель смертности, и пациенты были классифицированы как имеющие значения данного показателя выше или ниже медианного: высокое значение показателя было связано с более низкой кумулятивной выживаемостью (p < 0,0001), в 50 % случаев смерть наступала на 13-е сутки госпитализации.

Заключение. При тяжелом течении COVID-19, требующем ИВЛ, повышенные концентрации МПО и vWF:Ag в крови у пациентов с тяжелым COVID-19 коррелируют с низкой выживаемостью.

Ключевые слова: COVID-19, ADAMTS-13, фактор фон Виллебранда, vWF, миелопероксидаза, МПО, выживаемость

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Highlights

What is already known about this subject?

- Activation of myeloid cells and deep endothelial damage play a key role in the pathogenesis of severe COVID-19.

What are the new findings?

- High myeloperoxidase (MPO) activity and von Willebrand factor (vWF:Ag) served as independent predictors of poor survival in severe COVID-19 patients admitted to intensive care unit for mechanical lung ventilation.

How might it impact on clinical practice in the foreseeable future?

- MPO may have a prognostic value in severe COVID-19 patients undergoing mechanical lung ventilation.
- Further prospective studies are warranted to: i) verify such data in a prospective multicenter study, ii) evaluate an impact of current therapeutic advances on the risk factors noted above.
- Our data may be a prerequisite to test emerging von Willebrand factor inhibitors, granulocyte activation and MPO.

Основные моменты

Что уже известно об этой теме?

- Активация миелоидных клеток и глубокое повреждение эндотелия занимают ключевую роль в патогенезе тяжелого течения COVID-19.

Что нового дает статья?

- Высокая активность миелопероксидазы (МПО) и антигена фактора фон Виллебранда (vWF:Ag) были независимыми предикторами плохой выживаемости у пациентов с тяжелой формой COVID-19, поступающих в отделение интенсивной терапии и требующих искусственной вентиляции легких (ИВЛ).

Как это может повлиять на клиническую практику в обозримом будущем?

- МПО может иметь прогностическое значение у тяжелых пациентов с COVID-19, находящихся на ИВЛ.
- Необходимы дальнейшие проспективные исследования, во-первых, для проверки полученных результатов на проспективной многоцентровой основе, во-вторых, для оценки влияния текущих терапевтических разработок на эти 2 фактора риска.
- Наши данные могут быть предпосылкой для тестирования разрабатываемых ингибиторов фактора фон Виллебранда, активации гранулоцитов и МПО.

Introduction / Введение

The COVID-19 pandemic consequences have raised many new questions in medicine, including the study of inflammation and hemostasis disorders in the pathogenesis of its severe forms. Virus-induced hemostasis disorders are not uncommon mainly being presented as hemorrhagic manifestations similar to the well-known acute hemorrhagic fever, particularly Crimean fever (Congo fever), Ebola fever, etc. [1]. Coronavirus infection has become a vivid of the multiple virus-related thrombotic effects. Thus, in the severe form of COVID-19, the frequency of venous thromboembolic complications averages 45.6 %, whereas in non-severe forms it comprises around 23 % [2, 3]. Today, it is known that mortality in COVID-19 is mainly associated with severe impairment of lung function (severe pulmonary insufficiency) and concomitant diffuse thrombosis. Moreover, pulmonary insufficiency is mainly associated with local thrombosis of pulmonary vessels (mainly at the capillary level) [4]. The main mechanisms of the disorders caused by SARS-CoV-2 infection include generation of thrombin and intravascular coagulation in capillaries, severe endothelial damage and macrophage/monocyte activation, release of excessive amounts of pro-inflammatory cytokines, externalization of glycosaminoglycans on the endothelial surface, formation of extracellular neutrophil traps, and activated complement systems [5, 6]. Pathological NETosis that develops under super inflammation can play a decisive role in the disease severity and outcome. NETosis is an excessive release of so-called neutrophil extracellular traps (NETs) due to excessive neutrophil activation during inflammation. NETs are extracellular structures similar to networks of chromatin threads lined up with highly active proteases as well as proteins of nuclear, cytosolic, and granular origin. NETosis inducers can be presented by microorganisms, bacterial components, activated platelets, complementary peptides, and autoantibodies. Activated platelets initiate a powerful release of NETs by neutrophils, thereby providing a scaffold for fibrin deposition and thrombus stabilization. The uncontrolled release of pro-inflammatory cytokines resulting from activation of monocytes/macrophages/neutrophils, endothelium, and the complement system is called a cytokine storm, which can contribute to the development of a thrombotic storm with emerging thrombotic microangiopathy (TMA) [7]. The TMA may stem from diverse causes, but one of the most important etiological factors is the deficiency of ADAMTS-13 metalloproteinase (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) and, as a result, inadequate proteolysis of von Willebrand factor (vWF) multimers, which have a high potential to activate platelets. During SARS-CoV-2-mediated endothelial damage, ADAMTS-13 is consumed by excessive amounts of high-molecular-weight von Willebrand factor and accumulation of ultra-high-molecular-weight multimers, which, in combination with adherent and aggregated platelets, cause microcirculatory thrombosis along with developing organ failure [8].

The thrombotic microangiopathy during systemic inflammatory response and NETosis may be also caused by directly inhibited natural ADAMTS-13 anticoagulant properties by neutrophil traps. Thus, thromboinflammation and immunothrombosis are now considered as the main processes underlying severe forms of COVID-19 [4, 9]. There are few publications on potential role of ADAMTS-13 and vWF in severe forms of COVID-19, with a limited number of patients studied and providing results. However, much less information was collected about the prognostic role of some NETosis markers, particularly myeloperoxidase (MPO), a known marker of neutrophil activation [10]. Although a high level of serum D-dimer, thrombocytopenia, and prolongation of prothrombin time have been proposed as prognostically unfavorable markers in severe COVID-19 [11, 12], the search for independent predictors that determine survival in severe forms of COVID-19 is still of importance. Thus, there is a need to analyze a prognostic value for laboratory hemostasis and inflammation parameters in severe forms of COVID-19.

Aim: to investigate a pathogenetic role and predictive significance for serum markers of inflammation, endothelial dysfunction and hemostatis activation such as vWF, ADAMTS-13 and MPO for assessing in-hospital mortality in severe COVID-19 patients requiring mechanical lung ventilation.

Materials and Methods / Материалы и методы

Study design / Дизайн исследования

There was conducted a single-center retrospective observational study of patients with severe forms of COVID-19. The patients were hospitalized at the intensive care unit (ICU) of Vorokhobov City Clinical Hospital № 67 that was used as the COVID hospital during the pandemic from May 2020 to May 2021. The diagnosis of COVID-19 and clinical manifestations were laboratory-confirmed in all patients by performing a reverse transcription-

polymerase chain reaction (PCR) test with nasal and oropharyngeal swabs for SARS-CoV-2 of all patients.

