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Dear Colleagues!

The new issue 3 of the updated Extreme Medicine journal addresses one of the greatest challenges facing mankind today. The Federal Medical Biological Agency joined in the battle against the new threat in the earliest days of the coronavirus pandemic. The systemic approach, concerted teamwork and the rapid mobilization of clinical and scientific resources allowed the state to avoid disruption of its key sectors and industries.

So far, over 50 hospitals have been opened to deliver medical care to those afflicted with the novel coronavirus infection; clinical protocols have been revised, and healthcare facilities have been reequipped. A new reference center has been opened to provide expertise and support to all FMBA affiliates. Due credit should be given to the well-coordinated work of the FMBA Blood Service, including preparation of blood products, which contributed tremendously to containing the spread of COVID-19 in the subordinate organizations.

Despite of challenging conditions, research institutions and branches of FMBA were committed to their work. We were able to redistribute their capacities and thus make significant advances in understanding the new threat. Our researchers have created a variety of coronavirus detection systems based on PCR, isothermal amplification and microfluidic chips. Original diagnostic platforms have been designed to measure antibody titers to SARS-CoV-2. Preclinical trials of innovative therapies against the novel coronavirus are currently in progress, including those investigating the use of small interfering RNA. Basic research studies of the viral genome are underway. The pathogenesis of COVID-19 complications and the efficacy of novel therapies are being investigated using new experimental models. The ongoing large-scale epidemiological will give answers for the questions we are struggling to solve now. The FMBA of Russia has proved its role of a frontline special forces unit that can effectively respond to manmade disasters and biological threats and mobilize scientific resources. This issue of the journal features articles about different aspects of FMBA work: from research studies to clinical diagnosis, therapy and rehabilitation.

My best wishes of good health and success to all of you,
Veronika Skvortsova, Editor-in-Chief

STUDY OF THE EFFECTIVENESS OF METHYLPREDNISOLONE AT DIFFERENT STAGES OF INPATIENT CARE FOR PATIENTS WITH PNEUMONIA CAUSED BY A NEW COVID-19 CORONAVIRUS INFECTION

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Glucocorticoid therapy for a cytokine storm is one of the mainstays of managing the novel coronavirus disease COVID-19. The aim of this study was to evaluate the efficacy of methylprednisolone at different stages of medical care: in an intensive care unit (ICU) vs. a medical ward setting. Methylprednisolone therapy was delivered to 54 patients, amounting to 9% of the total patients hospitalized to the Federal Center of Brain Research and Neurotechnology of FMBA, Russia. Twenty-eight patients received methylprednisolone in the ICU setting; 26 patients, in a medical ward setting. The control group comprised 14 patients. Methylprednisolone was administered continuously, intravenously at 250 mg per day over the course of 3 days; the total dose was 750 mg. The analysis revealed a significant reduction in mortality in the group receiving methylprednisolone in a medical ward setting (7.7%) in comparison with the group receiving the drug in ICU (67.9%) and the control group (42.9%, $p < 0.001$). The need for mechanical ventilation was lower in the group receiving methylprednisolone in a medical ward (2 (7.7%), 20 (71.4%) and 7 (50%) cases, respectively, $p < 0.001$). Thus, preventive anti-inflammatory methylprednisolone therapy for delivered in a medical ward setting reduces hospital mortality and the need for MV in patients with COVID-19-induced pneumonia.

Keywords: coronavirus infection, COVID-19, corticosteroids, viral pneumonia

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ИССЛЕДОВАНИЕ ЭФФЕКТИВНОСТИ МЕТИЛПРЕДНИЗОЛОНА НА РАЗНЫХ ЭТАПАХ ОКАЗАНИЯ СТАЦИОНАРНОЙ МЕДИЦИНСКОЙ ПОМОЩИ ПАЦИЕНТАМ С ПНЕВМОНИЕЙ, ВЫЗВАННОЙ НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИЕЙ COVID-19

Е. Е. Аринина[1], Р. Т. Таирова[1], А. Б. Бердалин[1], С. С. Гужев[1], Н. А. Глотова[1], Ю. В. Рублева[1], М. А. Булатова[1], Б. Б. Поляев[1], Д. А. Терехов[2], В. В. Белоусов[1], Н. А. Шамалов[1]

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Одним из основных направлений терапии пневмонии, вызванной новой коронавирусной инфекцией COVID-19, является применение средств, направленных на борьбу с цитокиновым штормом, в том числе глюкокортикостероидов. Целью настоящего исследования явилось изучение эффективности применения метилпреднизолона на разных этапах оказания стационарной медицинской помощи — в условиях отделения реанимации и интенсивной терапии (ОРИТ) и в терапевтических отделениях (ТО). Терапия метилпреднизолоном была проведена 54 пациентам, что составило 9% от общего количества госпитализированных больных в ФГБУ «ФЦМН» ФМБА России. В условиях ОРИТ терапия проводилась 28 пациентам, в условиях ТО — 26 больным, контрольную группу составили 14 пациентов. Метилпреднизолон вводили в суточной дозе 250 мг непрерывно внутривенно в течение трех суток, суммарная доза составила 750 мг. Результаты исследования показали достоверное уменьшение показателя летальности в группе, терапия метилпреднизолоном которым проводилась в ТО (7,7%) по сравнению с группой, в которой терапия проводилась в ОРИТ (67,9%) и контрольной группой (42,9%, $p < 0,001$). Также для группы с проведенной терапией в ТО была характерна меньшая частота проведения ИВЛ (в 2 (7,7%), 20 (71,4%) и 7 (50%) случаев, соответственно, $p < 0,001$). Таким образом, введение метилпреднизолона в условиях терапевтического отделения в качестве упреждающей противовоспалительной терапии способствует снижению показателей больничной летальности и частоты использования ИВЛ у пациентов с пневмонией, вызванной новой коронавирусной инфекцией COVID-19.

Ключевые слова: коронавирусная инфекция, COVID-19, глюкокортикостероиды, вирусная пневмония

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The novel coronavirus SARS-CoV2 was first isolated and identified in Wuhan, China, in 2019. On March 11, 2020 WHO declared a pandemic of the novel coronavirus disease [1].

The excessive immune response to COVID-19 culminating in a cytokine release syndrome, also known as cytokine storm, plays the leading role in the pathogenesis of severe pneumonia caused by COVID-19. The devastating consequences of uncontrolled cytokine release include damage to the lungs, diffuse alveolar damage, acute respiratory distress syndrome (ARDS), and death [2–5].

So far, there is no effective etiotropic therapy against COVID-19. Improving oxygenation by prone positioning, oxygen therapy or mechanical ventilation, preventing and treating bacterial complications and using drugs to suppress the cytokine storm (glucocorticoids, inhibitors of proinflammatory factors and Janus-kinases) are the mainstay of anti-COVID-19 treatment [6–8].

Early reports on the benefits of glucocorticoid therapy in patients with COVID-19 were controversial [8-13]; however, later studies provided evidence of its efficacy [14, 15].

The aim of this study was to investigate the efficacy of methylprednisolone in reducing patient mortality in intensive care units (ICU) and medical wards.

METHODS

This study was a prospective quasi-experimental single-center open nonrandomized clinical trial. The study protocol was approved by the Academic Board of the Federal Center of Brain Research and Neurotechnology (FMBA, Russia) and the local Ethics Committee. The initial plan laid out in the first version of the protocol was that methylprednisolone would be administered only in an intensive care setting to eligible patients transferred to ICU from ER or a medical ward. Eligibility criteria

for transfer to ICU are specified in the guidance of the Russian Ministry of Healthcare (ver. 5 and 6). [16, 17]. However, due to the increasing number of patients admitted to medical wards with signs of lung damage similar to those in patients already transferred to ICU, the protocol was revised. Importantly, patients on the hospital floor had similar comorbidities to patients already transferred to ICU. So, considering the first-hand experience in treating patients with COVID-19, amendments were proposed to the first version, and the Academic Board of the Federal Center of Brain Research and Neurotechnology revised the protocol. The second version of the protocol permitted administration of methylprednisolone via continuous 3-day IV infusions using Infusomat/Perfusor Space systems in a medical ward setting.

From April 13, 2020 to May 25, 2020, 603 patients with community-acquired COVID-19-induced pneumonia were hospitalized to the Federal Center of Brain Research and Neurotechnology. Methylprednisolone therapy was administered to 54 patients (9% of all hospitalized patients). Group 1 (n = 28) included patients receiving methylprednisolone therapy in the ICU setting only between April 24, 2020 and May 6, 2020. Group 2 (n = 26) consisted of patients who received methylprednisolone in a medical ward setting between May 7, 2020 and June 12, 2020. The historical control group (group 3, n = 14) comprised patients hospitalized to the Federal Center of Brain Research and Neurotechnology from April 13, 2020 to April 23, 2020; these patients did not receive hormonal or other therapy for the cytokine storm (monoclonal antibodies, JAK inhibitors, etc.) and were comparable in terms of their clinical characteristics to the patients on methylprednisolone therapy.

The following inclusion criteria were applied:

1. Male and female patients aged over 18 years;
2. Positive PCR test results for SARS-CoV-2 RNA;
3. Clinical signs of pneumonia (fever >38.5 °C, respiration rate over 22 breaths per min, shortness of breath on exertion, SpO_2 < 95% at room air);
4. Chest CT findings suggestive of pneumonia.

Exclusion criteria:

1. Signs of bacterial or fungal infection confirmed by procalcitonin and/or presepsin test and full blood count;
2. HIV/AIDS;
3. Active or latent TB infection;
4. Congestive heart failure;
5. Recent myocardial infarction
6. Severely impaired liver and/or kidney function;
7. Recent intestinal anastomosis
8. Esophagitis, gastritis, active or latent peptic ulcer
9. Myasthenia gravis;
10. Glaucoma
11. Severe osteoporosis
12. Hypothyroidism
13. Psychiatric disorders
14. Poliomyelitis (except bulbar poliomyelitis and polioencephalitis)
15. BCG lymphadenitis;
16. Recent vaccination.

Continuous IV infusions of methylprednisolone (Solu-Medrol; 250 mg per day) were administered to the patients over the course of 3 days; the total dose was 750 mg. The patients were monitored for blood pressure and glycemia; proton pump inhibitors were prescribed for gastric protection. The patients also received standard therapy for COVID-19 recommended by the guidance of the Russian Ministry of Healthcare (ver. 5 and 6): antibacterial and detoxicating agents, antipyretic drugs, and anticoagulants [16, 17]. Antimalarial and anti-HIV medications were not included in the regimen. Oxygen was delivered to patients with SpO_2 < 93% through nasal cannulas or a mask at 15 L/min.

Illness severity was assessed on the NEWS scale [18]; lung damage was evaluated based on the chest CT scans following the guidance [16, 17]. The efficacy of the administered therapy was determined based on the mortality rate; we also looked at the need for and duration of mechanical ventilation, the length of ICU and overall hospital stay. Body temperature dynamics were evaluated in groups 1 and 2 on days 3 and 5 after the beginning of methylprednisolone therapy; the respiration rate (RR) and SpO_2 were measured in group 1 and 2 patients who were not on MV. C-reactive protein (CRP) levels were measured to evaluate the severity of inflammation.

Statistical analysis

Categorical variables were compared using the chi square test; continuous variables were compared using the Kruskal-Wallis test (differences between all groups) and the Mann-Whitney U test (for groups 1 and 2).The Friedman test was applied to compare linked samples (SpO_2, RR and CRP before methylprednisolone therapy and on days 3 and 5 into therapy).To evaluate the effect of methylprednisolone on patient mortality adjusted for confounding factors (parameters that differed between the groups, including age, chronic heart failure, chronic renal failure, severity of lung damage on CT scans), binary logistic regression was applied. Quantitative variables are presented below as medians and upper and lower quartiles. All computations were performed in IBM SPSS ver. 16.0. The null hypothesis was rejected at p < 0.05. Two-tailed tests were used in all cases.

RESULTS

The basic characteristics of the patients included in the study are provided in Table 1. All groups were comparable in terms of demographics (age, sex), time from disease onset to hospitalization, time from hospitalization to the initiation of methylprednisolone therapy (for groups 1 and 2).

The analysis of comorbidities revealed that group 1 was dominated by patients with chronic heart failure (CHF) and chronic kidney disease (CKD); these conditions were observed in 39.3% (p = 0.009) and 25% (p = 0.017) of patients, respectively. For other comorbidities, no significant differences were detected between the groups. Similarly, no significant differences were observed between the groups in terms of presenting symptoms: the majority of patients presented with cough, fever and labored breathing. The severity of pneumonia on the NEWS scale was comparable between all patient groups. However, there were significantly more patients with severe lung damage in group 1 (p = 0.012). Thus, on admission the radiographic findings were indicative of a much more severe lung damage in group 1, which was also characterized by more frequent comorbidities, including CHF and CKD.

In group 1, 2 (7.1%) patients were transferred to ICU straight from ER; the remaining 26 patients were transferred from their medical wards to ICU due to disease progression, as recommended by the guidance [16, 17]. In group 2, only 2 patients (7.7%) were transferred to ICU after methylprednisolone therapy and then placed on MV. In spite of intensive care, these 2 patients died. Eight (57.1%) patients from group 3 were transferred from their medical wards to ICU and placed on MV; of them 6 (42.9%) individuals died.

Analysis of clinical outcomes (Table 2) revealed a significantly lower mortality rate in group 2 (2 (7.7%) cases), as compared to groups 1 (19 (67.9%) cases) and 3 (6 (42.9%) cases, $p < 0.001$). In group 2, ME was less frequent than in groups 1 and 3 (2 (7.7%), 20 (71.4%) and 7 (50%) patients, respectively, $p < 0.001$).

The longest ICU stay (median: 9 days) and the highest MV duration (median: 9 days) were observed in group 1 ($p = 0.025$ and $p = 0.023$, respectively). In group 2, these parameters equaled 5 and 5 days, respectively, and in group 3, 5 and 3 days, respectively. No significant differences were detected between the groups in terms of total hospital stay.

Considering that our groups differed in a number of factors that could potentially affect the outcome (CHF, CKD, severity of lung damage on a CT scan, age — all presenting a pronounced trend, see Table 1), we applied binary regression in order to make adjustments for these confounding factors. Results are provided in Table 3.

Adjusted for confounding factors, the odds of death in group 2 were significantly lower than in 2 other groups. Notably, the contribution of age was substantial whereas the contribution of other predictors was insignificant. Perhaps, CHF and CKD are more prevalent and lung damage visible on CT is more severe in patients of advanced age, i.e. these parameters are not significant outside the context of age. The overall quality of our regression model was satisfactory: Nigel-Kirk's pseudo-R-squared was 0.612, and the Hosmer-Lemeshow goodness of fit was 0.499.

Comparative analysis of clinical and laboratory parameters between the groups of patients receiving methylprednisolone therapy in ICU vs. a medical ward setting is provided in Table 4. By day 5 after methylprednisolone therapy was initiated, group 2 was dominated by patients without hyperthermia (23 (92%) cases), as compared to group 1, where only 13 patients (50%) ($p = 0.002$) had normal body temperature at this time point. Also, shortness of breath became less pronounced in group 2 on day 3 (end of methylprednisolone therapy): median RR was 19 breaths per min, whereas in group 1, RR was 24 breaths per min ($p = 0.043$). No significant differences in SpO_2 were detected between the groups.

Prior to methylprednisolone therapy, CRP levels were high in both groups treated with this drug (median 136 [94; 213] mg/L in group 1 and 148 [68; 183] mg/L in group 2). By day 3 into treatment, CRP levels started to decline, reaching 68 [41; 126] mg/L in group 1 and 52 [23; 142] mg/L in group 2; the differences between the groups were insignificant. On day 5, CRP levels differed significantly between groups 1 and 2 (68 [35; 210] mg/L and 29 [12; 52] mg/L, respectively, $p = 0.005$).

Table 1. Patient demographics

Parameter		Group 1 ($n = 28$)	Group 2 ($n = 26$)	Group 3 ($n = 14$)	Significance of differences, p
Sex	Male (%)	17 (60.7)	17 (65.4)	12 (85.7)	0.251
	Female (%)	11 (39.3)	9 (34.6)	2 (14.3)	
Age, years Median [Q1; Q3]		66 [58; 77]	59 [53; 70]	57 [49; 68]	**0.05**
Time from disease onset to hospital admission, days Median [Q1; Q3]		7 [5; 10]	7 [6; 9]	8 [3; 11]	0.831
Time from hospital admission to beginning of methylprednisolone therapy, days Median [Q1; Q3]		3 [2; 5]	3 [2; 6]	–	0.936
Comorbidities					
Hypertension (%)		18 (64.3)	12 (46.2)	8 (57.1)	0.405
Smoking (%)		2 (7.1)	2 (7.7)	1 (7.1)	1.00
Diabetes mellitus (%)		6 (21.4)	4 (15.4)	2 (14.3)	0.788
Past history of stroke or TIA (%)		4 (14.3)	2 (7.7)	1 (7.1)	0.662
Past history of myocardial infarction (%)		5 (17.9)	1 (3.8)	1 (7.1)	0.217
Obesity (%)		9 (32.1)	4 (15.4)	4 (28.6)	0.180
Asthma (%)		1 (3.6)	1 (3.8)	0	0.764
Chronic obstructive pulmonary disease (%)		3 (10.7)	2 (7.7)	0	0.454
Chronic heart failure (%)		11 (39.3)	4 (15.4)	0	**0.009**
Chronic kidney disease (%)		7 (25)	1 (3.8)	0	**0.017**
Presenting complaints					
Cough (%)		26 (96.3)	24 (92.3)	14 (100)	0.516
Fever (%)		27 (96.4)	25 (96.2)	14 (100)	0.449
Shortness of breath (%)		26 (96.3)	20 (76.9)	10 (71.4)	0.063
Severity on admission					
NEWS score Median [Q1; Q3]		5 [5; 7]	4 [2; 7]	4 [2; 6]	0.173
Lung damage on CT on admission					
	Grade 1 (%)	3 (10.7)	2 (7.7)	0 (0)	**0.012**
	Grade 2 (%)	2 (7.1)	12 (46.2)	3 (21.4)	
	Grade 3 (%)	11 (39.3)	10 (38.5)	7 (50)	
	Grade 4 (%)	12 (42.9)	2 (7.7)	4 (28.6)	

Intragroup comparisons of these parameters revealed that CRP ($p = 0.033$), SpO_2 ($p < 0.0005$) and RR ($p < 0.0005$) dynamics were significant in group 1. For group 1, only CRP dynamics were significant ($p = 0.023$), unlike RR and SpO_2.

DISCUSSION

This study investigated effects of methylprednisolone therapy at different stages of inpatient medical care. We found that administration of methylprednisolone in a medical ward setting aimed at preventing inflammation improves survival, reduces the frequency of patient transfer to ICU and the need for MV in patients with COVID-19-induced pneumonia. Methylprednisolone therapy delivered in the ICU setting did not have a significant effect on mortality (which was 67.9%), in comparison with the control group, whereas its early application in a medical ward setting prevented most patients from worsening, transfer to ICU and placement on MV, resulting in lower mortality.

Being a potent anti-inflammatory drug, methylprednisolone can block secretion of proinflammatory cytokines and accelerate resolution of pulmonary and systemic inflammation in patients with pneumonia [11, 19]. At the same time, some studies demonstrated that glucocorticoids hampered pathogen elimination from the organism and increased mortality in patients with other viral infections [11, 20]. However, there have been no studies so far addressing the use of the proposed methylprednisolone regimen against COVID-19.

There are a number of publications investigating the efficacy of glucocorticoid therapy in patients with COVID-19 progressed to pneumonia. Specifically, a retrospective cohort study conducted in patients with confirmed COVID-19 and ARDS revealed that methylprednisolone administered intravenously at 1–2 mg/kg per day for 5–7 days reduced the risk of mortality (23 deaths in 50 (46%) patients who received methylprednisolone vs. 21 deaths in 34 patients (61.8%) who did not receive this drug) [21]. In another study conducted in 46 patients with severe COVID-19 progressed to respiratory failure, therapy with methylprednisolone was associated with better clinical benefits and reduced duration of the disease [22]. According to the Chinese Thoracic Society experts consensus, methylprednisolone should be administered at low to medium doses (≤ 0.5–1 mg/kg a day) [23]; it is reported that the most common methylprednisolone regimens in China are 40–80 mg of the drug per day for 3–6 days [24].

A study by Fadel Raef et al. [14] compared the effects of different methylprednisolone regimens in managing COVID-19: early (within 2 days after admission) vs. later (day 5 after admission therapy start. Methylprednisolone was delivered to patients at 0.5 to 1 mg/kg a day over the course of 3 days; the total daily dose was administered in two divided doses every 12 h. The study demonstrated the efficacy of early methylprednisolone therapy in achieving a primary composite endpoint (death from any causes + transfer to ICU + placement on ME). In the early therapy start group, the primary endpoint rate was 34.9%, whereas in the later start group, it was 54.3% ($p = 0.005$).

The methylprednisolone dosage and regimen used in our study differed from the cited studies: the drug was administered continuously at 250 mg per day for 3 days in a row; this allowed us to use a higher total methylprednisolone dose (750 mg).

Our study has a few limitations. Due its quasi-experimental design, there was no randomization and placebo control; the control group was formed based on the historical principle. Patients from group 1 were slightly older, had more comorbidities and more severe damage to the lungs on CT scans.

Summing up, this study demonstrates the need for a randomized double-blind placebo-controlled trial of the efficacy of methylprednisolone therapy in patients with pneumonia caused by the novel coronavirus infection. In our experience, preventive methylprednisolone therapy might be beneficial in terms of costs incurred by a medical facility in the absence

Table 2. Comparative analysis of clinical outcomes and some clinical variables

Parameter	Group №1 ($n = 28$)	Group №2 ($n = 26$)	Group №3 ($n = 14$)	Significance of differences, p
Death (%)	19 (67,9)	2 (7,7)	6 (42,9)	<0,001
ME (%)	20 (71,4)	2 (7,7)	7 (50)	<0,001
ME duration Median [Q1; Q3]	9 [6; 13]	5 [2; 7]	3 [1; 5]	0,023
Days in ICU Median [Q1; Q3]	9 [6; 17]	5 [2; 7]	5 [2; 6]	0,025
Total length of stay Median [Q1; Q3]	19 [12; 25]	16 [12; 19]	14 [6; 17]	0,074

Table 3. Binary logistic regression with confounding factors and a group factor, death is dependent variable. CI– confidence interval, OR– odds ratio. Significant predictors are highlighted. OR is not specified for the reference category in the case of categorial predictors

Predictor	Significance (p)	OR	95% CI for OR	
			Lower limit	Upper limit
Group 2	**0.003**			
Group 3	**0.002**	52.693	4.065	683.107
Group 1	**0.001**	47.824	4.690	487.640
CHF	0.165	0.202	0.021	1.929
CKD	0.140	6.191	0.549	69.790
Lung damage grade 1	0.654			
Lung damage grade 2	0.497	0.306	0.010	9.323
Lung damage grade 3	0.317	0.191	0.007	4.885
Lung damage grade 4	0.623	0.434	0.016	12.088
Age	**0.014**	1.104	1.021	1.194

Table 4. Comparative analysis of clinical and laboratory data between groups 1 and 2

Parameter	Group №1 (n = 28)	Group №2 (n = 26)	Significance of differences, p
Body temperature recovery on day 3 from therapy onset (%)	14 (51.9)	19 (73.1)	0.158
Body temperature recovery on day 5 from therapy onset (%)	13 (50)	23 (92)	0.002
RR before therapy Median [Q1; Q3]	24 [20; 28]	24 [22; 27]	0.754
RR on day 3 from therapy onset Median [Q1; Q3]	24 [20; 25]	19 [18; 20]	0.043
RR on day 5 from therapy onset Median [Q1; Q3]	20 [18; 30]	19 [18; 20]	0.117
SpO_2 before therapy, % Median [Q1; Q3]	90 [87; 94]	93 [90; 95]	0.340
SpO_2 on day 3 from therapy onset, % Median [Q1; Q3]	94 [89; 98]	95 [95; 97]	0.170
SpO2 on day 5 from therapy onset, % Median [Q1; Q3]	96 [84; 96]	97 [96; 98]	0.163
CRP before therapy Median [Q1; Q3]	136 [94; 213]	148 [68; 183]	0.436
CRP on day 3 from therapy onset Median [Q1; Q3]	68 [41; 126]	52 [23; 142]	0.307
CRP on day 5 from therapy onset Median [Q1; Q3]	68 [35; 210]	29 [12; 52]	0.005

of sufficient funding. The obtained data can be used in further studies looking into the effect of different therapeutic anti-COVID-19 regimens on public health budget at many levels.

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EXPERIMENTAL EVALUATION OF THE ACTIVITY OF THE PRODUCT MEFLOCHINE AGAINST CORONAVIRUS SARS-COV-2

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When evaluating the effectiveness of the drug Mefloquine against SARS-CoV-2 coronavirus, *in vitro* experiments examined its toxicity for African green monkey kidney cell culture — Vero C1008, as well as antiviral activity against SARS-CoV-2, which was evaluated by suppressing the cytopathic effect the virus. A study of the toxicity of the drug Mefloquine showed that the concentration at which the drug exerts a cytopathic effect against 50% of Vero C1008 cells is 4.5 μg / ml. The maximum tolerated concentration (MTD) of Mefloquine is 2.25 μg / ml. A study of the effectiveness showed that 1 day after infection, the antiviral effect of Mefloquine was recorded when the drug was added 24 hours and 1 hour before infection with SARS-CoV-2, as well as when it was added 1 hour after infection, the cell culture was already at a concentration of 0.5 μg / ml Mefloquine at a concentration of 2 μg / ml, added to the Vero C1008 cell culture 1 hour after the introduction of SARS-CoV-2, completely blocked the action of the virus for 2 days after infection.

Keywords: coronavirus, COVID-19, SARS-CoV-2, SARS-COV-2, Mefloquine, antiviral activity

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ЭКСПЕРИМЕНТАЛЬНАЯ ОЦЕНКА АКТИВНОСТИ ПРЕПАРАТА МЕФЛОХИН В ОТНОШЕНИИ КОРОНАВИРУСА SARS-COV-2

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В ходе оценки эффективности препарата Мефлохин в отношении коронавируса SARS-Cov-2 в экспериментах *in vitro* исследована его токсичность для культуры клеток почки африканской зеленой мартышки — Vero C1008, а также противовирусная активность в отношении SARS-Cov-2, которую оценивали по подавлению цитопатического действия вируса. Изучение токсичности препарата Мефлохин показало, что концентрация, в которой препарат проявляет цитопатическое действие в отношении 50 % клеток Vero C1008 (ЦПД50), составляет 4,5 мкг/мл. Максимальная переносимая концентрация (МПК) Мефлохина составляет 2,25 мкг/мл. Изучение эффективности показало, что через 1 сут после инфицирования противовирусное действие Мефлохина регистрировали при внесении препарата за 24 ч и 1 ч до заражения SARS-CoV-2, а также при его добавлении через 1 ч после инфицирования культуры клеток уже в концентрации 0,5 мкг/мл. Мефлохин в концентрации 2 мкг/мл, добавленный к культуре клеток Vero C1008 через 1 ч после внесения SARS-CoV-2, полностью блокировал действие вируса в течение 2-х сут после инфицирования.

Ключевые слова: коронавирус, SARS-CoV-2, COVID-19, SARS-COV-2, Мефлохин, противовирусная активность

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Coronaviruses (Coronaviridae) are RNA viruses that can infect humans and some animals. In human beings, coronaviruses can cause a range of diseases, from mild acute respiratory infections to severe acute respiratory syndrome (SARS). Currently, there are four coronaviruses (HCoV-229E, -OS43, -NL63 and -HKU1) known to circulate year-round and cause acute respiratory viral infections. As a rule, these infections translate into mild and moderate damage to the upper respiratory tract (URT) [1].

Until 2002, coronaviruses were regarded as pathogens causing URT diseases that extremely rarely ended in death. At the end of 2002, the SARS-CoV coronavirus was registered. It is the causative agent of SARS, severe acute respiratory syndrome. During this epidemic, over 8000 cases were registered in 37 countries, of which 774 were fatal. Since 2004, no new cases of SARS caused by SARS-CoV have been registered [2].

In 2012, the world encountered MERS-CoV, a new coronavirus causing Middle East respiratory syndrome. There have been 2494 cases of this infection registered since 2012, with 858 of them fatal. Geographically, all these cases were associated with the Arabian Peninsula. Currently, MERS-CoV continues to circulate and cause new cases of the disease [3, 4].

In December 2019, a new disease (that was later named COVID-19) was registered in Wuhan, Hubei province, China. The causative agent of this disease is SARS-CoV-2, a new coronavirus. COVID-19 posed new healthcare challenges; in particular, it called for rapid testing techniques and clinical case management tactics [5]. The currently available epidemiological, clinical, prevention and treatment-related information on COVID-19 is limited and inconsistent. The virus was put into the II pathogenicity group, like some other representatives of this genera (SARS-CoV-1, MERS-CoV). The most common clinical manifestation of the new variant of coronavirus infection is pneumonia, although a significant number of patients have developed acute respiratory distress syndrome.

Chloroquine and hydroxychloroquine, derivatives of quinoline, are the drugs selected for 2019-nCoV therapy [6]. Previously, antiviral activity of chloroquine and hydroxychloroquine against SARS-CoV was demonstrated in cell culture studies [7]. Chloroquine was about fivefold more active than hydroxychloroquine (EC50 in cell culture was 6.5 ± 3.2 μM and 34±5 μM, and the selectivity index was -> 15 and >3, respectively). Chloroquine was found even more active against the HCoV-OC43 strain (the causative agent of SARS). Its viral replication suppression EC_{50} was 0.306 ± 0.091 μM.

In experiments on mice, it was established that a 15 mg/kg dose of chloroquine (about 80 mg for human beings) ensured survival of mice infected with 103 copies of HCoV-OC43 [8]. Chloroquine was also found to produce an effect associated with MERS virus replication blocking at concentrations of 3–8 μM [9].

An *in vitro* comparative study of antiviral activity of chloroquine and hydroxychloroquine [10] yielded dose-effect curves for four different multiplicities of infection (MOI) by counting viral RNA copies in cell supernatant 48 hours after infection. The preparations were introduced into the cell culture 1 hour before the virus.

Depending on the infective dose, hydroxychloroquine's EC_{50} was 4.51–12.96 μM. Cytotoxic dose of hydroxychloroquine in cell culture was more than 100 times higher than the average effective dose against the virus.

In another study [11], at MOI = 0.01 and therapeutic use of hydroxychloroquine (it was introduced into the medium 2 hours after incubation of the cell culture with viral particles at 37 °C), its EC_{50} after 24 hours was 6.14 μM (2060 μg/ml), after 48 h — 0.72 μM (258 μg/ml).

These drugs, along with other medicines, were included in the 5th and 6th editions of the COVID-19 Prevention, Diagnostics and Treatment Guidelines published in China in January-February 2020. Hydroxychloroquine was included in the COVID-19, New Coronavirus Infection Interim Prevention, Diagnostics and Treatment Guidelines (versions 4-6) published by the Ministry of Health of the Russian Federation in 2020.

Mefloquine is another antimalarial drug that can potentially be used to treat the SARS-CoV-2 infection. The drug was developed to treat forms of malaria resistant to chloroquine and hydroxychloroquine.

Research into Mefloquine's capability to fight viruses is limited. Nevertheless, it was found to show antiviral activity against Ebola [12], Dengue and Zika viruses [13]. The activity of Mefloquine against various coronaviruses was discovered through in vitro research. Comparative studies that modeled infecting a cell culture with FCoV (feline coronavirus) have shown chloroquine and Mefloquine among the most active drugs out of 19 preparations researched. The antiviral activity of Mefloquine is 2–5 times higher than that of chloroquine, and its average effective dose, which suppresses the cytopathic effect of the virus in cell culture, was 7.5–8.31 μM. The average effective viral replication suppressing dose was 4.43–7.36 μM [14]. When combined with interferon, Mefloquine becomes even more potent against viruses [15].

Mefloquine was also found effective against the 2019-nCoV coronavirus. The drug was discovered to block the coronavirus' cytopathic effect in a cell culture and prevent its replication in concentrations of no more than 10 μM (4 μg/L) [16]. However, no effort was made to determine the average effective dose of Mefloquine more accurately.

MATERIALS AND METHODS

We used samples from three lines of Mefloquine (№ 010719, № 020719, № 030719) developed at the Farmzaschita R&D and production center, Federal state unitary company under the Federal Medical-Biological Agency of Russia. The virus used was variant B of SARS-nCoV, obtained in 2020 from Vector Virology and Biotech Research Center (Federal State Budgetary Institution under Rospotrebnadzor) without isolation data; the variant is stored in the Specialized collection of the 48th Central Research Institute, Federal State Budgetary Institution under the Ministry of Defense of the Russian Federation. For the experimental stage, we used Vero C1008, a permanent culture of African green monkey kidney cells. Eagle's minimal essential medium (MEM) in Hanks saline solution containing 7.5% and 2% fetal calf serum, respectively, were used as growth and maintenance cultures.

Biological properties of the SARS-CoV-2 pathogen were assessed by titrating a virus containing suspension in a Vero C1008 cell culture relying on the cytopathic action of the virus.

The cytotoxicity of the Mefloquine samples was assessed by visual observation of the state of Vero C1008 cell culture with the help of a light microscope at low magnification. Drug concentrations that had cytopathic effect on the cells (destruction of cell monolayer and their overall destruction as confirmed through visual observation) were considered toxic. At the same time, we determined the maximum tolerated dose (MTD), i.e. concentration that did not destroy the cell culture used. In determining the MTD, the optimal time of contact between the studied compound and the cell culture corresponded to the cell cultures' maximum functioning period (4 days on average) [17–19].

We followed recommendations released by the Scientific Centre for Expert Evaluation of Medicinal Products (Federal State Budgetary Institution under the Ministry of Health and Social Development of Russia) in assessment of antiviral efficacy of the experimental substances. The antiviral efficacy of Mefloquine was assessed when its tenfold concentration was administered, a concentration that has a 50% cytopathic effect (10 CPE50) before infection (1 hour and 24 hours) and 1 hour after infection. The effect on cytopathogenicity of the virus was assessed 24 hours and 48 hours after infection. Monolayer was registered completely destroyed after 48 hours, and after 24 hours we observed cell destruction in 75% of cases. For each Mefloquine concentration investigated, we used 4 tubes containing a mono-layer of cells; the number of independent experiments was three, which makes the sum total of the tubes twelve. Virus cytopathogenicity inhibition coefficient (IC, %) allowed assessment of the preparations' efficacy. This coefficient was calculated with the help of the following formula:

$$IC = \frac{A_{kontr} - A_{op}}{A_{kontr}} \cdot 100\%$$

where A_{kontr} is biological activity of the virus, determined in cells without introduction of the chemical drug; A_{op} is biological activity of the virus in cells with introduction of the chemical drug (CPE).

The results were statistically processed using Microsoft Office Excel 2007.

RESULTS AND DISCUSSION

Infecting preparations

To prepare the SARS-CoV-2 virus, variant B, infecting preparation, we used the Vero C1008 cell culture. A cell suspension with the density of 200 thousand/ml was introduced into sterile plastic cell culture flasks, incubated for 24 hours in a CO_2 incubator (5% CO_2 at 37.0 ± 0.5 °C) until a continuous monolayer was formed, as registered at low magnification of a light microscope. The virus culture was bred on a growth medium. The multiplicity of infection was 1 PFU per cell. The virus adsorption procedure lasted for 60 minutes at 37.0 ± 0.5 °C. When adsorption was over, we removed the inoculum, washed cells in three volumes of MEM and added 7–8 ml of fresh growth medium containing 2% fetal bovine serum to each vial. Flasks with the infected cell culture monolayer were placed in

a CO_2 incubator (5% CO_2 at 37.0 ± 0.5 °C). After 48 hours of incubation, we cryodestroyed the cells, clarified and packed for storage at –70 °C. The properties assessed were sterility of the resulting infecting preparation its infectious activity. We determined the SARS-CoV-2, variant B, experimental culture activity applying the negative colonies method and using a day old Vero C1008 cell culture monolayer (PFU/ml); another criterion was the virus' cytopathic effect (CPE_{50}/ml). Sowing on a 10-fold solution on a universal selective thioglycolic medium allowed assessing presence of foreign microflora in the preparations made.

Investigation of Mefloquine cytotoxicity to a Vero C1008 cell culture and antiviral activity against SARS-CoV-2 virus

The studied Mefloquine lines were introduced into tubes containing Vero C1008 cell monolayer and incubated at 37.0 ± 0.5 °C for 120 hours. After the incubation, we evaluated cellular damage caused by the lines.

Results of the experiment indicate that all the studied lines of the drug show the same cytotoxicity in vitro. For the cell culture used, their CPE_{50} was 4.5 μg/ml. At concentrations below 2.25 μg/ml the drug did not have a toxic effect on the cell culture, and at concentrations above 8.0 μg/ml it proved cytopathic to almost all cells of the monolayer. Thus, the maximum tolerated dose (MTD) of Mefloquine was 2.25 μg/ml, and the range of concentrations for evaluation of its antiviral activity was 0.5–2.0 μg/ml.

The results of investigation of effect of Mefloquine on the cytopathic activity of SARS-CoV-2, variant B, 24 hours post-infection are given in. The results were similar for all the drug lines tested.

The results obtained allow a conclusion that 24 hours after infection of the cells, cytopathic effect of the virus disappeared in case 0.5 to 2.0 μg/ml of Mefloquine were administered either before or after infection. In the control group, the cytopathic effect was 75%.

The results of investigation of effect of Mefloquine on the cytopathic activity of SARS-CoV-2, variant B, 48 hours post-infection are given in .

Forty-eight hours after infection of the cells, cytopathic effect of the virus disappeared in case 2.0 μg/ml of Mefloquine were administered after infection. In the control group, the cytopathic effect was 100% (12/12). Administration of any of the studied doses of Mefloquine 1 hour before infection yielded no suppression of cytopathic activity of the virus. Doses of 1 μg/ml and 0.5 μg/ml administered 24 hours before infection caused 50% and 25% suppression of CPE, respectively.

The results of Mefloquine effect on virus reproduction in a cell culture are presented in. Ribavirin and Rebif® (Interferon β1α) were used as comparator drugs.

The results obtained indicate that 2.0 μg/ml of Mefloquine administered post-infection suppress reproduction of SARS-CoV-2 virus at 1.7–1.9 lg, with the inhibition rate at about 99%.

CONCLUSION

The results obtained confirm that 2 μg/ml of Mefloquine administered to the Vero Cl008 cell culture 1 hour after introduction of SARS-CoV-2 completely block the effect of the virus within 2 days after infection. A day after infection, Mefloquine was registered to have antiviral effect when administered both 24 hours and 1 hour before and 1 hour after infection of the cell culture with SARS-CoV-2. As little as 0.5 μg/ml of Mefloquine yielded 100% suppression of viral activity. The Mefloquine toxicity investigation showed that the drug exhibits a cytopathic effect on 50% of Vero Cl008 cells (CPE_{50}) at 4.5 μg/ml. The maximum tolerated dose of Mefloquine is 2.25 μg/ml.

Thus, the chemotherapeutic index (an indicator of the breadth of therapeutic effect, the ratio of its minimum effective dose to the maximum tolerated dose) for Mefloquine was ≥ 2, which indicates a low specific activity of the drug. At the same time, it should be noted that the concentration at which Mefloquine becomes effective against SARS-CoV-2 can be accumulated when taking the drug in doses recommended for the prevention and treatment of malaria.

The generalized pharmacokinetic research data show that Mefloquine dose escalation in the range of 250–500–1000–1500 mg (as provided in the Mefloquine Medical Use Guidelines) translates into an almost linear increase of its maximum concentration in blood plasma: 0.25- 0.43–0.8-1.22 μg/ml, respectively, with the tmax value constant at 19.6 hours [20]. A one-time administration of Mefloquine in the doses of 750–1500 mg/day brings its maximum concentration in blood plasma to 1510 μg/l [21], while a course increases it 1.8–2.5 times [22]. Considering that the half-life of the drug is 15.5 ± 10.4 days, the concentration that ensures elimination of the virus can be achieved on the 2–3 day of administration, and it is maintained throughout the treatment period.

Table 1. Evaluation of antiviral activity of Mefloquine against the SARS-CoV-2, variant B, in a Vero C1008 cell culture, 24 hours after infection (infection dose 10 CPE, *n* = 9)

Preparation	Preparation concentration μg/ml	CPE detection rate	CPE inhibition coefficient, %
24 hours before infection	2	0/12	> 75
	1	0/12	> 75
	0.5	0/12	> 75
1 hour before infection	2	0/12	> 75
	1	0/12	> 75
	0.5	0/12	> 75
1 hour after infection	4	0/12	> 75
	2	0/12	> 75
	1	0/12	> 75
	0.5	0/12	> 75
Infectious dose control	–	9/12	–
Medium control	–	0/12	–

Table 2. Evaluation of antiviral activity of Mefloquine against the SARS-CoV-2, variant B, in a Vero C1008 cell culture, 48 hours after infection (infection dose 10 CPE, *n* = 9)

Preparation	Preparation dose, μg/ml	CPE detection rate	CPE inhibition coefficient, %
24 hours before infection	2	12/12	0
	1	6/12	50
	0.5	9/12	25
1 hour before infection	2	12/12	0
	1	12/12	0
	0.5	12/12	0
1 hour after infection	2	0/12	100
	1	12/12	0
	0.5	12/12	0
Infectious dose control	–	12/12	–
Medium control	–	0/12	–

Table 3. Evaluation of antiviral activity of Mefloquine against the SARS-CoV-2, variant B, in a Vero C1008 cell culture, 48 hours after infection (infection dose 10 CPE, *n* = 9)

Preparation	Preparation dose, μg/ml	Virus accumulation level, lg PFU/ml	Virus accumulation level drop, Δ lg	Inhibition coefficient, IC (%)
Mefloquine, series 010719	2.0	4.61 ± 0.07	1.83	98.93
Mefloquine, series 020719	2.0	4.50 ± 0.09	1.94	99.06
Mefloquine, series 030719	2.0	4.73 ± 0.13	1.71	98.83
Rebif® Interferon β1α	10^3	0.00 ± 0.00	6.44	100.00
	10^2	0.00 ± 0.00	6.44	100.00
Ribavirin, substance	100	4.23 ± 0.03	2.21	99.38
Infection dose control	–	6.44 ± 0.09	–	–

It was established that Mefloquine concentration in internal organs and blood cells is over 5 times higher than that in blood plasma [23–24], and its concentration in the brain tissues can be 10–30 times greater than blood plasma concentration and reach 20 μM [25]. Thus, in internal organs the drug may be eliminated as early as on the 1st day after beginning of administration.

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COVID-19: EXTRAPULMONARY IMPAIRMENTS (OWN DATA OF INFECTION HOSPITAL OF FSBI FSSCC FMBA OF RUSSIA) AND EXPERIENCE OF USE DIFFERENT PROFILE SPECIALISTS TO WORKING IN HOSPITALS

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Abstract. The article dwells upon the identification of extrapulmonary manifestations of COVID-19 using a time-saving, questionnaire specially designed by the authors to be filled out by the patients themselves. The introduction: sets out the relevance of exploratory studies of extrapulmonary lesions of this disease, identifies the main links in the pathogenesis of extrapulmonary lesions, and theoretically identifies possible targets in the body. It also includes the data, available in the literature at present, on the causative agent COVID-19 and other coronaviruses. Potential targets (in addition to the lungs) can be the nervous, digestive, cardiovascular and urinary systems, and the skin. The materials and methods: describe the questionnaire itself, its subdivision into domains, and include the data on the patient population. The results and discussion section sets out the researchers' own data. The most common symptoms in patients are apathy and asthenia, febrile syndrome, and respiratory symptoms. Formally, lesions of the nervous and digestive systems, as well as cardiovascular events, are less common. However, with a slight change in the counting technique (including apathy, asthenia and headache into it), the prevalence of neurological manifestations approaches 97.75%, and becomes the first in occurrence frequency rating. Symptoms indicating involvement of kidneys and skin were significantly less common. On the one hand, with the appearance of more severe cases of the disease, this percentage should increase, and on the other hand, its identification by the method of questioning in more severe patients is less important, especially since the main vital indicators of such patients are monitored. Conclusions: the authors outline directions for further search activity (confirmation of the data obtained by the results of laboratory and instrumental examinations, studying the connection to the therapy) and medical care organization for patients with COVID-19 (including particular specialists in the teams of infectious hospitals during the rise in the incidence and transition to counseling conducted by the specialists as the incidence subsides in the future).

Keywords: COVID-19, extrapulmonary manifestations, neurological lesions, pain syndromes, gastroenterological symptoms, apathy and asthenia, hyposmia, medical care organization

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COVID-19: ВНЕЛЕГОЧНЫЕ ПРОЯВЛЕНИЯ У ПАЦИЕНТОВ (СОБСТВЕННЫЕ ДАННЫЕ ИНФЕКЦИОННОГО ГОСПИТАЛЯ ФГБУ ФСНКЦ ФМБА РОССИИ)

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Введение: статья посвящена вопросам выявления внелегочных проявлений COVID-19 с использованием малозатратной по времени, специально разработанной для этой цели авторами анкетой, заполняемой самим пациентом. Материалы и методы: всего в исследование было включено 93 пациента, выразивших готовность сотрудничать. Анкета, включает в себя демографические данные (пол, возраст), 3 вопроса, касающихся симптомов, беспокоящих пациента в открытой (свободной форме), и 92 вопроса, указывающих на симптом в закрытой форме (пациент в случае наличия симптома должен был поставить галочку в соответствующем окошке). Фактически все симптомы (перечисленные в закрытом блоке) можно классифицировать на 9 больших доменов (групп): болевые, лихорадочные, респираторные, неврологические, гастроэнтерологические, дерматологические, нефроурологические, кардиологические, и отдельно выделена группа апатии и астении. Результаты и обсуждение: наиболее распространенными симптомами у пациентов являются апатия и астения, лихорадочный синдром, респираторные явления. Формально, несколько меньшую распространенность имеют поражение нервной и пищеварительной систем, а также сердечно-сосудистые явления. Однако, при небольшом изменении методики подсчета (включение апатии, астении и головной боли), распространенность неврологических проявлений приближается к 97,75%, и выходит на первое место. Симптомы, указывающие на вовлечение почек и кожи, имели существенно меньшую распространенность. Выводы: подавляющее большинство пациентов имеют внелегочные проявления заболевания. Авторами намечены направления для дальнейшей поисковой активности (подтверждение полученных данных результатами лабораторных и инструментальных обследований, изучение связи с проводимой терапией) и организации медицинской помощи пациентам с COVID-19 (включение разнопрофильных узких специалистов в состав бригад инфекционных госпиталей во время подъема заболеваемости и переход к консультированию узкими специалистами по мере спадания заболеваемости в дальнейшем).

Ключевые слова: COVID-19, внелегочные проявления, неврологические поражения, болевые синдромы, гастроэнтерологические симптомы, апатия и астения, гипосмия, организация медицинской помощи

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Introduction

The novel coronavirus disease (COVID-19) first reported in December 2019 has rapidly become a global public health emergency. Pneumonia and acute respiratory distress syndrome are commonly observed serious clinical manifestations of COVID-19. According to WHO, by mid-June 2020 over 6 million people worldwide had contracted the infection. The number of publications on the clinical course, diagnosis and therapy of COVID-19-induced pneumonia continues to grow. Yet little is known about the long-term effects of the infection on the respiratory tract. Even more understudied are the extrapulmonary complications of COVID-19. At the time of writing, there was very little information on the extra-pulmonary presentations of the disease; the scarce available data came from mass media sources, single clinical case reports or small-scale observational studies conducted in China and the European Union. At that time, there were no robust data representing the Russian population.

Coronaviruses use their spike proteins (SP) to bind to a receptor on the host cell membrane. At least 3 receptors are known that mediate the invasion, including the angiotensin-converting enzyme 2 (ACE2) [1], dipeptidyl peptidase 4 (DPP4) [2] and CD147 [3]. Once the virus latches onto its target, it

fuses with the host cell membrane and its RNA enters the cytoplasm for subsequent translation and protein replication. The tropism of the coronavirus is determined by the expression of the aforementioned receptors in different organs [4]. This means that damage inflicted by coronaviruses is not limited to the respiratory tract: the central nervous system (CNS) can also be attacked [5, 6, 7].

Once SARS-CoV-2, the causative agent of COVID-19, enters the bloodstream, it can cross the blood-brain barrier and spread to CNS. Another possible route of infection is via the olfactory bulb: the virus spreads to CNS by moving along the axons that course though the lamina cribrosa. There is experimental evidence that the mouse hepatitis virus (MHV), another representative of the Coronaviridae family, attacks CNS following the intranasal challenge. In an experiment conducted by Perlman S. et al. in 1990, surgical ablation of the olfactory pathway prior to nasal inoculation with MHV prevented CNS infection. Interestingly, SARS-CoV RNA was detected post-mortem in the brain tissue of 8 patients who died of atypical pneumonia in the early 2000s [5, 6, 7].

There are other entry points the virus can use to infect CNS. ACE2 is a defense factor for the cardiovascular system and the brain; it is found in many organs, including the nervous system and skeletal muscles, and plays the central role in regulating arterial blood pressure [8]. By binding to ACE2, viruses can cause elevated blood pressure and promote the risk of cerebral hemorrhages. Considering that the spike protein of SARS-Cov-2 is capable of interacting with ACE2 expressed in the capillary endothelium, the virus can attack the vascular system, breach the blood-brain barrier and invade CNS [4].

Neurological symptoms of COVID-19 are not limited to CNS, but can also develop in the peripheral nervous system (PNS). Impaired consciousness and other symptoms of brain damage are predictors of a very poor prognosis: 22% of non-survivors with COVID-19 vs. 1% of survivors had impaired consciousness [9]. Headache, dizziness, impaired consciousness, ataxia, acute cerebrovascular events, and seizures were the main clinical signs of neurological (CNS) damage observed in 53 of 218 (24.8%) Chinese patients with COVID-19. On the other hand, PNS involvement was observed in 19 patients (8.9%) in that cohort; hyposmia and dysgeusia were the most common symptoms affecting 11 (5.1%) and 12 (5.6%) patients, respectively.

A study conducted in a European population [10] reported hyposmia in 85.6% of patients with or without nasal congestion; 88% of patients had dysgeusia. In the short term, only 44% of patients recovered the sense of smell. Indeed, every systemic infection can cause damage to CNS or PNS, but this is also the reason why these phenomena need to be thoroughly studied.

In patients with COVID-19, distortion of the sense of smell or taste might arise from both CNS or PNS damage, requiring further investigation. The hypothesis about the loss of olfaction being the early symptom of the novel coronavirus infection is highly controversial. In 2006, there was a clinical case report of complete anosmia set in 3 weeks after the onset of the first SARS-CoV symptom. The patient was a 26-year-old female. She developed complete bilateral anosmia after her upper respiratory tract condition started to improve. This might indicate progression to chronic infection (persistence of the virus?) or delayed damage resulting from the activation of the immune system.

There are reports of 3 encephalitis cases associated with COVID-19. In one study, SARS-Cov-2 was detected in a patient's cerebrospinal fluid [11], suggesting that encephalitis was not the result of the immune response to infection. Similarly, Moriguchi T. et. al (2020) reported a case of meningitis/encephalitis, in which SARS-Cov-2 RNA was not detected in the nasopharyngeal swab of a patient but was present in the cerebrospinal fluid. The cerebrospinal fluid test was ordered because prior to that a CT scan had revealed ground glass opacities in the patient's lungs, which is a relatively specific sign of COVID-19 [12]. Another neurological manifestation of a coronavirus infection is acute disseminated encephalomyelitis (ADEM) [13].

Almost 40% of patients infected with the novel coronavirus suffer from headache, impaired consciousness and other symptoms of brain dysfunction. On autopsies, brain edema is a common finding in COVID-19 patients [7]. Therefore, it can be hypothesized that COVID-19 causes toxic encephalopathy.

With SARS-Cov-2, there is a potential risk of chronic CSN infection. CNS has a dense parenchyma and normally the blood-brain barrier can protect it from viral invasion. However, once the virus has entered CNS, its elimination becomes a challenge for the immune system [14]. Due to the lack of MHC in CNS, elimination of the virus in nerve cells is performed by cytotoxic T cells or through neuronal apoptosis. Besides, some aspects of nerve cell homeostasis also foster the survival of the virus [14]. Elimination of the virus through neuronal apoptosis raises a question about the long-term effects and potential risks of neurodegenerative conditions that develop independently of or are associated with chronic CNS damage induced by the coronavirus.