Inclusion and exclusion criteria / Критерии включения и исключения

Inclusion criteria: age > 18 years old; patients with severe COVID-19 requiring mechanical ventilation with a positive PCR result for SARS-CoV-2; acute respiratory distress syndrome caused by SARS-CoV-2.

Exclusion criteria: age < 18 years old; the admission to the ICU is due to non-SARS-CoV-2 secondary infection.

Study groups / Группы обследованных

There were examined 314 patients, among which 312 patients were selected into the study. Of these, 214 patients were at ICU with severe COVID-19 requiring mechanical lung ventilation.

During the selection process, out of 214 patients admitted to the ICU, only 129 were subsequently included in the study because relevant plasma samples were collected immediately upon admission to the ICU.

The control group consisted of 40 COVID-19-free healthy volunteers.

Study methods / Методы обследования

In 129 patients, parameters of hemostasis and inflammation such as vWF, ADAMTS-13 metalloproteinase (ADAMTS-13:Ag antigen, ADAMTS-13:Ac activity, ADAMTS-13:i inhibitor), D-dimer, C-reactive protein (CRP), ferritin, as well as platelet/lymphocyte ratios (PLT/LYM), ADAMTS-13:Ag/vWF:Ag, ADAMTS-13:Ac/vWF:Ag were analyzed.

In addition, serum MPO (MPO:Ag) level was assessed in 79 of them along with parameters indicated due to collecting sufficient number of plasma samples.

The blood plasma of healthy volunteers (control group) was also examined for such non-routine parameters.

Serum samples from patients obtained on day 1 of ICU admission before initiation of anticoagulant therapy were centrifuged and stored at – 80 °C. Subsequently, plasma samples were analyzed for routine parameters such as CRP and ferritin concentrations; prothrombin time (PT) and activated partial thromboplastin time (APTT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), as well as lactate dehydrogenase (LDH), hemoglobin concentration, total leukocyte count, platelet count, as well as whole blood platelet-to-lymphocyte ratio. Non-routine studies included analysis of platelet-poor plasma for von Willebrand factor antigen (vWF:Ag), ADAMTS-13 antigen (ADAMTS-13:Ag), ADAMTS-13 activity (ADAMTS-13:Ac), ADAMTS-13 inhibitor (ADAMTS-13:i) concentration using commercial TECHNOZYM® test kits (Technoclone Herstellung von Diagnostika und Arzneimitteln Gmb, Vienna, Austria). The reference normative ranges for these indicators, according to the manufacturer's recommendations, are: 0.41–1.41 IU/ml for ADAMTS-13:Ag; 0.4–1.3 IU/ml for ADAMTS-13:Ac; less than 15 U/ml for ADAMTS-13:i; 0.5–1.5 IU/mL (50–150 %) for WF:Ag.

The MPO antigen (MPO:Ag) was quantified by ELISA by using a commercial kit (Hycult Biotech, Netherlands). According to the manufacturer, the normative reference range for MPO:Ag is 2.56 ± 0.33 ng/ml.

D-dimer was determined using the TechnoLEIA kit (Technoclone, Austria). According to the manufacturer's recommendations, the normative reference range is less than < 250 ng/ml. Despite the manufacturer's recommendations for normative reference values, the results obtained in patients were also compared with those obtained in the control group.

Ethical aspects / Этические аспекты

The study was conducted under the Helsinki Declaration of Ethics in Human Research and was also approved by Ethics Committee at Vorokhobov City Clinical Hospital № 67, protocol № 14 dated of 06.09.2021.

Statistical analysis / Статистический анализ

Data from the patients admitted to the ICU underwent statistical analysis. Two disease outcomes were assessed: lethal outcome or discharge/transfer to another department. The duration of stay at the ICU was used as the time frame for survivors.

At stage 1, one-parametric data analysis was used. The optimal threshold was determined; the approach of searching for the optimal splitting variable in decision trees was used. The minimum p-value for the log-rank statistics from the survival time (staying in the ICU) was used as a splitting criterion, censoring by ICU discharge. Thus, a threshold was chosen that led to statistically significantly different survival functions in the two resulting samples.

After choosing a threshold for all indicators, contingency tables were built, and odds ratio (OR) and relative risk (RR) were calculated for each parameter. The p-value of the Cochran–Mantel–Haenszel statistics was used to assess the significance level.

For all the parameters studied, a one-dimensional regression was built, a point estimate of the regression coefficient and its standard error were obtained as well as relevant significance level, was estimated using the p-value of the Wald test.

2022 • Vol. 16 • № 3 Obstetrics, Gynecology and Reproduction

To assess each variable to predict lethal outcome, a concordant statistical method (c-statistics) was carried out with the construction of the ROC (receiver operating characteristic) curve and the ROC index or area under curve (AUC) was calculated further evaluated nonparametrically.

Spearman's rank correlation was used to assess inter-parameter correlation.

Separately, the most critical parameters – ADAMTS-13:Ag, ADAMTS-13:Ac, ADAMTS-13:i, vWF:Ag, and MPO:Ag were compared with a control group containing 40 healthy subjects. Univariate ANOVA with the Kruskal–Wallis test and univariate nonparametric ANOVA based on the Wilcoxon rank test allowed to test hypotheses about the coincidence of means and rank mean in the control and patient groups, and Boxplot plots were constructed with the ability to compare distributions on the baseline scale of parameter values and the rank scale. The main comparison tools were Boxplot charts showing the spread of values in groups and p-values for the corresponding statistics: Fisher statistics for ANOVA analysis and Wilcoxon for nonparametric analysis of means. The comparison result was considered significant at $p < 0.05$.

A comparison was also carried out for empirical distribution functions, showing a significant difference in distributions between the control and patient groups.

At stage 2, a multivariate logistic regression analysis was used to assess the prognostic significance of each factor studied. The multivariate logistic model included potential death-associated biological parameters (significance level $p < 0.20$). With a strong correlation of two variables (Pearson's correlation coefficient > 0.80), only one was retained according to the feasibility criteria. Informative parameters were selected using the inverse regression method. Parameters with $p < 0.10$ were included in the final multivariate model.

The most significant coefficients obtained from the inverse regression analysis were used to create a simplified mortality rate at the final stage of the statistical analysis.

The validity of this analysis was also tested using concordant statistics.

The use of the Yuoden index allowed to select the optimal parameters with the best ability to predict lethal outcome and assess sensitivity, specificity, positive predictive value (PPV), positive likelihood ratio (LR+), negative predictive value (NPV), as well as negative likelihood ratio (LR–).

The resulting simplified mortality rate allowed to further analyze survival using the Kaplan–Meier method. All survival periods were calculated from the ICU admission to the time of in-hospital lethal outcome (censored data). A log-rank test was used to compare survival curves between patients with a simplified mortality rate above or below the median.

Statistical analysis was performed using StatView®-windows software v.5.0 (SAS Institute Inc., USA) and XLSTAT® software v.2015.4.01.20116 (Addinsoft SARL, France).