A cytokine storm in response to infection is the immunological aspect of CNS invasion by the coronavirus; a cytokine storm can provoke acute cerebrovascular events [9, 15]. Patients with severe COVID-19 have elevated D-dimer and a low platelet count, which makes these patients susceptible to vascular catastrophes [3].

The effect of the coronavirus on PNS was described in a number of studies. For example, Zhao H. [16] discovered an association between the Guillain-Barre syndrome (GBS) and COVID-19. A 61-year-old female presented with complaints of acute weakness in both legs and fatigue. Remarkably, acute respiratory symptoms appeared 7 days afterwards. Her nasopharyngeal swabs were positive for SARS-Cov-2 (RT-PCR) [16]. It Italy, 5 individuals infected with the novel coronavirus developed GBS; nasopharyngeal swabs were positive for COVID in 4 out of 5 individuals at the time of GBS manifestations. Later, COVID-19 was serologically confirmed in all of those patients. PCR tests did not detect the virus in their cerebrospinal fluid. Time from the first symptoms of the coronavirus infection to the onset of GBS symptoms was 5 to 10 days, i.e. similar to other infections also leading to GBS [17]. Besides, 2 cases of the Miller-Fisher syndrome were reported in COVID-19 patients, who developed ophthalmoplegia, ataxia and areflexia [18]

Summing up, the following CNS pathology may be associated with COVID-19:

1. encephalitis (meningoencephalitis?) caused directly by the virus;
2. toxic encephalopathy;
3. cerebrovascular complications (stroke, TIA);
4. demyelinating disorders (ADEM);

The following PNS pathology can develop following SARS-CoV-2 infection:

1. Guillain-Barre syndrome;
2. Miller-Fisher syndrome.

Possible routes of infection include:

1. direct invasion of the nervous tissue (the olfactory route);
2. through the bloodstream by crossing the blood-brain barrier.

Possible mechanisms underlying CNS/PNS damage include:

1. direct cytopathic effect of the virus;
2. hypoxia (in severe cases);
3. cytokine storm;
4. changes in blood rheology and blood coagulation properties leading to a cerebrovascular catastrophe;
5. damage by acute phase antibodies;
6. damage by activated macrophages and microglia cells involved in chronic inflammation;
7. changes in arterial blood pressure as a result of the virus binding to ACE2, followed by a cerebrovascular catastrophe.

As mentioned above, the virus has tropism for any tissue expressing ACE2, therefore, it can cause damage to the intestine [19] and heart [20, 6, 21]. ACE2 is predominantly expressed in the lungs (alveolar type 2 cells), hepatic cholangiocytes, the large intestine, esophagus, ileum, and rectum, gastric epithelial cells, and proximal tubules of the kidney. Some patients develop signs of kidney/liver failure, which suggests that COVID-19 can affect these organs, too. There has been a report of collapsing glomerulopathy in a COVID-19 patient [22]. By analyzing the accumulated data, researchers were able to identify the organs at risk, including the lungs, heart, esophagus, kidneys, bladder, and ileum, and a few vulnerable cell types, including alveolar type 2 and myocardial cells, cells of the proximal tubules, ileal and esophageal epithelium, urothelial cells of the bladder. In a study conducted in 204 patients with confirmed COVID-19 undergoing treatment in Hubei hospitals (China), 99 patients (48.5%) had gastrointestinal complaints in the absence of respiratory symptoms [19]. There has been a lot of discussion on the interactions between the microbiota and the immune system and its effects on pro- and anti-inflammatory factors. The "gut-brain axis" has become a widely recognized term, and the role of microbiota in multiple sclerosis has been proved [23].

Today, there is evidence suggesting the existence of the gut-lung axis [24]. Presumably, the gut-lung axis is bidirectional, i.e. endotoxins and microbial metabolites can exert their effects on the lungs through blood and, in turn, inflammation in the lungs can affect the gut microbiota [25]. Hypothetically, SARS-Cov2 might have an effect on the gut microbiota. In fact, some studies have demonstrated a link between respiratory infections and changes in the gut microbiota composition. It would be only natural and logical to hypothesize that all complications and forms of COVID-19 might depend on the gut microbiota and that the virus can and does provoke gastrointestinal symptoms.

Materials and methods

Considering the abovesaid, it was only logical to study the actual prevalence of various symptoms in patients with COVID-19 admitted to the hospital for infectious diseases of the Federal Siberian Research Clinical Center (FMBA, Russia). A questionnaire was designed to collect patients' demographic data (age, sex), free-form answers to 3 open-ended questions and answers to 92 tick-a-box closed-ended questions about possible complaints and symptoms. All symptoms listed in the closed-ended section of the questionnaire can be classified into 9 major domains (groups): pain, febrility, respiratory symptoms, neurological symptoms, digestive symptoms, skin symptoms, renal and urological symptoms, cardiac symptoms, apathy and asthenia. Many of the symptoms were listed in duplicates; for example, a patient could select from "increased body temperature", "fever" and "chills" in the febrility domain. This was done on purpose because patients could use different semantic structures to describe their condition. Questions from other groups were presented in a similar fashion. The study was approved by the Local Ethics Committee. Hospitalized patients who gave consent to participate received questionnaires in May through June 2020. The study recruited 93 patients. In 4 cases, some of the questions in the questionnaires were skipped, so those questionnaires were excluded from the analysis. Women accounted for 64.04% (57) of the participants and men, for 35.96% (32). The mean age was 50.80 ± 13.55 years; the youngest participant was 20 years old; the oldest, 94 years old. The questionnaires were filled out at different time points from the onset of the disease. The "earliest" questionnaire was completed on the day of onset, the latest, on day 35. On average, the period between the onset of the disease and questionnaire completion was 15.55 ± 9.96 days.

Results and discussion

Considering that some symptoms from the closed-ended section overlapped, the most interesting part of the analysis was not the total score itself (in all domains or in one domain),

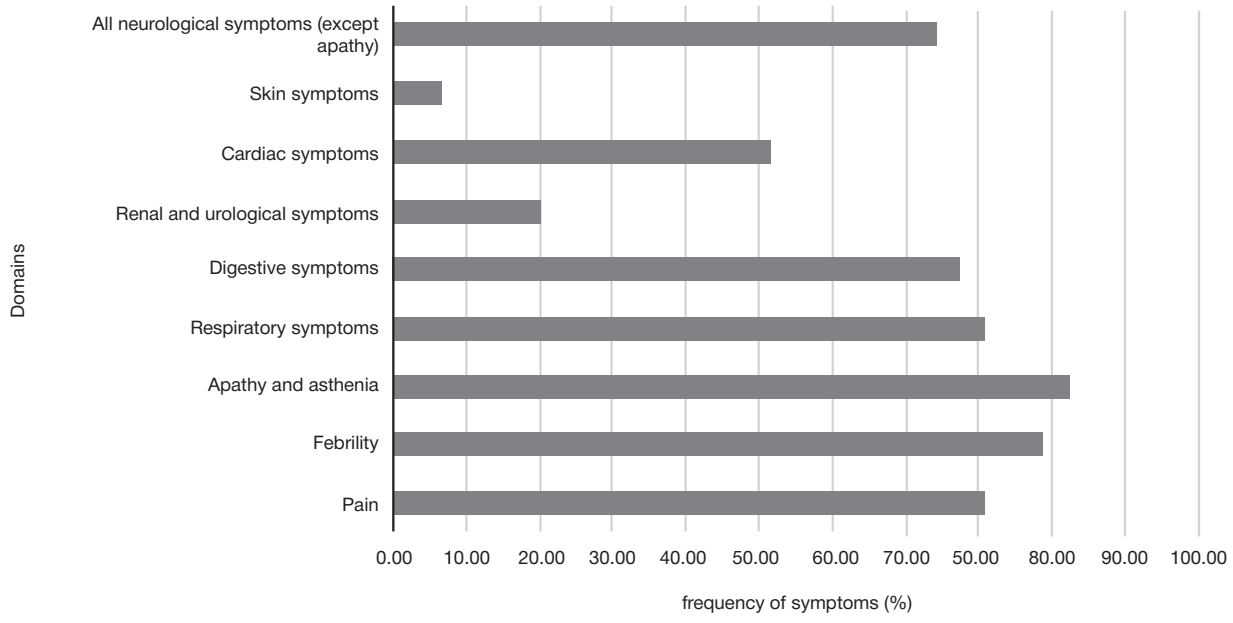

Fig. 1. The prevalence of symptoms from different domains

since the total number of positive answers does not necessarily suggest the severity of the condition, but the prevalence of symptoms from each domain. If a patient put a tick against at least one symptom listed in a group of symptoms, he/she was considered positive for this group of symptoms. The results are provided in Fig. 1.

The bar chart shows that the most common symptoms were apathy and asthenia (92.13%). These symptoms cannot be explained by febrility only because febrile manifestations were less frequent. Perhaps, other factors might be in play here, including the effect of the virus on CNS and the psychoemotional response of the patient. Fever ranked second (88.76%), referring to any increase in body temperature, including subfebrile, which is a common manifestation of infection. Pain and respiratory symptoms ranked third (80. 90% for both), followed by digestive (77.53%), neurological (74.16% excluding apathy, asthenia and headache), cardiac (51.69%), renal and urological (20.22%), and skin (6.74%) symptoms.

Compositionally, the asthenia and apathy domain was dominated by weakness (83.15%), followed by inertia (47.19%) and apathy (41.57%).

The febrility domain was dominated by febrile chills (55.06%).

The pain domain was dominated by headache (48.31%), indirectly suggesting CNS damage, chest pain (38.20%), which can be explained by the pulmonary manifestations of the disease, and myalgia (35.96%), which implies intoxication and/or an immune system reaction. There were a few interesting findings: loin pain (16.85%), which raises concerns about the possibility of kidney damage, subcostal pain on the ride side (11.24%) (liver damage?) and stomach pain (19.10%) (intestinal involvement?). In our future studies, we will attempt to retrospectively analyze possible correlations between these symptoms and the results of laboratory tests.

The respiratory domain was dominated by cough (55.06%), labored breathing (35.96%) and shortness of breath (30.34%). Interestingly, nasal cold (11.24%) was much less frequent than hyposmia (40.45%). This proves the predominantly neurogenic origin of hyposmia.

In the digestive domain, nonspecific symptoms were the most prevalent, including poor appetite (53.93%) and nausea (40.45%). Vomiting was present in 24.72% of patients. These figures suggest damage to the gastrointestinal tract hypothesized in previous research studies. Diarrhea was observed in 28.09% of patients, whereas bloating, in 19.10%. These symptoms might indicate the involvement of the gut microbiota or the intestine. Jaundice was reported in 4.49%, cases, while bitter taste in the mouth, in 20.22%, possibly indicative of liver damage. Currently, the authors are researching a possible correlation between these symptoms and the received therapy.

Special consideration should be given to neurological symptoms (see Table 1).

The table shows that olfactory and gustatory impairments (46.07%) are typical and widely-spread symptoms of COVID-19. Affective disorders (38.20%) and cognitive impairment (31.46%) are also common.

Other symptoms are significantly less frequent, but on the whole, they confirm the possibility of damage to ACE2-expresing structures, the brain stem in particular (the cytopathic effect of the virus?), and to peripheral structures (immune-mediated damage?). Notably, the total frequency of nervous system damage, without apathy, asthenia and headache is 74.16%; with these 3 symptoms included, the figure is 97.75%. Thus, neurological damage may be the leading complication of the disease in terms of frequency but not severity, depending on how the symptoms are distributed between different symptom domains.

Considering the pathogenesis of the disease, cardiac symptoms are unsurprisingly mainly represented by elevated blood pressure (24.72%) and tachycardia (20.22%). In 13.48% of cases, patients had a subjective sensation of cardiac pathology. Further research is needed to objectively confirm the underlying cause of the complaints and to evaluate the effect of therapeutic interventions. Low blood pressure was reported in 13.48% of cases. This might indicate autonomic dysfunction, which again brings up the question of the real prevalence of neurological symptoms in patients with COVID-19.

Among nephrological symptoms, the most prevalent were frequent urination (14.61%), difficulty urinating (4.49%) and painful urination (2.25%). On the whole, these symptoms were not so common. Likewise, in the skin domain, cyanosis or hyperemia were present in 4.49% of cases. Bruises, petechiae and the like were absent, which indirectly suggests the adequacy of the chosen regimen for supporting normal blood rheology and coagulation properties. However, as the number of severe patients grows, the proportion of renal/urological and skin symptoms might also increase.

The answers in the open-ended section of the questionnaire were systematized. Not in all cases, though, the sematic structures used by the patients to describe their condition allowed us to categorize the most nagging symptom (for example, “the simultaneous and extremely strong effect of all symptoms”). Approximately in 47.14% cases, febrility was reported as the most bothersome symptom. Weakness ranked second (14.29%), followed by pain and shortness of breath (11.43%). Other symptoms were less frequent. However, 2 patients specified nausea and 1 reported diarrhea as very distressing, which suggests the importance of these symptoms for the patients.

It was difficult for the patients to describe their complaints in an open-ended part of the questionnaire, which speaks in favor

Table 1. Neurological symptoms (excluding apathy, asthenia and headache)

Symptoms and possibly affected structures	Frequency, %
Dysgeusia and hyposmia (olfactory and gustatory analyzers)	46.07
Motor symptoms (localized weakness, transient facial asymmetry) (pyramidal tracts, peripheral nerves)	26.97
Extrapyramidal symptoms (predominantly tremor) (basal ganglia)	13.48
Visual impairment, oculomotor symptoms (CN II, III, IV, VI and midbrain structures)	13.48
Auditory impairment (CN VIII, cochlear branch)	12.36
Impaired coordination (ataxia) (CN VIII, vestibular branch, cerebellum, its connections, proprioceptive pathways)	31.46
Bulbar disorders: dysarthria, dysphagia (CN IX, X)	4.49
Sensory disorders, hypesthesia, paresthesia, cramps (brain stem, peripheral nerves)	16.85
Affective disorders (irritability, anxiety, sleep disorders, depression) (limbic system)	38.20
Cognitive impairment (attention or memory deficit, disorientation, sensory or motor aphasia) (cortex and its connections)	14.61

of using closed-ended questions as a rapid and effective tool for assessing the symptoms of the disease.

CONCLUSIONS

We conclude that for the majority of patients, especially for those with severe disease, respiratory complications (pulmonary, in particular) were the most threatening. However, most of our patients also had extrapulmonary symptoms. Apathy, asthenia, pain, digestive and neurological manifestations were the most common, followed by cardiovascular symptoms. Less often, the virus can cause damage to other organs and tissues. The medical staff providing care to patients with COVID-19 must be competent in identifying extrapulmonary symptoms of the disease. he fact that a lot of non-infectious disease specialists have been retrained to provide medical care to COVID-19 patients can be regarded as an advantage since it creates an opportunity to obtain valuable consultations on the extrapulmonary manifestations of the infection from an experienced specialist. When the epidemiologic situation improves and the number of infected individuals goes down, so will the hospital bed occupancy by COVID-19 patients and the number of medical personnel involved in delivering COVID-19-oriented care. Therefore, new approaches will be needed to provide consultations to such patients.

The authors believe that long-term sequelae of COVID-19, both pulmonary and extrapulmonary, need to be thoroughly studied. The majority of our patients were willing to participate in further research. This encouraged us to apply for a Russian Foundation for Basic Research Grant (Id 20-04-60548) to sponsor the project on the evaluation of the long-term effects of pulmonary and extrapulmonary (neurological, gastrointestinal, nephrological, and immunological) complications of COVID-19 considering the effect of the gut microbiota, their mathematical modeling, prediction and ways to minimize the inflicted damage. We also believe that raising awareness of the extrapulmonary symptoms of COVID-19 in healthcare workers specializing in different medical fields will improve the efficacy of medical care for in-and outpatients and ensure timely detection of individuals presenting with extrapulmonary symptoms of COVID-19.

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ACTUAL PROBLEMS OF PSYCHOLOGICAL SUPPORT OF HEALTHCARE WORKERS IN INFECTIOUS HOSPITALS FOR PATIENTS WITH COVID-19 IN THE TERRITORY OF THE KRASNOYARSK REGION

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Annotation. This article presents the experience of implementing psychological support measures for healthcare workers of infectious hospitals for patients with COVID-19 during a pandemic. The results of an empirical study of the prevalence, severity and specificity of the development of anxiety and depressive symptoms in healthcare workers of residents of a megalopolis (Krasnoyarsk), a closed territorial district (Zelenogorsk) and seconded to the North Yenisei district of the Krasnoyarsk Territory depending on social status and professional factors of burnout are presented. The stages of the implementation of measures of psychological support for the activities of healthcare workers are described. A comparative analysis of the involvement of employees of various infectious hospitals was carried out. Material and methods. The total sample of the study included 126 subjects (21 men and 105 women) engaged in the provision of medical care in three infectious diseases hospitals for patients with COVID-l9 in the Krasnoyarsk region. To achieve the goals and objectives of the study, the following methods were used: psychodiagnostic testing with the BDI scale, STAI test and MBI questionnaire, and statistical data processing (Spearman rank correlation coefficient, Mann–Whitney–Wilcoxon U-test). We invited 284 medical workers to participate in psychological support activities. Results and conclusions. The prevalence of subdepression among medical personnel varies from 5.5 to 30.9%, depending on the location of the infectious diseases hospital for patients with COVID-19. Severe depressive symptoms were detected in 4.46% of the total number of subjects. A low level of situational anxiety was detected in less than 30%. It has been confirmed that employees of a younger age and with less experience are more susceptible to the development of depersonalization and cognitive-affective symptoms of depression. And employees who do not have children show higher indicators of situational and personal anxiety. Moreover, despite the prevalence of alarming and depressive symptoms, there is a low involvement and unwillingness of healthcare workers to participate in psychological support activities.

Keywords: anxiety, depression, COVID-19, psychological support, burnout, occupational stress

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АКТУАЛЬНЫЕ ПРОБЛЕМЫ РЕАЛИЗАЦИИ МЕРОПРИЯТИЙ ПСИХОЛОГИЧЕСКОГО ОБЕСПЕЧЕНИЯ ДЕЯТЕЛЬНОСТИ МЕДИЦИНСКИХ РАБОТНИКОВ ИНФЕКЦИОННЫХ ГОСПИТАЛЕЙ ДЛЯ ПАЦИЕНТОВ С COVID-19 НА ТЕРРИТОРИИ КРАСНОЯРСКОГО КРАЯ

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Аннотация. В статье обобщен опыт проведения мероприятий психологического сопровождения медицинских работников инфекционных госпиталей для пациентов с COVID-19 в период пандемии. Представлены результаты эмпирического исследования распространенности, выраженности и специфики развития тревожной и депрессивной симптоматики у медицинских работников — жителей мегаполиса (г. Красноярск), ЗАТО (г. Зеленогорск) и командированных в Северо-Енисейский район Красноярского края в зависимости от социального статуса и факторов профессионального выгорания. Описаны этапы реализации мероприятий психологического обеспечения деятельности медицинских работников, проведен сравнительный анализ вовлеченности сотрудников различных инфекционных госпиталей. Материал и методы. Общая выборка исследования представлена 126 испытуемыми (21 мужчина и 105 женщин), занятых оказанием медицинской помощи в трех инфекционных госпиталях для пациентов с COVID-l9 на территории Красноярского края. Для реализации целей и задач исследования применялись методы анкетирования, психодиагностического тестирования (Шкала депрессии Бека (BDI), тест Спилбергера (STAI), опросник выгорания для медицинских работников Маслач (MBI) и статистической обработки данных (коэффициент корреляции рангов Ч. Спирмена, *U*-критерий Манна–Уитни–Уилкоксона). К участию в мероприятиях психологического обеспечения были приглашены 284 сотрудника. Результаты и выводы. Распространенность субдепрессии у медицинского персонала варьируется от 5,5 до 30,9% в зависимости от месторасположения инфекционного госпиталя. Выраженная депрессивная симптоматика выявлена у 4,46% от общего числа испытуемых. Низкий уровень ситуативной тревожности выявлен менее чем у 30%. Подтверждено, что сотрудники более младшего возраста и с меньшим трудовым стажем в большей степени подвержены развитию деперсонализации и когнитивно-аффективной симптоматики депрессии. А сотрудники, не имеющие детей, демонстрируют более высокие показатели ситуативной и личностной тревожности. При этом, несмотря на распространенность тревожной и депрессивной симптоматики, отмечается низкая вовлеченность и неготовность медицинских работников участвовать в мероприятиях психологического сопровождения.

Ключевые слова: депрессия, COVID-19, психологическое сопровождение, тревожность, профессиональное выгорание, производственный стресс

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The theme of psychological support for healthcare workers is particularly relevant during the COVID-19 pandemic. Numerous studies reported by domestic and foreign authors demonstrate the negative impact of various viral infections spread in epidemic and pandemic amounts both on the physical and psychological well-being of the population [4, 5, 6, 8, 9, 10, 13]. The risk of negative mental and emotional state in medical specialists working in direct contact with infected patients is emphasized [4, 5, 6]. The recent reports published by foreign colleagues from Italy and China summarize the results confirming the prevalence of depression (50.3%), anxiety (44.6%) and insomnia (34%) in healthcare workers during the COVID-19 pandemic. Concerns about the adverse psychological effects revealed are compounded by the high rate of pre-existing psychological problems, as well as by the high total suicide rate among physicians. The Chinese and Italian researchers also

point out that the long ignored, untreated depression together with difficult working conditions can be a deadly combination [4]. Another large-scale study performed by Chinese scientists revealed some psychological disorders in 39.1% of healthcare workers. For example, the psychological distress in specialists working in Wuhan was related to the risk of infection and insufficient protective measures [10]. Professor Neil Greenberg, psychiatrist from King's College London, expert in the field of diagnosis and treatment of psychological trauma, occupational stress, mental and post-traumatic stress disorders, and his team introduced the "moral injury" concept explaining the typical mental health problems the healthcare workers face during the pandemic [12]. The Chinese researchers determined that close contact of medical staff with COVID-19 patients combined with spending 2 hours and above daily on the news led to worsening of the anxiety and depression symptoms. However, the use of online platforms as an instrument of psychological support reduced the impact of discussed factors [13].

In Russia serious steps had been undertaken towards maintaining the healthcare workers' psychological well-being during the pandemic. Veronika I. Skvortsova, Head of the Federal Medical-Biological Agency, reported the need to establish a psychological support service in the federal medical centers transformed into hospitals for patients with COVID-19. The Federal Medical-Biological Agency (Federal Brain and Neurotechnologies Center) together with the team of the Faculty of Psychology of Lomonosov Moscow State University and the psychological service of the Armed Forces of the Russian Federation issued guidelines on the psychological support of healthcare institutions heads and heads of departments in the context of care provision to COVID-19 patients, guidelines on prevention of psychological ill-being in physicians and healthcare workers during the pandemic, and guidelines on the target groups psychological support in medical institutions during the COVID-19 pandemic [3].

The study was aimed to analyze the experience of psychological support provision to the healthcare specialists working in the infectious diseases hospitals during the novel coronavirus infection (COVID-19) pandemic caused by SARS-CoV-2. One of the key research tasks was to study the anxiety and depression signs development in medical professionals engaged in care provision to COVID-l9 patients in the Krasnoyarsk Territory in relation to various social and psychological factors (age, marital status, etc.), including the occupational burnout severity, in order to customize the psychological support strategy for healthcare workers during the pandemic.

The levels of state and trait anxiety, depression and occupational burnout in healthcare workers were studied as a part of the study. It should be noted that state anxiety is the emotional state related to current situation of testing. The trait anxiety is the individual's stable tendency to experience anxiety across many situations [2]. Occupational (emotional) burnout includes the following factors: emotional exhaustion, depersonalization, and reduced personal accomplishment. Emotional exhaustion is characterized by the emotional fatigue, emotional resources overextension and indifference. Depersonalization is a combination of various impaired relationships with others. Reduced personal accomplishment is distinguished by the tendency to negative self-appraisal, limiting of capabilities, decline in the feeling of competence and successful achievement [1]. Depression is considered a combination of cognitive-affective (low mood, pessimism, sense of failure, lack of satisfaction, guilt feelings, sense of punishment, self-dislike, self-accusation, suicidal wishes, crying, irritability, social withdrawal) and somatic symptoms (indecisiveness, distortion of body image, work inhibition, sleep disturbance, fatigability, loss of appetite, weight loss, somatic preoccupation, and loss of libido) [11].

METHODS

The survey sample included 126 subjects (21 men and 105 women), who provided medical care in the infectious diseases hospitals for COVID-19 patients of the Krasnoyarsk Territory. Of them 23 people were physicians, 55 were mid-level practitioners, and 48 were nursing staff. At the time of the survey all specialists worked in the "red zone" of the hospital (for an average of 10.3 ± 4.2 days) in close contact with the patients.

The following methods were used to achieve the goals and objectives of the study: 1) questionnaire survey; 2) psychodiagnostic testing using the Beck Depression Inventory (BDI), State-Trait Anxiety Inventory (STAI) customized by Khanin YuL, Maslach Burnout Inventory (MBI) customized by Vodop'janova NE and Starchenkova ES; 3) statistical analysis (correlation analysis using the Spearman's rank correlation coefficient and the Wilcoxon-Mann-Whitney U-test). The results were processed using the STATGRAPHICS Plus software package.

The study was carried out in three hospitals for patients with coronavirus infection: Federal Siberian Research Clinical Center under FMBA of Russia (hospital № 1), Krashoyarsk, branch of FSRCC FMBA of Russia, Clinical Hospital No. 42 in the closed territorial district Zelenogorsk (hospital No. 2), and in the field hospital deployed in the territory of "Olimpiadinsky" Ore Mining and Processing Enterprise in the Eruda settlement of Severo-Yeniseysky District of the Krasnoyarsk Territory for the Polyus Krasnoyarsk company employees infected with COVID-19 (hospital № 3). Inclusion criteria: submitted informed consent, and a conscious desire to participate in the study (it should be noted that initially 142 healthcare workers were offered to participate in the study, of them 16 people refused (11.2%)). For general survey sample characteristics see Table 1.

RESULTS AND DISCUSSION

The prevalence of sub-threshold (mild) depression among the study participants was 5.5–30.9% depending on the infectious hospital location, the maximum values were observed in specialists sent to Severo-Yeniseysky District (Table 2). Thus, 94.5% of Zelenogorsk hospital employees had no symptoms of depression, in the Krasnoyarsk and Eruda settlement hospitals the proportions of such workers were 81.1% and 65.4% respectively. Symptoms of depression (mild, moderate and severe) were diagnosed in 6 people (4.76% of total number of participants). Low levels of state anxiety were revealed in less than 30% of healthcare workers regardless of the infectious diseases hospital location (Table 2). In most respondents the moderate levels of state anxiety were diagnosed (55.5–61.81% of surveyed subgroups). The prevalence of high levels of state anxiety was 12.72–16.6%. The moderate level of trait anxiety was detected in more than 70% of surveyed workers, and the prevalence of high trait anxiety varied significantly (from 11.1% in Zelenogorsk to 20.75% in Krasnoyarsk).

Some differences in the degree of healthcare workers occupational burnout factors were observed depending on the infectious hospital location. High levels of emotional exhaustion were detected in 11.3% of employees in the Krasnoyarsk hospital for patients with COVID-l9 (by comparison, in other

hospital that parameter did not exceed 5.5%). At the same time, more than 20% of the infectious diseases hospitals № 1 and 3 employees had high levels of depersonalization (in Zelenogorsk there were 5.5% of such workers). Almost 45% of the hospital № 2 healthcare workers were diagnosed with high levels of reduced personal accomplishment, while in other hospitals the proportion of employees with high reduced personal accomplishment did not exceed 30% (Table 2).

The average scores for cognitive-affective (subscale C-A) and somatic (subscale S-P) symptoms of depression, anxiety and occupational burnout in physicians of infectious diseases hospitals for patients with COVID-19 are listed in Table 3. It is important to understand that when interpreting data using the "reduced personal accomplishment" scale, score 33 and higher is considered low, and score 22 and lower is considered high.

The relationship between the depression, anxiety and occupational burnout symptoms severity, and the healthcare workers' marital status was analyzed (partnership status (married) was compared with other statuses (divorced, single)). In the hospitals № 1 and 2 no significant correlation between the symptoms severity and the marital status was observed. Meanwhile, the cognitive-affective symptoms severity in married workers seconded to the hospital № 3 located in Eruda settlement of Severo-Yeniseysky District was significantly higher compared to their colleagues with other marital status. It can be concluded that marital status does not influence the anxiety, depression and occupational burnout symptoms in the infectious hospital employees working and living in the same city. Nevertheless, in "family" people working away from their home, being apart from the partner and related emotional experience were more acute, which led to worsening of cognitive-affective symptoms of depression (Table 4).

The relationships between anxiety, depression and occupational burnout levels, and age, professional experience, and number of children in the family revealed in healthcare workers using correlation analysis depended on the infectious diseases hospital location. Thus, negative correlations were observed between age, professional experience, and cognitive-affective symptoms of depression in the hospital No. 2 employees, as well as between the number of children in the family and the state anxiety ($k = –0.513$, $p \leq 0.05$; $k = –0.685$, $p \leq 0.01$; $k = –0.577$, $p \leq 0.05$). Negative correlations were also observed between age and depersonalization, number of children in the family and trait anxiety in the hospital № 1 employees ($k = –0.236$, $p \leq 0.05$; $k = 0.320$, $p \leq 0.05$). However, the tendencies revealed were not confirmed during the infectious hospital № 3 employees' survey. There were no correlations between age, professional experience and number

Table 1. General characteristics of studied subgroups

		Hospital № 1 ($n = 53$)	Hospital № 2 ($n = 18$)	Hospital № 3 ($n = 55$)
Average age of workers (years)		39.06 ± 18.3	44.2 ± 12.6	38.4 ± 15.8
Professional experience (years)		17.3 ± 12.4	18 ± 6.5	15.9 ± 9.1
Marital status	Married (%)	43.39	66.66	34.5
	Single (%)	37.7	33.3	36.36
	Divorced (%)	18.86	–	21.81
Number of children		An average of one child per family	An average of more than one child per family	An average of less than one child per family
Proportion of individuals having no children		26.4	5.55	44.6

Table 2. Comparison of state and trait anxiety, depression and occupational burnout levels in employees of infectious diseases hospitals for COVID-19 patients

		Hospital № 1 ($n = 53$), Proportion of people, %	Hospital № 2 ($n = 18$), Proportion of people, %	Hospital № 3 ($n = 55$), Proportion of people, %
Depression	No symptoms	81.1	94.5	65.4
	Mild (sub-threshold)	11.3	5.5	30.9
	Moderate	1.88	0	1.81
	Pronounced	5.66	0	0
	Severe	0	0	1.81
State anxiety	Low	28.3	27.7	25.45
	Moderate	56.6	55.5	61.81
	High	15.09	16.6	12.72
Trait anxiety	Low	5.66	16.6	16.36
	Moderate	73.58	72.2	72.7
	High	20.75	11.1	12.72
Emotional exhaustion	Low	49.06	55.6	58.19
	Moderate	39.62	38.8	38.18
	High	11.32	5.5	3.63
Depersonalization	Low	22.65	27.8	52.73
	Moderate	54.71	66.6	27.27
	High	22.64	5.55	20.00
Reduced personal accomplishment	Low	33.9	5.5	49.1
	Moderate	39.6	50	23.63
	High	26.4	44.5	27.27

of children. Thus, it can be concluded that when working in the infectious diseases hospitals for COVID-19 patients the younger and less experienced employees are more susceptible to depersonalization at work and to cognitive-affective depression symptoms development. Any children in the family and the number of children positively correlate with state and trait anxiety levels: with an increase in the number of children per family, these indicators decrease. According to currently available literary sources taking into account the survey sample sex/age composition this can be explained by the fertile age women's concerns about possible risk of miscarriage and pregnancy complications due to contact with infectious agents and preventive anti-infective medications [7].

The relationship between the anxiety and depression symptoms severity, and the occupational burnout factors, was analyzed (Table 5).

Multiple correlations revealed indicate the relationship between the occupational burnout phenomenon and the current psycho-emotional status of healthcare workers, particularly of those being the inhabitants of metropolises or being sent to

Table 3. Average levels of depression, anxiety and occupational burnout in physicians working in infectious diseases hospitals for COVID-19 patients

		Hospital № 1	Hospital № 2	Hospital № 3
Depression	C-A	3.83 ± 4.2	2.33 ± 2.9	3.85 ± 3.4
	S-P	1.92 ± 1.9	1.16 ± 1.65	2.27 ± 3.6
	Total	5.75 ± 5.4	3.5 ± 3.8	6.12 ± 6.5
Anxiety	State	37.75 ± 10.2	35.72 ± 7.7	36.01 ± 11.3
	Trait	39.43 ± 7.6	37.27 ± 6.8	37.4 ± 7.1
Occupational burnout	Emotional exhaustion	16.33 ± 7.3	14.11 ± 4.4	14.78 ± 6.3
	Depersonalization	8.39 ± 4.6	6.66 ± 2.3	6.2 ± 4.2
	Reduced personal accomplishment	34.13 ± 6.1	31.5 ± 3.7	35.32 ± 6.4

Table 4. Correlation between depression, state and trait anxiety, occupational burnout severity and the marital status

Studied factors of depression, state anxiety, trait anxiety, and occupational burnout		Average values for workers with different marital status		Significant differences (*U*-test)
		Married	Other status	
Hospital №1				
Depression	C-A	4.28 ± 4.0	3.46 ± 3.2	No
	S-P	1.56 ± 1.5	2.07 ± 1.9	No
	Total	5.84 ± 5.14	5.53 ± 5.0	No
Anxiety	State	36.72 ± 9.7	37.9 ± 10.6	No
	Trait	38.1 ± 7.7	40.4 ± 7.8	No
Occupational burnout	Emotional exhaustion	15.6 ± 6.9	16.6 ± 7.9	No
	Depersonalization	8.64 ± 4.5	7.9 ± 4.7	No
	Reduced personal accomplishment	34.2 ± 6.2	34.07 ± 6.3	No
Hospital № 2				
Depression	C-A	1.9 ± 1.4	3.4 ± 3.2	No
	S-P	1.07 ± 1.4	1.4 ± 1.1	No
	Total	3.0 ± 2.1	4.8 ± 4.5	No
Anxiety	State	36.1 ± 7.6	34.6 ± 8.7	No
	Trait	37.9 ± 6.6	35.6 ± 7.7	No
Occupational burnout	Emotional exhaustion	14.4 ± 4.8	13.2 ± 3.5	No
	Depersonalization	6.7 ± 2.0	6.4 ± 3.3	No
	Reduced personal accomplishment	31.6 ± 4.1	31.2 ± 2.8	No
Hospital № 3				
Depression	C-A	4.8 ± 3.2	3.6 ± 3.0	$U = 303.0^{**}$
	S-P	2.2 ± 2.0	2.4 ± 1.4	No
	Total	6.8 ± 5.1	5.9 ± 4.5	No
Anxiety	State	35.9 ± 11.03	37.03 ± 11.64	No
	Trait	36.6 ± 7.4	38.3 ± 7.02	No
Occupational burnout	Emotional exhaustion	14.9 ± 6.1	15.5 ± 6.2	No
	Depersonalization	6.5 ± 3.4	6.5 ± 4.6	No
	Reduced personal accomplishment	36.4 ± 5.3	34.2 ± 6.6	No

Note: * — sigificant differences ($p \leq 0.01$); ** — significant differences ($p \leq 0.05$).

another locality to perform their professional duties during the pandemic. Emotional exhaustion as a factor of occupational burnout played a vital part in development of depressive symptoms in all the infectious hospitals employees. In hospitals № 1 and 2 employees, both state and trait anxiety increased with the growth of occupational burnout manifestations levels. It is noteworthy that in all subgroups of healthcare workers state anxiety increased with reduced personal accomplishment level growth. In other words, state anxiety is based on lack of motivation to work, negative evaluation of one's work and its results, as well as prospects in the profession in general (the specialist is convinced that he deserves the best), including dissatisfaction with duties and the desire to shift responsibility onto his (her) colleagues.

Management of psychological follow-up for medical staff working in infectious hospitals for COVID-19 patients

Since April 10, 2020 the psychological service (4 medical psychologists and 1 psychologist) of the Federal Siberian Research Clinical Center under FMBA of Russia and the branch of FSRCC FMBA of Russia, the Clinical Hospital № 42 in Zelenogorsk, was partially diverted to solution of urgent tasks related to psychological support of healthcare workers involved in care provision to patients with novel coronavirus infection. Psychopreventive and psychocorrectional actions were carried out on a phased basis.

The main tasks of the first phase (prior to opening of the first infectious hospital) were as follows: creating an enabling environment and reduction of tension between co-workers resulting primarily from uncertainty and unpredictability of the current situation. Provision of information was the main working method during that phase (filling the information gaps, provision of adequate information). The memo "How to overcome anxiety and stress during the pandemic staying at work" for healthcare workers was issued and published on the medical institution information portal. Further, the medical managers and appropriate staff were familiarized with recommendations on prevention of psychological ill-being in physicians and medical staff during the pandemic, as well as with recommendations on the target groups' psychological follow-up under the context of COVID-19 pandemic [3]. The managers were given access to recommendations on psychological follow-up of the medical institutions heads and heads of departments in the context of care provision to patients with COVID-19.

The second phase started immediately after opening the first infectious hospital, and it is an ongoing phase. The main task of that phase was to create the enabling environment for effective adaptation of employees to the infectious hospital working conditions, including prevention, timely diagnosis and correction of neurotic disorders and states. All employees were informed about goals and tasks of forthcoming work, as well as about its voluntary nature.

During that phase the psychodiagnostic assessment of anxiety and depression symptoms was carried out at least once every 14 days, and for sure within first three days after starting work in the hospital and at the final stage. The current psycho-emotional status of at-risk employees was monitored weekly in order to reveal the neurotic symptoms worsening. The availability of psychological assistance was ensured by a number of measures. The 24-hour hotline was created for individual counseling. For healthcare workers of the field hospital located in Eruda settlement of the Severo-Yeniseysky District the face-to-face counseling by medical psychologist was available.

Training aimed at team-building, soft skills improvement and learning how to use the self-help techniques (self-regulation, self-organization, relaxation, etc.) was carried out in groups two times a week using the remote technologies (video conferencing).

Considering the certain employees' unwillingness to join the psychological support activities and understanding the inefficiency (uselessness) of using the coercive measures in further work with the team, we invited all specialists involved in working with COVID-19 patients to join the psychological support chat, giving them an opportunity to familiarize

Table 5. Relationship between anxiety and depression symptoms severity, and occupational burnout factors in healthcare workers of infectious diseases hospitals

Studied depression, state and trait anxiety levels	Occupational burnout factors		
	Emotional exhaustion	Depersonalization	Reduced personal accomplishment
Hospital № 1 (n = 53)			
C–A	0.285**	–	–
S–P	0.462*	–	–
Depression	0.377*	–	–
State anxiety	0.570*	0.352*	–0.307**
Trait anxiety	0.378*	0.367*	–
Hospital № 2 (n = 18)			
C–A	–	–	–
S–P	0.563**	–	–
Depression	0.551**	–	–
State anxiety	–	–	–0.535**
Trait anxiety	–	–	–
Hospital № 3 (n = 55)			
C–A	0.375*	0.333*	–0.263**
S–P	0.381*	–	–0.375*
Depression	0.405*	0.288**	–0.320**
State anxiety	0.495*	0.296**	–0.475*
Trait anxiety	0.518*	–	–0.365*

Note: * — significance level $p \leq 0.01$; ** — significance level $p \leq 0.05$

Table 6. ealthcare workers' involvement in psychological support activities during the pandemic

	Infectious hospitals in Federal Siberian Research Clinical Center, Krashoyarsk, and Clinical Hospital № 42, Zelenogorsk; Proportion of people, %	Field hospital deployed in the territory of "Olimpiadinsky" Ore Mining and Processing Enterprise, Eruda settlement, Severo-Yeniseysky District, Krasnoyarsk Territory; Proportion of people, %
Number of healthcare workers invited to participate in psychological support activities	*n* = 216	*n* = 68
Format of work	Remote	Face-to-face
Activities:		
Independent study of memos, guidelines and other information materials	92 (42.5%)	57 (83.8%)
Psychodiagnostic screening assessment	171 (79.1%)	55 (80.8%)
Individual counseling upon individual appointment (including calling the hotline)	4 (1.85%)	9 (13.23%)
Group training	26 (12.03%)	0 (0%)
Psychological support chat	74 (34.2%)	34 (50%)
"Psychological Thermometers"	3 (1.38%)	1 (1.47%)

themselves with informational materials and tasks on a regular basis. In particular, the healthcare workers were introduced to self-observation diaries with protocols of irrational thoughts (cognitive behavioural approach to modifying the dysfunctional beliefs), art therapy exercises for negative emotions management and psychoemotional stress relief after work, mindfulness-based writing practices (mindfulness-based cognitive therapy), and audio and video files (sessions of autogenic training, instilled rest, meditation, etc.) for rapid psychophysiological resources recovery and current functional state optimization. Thus, joining the chat allowed one to become involved in the process, execute the tasks at his (her) own pace, but required no active participation (which was likely to be an additional source of stress for some workers).

During that phase all employees were invited to join the "Psychological Thermometers" project of FMBA of Russia for physicians, mid-level practitioners and nursing staff developed in conjunction with the Faculty of Psychology of Lomonosov Moscow State University. The employees could use three "psychological thermometers" to measure their "emotional themperature" online and receive the immediate supportive feedback concerning the self-help measures, collegues' support and the need to call for professional psychological assistance. Self-examination was performed using no authentification, but the employee was allowed to submit the contact information requesting the targeted professional psychological assistance.

The total of 284 healthcare workers was invited to participate in the psychological support activities. The activities were carried out remotely in all hospitals, except the field hospital located in the territory of "Olimpiadinsky" Ore Mining and Processing Enterprise in the Eruda settlement of Severo-Yeniseysky District of the Krasnoyarsk Territory. The medical psychologist was sent to the field hospital as a part of the integrated team of specialists. The statistics of healthcare workers' participation in the psychological support activities is presented in Table 6.

The table demonstrates that higher degree of staff involvement is observed in the infectious diseases hospital providing face-to-face counseling. In our opinion, this is due to the fact that after the medical psychologist recruitment the direct participation in the meetings and planning conferences becomes possible. Thereby, the specialist may give more detailed information about goals and tasks of psychological support to colleagues during conversation, establish personal relationship, create the safe communication environment, answer the questions and to some extent overcome the psychological resistance during the initial contact.

CONCLUSION

In general, analysis of psychological support activities for healthcare specialists working in the infectious diseases hospitals for patients with coronavirus infection indicated that despite the prevalence of alarming and depression symptoms the willingness of employees to participate in the psychodignosic testing, individual counseling, training, etc., remained low. The conflict between the objective need (according to psychodignosic assessment results) and the professional psychological assistance availability on one hand, and the unwillingness to accept the assistance on the other hand, is obvious.

This fact does not conflict with the foreign colleagues' observations. Among others, the healthcare workers in some regions of China also refused to participate in the individual and group psychotherapy sessions and never requested psychological assistance despite the signs of irritability and high level of psychological distress [9]. The discovered phenomenon requires further detailed study. However, it is already clear that the modern medical worker's ability to understand his (her) psychological deficits while experiencing distress, to take timely measures in order to stabilize his (her) psycho-emotional state (self-help skills, calling the appropriate specialist) and activate the resources needed for effective health-preserving coping, is the essential component of professional competence. In our opinion, the development of such competence is important not only for adequate working tasks solution under the context of high professional risk, but also for effective professionalization of a specialist starting from the moment of study at a medical educational institution.

In view of the above, it would be better to discuss the need for the third phase of the healthcare workers psychological follow-up (after finishing working in the infectious diseases hospitals for patients with COVID-19). Together with psychophysiological rehabilitation, prevention of post-traumatic stress disorder, etc., the creation of environment for discussed professional competence development will become one of the main tasks of the third phase.

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EFFECT OF PHYSICAL ACTIVITY LEVEL ON THE COURSE OF PNEUMONIA CAUSED BY COVID-19

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Data from medical records of 144 COVID-19 patients who had completed inpatient treatment were analyzed, as well as the results of the subsequent survey using a modified questionnaire. The relationship between physical activity level, performance, quality of life (prior to infection and after treatment), age, therapeutic exercises execution rate while staying in the hospital, stool problems, high blood pressure episodes after treatment, and the course of the disease was evaluated. The patients were divided into a control and experimental group in accordance with the the initial subjective level of performance. The moderate form of the disease prevailed (69.44%). The mild form was typical for younger patients, the patients over 50 years of age made up 62.49% of the total number of severe cases. Severe patients reported lower quality of life and performance prior to the disease compared to those with moderate and mild course of the infection. The patients (mild and moderate cases) with more active initial lifestyle and higher initial performance who practiced therapeutic exercises while staying in the hospital had a more favorable course of the disease (reduced length of stay in a hospital, fast recovery of performance, reduced number of CT scans). The higher initial physical activity level contributed to milder course of the infection. It is necessary to raise public awareness, especially among the elderly, about the regular physical activity benefits and the correlation between physical activity level and the course of the disease, as well as to introduce exercise therapy at all treatment stages.

Keywords: physical activity, COVID-19, coronavirus disease, elderly people, sedentary lifestyle, physical exercises

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ВЛИЯНИЕ УРОВНЯ ФИЗИЧЕСКОЙ АКТИВНОСТИ НА ТЕЧЕНИЕ ПНЕВМОНИИ, ВЫЗВАННОЙ COVID-19

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Проведен анализ данных медицинской документации 144 пациентов с COVID-19, завершивших стационарное лечение, а также последующее анкетирование с помощью модифицированного опросника. Оценивались связь уровня физической активности, работоспособность, а также качество жизни (до заболевания и после окончания лечения), возраст, частота выполнения лечебной физкультуры на госпитальном этапе, наличие нарушений стула, эпизодов повышения артериального давления после окончания лечения с течением заболевания. Пациенты были разделены на контрольную и экспериментальную группу в зависимости от исходного субъективного уровня работоспособности. Преобладала среднетяжелая форма (69,44%). Легкая форма характерна для более молодых пациентов, пациенты старше 50 лет составляют 62,49% от общего числа случаев тяжелого течения. Пациенты, перенесшие тяжелую форму заболевания отмечали качество жизни и работоспособность до болезни на более низком уровне, в отличие от перенесших среднюю и легкую форму. Изначально ведущие более активный образ жизни пациенты с исходно более высокой работоспособностью и качеством жизни, выполнявшие лечебную физкультуру на госпитальном этапе (при легкой и среднетяжелой форме) имеют более благоприятное течение заболевания (сокращение сроков госпитализации, скорейшее восстановление работоспособности, уменьшение количества проведенных компьютерных томографий органов грудной клетки). Исходная более высокая физическая активность способствует более благоприятному течению заболевания. Необходимо повышать осведомленность населения о пользе регулярной физической активности и ее связи с течением болезни, особенно среди пожилых людей, а также внедрять методики лечебной физкультуры на всех этапах лечения.

Ключевые слова: физическая активность, COVID-19, коронавирусная инфекция, пожилые люди, гиподинамия, лечебная физкультура

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At the current stage of community development the increase in life expectancy is observed. It should be noted that the proportion of people aged over 60 in the general population grows faster than the total population. Thus, a continuous trend towards ageing of the world's population is reported. This led to the World Health Organization introducing the following new age classification in 2018: people aged 60–75 were considered ageing and elderly, people aged 75–90 were considered old, and people aged over 90 were considered centenarians.

At the same time, urbanization, continuous development of new technologies, improvements in transport are accompanied by the sedentary lifestyle rate growth, which negatively affects both people's physical health and economic development contributing to increased incidence of somatic pathologies and, consequently, to the public health costs increase [1, 12].

It has been revealed that regular physical exercises contribute to prevention and improvement of most common noncommunicable diseases being the chief causes of death in people all over the world, and also reduce the risk of falls and injuries from falls, which is extremely important for elderly people [2, 3]. According to the WHO, insufficient physical activity is one of the four top risk factors of noncommunicable diseases together with tobacco smoking, unhealthy diet and alcohol consumption [2]. Consequently, in 2018 the WHO issued the "Global Action Plan on Physical Activity 2018–2030: More Active People for a Healthier World", which underlined the importance of regular physical exercises for modern people. Also in 2018, in Moscow (and then in a number of regions) the extremely successful and popular Moscow Longevity project was launched aimed at increasing the older people's vitality and improving their quality of life.

In March 2020, during the American Heart Association EPI | LIFESTYLE 2020 Scientific Sessions it was reported that elderly people could live healthier lives by increasing their physical activity [4]. It should also be noted that elderly people have lower level of immunity, and physical exercises may help to improve the stress tolerance and activate the immune system.

Given the mostly airborne transmission of COVID-19 and high disease susceptibility and severity in people aged 65 and over, first in Moscow (since March 26) and then in other regions of Russian Federation the self-isolation regime was announced for people over 65 and people with some somatic pathologies aimed at prevention of the novel infection spread (order from Moscow Mayor №26-УМ dated March 23, 2020).

Under prevailing conditions regardless of their initial physical activity experience people aged 65 and over were forced to change their lifestyle to hypodynamic for a long period. The typical for Russian community sedentary lifestyle of elderly people became more complicated due to small living space (at the place of residence of self-isolation).

Under such circumstances the value of regular physical exercises in people aged 65 and over, both during self-isolation and during normal live, becomes not only the medical challenge (disease prevention) but also the social medicine objective [5, 6, 11].

The importance of care and health promotion in at-risk population is emphasized by the correlation revealed between high morbidity, mortality and insufficient level of vitamin D most typical for elderly people [7].

According to the WHO recommendations, elderly people should do at least 150 min of physical activity throughout the week (just over 20 min a day) [8]. However, at least the routine physical activity should be maintained in the changed life circumstances. Explaining the more active lifestyle benefits should be used to achieve at least the minimum activity even in elderly patients with sedentary lifestyle, provided their initial condition allows them to handle the load [9].

To cope with the current situation, in April 2020 the infectious hospital for patients with COVID-19 was established on the basis of Burnasyan Federal Medical Biophysical Center of Federal Medical Biological Agency. Patients with suspected and confirmed COVID-19 were admitted to hospital.

The study was aimed to evaluate the patients with novel coronavirus infection completed inpatient treatment cases in order to reveal the relationship between the initial physical activity level, patient's age, practicing therapeutic exercises therapy while staying in the hospital, and the course of the disease.

METHODS

Data from medical records of 144 COVID-19 patients who had completed inpatient treatment in the infectious hospital for patients with COVID-19 in April–May 2020 (discharged with improvement). All patients were diagnosed in accordance to ICD-10 U07.1 (coronavirus infection caused by COVID-19, virus identified) or U07.2 (coronavirus infection caused by COVID-19, virus not identified) [10]. During the impatient treatment at different stages of care provision the patients were introduced to various methods of breathing exercises and exercise therapy used to reduce the length of stain in a hospital and improve the disease prognosis. The patient received a discharge summary with a complex of exercises to be done during self-isolation (within 14 days after the impatient treatment completion). Patients with mild, moderate and severe infection were divided into experimental and control groups according to initial (prior to the disease) subjective performance level, quality of life and physical activity. The listed parameters were evaluated using the modified questionnaire after the inpatient treatment completion in order to assess the physical activity level, quality of life, physical performance, everyday skills prior to infection, and the degree of those parameters decrease after discharge. It was specified, if the patients practiced therapeutic of breathing exercises while staying in the hospital and at home after discharge, or not, and if they experienced high blood pressure episodes and stool problems after discharge from hospital. The patients with pronounced cognitive impairment and severe condition due to comorbidities were excluded from analysis, since it was difficult to acquire data on that category of patients (uncooperativeness, inability to answer questions). The study results were processed using the Microsoft Excel 2016 application. The average values were calculated as the arithmetic mean.

RESULTS

The analysis of data from medical records revealed the following: in most patients admitted to infectious unit (69.44%) the course of novel coronavirus infection was moderate, in 13.89% of patients in was mild, and in 16.67% of patients it was severe.

Analysis of the sex/age composition revealed that women predominated only among patients with moderate infection (54.00%). The proportions of men and women among patients with mild and severe infection were equal (50.00%).

Analysis of the proportion of confirmed and not confirmed COVID-19 revealed that patients with confirmed diagnosis prevailed among patients with mild and moderate course of the disease (65.00 and 92.00% respectively). The highest proportion (54.17%) of patients with the "virus not identified" status (negative SARS CoV-2 PCR test result) was observed in the group with severe disease compared to other groups. However, coronavirus infection (COVID-19) was diagnosed according to combined clinical and anamnestic data, epidemiological data and the results of instrumental and laboratory tests. Some of these patients were transferred from other hospitals or received etiotropic therapy on an out-patient basis. The virus could be eliminated under the impact of antiviral therapy.