Results / Результаты

Epidemiological data analyzed patient age, gender, and comorbidities (**Table 1**). The total hospital stay ranged from 5 to 80 days (except 1 patient who stayed at hospital for 108 days). The majority of patients (n = 70) died 13 days after admission to the hospital.

We evaluated laboratory markers of hemostasis and inflammation as potential diagnostic, prognostic, and control markers and predictors of lethal outcome in ICU patients with severe COVID-19 requiring mechanical ventilation. After a single parameter logistic regression analysis, parameters with $p < 0.20$ were identified (**Table 2**). Next, the optimal threshold values for each biological variable were set. The point estimate of the regression coefficient and its standard error, the p-value of the Wilde statistic, the odds ratio/relative risks, and the ROC index were also determined.

ROC curve analysis / Анализ ROC-кривых

The results of analyzing ROC curves and AUC (**Table 2**, **Fig. 1**), showed a strong correlation between the disease outcome and the following parameters such as vWF:Ag, ADAMTS-13:Ac, ADAMTS-13:Ag, MPO:Ag, D-dimer, ferritin, PLT/LYM ratio, ADAMTS-13:Ac/vWF:Ag and ADAMTS-13:Ag/vWF:Ac. The ROC curves for the variables that were further used for multivariate analysis were also calculated (**Fig. 1**).

A particular attention was addressed to altered ADAMTS-13/vWF-axis as a potential prognostic factor for ICU non-survivors. Analysis of the ROC curves showed the best correlation between the disease outcome and von Willebrand factor, ADAMTS-13 activity, and the ADAMTS-13:Ac/vWF:Ag ratio (**Fig. 1**). The ADAMTS-13 inhibitor ROC curve analysis showed no correlation between ADAMTS-13:i levels and outcomes.

Among the all parameters evaluated, MPO concentration showed the highest correlation with lethal outcome in patients with severe COVID-19, comparable to vWF:Ag, in contrast to other markers of inflammation (ferritin, CRP). An inverse regression analysis revealed a statistically

Table 1. Epidemiological data for 129 COVID-19 patients admitted to the intensive care unit.

Таблица 1. Эпидемиологические данные госпитализированных в отделение реанимации и интенсивной терапии 129 COVID-19 пациентов.

Parameter Параметр	Non-survivors Невыжившие (n = 93) n (%)	Survivors Выжившие (n = 36) n (%)
Age / Возраст: 18–30 years / 18–30 лет 30–50 years / 30–50 лет 50–70 years / 50–70 лет > 70 years / > 70 лет	 2 (2.2) 11 (11.8) 47 (47.3) 36 (38.7)	 2 (5.6) 8 (22.2) 17 (47.2) 9 (25.0)
Females / Женский пол	55 (59.1)	17 (47.2)
Males / Мужской пол	38 (40.9)	19 (52.8)
Diabetes mellitus / Сахарный диабет	25 (26.9)	10 (27.8)
Arterial hypertension / Артериальная гипертензия	51 (54.8)	16 (44.4)
Pulmonary hypertension / Легочная гипертензия	35 (37.6)	6 (16.7)
Ischaemic heart disease / Ишемическая болезнь сердца	52 (55.9)	19 (52.8)
Malignancies / Злокачественные заболевания	15 (16.1)	2 (5.6)
Obesity / Ожирение	13 (14.0)	6 (16.7)
Venous thromboses / Венозные тромбозы	9 (9.7)	3 (8.3)
Arterial thromboses / Артериальные тромбозы	10 (10.8)	4 (11.1)
Mental illness / Психиатрические заболевания	12 (12.9)	5 (13.9)
Autoimmune diseases / Аутоиммунные заболевания	10 (10.8)	3 (8.3)
Liver diseases / Заболевания печени	8 (8.6)	2 (5.6)
Renal diseases / Заболевания почек	9 (9.7)	1 (2.8)

Table 2. A summarized table with threshold values and all parameter data.

Таблица 2. Сводная таблица с пороговыми значениями и результатами для всех переменных.

Parameter Параметр	Total patient number with optimal cut-off level Общее кол-во пациентов с оптимальным порогом отсечки cut-off	Cut-off level Порог отсечки	Non-survivors Невыжившие	Cochran–Mantel–Haenszel test Cochran–Mantel–Haenszel тест p	Odds ratio Отношение шансов	Relative risk Относительный риск	ROC index ROC-индекс	Wald test Уальд-тест p
vWF:Ag, U/ml vWF:Ag, Ед/мл	91	> 2,1	80	0,0000	13,986	5,4426	0,769	0,0000
ADAMTS-13:Ac, U/ml ADAMTS-13:Ac, Ед/мл	98	> 0,31	66	0,0333	0,3056	0,3952	0,611	0,0718
ADAMTS-13:Ag, U/ml ADAMTS-13:Ag, Ед/мл	85	> 0,273	56	0,0295	0,3653	0,4663	0,604	0,0649
ADAMTS-13:i, U/ml ADAMTS-13:i, Ед/мл	49	> 9,38	38	0,2813	1,5702	1,3920	0,512	0,8393
MPO:Ag, ng/ml МПО:Аг, нг/мл	33	> 34	27	0,0002	7,0000	3,3478	0,809	0,0003
D-dimer, ng/ml D-димер, нг/мл	41	> 2260	38	0,0004	7,4643	5,0674	0,710	0,0415
C-reactive protein, mg/l С-реактивный белок, мг/л	25	> 235	21	0,4078	1,6154	1,4706	0,534	0,4352
Ferritin, μg/l Ферритин, мкг/л	57	> 950	51	0,0041	3,8387	2,9556	0,617	0,4261
PLT / LYM	73	> 268	62	0,0317	2,3209	1,9356	0,606	0,1500
ADAMTS-13:Ac/vWF:Ag	44	> 0,223	20	0,0000	0,1370	0,2588	0,740	0,0136
ADAMTS-13:Ag/vWF:Ac	88	> 0,113	56	0,0018	0,1892	0,2683	0,717	0,0433

2022 • Vol. 16 • № 3
Obstetrics, Gynecology and Reproduction

significant association between D-dimer levels and very poor survival.

Patient vs. control group comparison / Сравнение с контрольной группой

The most essential parameters ADAMTS-13:Ag, ADAMTS-13:Ac, ADAMTS-13:i, vWF:Ag, and MPO:Ag were compared with a control group by using univariate ANOVA and rank-based univariate non-parametric ANOVA Wilcoxon test for non-parametric mean analysis. The constructed Boxplot charts (**Fig. 2**) compared the parameter distribution on the baseline scale. The rank scale in the control and patient groups showed a significant difference in these values.

Kaplan-Meier survival curve analysis / Анализ кривых выживаемости Каплана-Мейера

Taking into consideration the splitting thresholds obtained (**Table 2**), Kaplan–Meier survival curves were constructed (**Fig. 3**).

The Kaplan-Meier survival curves demonstrate the threshold-dependent nature of the correlation level for parameters selected. An analysis of the Kaplan–Meier survival curve for vWF (**Fig. 3**) showed that the samples with a threshold value > 2.1 (highlighted in red), the curve is located much lower that significantly differed from the samples with unmet threshold condition (vWF < 2.1, highlighted in blue), which indicates that the survival probability for at least day 20 of hospitalization in group 1

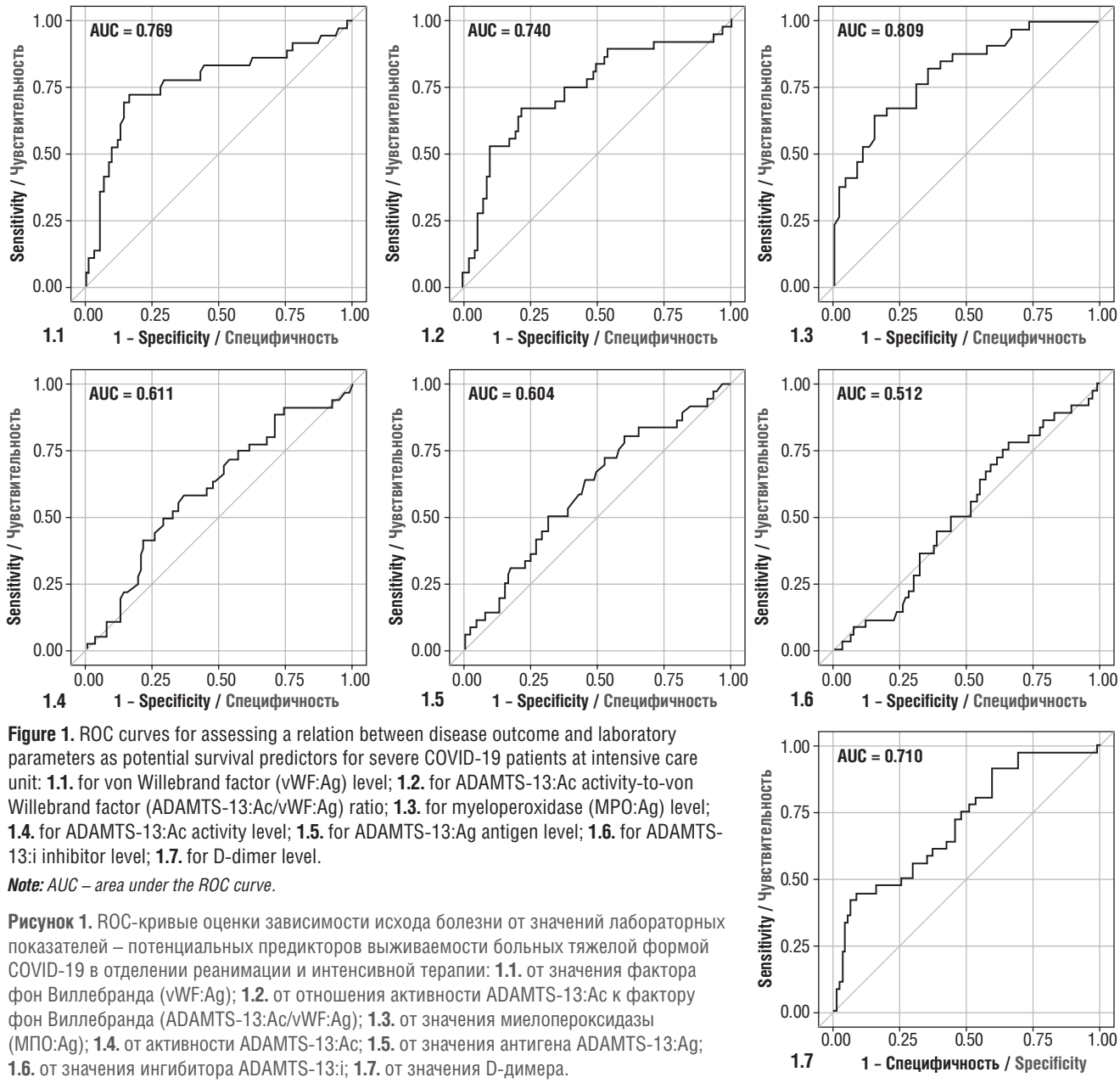


Figure 1. ROC curves for assessing a relation between disease outcome and laboratory parameters as potential survival predictors for severe COVID-19 patients at intensive care unit: **1.1.** for von Willebrand factor (vWF:Ag) level; **1.2.** for ADAMTS-13:Ac activity-to-von Willebrand factor (ADAMTS-13:Ac/vWF:Ag) ratio; **1.3.** for myeloperoxidase (MPO:Ag) level; **1.4.** for ADAMTS-13:Ac activity level; **1.5.** for ADAMTS-13:Ag antigen level; **1.6.** for ADAMTS-13:i inhibitor level; **1.7.** for D-dimer level.

Note: *AUC – area under the ROC curve.*

Рисунок 1. ROC-кривые оценки зависимости исхода болезни от значений лабораторных показателей – потенциальных предикторов выживаемости больных тяжелой формой COVID-19 в отделении реанимации и интенсивной терапии: **1.1.** от значения фактора фон Виллебранда (vWF:Ag); **1.2.** от отношения активности ADAMTS-13:Ac к фактору фон Виллебранда (ADAMTS-13:Ac/vWF:Ag); **1.3.** от значения миелопероксидазы (МПО:Ag); **1.4.** от активности ADAMTS-13:Ac; **1.5.** от значения антигена ADAMTS-13:Ag; **1.6.** от значения ингибитора ADAMTS-13:i; **1.7.** от значения D-димера.

Примечание: *AUC – площадь под ROC-кривой.*

(vWF > 2.1) vs. group 2 is almost 3-fold lower. The higher the vWF level, the higher the chance of lethal outcome.

Analysis of Kaplan–Meier survival curves for ADAMTS-13 activity showed that at a threshold > 0.31 (highlighted in red, **Fig. 3**), the curve is located higher that significantly differed from the sample with unmet threshold level (highlighted in blue, **Fig. 3**). Thus, the higher ADAMTS-13:Ac (> 0.31) level, the higher the chances of survival.

At the same time, no relation between survival and either discretized or continuous values of ADAMTS-13 antigen and ADAMTS-13 inhibitor level was found (**Fig. 3**).

An analysis of the Kaplan–Meier survival curves for the ADAMTS-13:Ac/vWF:Ag ratio showed that at a cut-off threshold of > 0.223 (highlighted in red), the curve is located higher that significantly differed from the sample with unmet threshold condition (highlighted in blue, **Fig. 3**). Hence, the higher ADAMTS-13:Ac/vWF:Ag ratio (> 0.223), the higher the chance of survival.

At splitting threshold of > 0.113 for the ADAMTS-13:Ag/vWF:Ag parameter (highlighted in red), the curve is observed higher that significantly differed from the sample with unmet threshold condition (highlighted in blue, **Fig. 3**). Therefore, the higher the ADAMTS-13:Ag/vWF:Ag ratio (> 0.113), the higher the chance of survival.