Analysis of the age composition showed that younger patients had milder COVID-19. Thus, 55.00% of patients with mild infection were under 40 years of age (45.45% of patients were less than 30 years of age).

Most patients with moderate course of the infection (69.00%) were aged 40–79.

Analysis of the age composition in patients who had overcome severe coronavirus infection revealed a clear trend towards the increase of the older patients' proportion. There were no young patients (aged 20–29) in that group. The highest proportion of severe cases (25.00%) was in the age group 40–49, next came the older age groups. In general, patients over 50 made up 62.49% of the total number of severe cases.

The patients spent 6–29 days in the hospital (an average of 14 days).

The diagnosis was verified inter alia using the computed tomography (CT). The number of scans varied from 1 to 6 (an average of 2.97 per individual).

Most patients had comorbidities (65.28%), generally cardiovascular and endocrine disorders (hypertension, diabetes mellitus, obesity, heart rhythm disturbances).

Most patients did therapeutic and breathing exercises while staying in the hospital (75.00%). However, the recommended therapeutic exercises during self-isolation (within 14 days after discharge) were practiced by insignificantly higher proportion of patients (77.77%).

The patients who had overcome the severe form of the disease reported the lower quality of life prior to infection (an average of 8.62 points out of 10) compared to patients with moderate (an average of 9.20 points out of 10), and mild forms of the disease (9.10 points out of 10). The initial performance was better in patients who had overcome the mild infection (9.65 — mild course, 9.15 — moderate course, 8.40 — severe course).

The analysis (experimental group) included patients with higher initial subjective level of performance, quality of life and physical activity, 50.00% in each group according to the severity of the disease.

Thus, the experimental group comprised:

1. Mild course: 10 people;
2. Moderate course: 50 people;
3. Severe course: 12 people.

Consequently, the control group included the same number of patients (10 — mild course, 50 — moderate course, 12 — severe course). The stool problems and the level of blood pressure control (episodes of high blood pressure) after discharge were evaluated as possible indirect signs of insufficient physical activity (-).

The largest group of patients with moderate infection had the following structure according to the types of comorbidities: 59.00% of patients had hypertension (stages 1–3), 18.00% of patients had excess body weight or obesity, 16.00% had type 2 diabetes mellitus, and 12.00% had heart rhythm problems (in the history or recorded after admission to hospital). Patients of experimental and control group were comparable according to comorbidities' types.

DISCUSSION

Thus, patients with mild form of the disease, as well as patients with moderate and severe forms having higher initial performance and quality of life prior to COVID-19 infection required fewer bed-days in the hospital (by 2.01 bed-days in patients with mild and moderate course, by 2.70 bed-days in severe patients). Recovering from the disease, the experimental group patients with mild and moderate infection also rated their quality of life and performance higher compared to the control group patients.

The experimental group patients with mild infection reported no high blood pressure episodes (vs. 50.00% of control group patients) and stool problems (vs. 25.00% of control group patients). The high initial level of physical activity was confirmed by the majority of patients doing therapeutic exercises for deterioration of condition and disease progression prevention (50.00% of experimental group vs. 25.00% of control group).

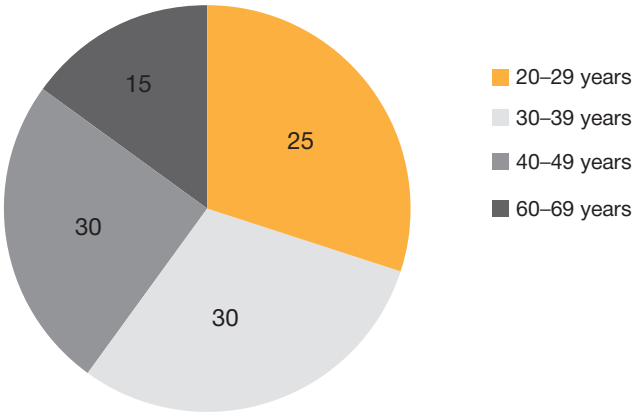

Fig. 1. Age structure of the group with mild form of the disease, %

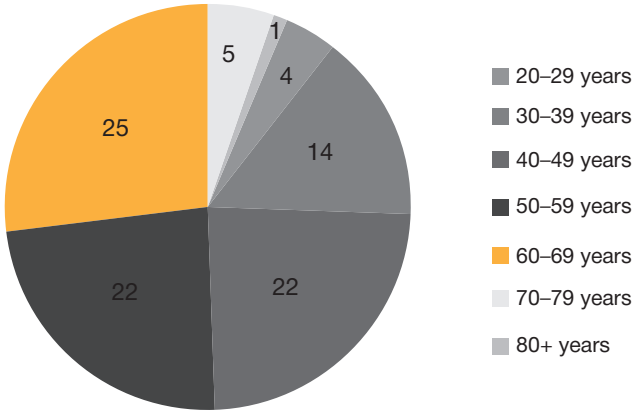

Fig. 2. Age structure of the group with moderate form of the disease, %

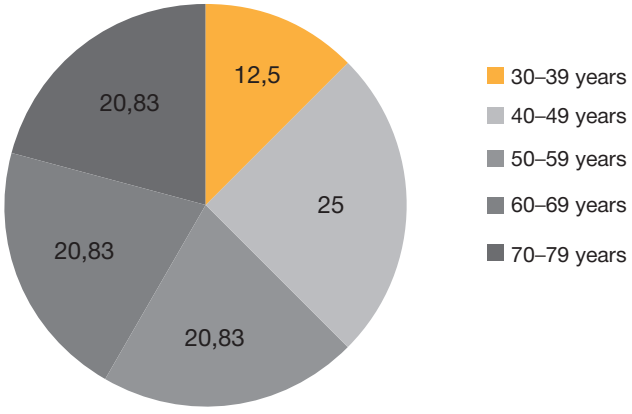

Fig. 3. Age structure of the group with severe form of the disease, %

That could contribute to the required total bed-days reducing and milder course of infection.

Therapeutic exercises, both in the hospital and after the inpatient treatment completion, were most actively practiced by patients with moderate course of the disease. Thus, 94.00% of experimental group patients did therapeutic exercises both while staying in the hospital and at home. In the control group, only 57.00% of patients did therapeutic exercises during the inpatient treatment (and 66.00% of patients practiced therapeutic exercises after the treatment completion). The use of exercise therapy methods correlated with reduced length of stay in a hospital, higher quality of life and performance rating, both initially and after discharge from hospital.

Despite the high blood pressure or hypertension diagnosed in 59.00% of that group, only 7.00% of patients from experimental group reported episodes of high blood pressure after discharge (vs. 17.00% of control group), and only 5.00% reported stool problems (vs. 13.00% of control group).

In patients with severe infection, not all parameters demonstrated such positive correlations. The initial quality of life and performance in the experimental group were higher. However, in patients who had overcome the disease, the

Table 1. Patient characteristics: mild form of the disease (*n* = 20)

Parameter/Group	Experimental	Control
Average number of bed-days	8.74	10.76
Average chest CT scans	1.76	1.76
Average age, years	37.50	50.15
Quality of life prior to infection*	9.80	8.40
Quality of life after infection*	9.10	6.65
Performance prior to infection*	9.65	9.65
Performance after infection*	9.00	7.50
High blood pressure after discharge, %	0	30.00
Stool problems after discharge, %	0	50.00
Executed therapeutic exercises in the hospital, %	50.00	25.00
Executed therapeutic exercises at home after discharge, %	25.00	25.00

Note: * — по 10-балльной шкале.

Table 2. Patient characteristics: moderate form of the disease (*n* = 100)

Parameter/Group	Experimental	Control
Average number of bed-days	12.97	14.98
Average chest CT scans	2.66	3.22
Average age, years	47.10	56.74
Quality of life prior to infection*	9.72	8.68
Quality of life after infection*	8.34	7.42
Performance prior to infection*	9.82	8.47
Performance after infection*	8.26	7.20
High blood pressure after discharge, %	7.00	17.00
Stool problems after discharge, %	5.00	13.00
Executed therapeutic exercises in the hospital, %	94.00	57.00
Executed therapeutic exercises at home after discharge, %	94.0	66.00

Note: * — по 10-балльной шкале.

Table 3. Patient characteristics: severe form of the disease (*n* = 24)

Parameter/Group	Experimental	Control
Average number of bed-days	16.40	19.10
Average chest CT scans	4.24	4.44
Average age, years	55.60	55.90
Quality of life prior to infection*	9.15	8.10
Quality of life after infection*	6.60	6.80
Performance prior to infection*	8.70	8.10
Performance after infection*	5.80	6.40
High blood pressure after discharge, %	41.67	0
Stool problems after discharge, %	20.84	25.01
Executed therapeutic exercises in the hospital, %	62.50	100.00
Executed therapeutic exercises at home after discharge, %	83.33	62.50

Note: * — по 10-балльной шкале.

values of the same parameters appeared to be lower than in control group. Furthermore, more patients in the experimental group reported high blood pressure episodes (41.67% vs. 0% in the control group). Meanwhile, the length of stay in a hospital and the number of CT scans were lower in the experimental group. The higher proportion of the experimental group patients practiced therapeutic exercises at home (83.33% vs. 62.50% in the control group), which confirmed the higher initial level of physical activity. However, all control group patients did therapeutic exercises while staying in the hospital (vs. 60.00% of experimental group patients). That could be due to various comorbidities limiting the therapeutic exercises execution or lack of knowledge about exercise therapy.

CONCLUSION

Based on the data obtained and the analysis performed, it can be assumed that in patients with more active initial lifestyle, higher performance and better quality of life, the more favourable course of the disease was observed leading to reduced length of stay in a hospital, fast recovery of performance after the SARS CoV-2 infection, and reduced number of CT scans due to milder course of the disease. Such patients with mild and moderate course of the disease reported fewer episodes of high blood pressure after discharge. Moreover, the patients with mild and moderate infection who did therapeutic exercises while staying in the hospital had more favourable outcome and required shorter period of treatment.

All of this confirms the importance of regular physical activity being a proven method of most common noncommunicable diseases prevention. Regarding the current epidemiological situation and the COVID-19 pandemic, it is nesessary to increase awareness about the importance of regular physical exercises and the relationship between physical activity and the novel coronavirus infection prognosis and course generally among elderly people, as well as to introduce the exercise therapy methods both during inpatient treatment and after discharge from hospital.

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CLINICAL AND EPIDEMIOLOGICAL FEATURES OF THE NEW CORONAVIRUS INFECTION COVID-19 IN THE CENTRAL BLACK REGION

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The article provides an analysis of a large-scale epidemic outbreak caused by human coronaviruses. The epidemiological situation in the world and the Russian Federation is analyzed, which forced specialists to significantly increase the level of epidemiological danger from coronaviruses. The epidemic situations of the incidence in six regions of the Central Black Earth Region are described, the dynamics and regional features of the spread and nature of the course of the new coronavirus infection Covid-19 are generalized and systematized. Some epidemiological aspects of this infection in the territory of the Voronezh region are described. The dynamics of the epidemic process is described and a number of epidemiological indicators are analyzed (daily increase in morbidity and mortality, distribution of the duration of lethal diseases, risk groups, etc.). The clinical and epidemiological features of the combined forms of infections are analyzed: the prevalence of a moderate course, the risk of complications in risk groups. The difficulty of verifying this infection from other viral infections based on the clinical picture, the high virulence and severity of the course has been established. It was shown that the mobilization of health care to combat coronavirus infection revealed the main thing: the health care system has resources and mechanisms through which it is possible to quickly switch to work in extreme conditions. New hospitals and beds, re-equipment with diagnostic and resuscitation equipment, accelerated retraining of doctors. All this was effective evidence that an adequate potential supply of resources will not only reduce the consequences of possible epidemics in the future, but also during the period outside the epidemic will help accelerate the adoption of effective decisions and improve the quality of medical care for the population.

Keywords: coronavirus; coronavirus infection; pneumonia; treatment; safety in extreme situations

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КЛИНИКО-ЭПИДЕМИОЛОГИЧЕСКИЕ ОСОБЕННОСТИ НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИИ COVID-19 В ЦЕНТРАЛЬНО-ЧЕРНОЗЕМНОМ РЕГИОНЕ РОССИИ

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В статье приведен анализ масштабной эпидемической вспышки, обусловленой коронавирусами, патогенными для человека. Проанализирована эпидемиологическая ситуация в мире и Российской Федерации, которая заставила специалистов существенно повысить уровень эпидемиологической опасности со стороны коронавирусов. Описаны эпидемические ситуации по заболеваемости в шести областях Центрально-Черноземного региона, обобщены и систематизированы динамика и региональные особенности распространения и характера течения новой коронавирусной инфекции Covid-19. Описаны некоторые эпидемиологические аспекты данной инфекции на территории Воронежской области. Описывается динамика эпидемического процесса и анализируется ряд эпидемиологических показателей (ежедневный прирост заболеваемости и смертности, распределение продолжительности летальных заболеваний, группы риска и т.д.). Проанализированы клинико-эпидемиологические особенности сочетанных форм инфекций: преобладание среднетяжелого течения, риск развития осложнений в группах риска. Установлена сложность верификации данной инфекции от других вирусных инфекций на основе клинической картины, высокая вирулентность и тяжесть течения. Показано, что проведенная мобилизация здравоохранения для борьбы с коронавирусной инфекцией выявила главное: у системы здравоохранения есть ресурсы и механизмы, благодаря которым можно быстро перестроиться на работу в экстремальных условиях. Новые госпитали и койки, дооснащение диагностическим и реанимационным оборудованием, ускоренное переобучение медиков. Все это являлось действенным доказательством того, что адекватный потенциальный запас ресурсов не только позволит уменьшить последствия от возможных эпидемий в будущем, но и в период вне эпидемии поможет ускорить принятие эффективных решений и улучшить качество медицинской помощи населению.

Ключевые слова: коронавирус, коронавирусная инфекция, пневмония, лечение, безопасность в экстремальных ситуациях

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Throughout its history, mankind has seen countless epidemics and pandemics take millions of lives. In the new millennium, typhoid fever and plague have become a thing of the past. But climate change and other environmental factors have given rise to novel viruses whose swift spread across the globe is driven by high population density and migration [6].

In late 2019, the World Health Organization declared an outbreak of a novel coronavirus disease (COVID-19). According to John Hopkins University, USA, there were over 11 million confirmed COVID-19 cases worldwide in early July 2020 [2]. Currently, the United States ranks first in the total number of infections (2.8 million) [3, 4], followed by Brazil (1.5 million) and Russia (670, 000). In Russia, there has been 10,027 confirmed deaths so far, with the case fatality rate being 1.49% [5, 6].

By March 2020, the pandemic had reached the Black Earth Belt of central Russia, where the first COVID-19 cases were reported in Lipetsk region. The infection soon spread to 5 other regions of the Russian Black Earth Belt, including Voronezh, Kursk, Oryol, Tambov, and Belgorod [7, 8].

The aim of this study was to systematize data on COVID-19 collected in the Black Earth Belt region of Central Russia.

METHODS

The study was conducted using the established methodology for epidemiological surveillance which relies on analytical, descriptive, evaluative, and statistical techniques, mathematical modeling and prediction.

For the purpose of this study, we developed a framework based on the conceptual premises of basic and applied research in virology and highly infectious diseases proposed by Russian and foreign authors.

RESULTS AND DISCUSSION

We have analyzed data collected in 6 regions of the Black Earth Belt in central Russia. This analysis allowed us to identify both countrywide and regional patterns of COVID-19 spread (Fig.1).

The first cases of the novel coronavirus infection in Voronezh region were reported in March 2020. By July 2020, the total number of cases had gone beyond 8, 000 (Fig. 2), growing by 22.1% in comparison with March (Fig.3).

In June, the number of new cases peaked, reaching 3,351 (Fig.4).

From June, 15 to June, 21 there were over 200 confirmed daily cases in Voronezh region. By complying with stringent containment measures imposed by the government, the region was able to bring the spread of the disease under control. Since the end of June, there has been a downward trend in daily cases. In the last few days, the number of daily cases was just slightly over 100 (Fig. 5).

However, the risk of coronavirus spread in Voronezh region is still high. The average reproductive number is 4. Despite an insignificant decline in daily cases, the total number of confirmed infections continues to grow (Fig.6).

Having analyzed the medical histories and autopsy reports for suspected COVID-19 cases, the task force working group for Voronezh region concluded that the novel coronavirus infection affects the course of some chronic conditions and can cause exacerbations. Traces of the virus were detected in patients' nasal and pharyngeal specimens, kidneys, liver, pancreas, myocardium, tears, and feces. According to the

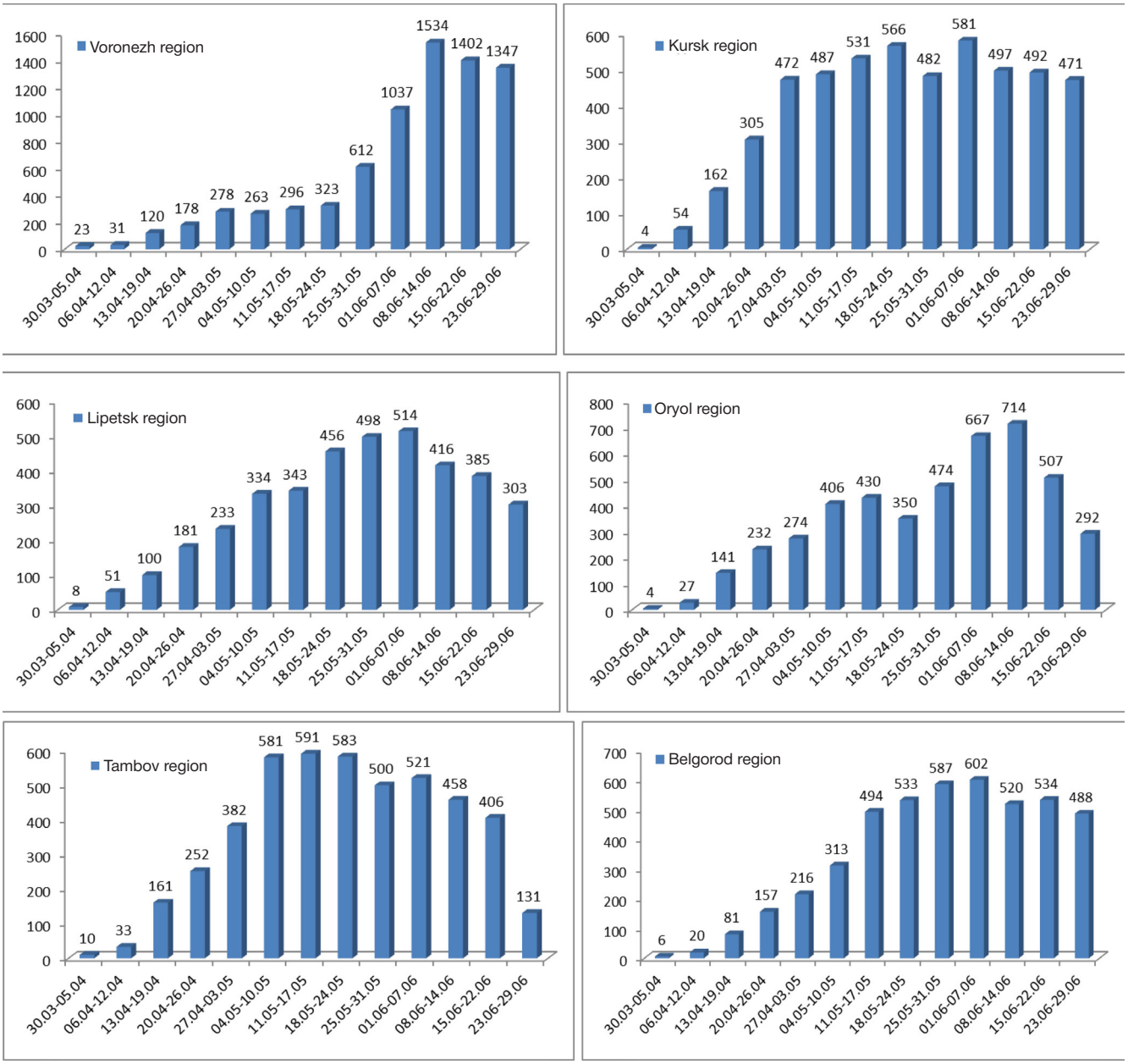

Fig. 1. Total confirmed cases of COVID-19 per week

findings of our colleagues from Sichuan University of Science & Engineering, the virus attacks ACE2, which is present in the vascular wall, myocardial tissue and intestinal epithelium [9]. This protein is abundant in the alveolar epithelium, that is why the virus often causes damage to the lungs. The course of the disease differs among individuals. In Voronezh region residents, the lungs are the most frequently affected organ (97%), followed by the heart (67%), blood vessels (54%), the intestine (23%), the kidneys (14%), and the central nervous system (8%). The diagnosis is often complicated by the lack of clear symptoms. The virus breaks the canons of classic epidemiology: the typical clinical picture of COVID-19 often contradicts the presenting complaints of a patient. In many cases, an infected individual deteriorates dramatically in no time and is at high risk of death [10].

Rapidly progressing acute respiratory distress and sepsis are not uncommon in patients with COVID-19. Many severe patients survived due to the multifaceted therapeutic approach, high-quality resuscitation and extended time spent in intensive care units. In Voronezh region, 24 hospitals were repurposed to accommodate 3,323 COVID-19 patients. This helped to prevent the catastrophic scenarios unfolding in other countries. Besides, Voronezh was one of 15 Russian cities where medical centers for infectious diseases were deployed in as little as 65 days to treat patients with COVID-19. The design was proposed by the Ministry of Defense of the Russian Federation. Such hospitals can accommodate up to 200 patients and are equipped with over 3,500 medical devices for infected patients.

Unfortunately, there is no convincing evidence proving the efficacy of medication therapy in patients with COVID-19.

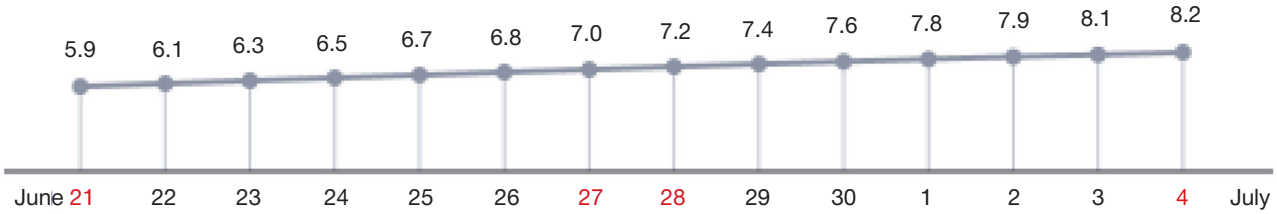

Fig. 2. Total number of COVID-19 cases in Voronezh region (according to Rospotrebnadzor and John Hopkins University)

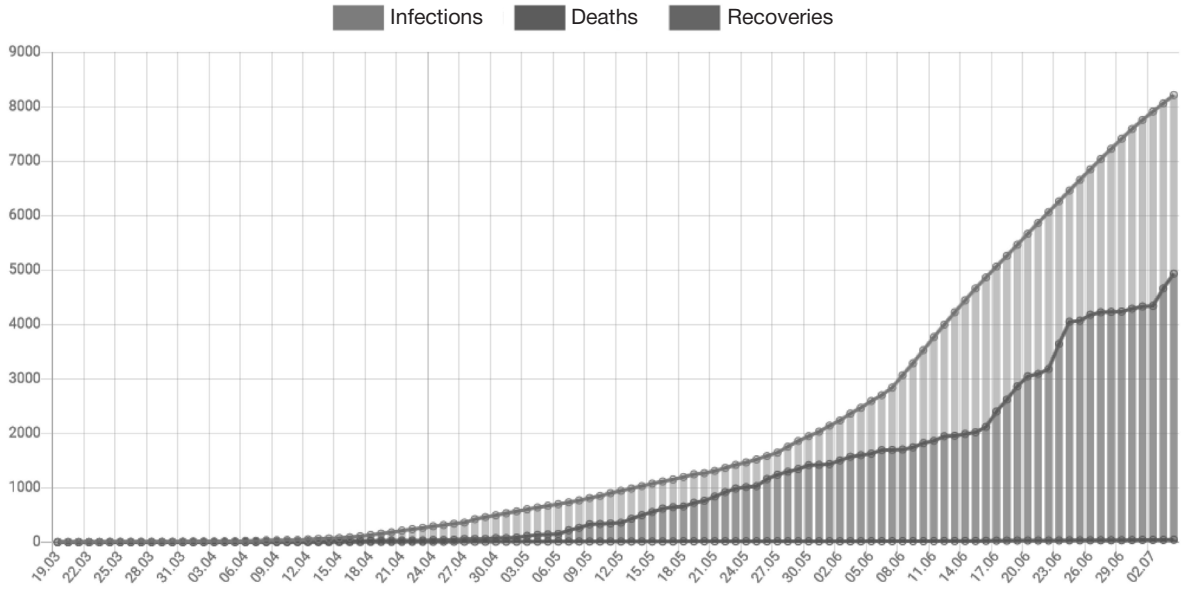

Fig. 3. cidence, recoveries and fatality rates for patients with COVID-19 (March-July 2020). Source: https://coronavirus-monitoring.info/v-voronezhskoj-oblasti/

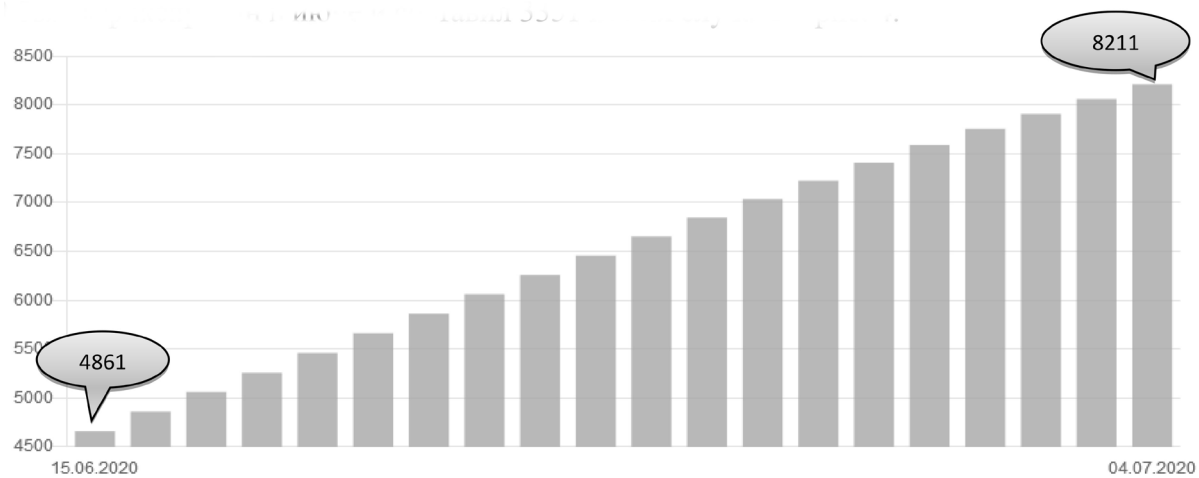

Fig. 4. Infection growth rates (March–July 2020.) Source: https://coronavirus-monitoring.info/v-voronezhskoj-oblasti/

According to the literature, there are a few drugs that target the underlying cause of SARS-CoV- and MERS-CoV-associated atypical pneumonia and are normally used in combination [11]. They are ribavirin, lopinavir+ritonavir and synthetic interferons. However, it is difficult to draw any firm conclusion about their efficacy based on patients' outcomes; therefore, they are used only at the approval of a medical consultative board if the potential benefit of the drug outweighs the risks. Besides, the virus destroys one of hemoglobin chains in red blood cells, i.e. it damages blood cells engaged in oxygen transport, leading to hypoxia [12, 13]. In case when SARS-COV-2 simultaneously attacks different organs, it gets harder to find an effective cure.

Another promising approach to treating COVID-19 is oxygen infusion treatment; the supplied oxygen is then carried by the blood to all bodily tissues and organs. Great hopes are pinned on drugs used to treat malaria and BCG vaccines but their effect still needs to be thoroughly studied.

The Department of Healthcare of Voronezh region is taking steps to contain the epidemic; it is also concerned about the possible disease sequelae for convalescent individuals or how the epidemic will affect those who are not infected yet or whether there are latent carriers, etc. Two Voronezh city clinics (№ 11 and № 16) have opened new post-acute transitional care and rehabilitation units for patients with COVID-19. The units can accommodate up to 160 patients with mild disease, those who do not require intensive care or ventilators and those who were severely ill and are now improving.

For COVID-19 patients, recovery and death rates differ across regions. According to the official reports published on Стопкоронавирус.рф, the total death toll over the entire surveillance period was 0.54% (a total of 51 cases) for Voronezh region. In Kursk region, this figure was 0.7%. The death toll was the lowest in Lipetsk and Tambov regions (0.4% for both) and the highest in Oryol (1.4%) and Belgorod (1%) regions (Fig. 7).

The first post-mortem confirmed case of COVID-19 in Voronezh region was reported on April 12. The patient was a 65-year old female residing in Voronezh region. Until June, the fatality rate did not exceed 0.35% but then spiked to 0.54% in just 15 days (Fig. 8 and 9).

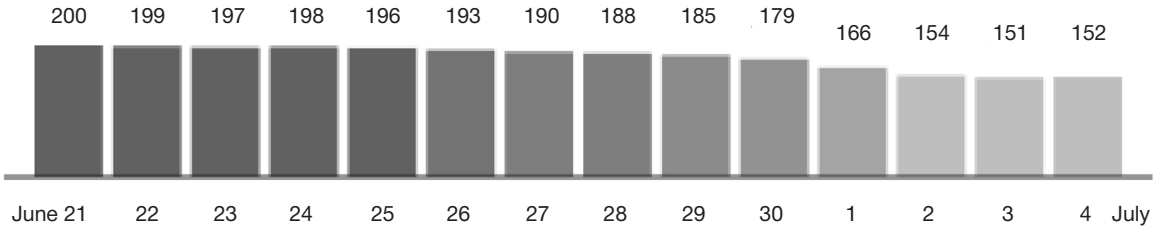

Fig. 5. New daily cases of COVID-19 (June 21–July 4, 2020)

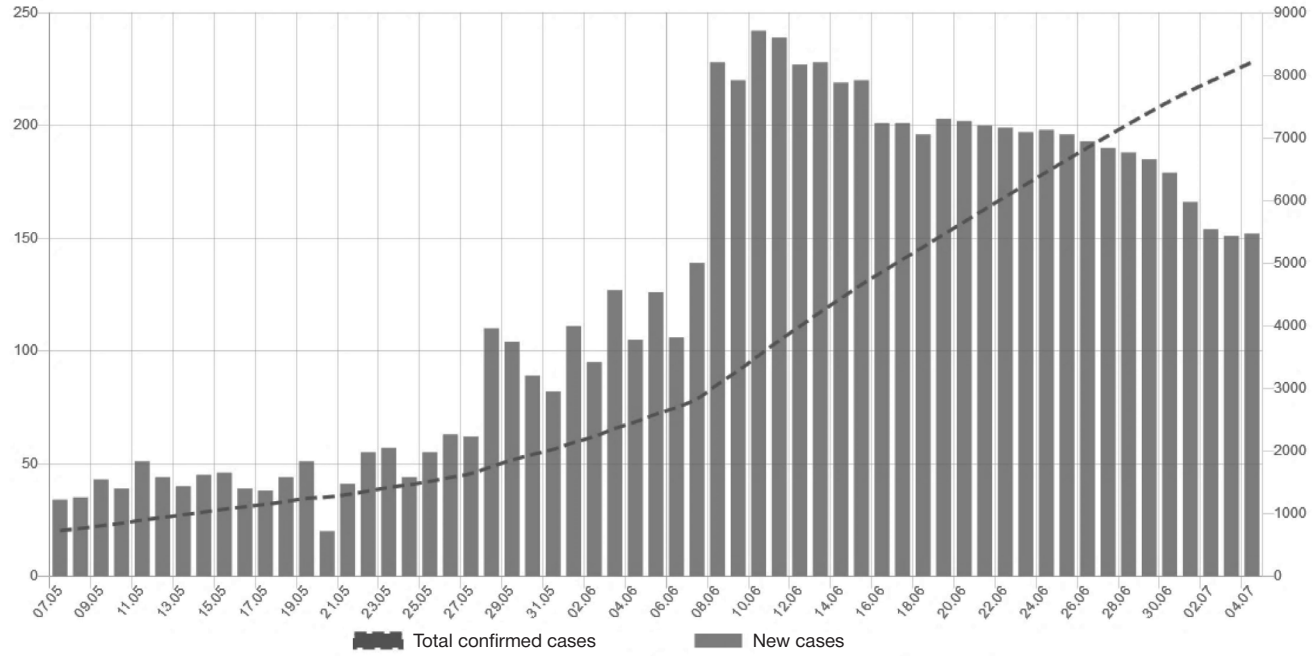

Fig. 6. Ratio of new cases to confirmed cases. Source: https://coronavirus-monitoring.info/v-voronezhskoj-oblasti/

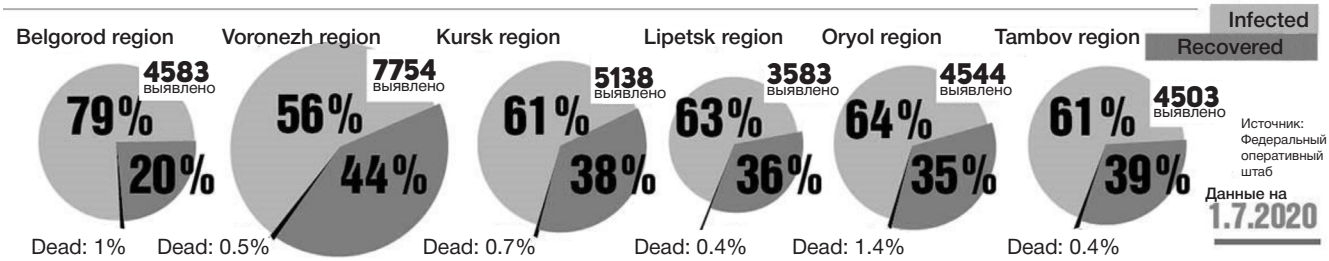

Fig. 7. Infections, recoveries and deaths in the studied bordering regions

Normally, the fatality rate is at its highest at the beginning of an outbreak and drops to a minimum at the end of it when more mild cases are increasingly diagnosed and advanced diagnostic tests have been already introduced. At the beginning, the COVID-19 epidemic unfolded as a nosocomial outbreak. Since Russian hospitals are equipped with specific diagnostic tools, no extra time was needed for their deployment and the rising fatality rate was attributed to an increase in the number of infected individuals.

The average age of COVID-19 fatalities is 68.6 years. Statistically, individuals of advancing age are at increased risk for the infection. Co-existing chronic conditions aggravate the course of COVID-19. Importantly, the disease can be fulminating. Lethal pneumonias comprise primary viral pneumonias (days 1–7 from the onset) and secondary bacterial or viral pneumonias (weeks 2 and 3 following the onset), which is also common for other severe acute respiratory viral infections.

In light of this, measures taken by the Voronezh region administration look reasonable and far from extreme. Despite the large population size, the epidemic in Voronezh region plateaued 3.5 months after its onset, which is still not fast enough: the spread of the virus in the region was affected by a few important factors. First, from February till April there were a lot of imported coronavirus cases. Between March 24 and April 6, over 1,470 individuals came to the Central Black Earth Belt region from 58 countries. Most of coronavirus cases in the region were imported from Thailand (265), Ukraine (179), Abkhazia (76), Turkey (57), and Armenia (51).

Another significant contributor to the spread of SarsCoV-2 infection was poor commitment to lockdown. Starting from March 20, public events were canceled and the region was on high alert. Starting from May 12, all Voronezh region residents had to wear face masks while on public transport and in shops and practice 1.5–2 m social distancing. On July 7, all restrictions were lifted, except for 65+ year old individuals, for whom the lockdown continued.

Although the official reports provide general understanding of the situation, they still fail to answer a number of important questions. A possible correlation between the imposed containment measures and the number of COVID-19 cases is not that obvious. In Voronezh, the disease incidence peaked in mid-June after containment measures had been loosened. At the same time, according to Yandex monitoring services, adherence to lockdown restrictions was very poor in Belgorod (1.4 points on the 5-point scale), but the number of daily cases there was relatively low.

There has been a lot of change in many public sectors since the start of the epidemic in Russia, from retraining of medical personnel to timely decision making and amendments to the current legislation.

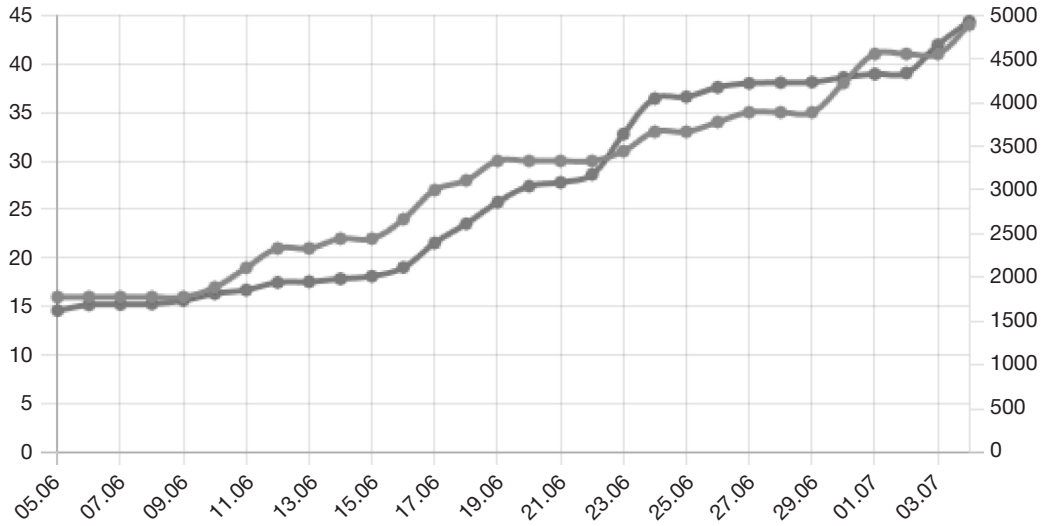

Fig. 8. Deaths and recoveries in Voronezh region

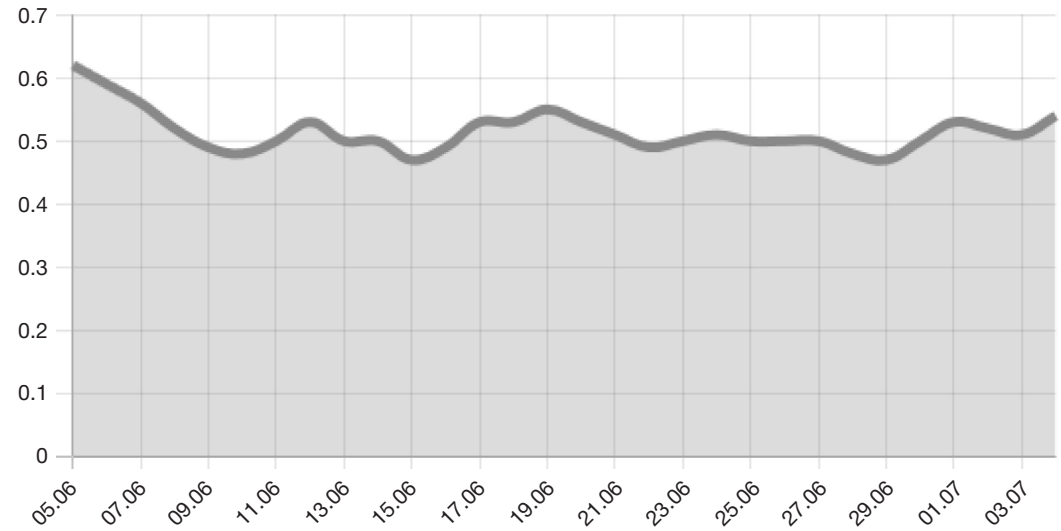

Fig. 9. Changes in the percentage of deaths from COVID-19 in Voronezh region over the 30-day period

In Voronezh region, standard hospital wards were repurposed into COVID-19 wards; patient flow was adequately managed; a centralized remote monitoring and control system was created at the Consultative Unit for Anesthesiology and Critical Care of the Regional Center for Disaster Medicine; guidelines were elaborated to facilitate interaction between different agencies; anatomic pathology services were provided for hospitals dealing with COVID-19 patients.

Voronezh State Medical University actively took part in countering the epidemic and preventing Sars-CoV-2 spread. A resource and consultation center was opened at the Regional Department for Healthcare Development. The main goals of the Center included providing consultations to medical institutions of Voronezh, Lipetsk and Tambov regions on the diagnosis and treatment of the novel coronavirus infection and pneumonias in adult patients; analyzing the efficacy of treatment for COVID-19 patients; organizing webinars for medical personnel and educate them about the disease course, diagnostic challenges and treatment; providing consultations to pulmonologists, infectious disease specialists, anesthesiologists, and critical care specialists.

The Center worked in collaboration with the Consultative Unit for Anesthesiology and Critical Care for adult patients with COVID-19 and pneumonias created at the facilities of the remote Unit for Critical Care and Consultations and of Voronezh Region Clinical Center for Disaster Medicine and the Department of Healthcare.

Practical training for doctors, interns and other medical personnel aimed at COVID-19 prevention, diagnosis and treatment will allow the participants to improve their expertise and acquire the skills needed for future emergencies.

CONCLUSION

Resource mobilization in the wake of the Sars-CoV-2 epidemic revealed that the Russian healthcare system can successfully respond to public health emergencies. New hospitals and wards, rapid deployment of diagnostic and intensive care equipment, quick retraining of medical staff are all compelling evidence of how sufficient human and material resources can minimize the devastating effects of future epidemics, ensure quick decision making and improve the quality of medical care in “times of peace”.

At the same time, the novel coronavirus pandemic unveiled the need for making amendments to the current public health legislation. This is especially true for the regulations applied to medicinal products, which do not work effectively in an emergency situation. In Russia, the law on biosecurity and biosafety is still in development. Biological threats have never been so intimidatingly real. Humankind is waging a war on pathogens at tremendous costs and with countless fatalities. The current status quo in the international relations underscores the role of fierce competition between superpowers for the right to possess cutting-edge technologies that ensure security and leadership in military, information, economic and biological sectors. But the ambitions of one state can jeopardize the security of another or even the whole group of countries, which often create political and military alliances in the face of an international or a local threat. The concept of collective security proposed by the Collective Security Treaty Organization is rooted in the idea that indivisible security is the key principle protecting interests, sovereignties and territorial integrity of member states [14, 15]. Therefore, protecting the population from the novel biological weapons of mass destruction is currently a crucial challenge for Russia.

The pandemic uncovered a number of problems facing the organization of effective public health services, especially strategic ones. It is not enough to merely state the importance of modernization — action should be taken to expedite improvement. We need a single center for decision making instead of 5 separate agencies. For example, a need arose to increase production of face masks and a range of drugs during the pandemic. Different agencies were assigned to this task, including the Ministry of Healthcare, the Ministry of Industry and Trade, Rospotrebnadzor (Russian Agency for Health and Consumer rights), Roszdravnadzor (Federal Service for Surveillance in Healthcare), and FMBA. There is a lack of cooperation between these agencies, which eventually affects the outcome.

There is a need for the overhaul of the entire strategy for the development of pharmaceutical industry in Russia. It is not enough to create an exhaustive list of drugs and their pharmaceutical ingredients that are supposed to be produced by domestic manufacturers. The pandemic revealed that many drugs used across the world are not registered in the Russian Federation. It usually takes at least half a year for a drug to be approved in Russia, which is way too long in case of an epidemic. This means that the rules regulating the use of unregistered drugs should be revised. The registration procedure should be simplified. It is reasonable to test the safety and clinical efficacy of a drug prior to the registration procedure and to conduct other phase trials afterwards. Those post-registration trials should include reporting of adverse effects; the doctors involved should be compensated for the paper work, etc. It is crucial to ramp-up production of pharmaceutical drugs for domestic use and export, conduct preclinical and clinical trials, stimulate development of effective next-generation drugs and the research potential of the Russian public health in order to be able to deal with challenges and look in the future with confidence.

Thus, there are 3 strategic priorities. The first one is reinforcing the commitment and capacities of medical institutions to effectively counter current and potential attacks. The second is creation of a self-sustainable system for providing the country and its separate regions with medical equipment and professional staff. The third priority is to provide the public health industry with cutting-edge technologies and effective drugs, along with ingredients for their production.

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DEVELOPMENT OF THE KIT FOR DIAGNOSTICS OF COVID-19 BY REAL TIME RT-PCR

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Late in December 2019, an outbreak of an unknown coronavirus, later identified as SARS-CoV- 2, emerged in the city of Wuhan, China. It causes a dangerous respiratory coronavirus disease in humans — COVID-19. Objective. To detect cases of the disease and prevent its spread across the Russian Federation it is necessary to create an effective diagnostic test system. Material and methods. Based on the analysis of the alignment of the SARS-CoV-2 nucleotide sequences, primers and a probe for RT-PCR were selected, and the analysis conditions were optimized. Results. The diagnostic system was developed and registered in the shortest possible time in real-time RT-PCR format for detecting SARS-CoV-2 coronavirus RNA in smears from the nasopharynx and oropharynx, sputum and feces. The high specificity of the system was verified on a representative set of viruses and microorganisms, the analytical sensitivity was 1x103 copies / ml in smears from the mucous membrane of the nasopharynx and oropharynx and sputum, 5x104 copies / ml in fecal samples. Diagnostic sensitivity and specificity established during clinical trials on samples from patients with confirmed COVID-19 infection, from patients with a different etiology of a disease and clinically healthy people were to 100% (range 94.2-100% with a confidence level of 95%).

Keywords: coronavirus, molecular diagnostics, COVID-19, SARS-CoV-2, SARS-COV-2, real-time RT-PCR, diagnosis of infectious diseases

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РАЗРАБОТКА ТЕСТ-СИСТЕМЫ ДЛЯ ДИАГНОСТИКИ COVID-19 В ФОРМАТЕ ОТ-ПЦР В РЕЖИМЕ РЕАЛЬНОГО ВРЕМЕНИ

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В конце декабря 2019 года в городе Ухань, Китай, возникла вспышка неизвестного коронавируса, позднее идентифицированного как SARS-CoV-2. Вирус вызывает опасное респираторное коронавирусное заболевание человека - COVID-19. Цель. Для выявления случаев заболевания и предотвращения его распространения на территории Российской Федерации необходимо создание эффективной диагностической тест-системы. Материалы и методы. На основании анализа выравнивания нуклеотидных последовательностей SARS-CoV-2 были выбраны праймеры и зонд для ОТ-ПЦР, оптимизированы условия проведения анализа. Результаты. В кратчайшие сроки разработана и зарегистрирована диагностическая система в формате ОТ-ПЦР в реальном времени для выявления РНК коронавируса SARS-CoV-2 в мазках со слизистой оболочки носоглотки и ротоглотки, мокроте и фекалиях. Высокая специфичность системы показана на репрезентативной выборке генетического материала вирусного и бактериального происхождения, аналитическая чувствительность составила 1×10^3 ГЭ/мл в мазках со слизистой носоглотки и ротоглотки и мокроте, 5x104 ГЭ/мл в образцах фекалий. Диагностические показатели (чувствительность и специфичность), установленные при клинических испытаниях на образцах, полученных от пациентов с подтвержденной инфекцией COVID-19, от пациентов с иной этиологией заболевания и клинически здоровых людей, составили 100% (диапазон 94,2–100 % с доверительной вероятностью 95 %).

Ключевые слова: коронавирус, молекулярная диагностика, SARS-CoV-2, COVID-19, SARS-COV-2, ОТ-ПЦР в реальном времени, диагностика инфекционных заболеваний

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SARS-CoV-2 is the new strain of coronavirus identified in late 2019 in the context of the outbreak of pneumonia in China [1, 2, 3]. The virus causes COVID-19, a dangerous human respiratory coronavirus disease. Severe COVID-19 has pneumonia with acute respiratory failure as complications, which explains high mortality.

SARS-CoV-2 is a single-stranded RNA virus that belongs to the beta coronavirus genus, genetically close to SARS [4, 5, 6]. Today, beta coronaviruses OC43, HKU1, SARS, MERS, SARS-CoV-2 and 229E and NL63 alpha coronaviruses are considered to be of clinical importance [2, 5, 7].

The World Health Organization (WHO) declared COVID-19 a public health emergency of international concern (PHEIC); a pandemic was declared in March 2020 [6]. The spread of the disease in the world is regarded as very intense. The number of cases, including fatalities, and the number of affected countries are increasing steadily, and therefore the governments are taking unprecedented measures to prevent spread of the virus. As of June 30, 2020, there were 10360882 SARS-CoV-2 infection cases registered in the world, with 507014 of them ending in fatality. Considering the overall number of cases, Russia is third to USA and Brazil with 646929 confirmed infections and 9306 fatalities as of the mentioned June 30, 2020 [8].

Timely detection of the disease and prevention of its further spread on the territory of the Russian Federation necessitates urgent development of a highly sensitive and specific diagnostic system for detection of SARS-CoV-2 coronavirus RNA in biological samples. The goal of this research effort was to develop such a system.

MATERIALS AND METHODS

The development started on 19.01.2020; at that time, GISAID database contained nucleotide sequences of 8 full-length genomes of the SARS-CoV-2 (formerly CoV Wuhan), which had minor genetic differences: (BetaCoV/Nonthaburi/74/2020|EPI_ISL_403963-crop, BetaCoV/Nonthaburi/61/2020|EPI_ISL_403962, BetaCoV/Wuhan/IVDC-HB-01/2019|EPI_ISL_402119, BetaCoV/Wuhan/IVDC-HB-04/2020|EPI_ISL_402120, BetaCoV/Wuhan/IVDC-HB-05/2019| EPI_ISL_402121, BetaCoV/Wuhan/IPBCAMS-WH-01/2019|EPI_ISL_402123, BetaCoV/ Wuhan/WIV04/2019 |EPI_ISL_402124, BetaCoV/Wuhan-Hu-1/2019|EPI_ISL_402125 и короткий фрагмент BetaCoV/Kanagawa/1/2020|EPI_ISL_402126). We used Mega X software (Clustal W algorithm) to align genome of the new SARS-CoV-2 coronavirus and those of other coronaviruses. To select diagnostic primers and a probe, we identified RdRp, a genome region around the RNA-dependent RNA polymerase of the coronavirus, position 15643-15778 under the MN985325 sequence. At the time of development of the system, this region was conservative to all the known SARS-CoV-2 genomes. Moreover, it differed significantly (nucleotide differences) from the genome sequences of other closely related coronaviruses, including SARS-CoV.

We designed the primers and the probe in conformity with the standard oligonucleotide primers and TaqMan probes selection requirements [9, 10], relying on the Oligo Calc online resources: Oligonucleotide Properties Calculator [11] and OligoAnalyzer Tool [12]. Thermodynamic characteristics of fluorescent probes and their secondary structures were assessed with the help of The mfold Web Server online service [13]. 6-Carboxyrodamine (R6G) with a black-hole quencher 1 (BHQ1) and carboxyfluorescein (FAM) with BHQ1 were used as fluorophores for the probes. AO Genterra synthesized primers and probes.

AmpliTest SARS-CoV-2, the set of SARS-CoV-2 RNA detection reagents under development, covers all stages of testing: virus RNA extraction from samples, reverse transcription and PCR. The disease caused by the new SARS-CoV-2 virus mostly affects the respiratory tract, but in some cases patients suffered disorders in their intestines. Therefore, we examined three types of clinical material: nasopharynx and oropharynx mucous membrane swabs, sputum and feces.

Control samples were used to assess efficacy of the system at all stages of testing. The internal control sample (ICS) is an artificially synthesized recombinant RNA sequence, about 500 bp in length, enclosed in the ms2 phage envelope [14, 15]. ICS is added at the RNA extraction stage to all samples tested, which allows controlling the success of RNA extraction, reverse transcription and amplification. A positive control sample (PCS) is a recombinant RNA containing the SARS-CoV-2 genome target region measuring ~500 bp, in the ms2 bacteriophage envelope [14, 15]. PCS is introduced as a separate sample at the nucleic acid extraction stage. QX200 Droplet Digital PCR System (Bio-Rad Laboratories, USA) enabled measurement of ICS and PCS concentrations. Cleanliness of ICS and PCS of residual DNA was established through PCR without reverse transcription.