At a splitting threshold of > 34 ng/mL for the MPO antigen (highlighted in red), the curve is positioned much lower that significantly differed from the sample with unmet threshold condition (highlighted in blue, **Fig. 3**). Therefore, the higher MPO (> 34 ng/ml) activity, the higher the probability of lethal outcome.

Thus, MPO activity, vWF:Ag, and ADAMTS:13:Ac/vWF:Ag ratio were both continuous and cut-off-dependent predictors, whereas ADAMTS-13 inhibitor and CRP level were insignificant at both cut-offs, as well as without them. Other variables, such as D-dimer and ferritin, were significant cut-off-dependent predictors and, at the same time, were insignificant if they were treated as continuous in the regression model (**Table 2**).

Simplified survival rate and survival estimate / Упрощенный показатель выживаемости и оценка выживаемости

After a univariate logistic regression analysis (**Table 2**), from a variety of laboratory markers of hemostasis activation and inflammation, several of them were selected as potential predictors for in-hospital lethal outcome, which were considered as significant risk factors for lethal outcome. Subsequent multivariate analysis adjusted for all variables with p < 0.20 in univariate analysis showed that increased concentrations of parameters such as vWF:Ag and MPO:Ag were conclusively and independently associated with mortality. A "simplified mortality rate" was calculated as follows: 1.212×vWF:Ag + 0.06×MPO:Ag. The magnitude of this parameter was significantly higher in ICU non-survivor vs. survivor patients (p < 0.0001) (**Table 3**).

Subsequent concordant statistics method allowed to assess the predictive power of the "simplified mortality rate" for survival level: AUC comprised 0.851 (95 % CI = 0.758–0.934), p < 0.0001 (**Fig. 4**). Using the Youden score, the best discriminatory value was 4.46: its sensitivity for predicting mortality was 0.800 (0.659–0.892), specificity – 0.837 (0.696–0.921), PPV = 0.837, LR+ = 4.914, NPV = 0.800, LR– = 0.239. This was in line with the mean simplified mortality rate observed in patients.

Next, comparing patient survival between a "simplified mortality rate" above its median value (high estimate value) and a simplified mortality estimate value below the median value (low estimate value) (**Fig. 5**) revealed that patients with a mortality rate above 4.46 had a significantly lower overall survival than patients with a simplified mortality estimate below 4.46 (log-rank test: p < 0.0001), 50 % of them died on day 13 post-hospitalization.

Discussion / Обсуждение

From the beginning of the COVID-19 pandemic, the medical publications have been literally flooded with reports about COVID-19. Of these, more than 3,000 publications are related solely to issues of hemostasis disorders and thrombosis. A substantial portion of them is presented by reviews or described clinical cases. Decompensated systemic inflammatory response syndrome and thrombotic disorders (including micro thrombosis and thrombotic microangiopathy) are now recognized as underlying pathological processes in severe COVID-19. The terms thromboinflammation, endothelial dysfunction, and immunothrombosis have increasingly mentioned in the literature concerning COVID-19 [9, 13, 14]. Therefore, the role of the ADAMTS-13/vWF axis and various inflammatory markers in the pathogenesis of SARS-CoV-2-infection is being actively studied. We found more than 20 studies focusing on the role of the von Willebrand factor and ADAMTS-13, and even fewer studies examining multiple factors simultaneously, including markers of netosis, with a limited number of patients enrolled and often presenting controversial results [15–17]. One of the central issues of the present investigation was to study the pathogenetic role and prognostic value of

2022 • Vol. 16 • № 3
Obstetrics, Gynecology and Reproduction

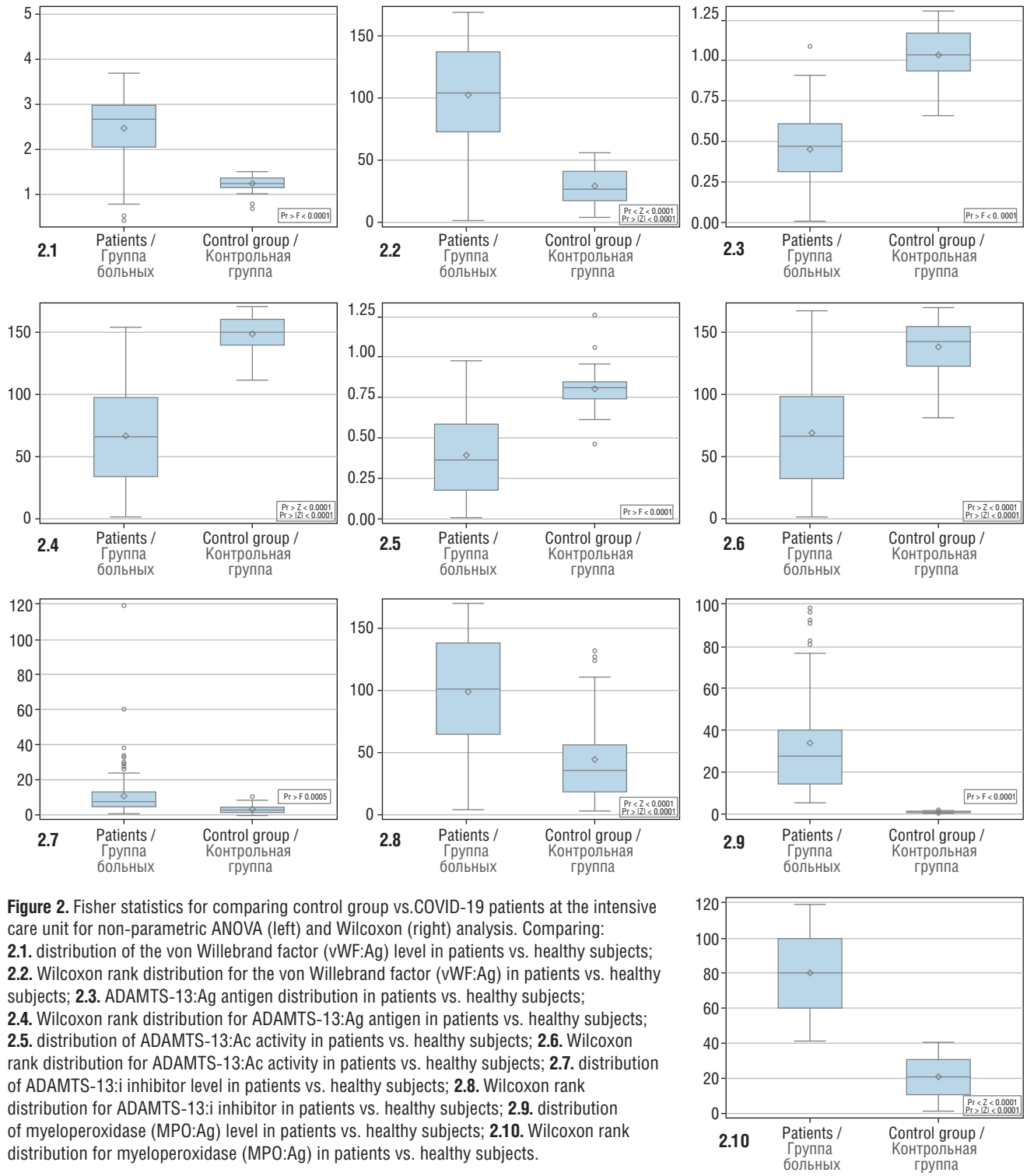
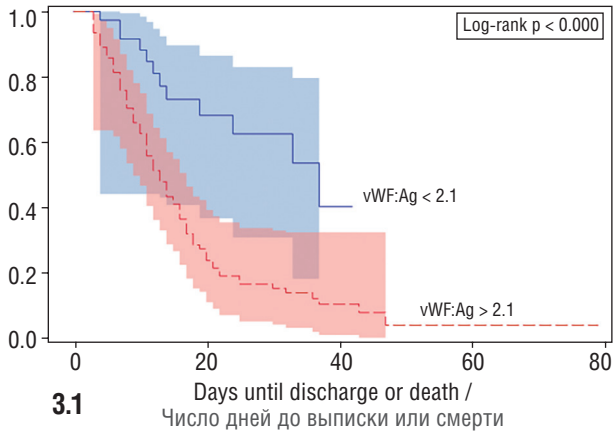