We followed the published clinical guidelines [16] in preparation of the samples of clinical material (smears and sputum). A slightly modified protocol was followed in preparation of feces: the clarified extract was obtained through thorough resuspension of 0.1 g (0.1 ml) of the material in 0.9 ml of phosphate buffer, then it was centrifuged for 5 min at 7000 g (MiniSpin, Eppendorf), with subsequent collection of the upper phase.

Treatment with guanidine isothiocyanate at 65 °C enabled extraction of nucleic acids, which was followed by the total DNA/RNA precipitation with isopropanol and glycogen as coprecipitation agent. The precipitate was washed to remove impurities and salts and then dissolved in a TE buffer with 0.02 mg/ml of potassium polyadenitate.

Reverse transcription and PCR were performed in one step. The volume of the reaction mixture was 50 µL. It contained the following components: 25 µL of RNA sample, 0.6 mM of each primer (AO Genterra, Russia), 0.3 mM of each probe (AO Genterra, Russia), 0.5 mM of each dNTP (Biosan, Russia) , 1 µl of TaqF polymerase (AO Genterra, Russia), 0.5 µl of TM-revertase (Mmlv) (AO Genterra, Russia), random primers — 0.15 mM (AO Genterra, Russia), polyA — 0.01 mg/ml (AO Genterra, Russia), sodium azide 0.05% (Sigma-Aldrich, USA), tris-HCl buffer (pH 8.3) with 70 mM of tris (oxymethyl)-aminomethane (Sigma-Aldrich, USA), magnesium chloride — no more than 5 mM (Sigma-Aldrich, USA), potassium chloride — no more than 80 mM (Sigma-Aldrich, USA), enzyme stabilizer — no more than 0.2 mg/ml (AO Genterra, Russia), sterile H_2O — up to 25 µl.

The format of RT PCR was multiplex, with the ICS fluorescence accumulation signal registered at the FAM fluorophore channel and the fluorescence accumulation signal associated with amplification of the target SARS-CoV-2 nucleic acid at the HEX fluorophore channel.

The amplification program included the following thermal cycling stages: 50 °C — 30 min; 95 °C — 15 min. The following stages were repeated for 45 cycles: 95 °C — 15 s, 60 °C — 30 s, 72 °C — 15 s. The temperature for detection at FAM/HEX fluorophore channels was 60 °C. Overall, the RT PCR process lasted about 2 hours. The result was evaluated with the help of the threshold method: Ct was determined by the intersection of the fluorescence curve and threshold line set in the middle of the fluorescence increase graph's exponential section (logarithmic scale). The amplification results were interpreted as positive if fluorescence curve crossed threshold line set at the needed level.

The analytical specificity of RT PCR with the selected primers and probe was evaluated in the study of RNA strains of human coronavirus 229E (ATCC® RV-740TM), Betacoronavirus 1 OC43 (ATCC® VR-1558™), influenza A virus (H1N1) (ATCC® VR-1469), influenza A virus (H3N2) (ATCC® VR-776) and influenza B virus (Victoria Lineage) (ATCC® VR-1930) from the ATCC® collection (American Type Culture Collection, USA), HCoV 229E, HCoV OC43, HCoV NI63, SARS-CoV HKU39849, MERS-CoV (European Virus Archive Global 011N-03868 — Coronavirus RNA specificity panel), DNA of Streptococcus pneumoniae strains (№ 131116), Streptococcus pyogenes (№ 130001), Haemophilus influenza (№ 151221), Staphylococcus aureus (№ 201108), Klebsiella pneumoniae (№ 180129) from the State Collection of Pathogenic Microorganisms of Scientific Centre for Expert Evaluation of Medicinal Products at the concentration of at least $1x10^6$ genomic equivalents in 1 ml (GE/ml).

Analytical sensitivity (detection threshold) was assessed on model samples of biological material (oropharynx and nasopharynx mucosa swabs, sputum, feces) with the addition of dilutions of the standard sample — protected recombinant RNA containing the target region of SARS-CoV-2 coronavirus genome, in the ms2 bacteriophage envelope. The following dilutions were used: 1×10^4, 5×10^3, 2×10^3, 1×10^3, 5×10^2, and 1×10^2 GE/ml. Each dilution was tested with 3 samples of each material, twice. The sensitivity threshold was set based on the minimum dilution detected in three takes.

We evaluated diagnostic indicators while analyzing all types of clinical material (oropharynx and nasopharynx mucosa swabs, sputum, feces) that was previously found to contain

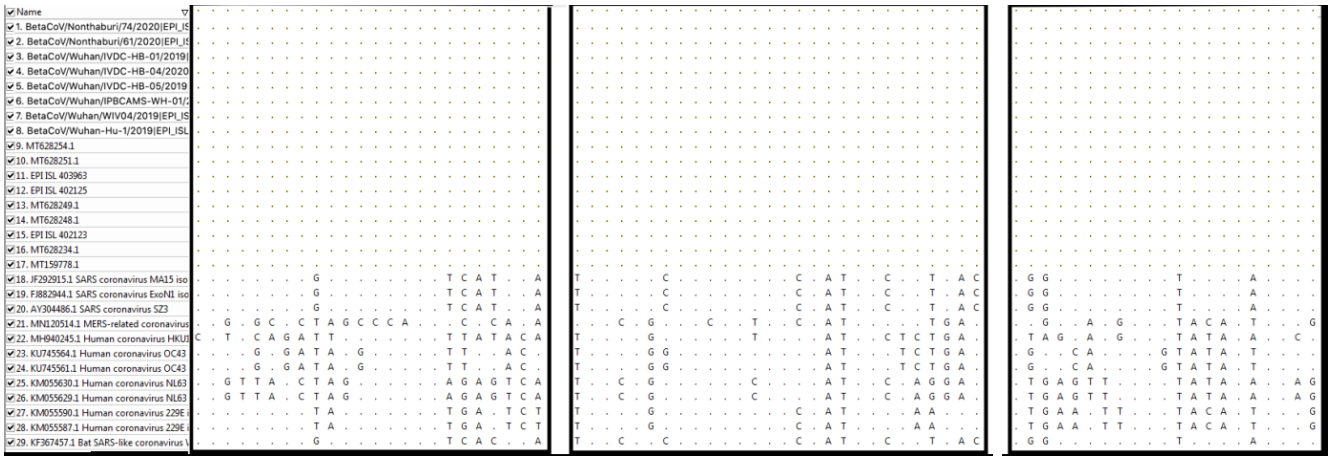

Fig. Genome sequence alignment, coronaviruses SARS-CoV-2, OC43, HKU1, SARS, MERS, 229E and NL63, in the area of primer and probe design.

or not contain SARS-CoV-2, as well as the material obtained from healthy people and patients with a different etiology of the disease, which was contaminated with the standard sample at a concentration of at least 10^3 GE/ml.

RESULTS AND DISCUSSION

The target we selected to enable SARS-CoV-2 RNA was RdRP, a gene of RNA-dependent RNA polymerase. The figure shows alignment of different sequences of SARS-CoV-2 and other coronaviruses at the site of primer and probe annealing. The nucleotide sequences in this region of SARS-CoV-2 genomes known at the time of development are completely identical; moreover, they have many differences with the genomes of other closely related coronaviruses, which ensures high specificity of the selected primers in terms of amplification of SARS-CoV-2 coronavirus RNA (Figure).

When we started developing this diagnostic system, there was no clinical material available in the Russian Federation (not a single case of COVID-19 was registered at the time). In this connection, we initially synthesized ~500 bp of RdRp gene region of the coronavirus genome. The target fragment of the coronavirus was cloned into a plasmid construct that allows obtaining recombinant RNA containing target region of genome of SARS-CoV-2 in the ms2 bacteriophage envelope [14, 15]. This recombinant RNA in the protein envelope served as PCS in the developed diagnostic system, which allowed evaluating effectiveness of all stages of testing.

To optimize the set of reagents part of the AmpliTest SARS-CoV-2 diagnostic system, we used the following PCR diagnostic devices registered in the Russian Federation as medical devices: Rotor-Gene Q (QIAGEN, Germany), CFX96 (Bio-Rad Laboratories, USA), Applied Biosystems QuantStudio 5 (Life Technologies Holdings Pte. Singapore), DTprime (DNK Tekhnologiya, Russia).

We used genetic material of other viruses and bacteria to assess specificity of AmpliTest SARS-CoV-2; these tests returned no cross-reactions, which confirmed 100% analytical specificity of the system. To control analytical sensitivity (detection threshold) of the reagents, we used model samples of biological material contaminated with the standard sample of ms2 recombinant bacteriophage containing a fragment of the SARS-CoV-2 genome. In case of nasopharynx and oropharynx membrane swabs, as well as sputum, the SARS-CoV-2 detection threshold was $1x10^3$ GE/ml, that for feces — $5x10^4$ GE/ml.

To assess diagnostic sensitivity and specificity, we used a sample of 115 model samples of various biological material (oropharynx and nasopharynx mucosa swabs, sputum, feces) contaminated with the standard sample to a concentration of at least 10^3 GE/ml, as well as 195 samples of biological material obtained from healthy people and patients suffering other pathologies. Later, with the spread of coronavirus infection in the Russian Federation, clinical trials were repeated. We examined 150 nasopharynx and oropharynx mucosa swabs, sputum and feces containing SARS-CoV-2 (samples obtained from patients with established COVID-19 infection), as well as 166 samples of biological material (same swabs, sputum, feces) that did not contain SARS-CoV-2 RNA (table 1). Diagnostic indicators (sensitivity and specificity) were at 100% (range from 94.2 to 100%, confidence level of 95%). Thus, we detected no false-positive and false-negative cases when assessing diagnostic sensitivity and specificity on samples from patients.

Coronaviruses are known to easily acquire new mutations [17]. Mutations in the regions of SARS-CoV-2 genome that are complementary to the primers and probe can translate into false-negative results or reduce sensitivity in detecting clinical isolates with nucleotide substitutions. To assess accumulation of mutations in the primer and probe regions, we compared them with the SARS-CoV-2 isolate sequences published in the GISAID database (multiple alignment of 50386 sequences

Table 1. Results of repeated clinical trials (assessment of diagnostic sensitivity and specificity) of the testing system

Sample type	Total samples tested	Device application results	
		Positive	Negative
Nasopharynx and oropharynx swabs	113	50	0
		0	63
Sputum	103	50	0
		0	53
Feces	100	50	0
		0	50

Table 2. Results of multiple alignment of 50386 known genome sequences of SARS-CoV-2 presented in the GISAID database, selected primers and probe regions. The columns indicate: 1 — sequence number of the nucleotide according to the MN985325 reference sequence, 2–5 identified polymorphisms among the analyzed sequences at this position, 6 — number of SARS-CoV-2 genome sequences in the GISAID database that do not differ at a given MN985325 position, 7 — number of sequences of SARS-CoV-2 GISAID genomes that differ in the given position, 8 — number of sequences of SARS-CoV-2 GISAID genomes that had no nucleotide established at the given position

sum.count GISAID=50386; ref MN985325					Number of GISAID seqs matching MN985325	Number of GISAID seqs differing from MN985325	Number of GISAID seqs for which there is no reliable reading
MN985325 nucleotide sequence	Polymorphisms						
	A	T	G	C			
1	2	3	4	5	6	7	8
Forward primer region							
15643	50359	0	0	0	50359	0	28
15644	2	0	50356	0	50356	2	29
15645	50361	0	0	0	50361	0	26
15646	50358	2	1	0	50358	3	26
15647	50358	0	1	1	50358	2	27
15648	0	50361	0	0	50361	0	26
15649	50370	0	0	0	50370	0	17
15650	0	0	50377	0	50377	0	10
15651	50380	0	0	0	50380	0	7
15652	2	0	50380	0	50380	2	5
15653	50376	0	5	0	50376	5	6
15654	0	50377	0	4	50377	4	6
15655	0	1	50378	0	50378	1	8
15656	0	50379	0	0	50379	0	8
15657	0	50379	0	0	50379	0	8
15658	0	2	50375	0	50375	2	10
15659	50380	0	0	0	50380	0	7
15660	0	16	0	50361	50361	16	10
15661	50378	0	0	0	50378	0	9
15662	0	7	0	50367	50367	7	13
15663	50374	0	3	0	50374	3	10
15664	0	0	50374	0	50374	0	13
15665	50377	0	0	0	50377	0	10
15666	0	0	0	50376	50376	0	11
Probe region							
15726	0	55	0	50317	50317	55	15
15727	0	0	50379	0	50379	0	8
15728	50381	0	0	0	50381	0	6
15729	0	50381	0	0	50381	0	6
15730	0	0	50381	0	50381	0	6
15731	0	0	0	50381	50381	0	6
15732	0	50381	0	0	50381	0	6
15733	1	0	50379	0	50379	1	7
15734	0	50379	0	0	50379	0	8
15735	0	50378	0	0	50378	0	9
15736	0	0	50379	0	50379	0	8
15737	0	50379	0	0	50379	0	8
15738	0	6	50373	0	50373	6	8
15739	0	50379	0	0	50379	0	8
15740	0	0	50378	0	50378	0	9
15741	0	50379	0	0	50379	0	8
15742	0	50379	0	0	50379	0	8
15743	0	50378	0	0	50378	0	9

15744	0	26	0	50340	50340	26	21
15745	50379	0	0	0	50379	0	8
15746	50379	0	0	0	50379	0	8
15747	0	50379	0	0	50379	0	8
15748	50378	0	0	0	50378	0	9
15749	1	0	50376	0	50376	1	10
15750	0	0	0	50377	50377	0	10
15751	50377	0	0	0	50377	0	10
15752	0	0	0	50377	50377	0	10
15753	0	50376	0	0	50376	0	11
Reverse primer region (complementary)							
15758	0	13	0	50361	50361	13	13
15759	50370	0	2	0	50370	2	15
15760	0	50371	0	0	50371	0	16
15761	1	1	0	50369	50369	2	16
15762	0	50370	0	1	50370	1	16
15763	0	0	0	50371	50360	0	16
15764	50370	1	0	0	50371	1	16
15765	50371	0	0	0	50371	0	16
15766	45	1	50325	0	50325	46	16
15767	0	0	50371	0	50371	0	16
15768	0	50371	0	0	50371	0	16
15769	0	10	0	50360	50360	10	17
15770	0	50370	0	0	50370	0	17
15771	50370	0	0	1	50370	1	16
15772	0	2	50368	0	50368	2	17
15773	1	50368	0	0	50368	1	18
15774	0	1	50371	0	50371	1	15
15775	1	1	50362	0	50362	2	23
15776	0	0	0	50371	50371	0	16
15777	0	50344	0	0	50344	0	43
15778	50366	0	0	0	50366	0	21

using MAFFT algorithm, available in the GISAID database as of June 30, 2020).

Table 2 shows presence of nucleotide polymorphisms in the primer and probe regions according to the alignment data for known SARS-CoV-2 genome sequences (as of June 30, 2020).

For the forward primer, 45 out of the known 50386 sequences were identified to have single polymorphisms (48 nucleotide differences; no more than 0.1% of the total). The substitutions are localized in the central region of the oligonucleotide (mainly one substitution per primer) and are not critical.

As for the reverse primer, there were 80 sequences (out of 50386) identified with one polymorphism in its region, of which 13 sequences have a G/A substitution at the 3' end (the substitution leads to the formation of C/A effective noncanonical interaction [18]).

There were also 82 sequences with polymorphisms in the region of the probe identified (C/T substitutions).

All the polymorphisms identified belong to SARS-CoV-2 isolates found all over the world, mainly in the USA, Australia, England, the Netherlands, Switzerland, and China. The analysis of 237 known Russian isolates found in GISAID in the regions of forward and reverse primers revealed no nucleotide differences. One local isolate (hCoV-19 / Russia / StPetersburg-RII8955S / 2020 | EPI_ISL_450) has a G/A nucleotide difference in the region of the probe, but this substitution is not critical, since it allows a fairly stable noncanonical C/A interaction of the probe with the matrix [18], and lies close to the 3' end of the probe.

The results obtained indicate there are no nucleotide differences critical to PCR diagnostics in the regions of primers and probes peculiar to all known SARS-CoV-2 isolates (low prevalence of polymorphisms, maximum of one or two substitutions for an isolate, formation of stable noncanonical pairs).

Thus, the analysis of genomes of all known isolates of SARS-CoV-2 revealed current high reliability of the developed AmpliTest SARS-CoV-2 diagnostic PCR system, which detects RNA of coronavirus in the vast majority of cases.

However, it should be noted that the high variability of coronavirus genomes suggests the need for constant monitoring of the accumulation of mutations in the primer and probe regions of new isolates, this monitoring allowing timely introduction of changes to the sequence of the oligonucleotides used ensuring high sensitivity of the system.

CONCLUSION

Specialists of Strategic Planning Center, Federal State Budgetary Institution under the Federal Medical-Biological Agency of Russia, developed AmpliTest SARS-CoV-2, a system (including a set of reagents) to detect RNA of SARS-CoV-2, a coronavirus causing severe acute respiratory syndrome (COVID-19). The set makes use of RT PCR in real time and enables control over all stages of testing. When the system

was developed, there were no sets of reagents detecting RNA of the new coronavirus registered in Russia. The system was registered as a medical device on 06.03.2020, registration certificate № RZN 2020/9765; on 30.06.2020 changes were made to the registration documents.

Technical and clinical laboratory investigation, as well as clinical practice of its use for testing purposes, confirmed high analytical and diagnostic sensitivity of the system, which makes it a promising device for timely detection of COVID-19. Today, the system is widely used in the Russian Federation.

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GENETIC DETERMINANTS OF THE RESPONSE TO CORONAVIRUS INFECTION COVID-19

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The heterogeneity of the COVID-19 clinical manifestation may be associated with the characteristics of the genome of both humans and the virus. A combination of allelic variants of genes associated with viral life cycle can determine susceptibility to SARS-CoV-2 infection. Allelic variants in genes ACE1, ACE2, TMPRSS2, IL6, SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6, and XCR1 can determine the severity of COVID-19. Analysis of the genomes of COVID-19 patients with different clinical course and development on their basis of model for stratification of people according to the degree of susceptibility and severity of manifestation will make it possible to develop a personalized approach for the prevention and treatment of COVID-19.

Keywords: COVID-19, SARS-CoV-2, genomic determinants, genome, genomic variants, alleles, pathogenesis, SARS-COV-2

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ГЕНЕТИЧЕСКИЕ ДЕТЕРМИНАНТЫ ОТВЕТА НА КОРОНАВИРУСНУЮ ИНФЕКЦИЮ COVID-19

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Гетерогенность клинического проявления COVID-19 может быть связана с особенностями генома как человека, так и вируса. Сочетание аллельных вариантов генов, связанных с жизненным циклом вируса, могут определять чувствительность к инфекции SARS-CoV-2. Аллельные варианты в генах ACE1, ACE2, TMPRSS2, IL6, SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6, и XCR1 могут определять тяжесть течения COVID-19. Анализ геномов разных по клинической картине пациентов с COVID-19 и создание на их основе модели стратификации людей по степени чувствительности и тяжести проявления позволят разработать персонализированный подход для профилактики и лечению COVID-19.

Ключевые слова: SARS-COV-2, COVID-19, геномные детерминанты, геном, геномные варианты, аллели, патогенез

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The novel infection caused by the SARS-CoV-2 coronavirus has become one of the global challenges to mankind in the 21st century. Since mid-February 2020 the infection started spreading quickly across the globe. In March the WHO announced the COVID-19 outbreak a pandemic. By June (at the time of writing), over 13 million confirmed cases were recorded, more than half a million infected people died.

The causative agent for COVID-19 is the novel SARS-CoV-2 virus belonging to the betacoronaviruses group, also comprising SARS-CoV. In 2002, SARS-CoV caused an outbreak of coronavirus infection resulting in atypical pneumonia and severe acute respiratory syndrome (SARS).

High transmission rate and tissue tropism make SARS-CoV-2 a dangerous pathogen. Furthermore, the virus is capable of inflicting damage to other tissues and organs (blood vessels, kidney, central nervous system, intestines). One of the coronavirus infection properties is a markedly varied clinical course: from asymptomatic to extremely severe, associated with multiple organ failure. Presumably, the clinical course of COVID-19 in each individual patient result from the genetic characteristics of both patient and virus determining the nature of their interaction. Identification of these characteristics will make it possible to develop the COVID-19 complications risk stratification model and will become a basis for the tailor-made prevention and treatment of the infection caused by SARS-CoV-2.

1. SARS-CoV-2 genomic structure

The SARS-CoV-2 coronavirus genome is ~ 30,000 nucleotides long. Unlike other highly pathogenic viruses causing severe disease in humans (SARS-CoV and MERS-CoV) this virus has a higher rate of transmission. The origin of the virus still remains unclear. The comparative data analysis has shown that SARS-CoV-2 could emerge due to recombination between the pangolin coronavirus Pangolin-CoV and the bat coronavirus RaTG13 [1]. The receptor binding domain of the Pangolin-CoV spike (S) protein has high sequence similarity with SARS-CoV-2: six inputs of the virus responsible for the major cell receptor binding have essentially identical sequences. The primary amino acid sequence of the novel coronavirus receptor binding domain is different from those of SARS-CoV, therefore, the receptor binding affinity of the SARS-CoV S protein is 10 times higher compared to the SARS-CoV S protein [2].

2. SARS-CoV-2 life cycle

SARS-CoV-2 employs a number of receptors for cellular entry. The angiotensin-converting enzyme 2 (ACE2) is a major receptor for SARS-CoV-2 [3]. In addition to the major receptor, the virus may employ other cell proteins, such as CD147 and GRP78, as additional receptors. For efficient host cell penetration, the SARS-CoV-2 spike (S) protein should undergo the proteolytic activation by the following cell proteases: furin and cellular serine protease TMPRSS2 [4].

Apart from that, SARS-CoV-2 may use the endosomal way of cell entry involving the cathepsin L protease [5]. Once the virus gets inside the cell, it reprograms the host cell's biosynthetic pathways for its own use, exploiting various cell proteins and forming the interactome (whole set of molecular interactions in a particular cell) of viral proteins and RNA with host factors [6].

3. Polymorphisms in SARS-CoV-2 genome and their impact on biological properties of the virus

Since the first SARS-CoV-2 genome sequencing until present, tens of thousands SARS-CoV-2 full-genome sequences have been obtained from different regions of the world. The data on the newly sequenced SARS-CoV-2 isolates is deposited in

the GISAID dataset (https://www.gisaid.org/) containing over 63,000 SARS-COV-2 full-genome sequences (at mid-July).

Two major clades were identified based on the differences between the nucleotide sequences of the viruses which circulated in late 2019–early 2020. Clade I included subclades characterized by amino acid substitutions in the proteins ORF3a: p.251G>V or S: p.614D>G. Clade II was distinguished from clade I by the following mutations: substitution in protein ORF8: p.84L>S (28144T>C) and protein ORF1ab: p.2839S (8782C>T) [7].

The S protein mutation characterized by aspartic acid to glycine shift at the amino acid position 614 (614G) related to clade I attracted more and more attention as more data were accumulated. The explosive outbreak of the described variant of the virus was observed in Italy in late February. Currently, the viruses carrying mutation G614 are widespread all over the world. While in March the described substitution rate in viral genomes was 26%, in April it was 65%, and in May the mutation rate reached 70%. The G614 genotype may be associated with higher viral load in infected patients resulting in higher transmissibility of the virus. Currently, the role of the G614 variant, and its biological properties (including transmissibility) are being actively studied [8].

During the pandemic, various polymorphisms in both structural and non-structural proteins possibly affecting the biological properties of the virus were reported. For example, the nucleocapsid (N) protein polymorphism at positions 203 and 204 (R203K/G204R) was able to reduce the binding of antigenic peptide to HLA-C*07 prevailing in European population [9]. It has been reported that mutation N501T in the SARS-CoV-2 S protein can significantly enhance binding to ACE2 [10]. Based on 10 most common mutations (mutation rate over 5%), the SARS-CoV-2 genomes can be divided into several major groups:

- Group 1 carries both a missense mutation (ORF8:c.251tTa>tCa) and a synonymous mutation (orf1ab:c.8517agC>agT).
- Group 2 carries three mutations, including the missense variant S (c.1841gAt>gGt), the ORF1AB upstream gene variant and the synonymous variant ORF1AB: c.2772ttC>ttT.
- Group 3 carries a nucleotide substitution (orf1ab:c.10818ttG>ttT).
- Group 4 carries a new missense mutation (ORF3a:c.752gGt>gTt) first detected in China.

Isolates from France and other countries carry mutations ORF3a: c.752gGt>gTt, often tohether with mutation S: c.1099Gtc>Ttc [11]. To date, hundreds of SARS-CoV-2 gene polymorphisms have been reported, and the new polymorphisms are still being identified. This may indicate the continuous process of evolution and adaptation of the virus to new host species.

4. Pleiotropic spectrum of COVID-19 manifestations is associated with distinct human genome features

One of the major COVID-19 infection features is a markedly varied clinical course: from asymptomatic patients to patients with acute respiratory distress syndrome (ARDS) and multiple organ failure. Such clinical manifestations diversity could be hardly explained by the variability of the virus, taking into account its negligible genetic variability compared to other RNA viruses. Many studies have been conducted aimed at the search for host factors essential for the life cycle of the virus, especially for the host cell entry. For example, the studies of the ACE2 and TMPRSS2 receptors expression patterns in various tissues and organs were carried out at single-cell resolution, which demonstrated that the described receptors expression could be observed not only in the cells of respiratory epithelium and lungs, but also in the intestinal epithelial cells, cardiomyocytes, hepatocytes and neurons [12]. Apparently, specific expression patterns of these receptors may determine the COVID-19 course.

The course of COVID-19 may be also defined by many other factors, such as comorbidities and environmental factors. It is hypothesised that genetic determinants (sets of gene variants responsible for body's response to SARS-CoV-2 infection) play a vital part in susceptibility to the virus.

5. Genetic determinants of susceptibility to COVID-19

Expression of receptors and host factors essential to follow the basic viral life cycle stages is a major factor in the body's susceptibility to coronavirus. The presence of receptor together with co-receptors is important for the effective viral penetration into the target cell. Thus, co-expression of ACE2 and TMPRSS2 may define the target cells for coronavirus. A number of studies using scRNA have demonstrated that different ACE2 and TMPRSS2 co-expression patterns are observed in various human cells, tissues and organs. This can explain the heterogeneity of the COVID-19 clinical manifestation, when the pathogenesis involves not only lungs, but also other organs: liver, kidney, intestines, blood vessels, myocardium and brain [13, 14].