Figure 2. Fisher statistics for comparing control group vs.COVID-19 patients at the intensive care unit for non-parametric ANOVA (left) and Wilcoxon (right) analysis. Comparing: **2.1.** distribution of the von Willebrand factor (vWF:Ag) level in patients vs. healthy subjects; **2.2.** Wilcoxon rank distribution for the von Willebrand factor (vWF:Ag) in patients vs. healthy subjects; **2.3.** ADAMTS-13:Ag antigen distribution in patients vs. healthy subjects; **2.4.** Wilcoxon rank distribution for ADAMTS-13:Ag antigen in patients vs. healthy subjects; **2.5.** distribution of ADAMTS-13:Ac activity in patients vs. healthy subjects; **2.6.** Wilcoxon rank distribution for ADAMTS-13:Ac activity in patients vs. healthy subjects; **2.7.** distribution of ADAMTS-13:i inhibitor level in patients vs. healthy subjects; **2.8.** Wilcoxon rank distribution for ADAMTS-13:i inhibitor in patients vs. healthy subjects; **2.9.** distribution of myeloperoxidase (MPO:Ag) level in patients vs. healthy subjects; **2.10.** Wilcoxon rank distribution for myeloperoxidase (MPO:Ag) in patients vs. healthy subjects.

Рисунок 2. Статистика Фишера в контрольной группе и группе COVID-19 пациентов в отделении реанимации и интенсивной терапии для непараметрического анализа ANOVA (слева) и Уилкоксона (справа). Сравнение распределений: **2.1.** значения фактора фон Виллебранда в группах больных и здоровых; **2.2.** рангов Уилкоксона для фактора фон Виллебранда (vWF:Ag) в группах больных и здоровых; **2.3.** значения антигена ADAMTS-13:Ag в группах больных и здоровых; **2.4.** рангов Уилкоксона для значений антигена ADAMTS-13:Ag в группах больных и здоровых; **2.5.** значения активности ADAMTS-13:Ac в группах больных и здоровых; **2.6.** рангов Уилкоксона для значений активности ADAMTS-13:Ac в группах больных и здоровых; **2.7.** значения ингибитора ADAMTS-13:i в группах больных и здоровых; **2.8.** рангов Уилкоксона для значений ингибитора ADAMTS-13:i в группах больных и здоровых; **2.9.** значения миелопероксидазы (МПО:Ag) в группах больных и здоровых; **2.10.** рангов Уилкоксона для значений миелопероксидазы (МПО:Ag) в группах больных и здоровых.



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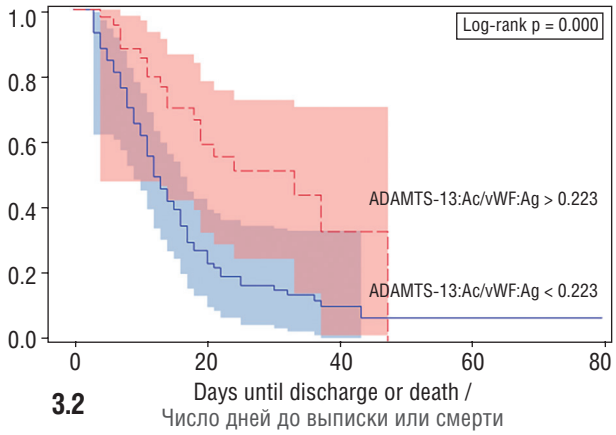

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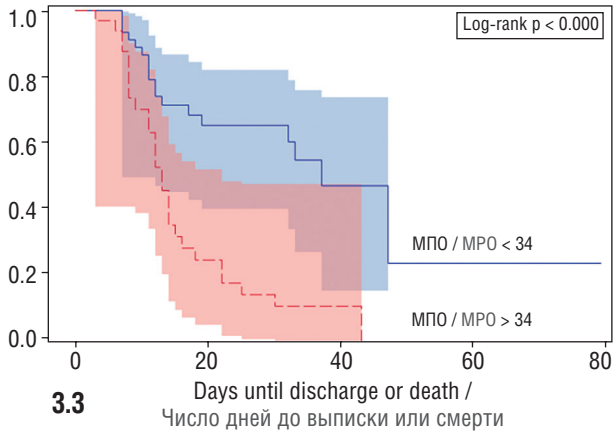

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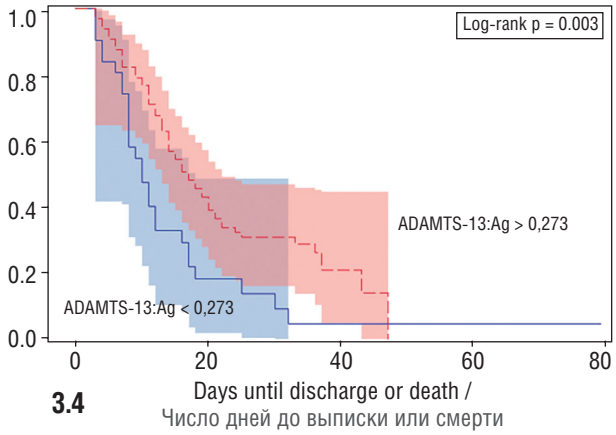

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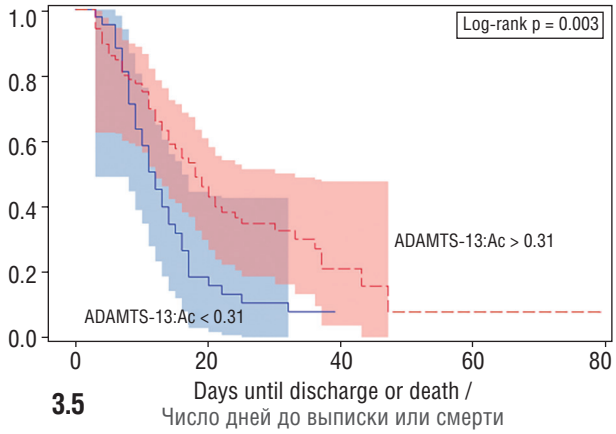

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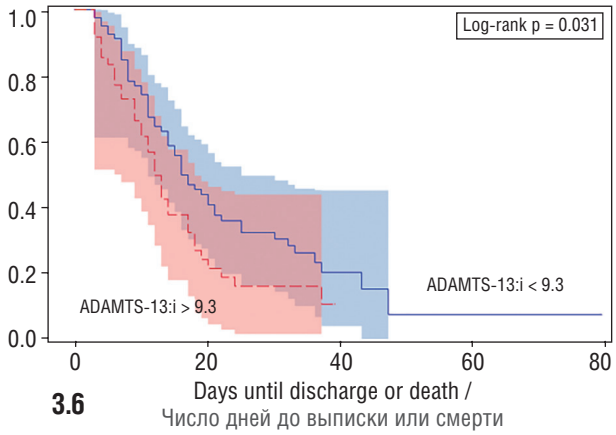

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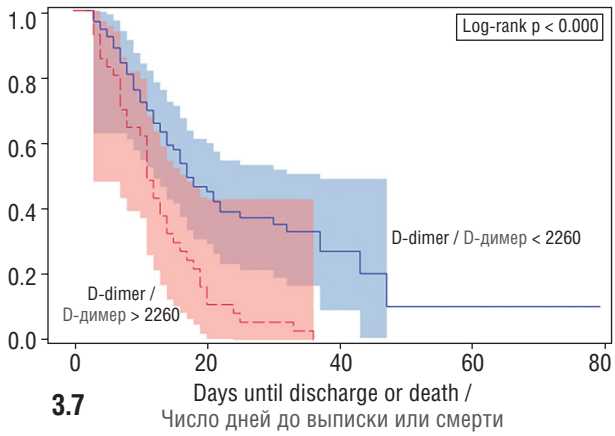

3.7

Figure 3. Kaplan-Meier survival curve analysis. Comparing survival function (with 95 % CI) for patient groups (values above and below the threshold are highlighted in red and blue, respectively): **3.1.** according to von Willebrand factor (vWF:Ag) level, at threshold 2.1 U/ml; **3.2.** according to ADAMTS-13:Ac activity-to-von Willebrand factor (ADAMTS-13:Ac/vWF:Ag) ratio, at threshold 0.223; **3.3.** according to myeloperoxidase (MPO:Ag) level, at threshold 34 ng/ml; **3.4.** according to ADAMTS-13:Ag antigen level, at threshold 0.273 U/ml; **3.5.** according to ADAMTS-13:Ac activity level, at threshold 0.31 U/ml; **3.6.** according to ADAMTS-13:i inhibitor level, at threshold 9.3 U/ml; **3.7.** according to D-dimer level, at threshold 2260 ng/ml.

Рисунок 3. Анализ кривых выживаемости Каплана–Мейера. Сравнение функций выживаемости (с 95 % ДИ) для групп пациентов с разделением по пороговому значению (больше порога – красный, меньше – синий): **3.1.** по уровню фактора фон Виллебранда (vWF:Ag) с разделением по пороговому значению 2,1 Ед/мл; **3.2.** по отношению активности ADAMTS-13:Ac к фактору фон Виллебранда (ADAMTS-13:Ac/vWF:Ag) с разделением по пороговому значению 0,223; **3.3.** по уровню миелопероксидазы (МПО:Ag) с разделением по пороговому значению 34 нг/мл; **3.4.** по уровню антигена ADAMTS-13:Ag с разделением по пороговому значению 0,273 Ед/мл; **3.5.** по уровню активности ADAMTS-13:Ac с разделением по пороговому значению 0,31 Ед/мл; **3.6.** по уровню ингибитора ADAMTS-13:i с разделением по пороговому значению 9,3 Ед/мл; **3.7.** по уровню D-димера с разделением по пороговому значению 2260 нг/мл.

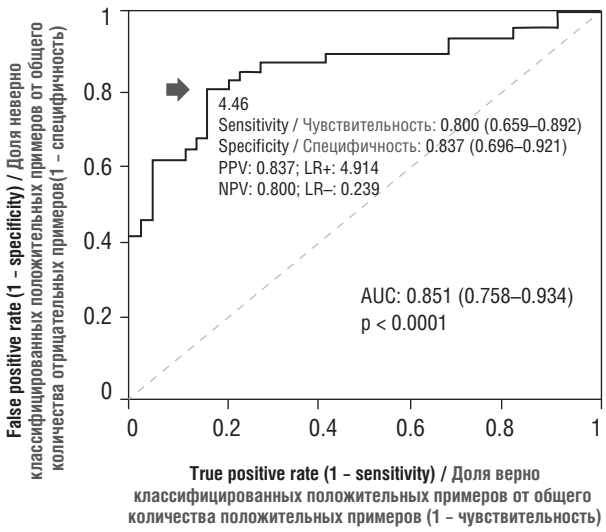

Figure 4. ROC curve for association between simplified mortality score calculated as 1.212×(vWF:Ag, IU/ml) + 0.06×(MPO:Ag, ng/ml) and in-hospital mortality rate.

Note: *PPV – positive predictive value; NPV – negative predictive value; AUC – area under curve; the black arrow points to the calculated Youden coefficient (simplified mortality score): a score above 4.46 is associated with poor survival.*

Рисунок 4. ROC-кривая для ассоциации значений упрощенной оценки смертности, рассчитанной как 1,212×(vWF:Ag, МЕ/мл) + 0,06×(МПО:Аг, нг/мл), с внутрибольничной смертностью.

Примечание: *PPV – точность положительного прогноза классификации; NPV – точность отрицательного прогноза классификации; AUC – площадь под кривой; черная стрелка указывает на рассчитанный коэффициент Юдена (упрощенный показатель смертности): значение показателя выше 4,46 ассоциируется с низкой выживаемостью.*

circulating blood markers linked to endothelial activation and damage owing to recruitment and stimulation of innate immunity cells particularly myeloid cells assessing a role of vWF and ADAMTS-13 for endothelium as well as MPO for circulating myeloid cells.

The results of our study show that laboratory parameters such as vWF:Ag, ADAMTS-13:Ac, ADAMTS-13:Ag, MPO:Ag, D-dimer, ferritin, PLT/LYM ratio, ADAMTS-13:Ac/vWF:Ag as well as ADAMTS-13:Ag/vWF:Ac ratios significantly correlate with ICU hospitalized non-survivors during severe COVID-19 that also reflects the pathogenetic role of inflammation and hemostasis disorders particularly the ADAMTS-13/vWF-axis and granulocyte activation with the released enzymes including myeloperoxidase. Along with neutrophil elastase (NE), MPO is also an important trigger for NETs formation resulting in destruction of intracellular proteins and the formation of reactive oxygen species as well as free radicals damaging the host endothelium [18].

In the control group of healthy volunteers, the magnitude for the selected laboratory parameters significantly differed from those in the patient group and exceeded no reference ranges (**Fig. 2**).

ADAMTS-13 deficiency and disorders of ADAMTS-13/vWF-axis during systemic inflammatory response include the activation of endothelium-secreted vWF multimers due to pro-inflammatory cytokines and consumption of ADAMTS-13 metalloproteinase, inhibition of ADAMTS-13 transcription, and its directly blocked activity. ADAMTS-13 deficiency has been shown to correlate with outcomes in patients with sepsis and multiple organ failure [19].

Thrombotic thrombocytopenic purpura and thrombotic microangiopathy in the absence of inflammation may be due to congenital ADAMTS-13 deficiency or, more commonly, ADAMTS-13 inhibitor (antibodies to ADAMTS-13) deficiency. Despite profoundly altered immunity and immunothrombosis typical to COVID-19, our study revealed no correlation between ADAMTS-13 inhibitor level and patient survival (**Fig. 2**). As a laboratory marker of inflammation, CRP also showed insignificant correlation with survival.

Analysis of the Kaplan–Meier survival curves demonstrates the threshold-dependent nature for the correlation between the parameters selected in the one-dimensional analysis (**Fig. 3**). Cut-off threshold exerted no effect on significant relation between ADAMTS-13 inhibitor or CRP level and survival.

Currently, a predictive value for D-dimer level as an unfavorable marker of survival is controversial. Our data are partly consistent with the ISTH (International Society on Thrombosis and Haemostasis) recommendations [20], wherein a high D-dimer level is recognized as an

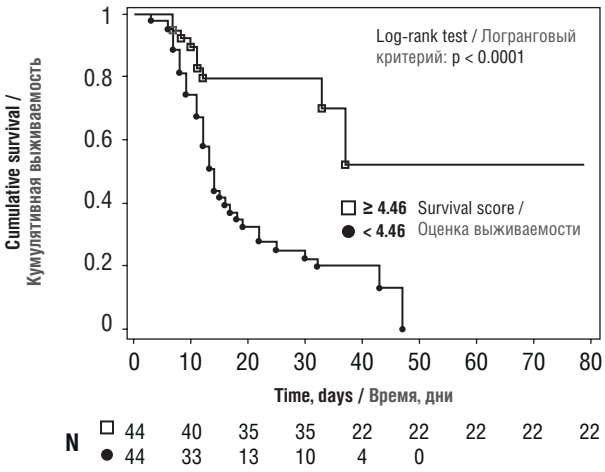

Figure 5. A cumulative survival for COVID-19 patients with high (□) and low (●) mortality score; N – number of survivors.

Рисунок 5. Кумулятивная выживаемость пациентов с высоким (□) и низким (●)значением показателя смертности; N – число выживших.

Table 3. Multivariate analysis for comparing laboratory parameter-based survival predictors.

Таблица 3. Результаты мультивариантного анализа предикторов выживания среди лабораторных параметров.

Parameter Показатель	M ± SD	Adjusted OR (95 % CI)* Скорректированное ОШ (95 % ДИ)*	p
vWF:Ag, IU/ml / vWF:Ag, МЕ/мл	1,212 ± 0,391	3,360 (1,562–7,228)	0,0019
MPO:Ag, ng/ml / МПО:Аг, нг/мл	0,060 ± 0,018	1,062 (1,024–1,101)	0,0011

Note: *OR – odds ratio; *adjusted for all laboratory parameters at p < 0.20 in univariate analysis.*

Примечание: *ОШ – отношение шансов; * с поправкой на все лабораторные показатели при p < 0,20 при однофакторном анализе.*

unfavorable prognostic factor in COVID-19 patients. According to our data, a threshold-dependent effect of D-dimer (9-fold higher than normal reference range) on the survival of ICU patients was found indeed, but this marker is not an independent risk factor for lethal outcome.

One of the main objectives of our study was to find independent prognostic markers for survival of patients with severe COVID-19.

From the entire spectrum of possible predictor candidates, only MPO and vWF:Ag remained independent predictors of lethal outcome in the multivariate survival model. Evaluation of MPO concentration resulted in the disappearance of markers associated with ADAMTS-13. Thus, it can be concluded that in severe COVID-19, pathological activation of myeloid cells with degranulation and release of enzymes into the bloodstream (MPO, neutrophil elastase), NETs formation, and severe damage to the endothelium are of greater importance. An increase in von Willebrand factor antigen level is the second independent predictor of lethal outcome. At the same time, the role for ADAMTS-13 as an independent predictor of adverse outcomes is eliminated, because the intensity of the released high-molecular von Willebrand factor in endothelial damage exceeds the regulatory potential of ADAMTS-13 molecules (depletion by the consumption of ADAMTS-13) and puts ADAMTS-13 into the category of dependent factors.

Study strength / Сильные стороны исследования

For the first time, it was discovered that:

– measuring serum MPO is of prognostic value in patients with severe COVID-19 requiring mechanical ventilation;

– MPO and vWF:Ag concentrations are independent predictors of lethal outcome in intubated patients with severe COVID-19.

Study limitations / Ограничения исследования

The study was carried out as monocentric and retrospective.

No role for vWF-related activities such as vWF:RCo and vWF high molecular weight multimers, which could potentially serve as better predictors of clinical outcomes, has been studied.

Prospects for further investigation / Перспективы дальнейших исследований

Conducting a prospective, multicenter, blinded study with a large number of participants.

The study of the role for pro-inflammatory cytokines and assessment of their relationship with vWF and MPO.

Conclusion / Заключение

Our study showed that high serum concentrations of MPO:Ag and vWF:Ag were independent predictors of poor survival in patients with severe COVID-19 admitted to the ICU for mechanical ventilation. Further prospective multicentre studies are necessary to confirm such findings. In case our data might be confirmed in a multicenter prospective study, it could open up new opportunities for treating patients with severe COVID-19 with inhibitors targeting von Willebrand factor activity, granulocytes, and MPO.

2022 • Vol. 16 • № 3
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The data on individual participants that underlie the results presented in this article, after de-identification (text, tables) will be available beginning 3 months and ending 3 years following article publication at the request of researchers who will provide a methodologically reasonable proposal. Proposals should be sent to the mailbox vikabits@mail.ru. To gain access, data requestors will require to sign a data access agreement.	Данные об отдельных участниках, лежащие в основе результатов, представленных в этой статье, после деидентификации (текст, таблицы) будут доступны спустя 3 мес и до 3 лет после публикации статьи по запросу исследователей, которые предоставят методологически обоснованное предложение. Предложения должны быть направлены на почтовый ящик vikabits@mail.ru. Чтобы получить доступ, лица, запрашивающие данные, должны будут подписать соглашение о доступе к данным.
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