The ACE2 and TMRSS2 expression in normal cells is low. However, pulmonary disorders and exposure to pollutants and toxic chemicals are usually associated with increased expression of these receptors. Susceptibility of such cells to viral infection is higher compared to normal cells. That can explain the higher proportion of infection and more severe course of the disease in people with comorbidities.

One of the main properties of the virus is the ability to infect cells of the immune system and cause the immunodeficiency disorders [15]. Allelic variations determining the structure of proteins encoded by these genes, as well as the variants in the regulatory non-coding regions affect the expression, contribute to the body's antiviral response and determine the severity of the disease.

The following international consortia facilitating the response to SARS-CoV-2 genetics basis research has been created for identification of such factors:

– COVID-19 Host Genetics Initiative [16];

– COVID-19 Genomics UK (COG-UK) Consortium [17], etc.

Genes and allelic variants most probably associated with COVID-19 susceptibility and severity are listed in the :tab_1;.

ACE2 gene variants and susceptibility to SARS-CoV-2

Carriers of different allelic variants in the ACE2 gene coding region demonstrate different viral spike (S) protein binding affinity. For example, alleles rs73635825 (S19P) and rs143936283 (E329G) significantly differ in the SARS-CoV-2 S protein binding affinity [17]. Both "dangerous" ACE2 alleles increasing the binding affinity of ACE2 to the S protein (S19P, I21V, E23K, K26R, T27A, N64K, T92I, Q102P and H378R) and "protective" ACE2 variants (K31R, N33I, H34R, E35K, E37K, D38V, Y50F, N51S, M62V, K68E, F72V, Y83H, G326E, G352V, D355N, Q388L and D509Y) decreasing the efficiency of receptor binding to the SARS-CoV-2 S protein have been reported [18]. The study of Italian population revealed some rare "protective" missense variants of ACE2 gene: p.Asn720Asp, p.Lys26Arg, p.Gly211Arg (MAF 0.002 to 0.015). These variants were able

to interfere with binding to the viral S protein. However, it should be remembered that recent analysis of 1000 genomes from the UK Biobank revealed no relationship between the COVID-19 severity and the variants in the ACE2 and TMPRSS2 genes [19].

TMPRSS2 gene variants and susceptibility to SARS-CoV-2

The TMPRSS2 cellular serine protease is essential for the SARS-CoV-2 S protein proteolytic activation needed for host cell entry. Differential expression of TMPRSS2 may determine the tissue specific virus–host interaction playing a vital part in susceptibility to viral infection.

Thus, the lung-specific loci variants affecting the expression profiles (eQTL) associated with the TMPRSS2 expression may be responsible for different susceptibility and response to SARS-CoV-2 infection. It has been shown that the eQTL variant rs35074065 is associated with higher expression of TMPRSS2 and low expression of the interferon-induced MX1 gene [20]. A number of alleles associated with increased expression of TMPRSS2 (for example, rs2070788, rs9974589, rs7364083) are common in European population [21]. The eQTL most common in Europeans (rs8134378) located near the androgen-dependent enhancer TMPRSS2 may be associated with increased TMPRSS2 expression in men [22]. Despite the predicted associations between the ACE2 and TMPRSS2 allelic variants, the recently published report has confirmed no association between the described variants and the COVID-19 susceptibility [23].

HLA and immune response features, immunodepletion in patients with coronavirus infection

It has been reported that the ability to bind and present antigens is a key point of effective immune response mobilization. Various MHC molecules (HLA molecules) bind to the viral proteins' antigenic peptides emerging in different way during the disease. This may explain the differences in the ability to develop an immune response between individuals.

Carriers of the HLA-B*46:01 variant had a few predicted binding peptides for SARS-CoV-2, suggesting that individuals with that allele might be particularly vulnerable to COVID-19. For example, it was shown that in people with such genotype the SARS-CoV course was more severe [24]. Among all HLA Class 1 alleles, HLA-A*02:02, HLA-B*15:03 and HLA-C*12:03 bind to the maximum range of the SARS-CoV-2 conserved antigenic peptides. On the contrary, alleles A*25:01, B*46:01, 150 C*01:02 HLA-A, -B, and -C are responsible for binding to the minimum range of antigenic peptides.

It should be noted that when presenting the antigenic peptides of 8–13 amino acids in length, the 44 peptides are highly conserved and are found in all coronaviruses, including the common human coronaviruses (OC43, HKU1, NL63 and 229E) [24].

6. Genetic determinants of COVID-19 severity and comorbidities

As mentioned above, the heterogeneity of the COVID-19 clinical manifestation may be associated with the differences between the allelic variants of genes not required for the viral life strategy implementation. The recent genome-wide association study (GWAS) aimed at the search for relationship between genes and COVID-19 severity in patients from Italy and Spain has revealed loci and genomic variants responsible for discrimination of patients based on the disease severity. One of these is locus 3p21.31, which comprises genes SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6 and XCR1. Genes of the locus 3p21.31 are associated with chemokines and the movement of immune cells toward sites of inflammation. It should be noted that gene SLC6A20 located within the described locus physically and functionally interacts with ACE2 and is able to modulate the properties of the receptor. The other detected locus (9q34) is associated with inheritance of AB0 blood type antigens [26]. The relationship between the COVID-19 severity and the rs8176747, rs41302905 and rs8176719 alleles defining the blood type in Chinese population has been previously reported. It has been shown that patients with blood type O have a decreased risk of severe infection, and patients with blood type A are vulnerable to severe COVID-19 [27].

Genetic variants involved in inflammatory response

In patients with COVID-19, a complex of factors contributes to the excessive inflammatory response and the cytokine storm syndrome. That has been confirmed by recent histological analysis of samples acquired from the post-mortem examination of patients with fatal COVID-19. The study has demonstrated that excessive inflammatory response is the most common cause of death in COVID-19 patients [34].

One of the major clinical manifestations of pneumonia is high level of proinflammatory cytokines (IL-6, TNF-α and IL1-β) and acute phase proteins (APPs). Allelic variants in genes responsible for inflammatory response may affect the disease severity. For IL-6, the correlation with IL-6-174C allele associated with high IL-6 level and severe course of pneumonia has been revealed in patients with severe COVID-19 [28]. Polymorphism of the C3 gene encoding the complement

Table 1. Genes and allelic variants possibly associated with susceptibility to COVID-19

Molecular pathway	Genes/genomic variants
Cell entry (receptor)	ACE2 rs73635825 (S19P) и rs143936283 (E329G)
Cell entry (receptor-protease)	TMPRSS2 rs2070788, rs9974589, rs7364083, eQTL (rs8134378),
Cell entry (protease)	TMPRSS4
Cell entry (protease)	Cathepsin L
Cell entry (protease)	Furin
Protease	PLG (plasmin)
Cell entry (co-receptor)	CD147
Cell entry (antiviral restriction factor)	GILT
Presentation of viral antigens	HLA -A*02:02, HLA -B*15:03 ,HLA -C*12:03, , HLA-A*25:01, HLA-B*46:01, HLA- C*01:02
Inflammatory response	IL6
Inflammatory response	IL1B

component 3 (C3) together with ACE1 allelic variant may also contribute to the COVID-19 severity [29].

Single nucleotide polymorphism (SNP) allele frequencies may vary among people of different ethnic groups. These are also associated with various COVID-19 susceptibility and severity. For example, the CCR5 Δ32 variant is associated with severe COVID-19 in patients of European origin [30]. The study of gene expression profiles in the infected lung cells has revealed a number of genes related to monocyte colony-stimulating factor 2 (CSF2), pro-inflammatory cytokine cascades and calcium-binding proteins S100A8 and S100A9 [31].

Genetic variants involved in coagulation pathway

The prevalence of coagulation disorders in patients with COVID-19 may be associated with gene variants involved in blood coagulation cascade. Elevated plasmin and plasminogen known to potentially promote the coronavirus S protein proteolytic activation are also associated with increased susceptibility to COVID-19 [32].

Genetic variants involved in antiviral response

The virus–host cell interaction induces the specific antiviral response by the viral RNA sensors activation, which leads to activation of the interferon synthesis pathway. The secreted interferons induce the interferon-stimulated genes (ISG) activation via interaction with receptors. This confers resistance to viral infection. The described response involves more than a hundred factors, both sensors RIG-I, MDA5, MAVS, STING, cGAS, TLR3, TLR9, TRIM25, RNF166, and effectors IFNa, IFNb, IFN-λ, OAS1, MX1 and IFITM3. The role of these genes' allelic variants currently remains unknown. It has been shown that the variant rs12252 of the IFITM3 gene may be associated with excessive inflammatory response and severe COVID-19 [33].

CONCLUSION

The extensive genome-wide association studies launched by the international consortia are important steps in the process of the novel coronavirus infection pathogenesis investigation. Sample size increase together with various ethnic groups' analysis will make it possible to identify the unique rare allelic variants responsible for susceptibility to COVID-19. Reconstructing the allelic variants' cumulative contribution to complex gene networks regulating the antiviral response might shed some light on the COVID-19 pathogenesis and help to create a genetic risk prediction model allowing one to define the probability of severe COVID-19

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JUSTIFICATION OF THE POSSIBLE DIRECTIONS OF PATHOGENETIC THERAPY OF A NEW CORONAVIRUS INFECTION

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The article analyses different stages of COVID-19 pathogenesis that drive development of severe complications, including acute respiratory distress syndrome, multiple organ failure and endotoxicosis. COVID-19 progression is described, from the first moments of infection to the disruption of the alveolar-capillary barrier and acute respiratory distress syndrome. The article looks at the causes initiating pathological processes that lead to acute respiratory distress syndrome. The special focus is on oxidative stress, hyperreactivity of the immune system, endothelial dysfunction, and cytotoxic effects of the virus. The article discusses tentative treatments for COVID-19 at different stages of its pathogenesis.

Keywords: coronavirus, medication therapy, endothelial dysfunction, SARS-CoV-2, COVID-19, acute respiratory distress syndrome, oxidative stress, SARS-COV-2

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ОБОСНОВАНИЕ ВОЗМОЖНЫХ НАПРАВЛЕНИЙ ПАТОГЕНЕТИЧЕСКОЙ ТЕРАПИИ НОВОЙ КОРОНАВИРУСНОЙ ИНФЕКЦИИ

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В статье анализируются звенья патогенеза новой коронавирусной инфекции, приводящие к тяжелым клиническим проявлениям заболевания — острому респираторному дистресс-синдрому, полиорганной недостаточности и эндотоксикозу. Представлена последовательность развития инфекционного процесса с момента попадания вируса в организм из внешней среды до повреждения альвеолярно-капиллярного барьера и развития острого респираторного дистресс-синдрома. Описаны факторы инициации патологических процессов, приводящих к развитию острого респираторного дистресс-синдрома, среди которых особое внимание уделено оксидативному стрессу, гиперреактивности иммунной системы, эндотелиальной дисфункции и цитотоксическому действию вируса. Обсуждаются возможные фармакотерапевтические направления лечения COVID-19 с учетом разных звеньев патогенеза.

Ключевые слова: коронавирус, фармакотерапия, эндотелиальная дисфункция, SARS-CoV-2, COVID-19, острый респираторный дистресс-синдром, оксидативный стресс, SARS-COV-2

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At the end of December 2019, the first cases of atypical pneumonia that was clinically different from all previously known viral pneumonias were reported in Wuhan, China. The rapid spread of the novel RNA SARS-CoV-2 (Severe Acute Respiratory Syndrome-related Coronavirus 2) to other countries in March 2020 pressured the World Health Organization to declare a pandemic [1]. According to the data collected by the WHO's monitoring center, the incidence of the novel coronavirus infection (COVID-19) is obstinately high. Diagnostic and therapeutic approaches used in patients with COVID-19 are largely standardized and work well in most cases; however, the unrelenting death rates observed in patients with severe infection raise some questions about their efficacy [2]. So far no consensus has been reached in the scientific community about the pathogenesis of COVID-19, and the mechanisms underlying progression to irreversible complications are not fully clear [3]. To contain the spread of the disease and minimize its consequences, systematic updates are needed on the international literature about the clinical course variations of COVID-19 and the efficacy of proposed treatments, including medication therapy at different stages of its pathogenesis. According to the experts of the Russian Ministry of Healthcare, the main approach to the management of patients with COVID-19 should be preventive therapy aimed at avoiding disease progression to overt life-threatening symptoms, such as pneumonia, acute respiratory distress syndrome (ARDS) and sepsis.

The aim of this study was to determine promising approaches to COVID-19 treatment at different stages of the disease based on the analysis and systematization of the accumulated data about the pathogenesis of the novel coronavirus infection.

Main part

The primary routes of human-to-human SARS-CoV-2 transmission are via respiratory droplets or dust particles and indirect physical contact mediated by hands or fomites followed by the virus landing on mucous membranes. The fecal-oral route is also possible when the virus enters the gastrointestinal tract with food or following hand contact with contaminated surfaces; there, SARS-CoV-2 adheres to the mucous membrane of the esophagus, stomach or upper small intestine

[4]. Besides, transplacental transmission cannot be excluded [5]; however, there have been no reports of intrauterine SARS-CoV-2 infection in Russia; a few reported cases of neonatal COVID-19 were attributed to the postnatal exposure to the virus [6].

Exogenous factors play a significant role in promoting infection, including chronic intoxication with psychoactive drugs that substantially increases the risk of SARS-CoV-2 infection and worsens its course [7]. For example, smoking spurs progression of the novel coronavirus infection and increases the risk of its aggravated course [8]; alcohol abuse can compromise the immunity and increase both the risk of infection and its complications [9]. Apart from exogenous factors, severity of COVID-19 is determined by pre-existing conditions, including cancer, hypertension, diabetes mellitus, chronic obstructive pulmonary disease and other disorders that under certain conditions cause irreversible systemic damage [10].

Standard antiviral prophylaxis is very effective in preventing the spread of the infection. Social distancing (1.5–2m) and good personal/respiratory hygiene are simple yet effective measures recognized worldwide. However, their effect should be reinforced with viricidal disinfectants [11].

The infection starts to unfold once SARS-CoV-2 comes in contact with the sialic acid-producing mucosal epithelium of the conjunctiva, the nasal/oral cavity, the respiratory tract, and the upper gastrointestinal tract [12]. Sialic acids constituting transmembrane glycoproteins are targets for the viral hemagglutinin-esterase, which is a surface protein of the viral envelope that facilitates viral entry into the cell and is required for virus replication. When the infected mucosal cells release a massive amount of new virions and proinflammatory factors, the virus can enter the bloodstream and travel to tissues containing its cellular targets; this leads to the development of clinical symptoms following the incubation period [13].

Transmembrane glycoproteins are the cellular gateway for SARS-CoV. Angiotensin-converting enzyme 2 (ACE2) has been identified as the main receptor for the novel coronavirus, as well as for the older SARS-CoV strain [5, 14, 15]. Its primary function is to regulate the activity of angiotensin II, which increases smooth muscle tone and affects heart and kidney function. ACE2 cleaves off one amino acid from angiotensin II, thereby altering its properties: the resultant molecule interacts more actively with angiotensin II membrane receptors, causing pronounced short-term local vasoconstriction. Besides, ACE2 modulates amino acid transport across the cell membrane by acting as a chaperone for one of amino acid transporters. The interaction between the membrane domain of ACE2 and the amino acid transporter on the internal side of the cell membrane facilitates viral invasion. ACE2 is predominantly expressed on the surface of type II alveolar cells, making them susceptible targets for SARS-CoV-2 [16]. Other ACE2-expressing cells (type I alveolar cells, macrophages, pulmonary/cerebral/cardiac/renal vascular endothelial cell, epithelial cells of the bronchi and bronchioles, esophagus, duodenum, ileum, and bladder, pancreatic cells, cortical and brain stem neurons) total to 20% of potential targets that the novel coronavirus can infect using ACE2 as an entry point.

According to some studies, one more glycoprotein from the transmembrane serine protease family is needed to mediate viral entry into the cell through ACE2 [17]. This protease is found in the vicinity of calcium channels, close to ACE2, and is activated through contact with a receptor-binding domain of the virus, cathepsin or under lowered pH; the protease fosters fusion of the virus envelope with the cell membrane [18]. Another mediator of the fusion process is furin, the protease that cleaves proteins at paired basic amino acids sites and participates in viral protein processing (maturation) [19].

Basigin, the transmembrane glycoprotein found in most cells, is another entry point for SARS-CoV-2. It is also known as cluster of differentiation 147 (CD147) or extracellular matrix metalloproteinase (MMP) inducer [17, 19]. The spike proteins of the coronavirus (spike-shaped protrusions from its surface) bind to basigin, as is also the case with ACE2. The main functions of basigin are MMP activation, participation in cell-cell interactions and spermiogenesis. Basigin effects are exerted through pronounced activation of blood monocytes, platelets and S-selectin, release of positively charged collagen and formation of parietal thrombi, followed by a reduction in permeability of capillary tissue barriers and macrophages migration from the bloodstream to the site of inflammation. This protein is chiefly found in erythrocytes, lymphocytes, retinal cells, fibroblasts, epithelial, endothelial and prostate cells [20]. Perhaps, this mechanism of viral entry and subsequent virus replication is implicated in coagulation disorders and formation of intravascular thrombi in organs affected by the virus, especially in the setting of endothelial dysfunction.

Another transmembrane glycoprotein that SARS-CoV-2 can bind to is a CD26 surface antigen, also known as DDP4 (dipeptidyl peptidase 4); it is a serine exopeptidase that cleaves proline- and alanine-containing dipeptides from the N-terminus of a protein molecule [21]. The ability of this glycoprotein to participate in coronavirus trafficking into the intracellular compartment was previously confirmed for MERS-CoV, the causative agent of the Middle East Respiratory Syndrome [22].

The mechanism of the novel coronavirus infection is shown in Fig. 1.

In case of any respiratory viral infection, it is essential to boost the local immune defenses as early as possible at the sites of primary contact of the virus with mucous membranes [23]. Therefore, it is advisable to use therapeutic agents that stimulate local and systemic immunity so as to inhibit replication of SARS-CoV-2 in the earliest stages of infection, before the onset of pronounced clinical symptoms. The list of such medications includes interferons exerting antiviral, immunostimulatory and antiproliferative effects, peptide and synthetic immunomodulators enhancing the bactericidal activity of neutrophils, and sodium nucleinate-based immunostimulatants that activate non-specific resistance. In his regard, the Russian Ministry of Health recommends nasal formulations of recombinant interferon α2b and interferon β1b in combination with lopinavir+ritonavir. At the site of inflammation, the virus can be inactivated with viricidal drugs used in combination therapy against viral pneumonias in patients without respiratory failure; such drugs might hold some promise for treating COVID-19. Fusion inhibitors and/or angiotensin II receptor blockers can prevent the virus from entering the target cell: their mechanism of action renders these drugs promising candidates for the combination therapy of viral pneumonias complicated by respiratory distress [24]. Soluble genetically engineered traps for the virus, which are currently in development, are another promising therapeutic option; these drugs are based on ACE2 protein fragments attached to the Fc region of human immunoglobulin IgG1 [25]. Viral replication can be stopped with inhibitors of viral proteases and RNA polymerases. However, the efficacy of medication therapy at the stage of viral replication is determined by a variety of factors, and at this point disease progression is not a rare thing. Considering the absence of validated treatments against the novel coronavirus, off-label drugs with antiviral potential might be worth giving a try [26]; their use must comply with

standards for ethical practice, WHO recommendations and current legislation.

Damage or death of a target cell is followed by the activation of alveolar macrophages and neutrophils, triggering chemokine release and migration of inflammatory cells into the interstitium. These events are followed by neutrophil infiltration of the affected site. The alveolar-capillary barrier becomes increasingly permeable, allowing some fluid to build up in alveoli and the pulmonary interstitium. At this stage, radiographic (CT) findings are consistent with specific pneumonia [27]. Further evolution of the disease will probably depend on a number of exogenous and endogenous factors.

Clinical course variations of the novel coronavirus infection progressing to pneumonia have been described in the guidance released by the Russian Ministry of Healthcare [11], FMBA guidelines [28] and other sources [1, 17, 29, 30]. COVID-19 can progress to pneumonia if the virus transmitted through the air invades the alveolar space (in this case, the target is type II alveolar cells) or when the course of the disease resembles that of influenza and the virus enters the bloodstream (in this case, it targets endothelial cells of pulmonary capillaries). Inflammation develops in parallel with oxidative stress, which sets in when the intensity of peroxidation and free radical oxidation induced by inflammation overpowers the capacity of enzymic and substrate mechanisms of anti-free radical and antioxidant defenses to negate detrimental effects on cell membranes; eventually, this leads to cell damage or death [31]. Mitochondrial dysfunction that plays a significant role in the development of neurodegenerative disorders is another contributor to cell damage under oxidative stress [32]. Here, medication therapy should be reinforced with antioxidants. Oxidative stress can be reduced by administering high doses of ascorbic acid, lipid and mitochondrial antioxidants, succinates, sulfhydryl donors, antioxidants with enzymic activity and reactive oxygen species inhibitors.

Pneumonia induced by SARS-CoV-2 is often accompanied by respiratory failure; once the infection becomes systemic, it initiates the acute respiratory distress syndrome (ARDS). ARDS is promoted by a combination of different factors, the leading factor being massive release of cytokines (the cytokine storm) from activated alveolar macrophages, neutrophils, endothelial and alveolar cells at the site of the primary infection of lung tissue; this leads to the rapid escalation of cell-membrane damage induced by free radicals [33, 34]. Generalized damage to target cells is accompanied by swift, pronounced activation of alveolar macrophages and

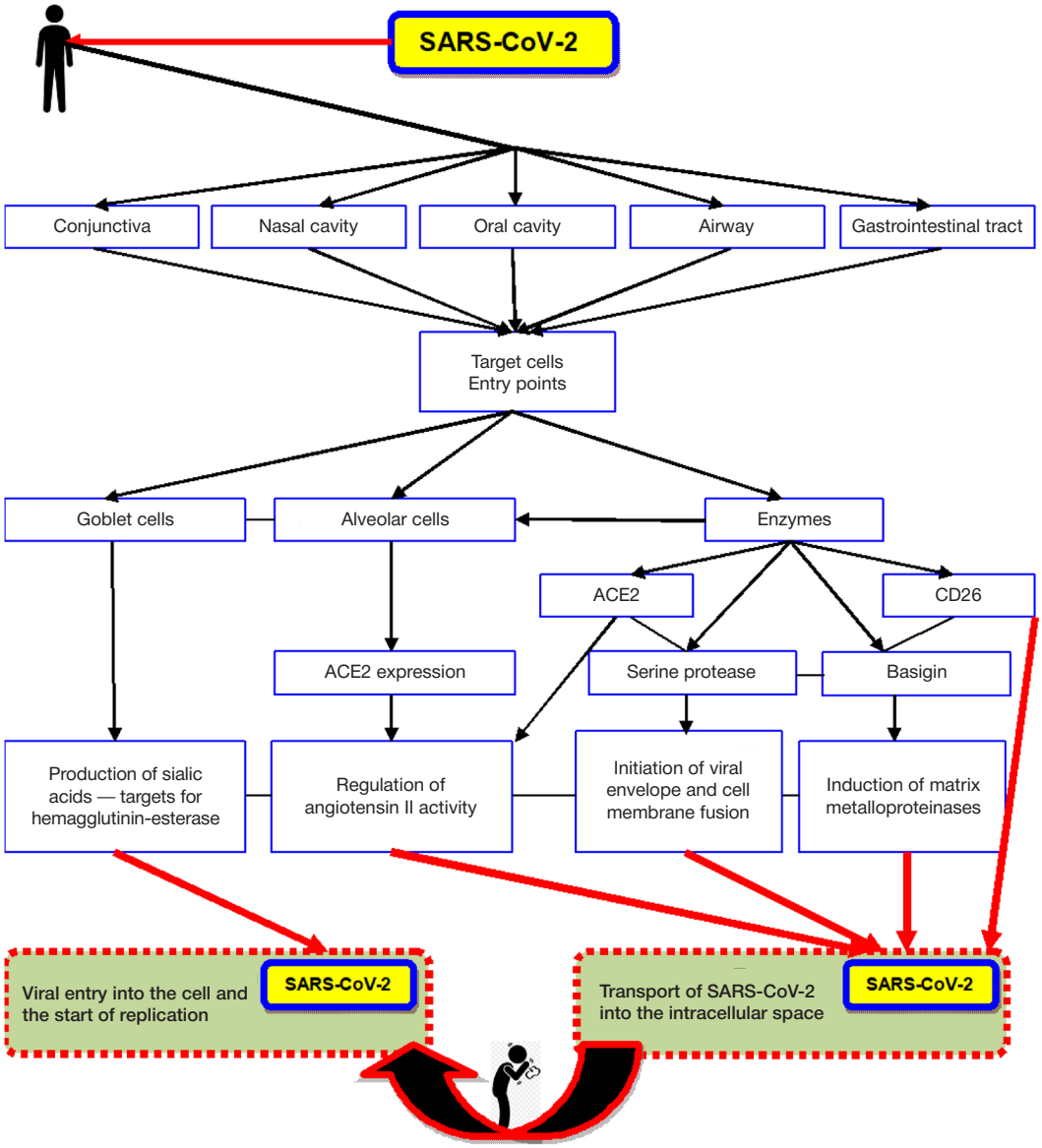

Fig. 1. Initiation of by the novel SARS-CoV virus

neutrophils, expression of proinflammatory cytokines (IL-1, IL-6. IL-10, tumor necrosis factor), production of prostaglandins and leukotrienes. Activated hyaluronidase and MMP digest the ground substance of the pulmonary interstitium, thereby disrupting the alveolar-capillary barrier. This results in a local microvascular spasm, elevated pulmonary blood pressure and vascular leakage into the interstitial space, causing interstitial edema and hampering gas exchange between alveoli and capillaries. Impaired gas exchange exacerbates hypoxemia; the patient develops respiratory acidosis. Carbon dioxide retention in the blood causes hyperstimulation of brainstem centers controlling respiratory and other autonomic functions of the body. Damaged pulmonary vascular endothelial cells begin to produce more endothelin, triggering the uncontrolled cascade of pathological events and leading to endotoxicosis and multiple organ failure [17].

The schematic representation of complications of the novel coronavirus infection is provided in Fig. 2

Direct damage to type II alveolar cells inflicted by SARS-CoV-2 disrupts pulmonary surfactant synthesis and destroys its monolayer as the surfactant is washed off from the alveolar surface by excess tissue fluid coming from the fluid-enriched interstitium [35], triggering alveolar collapse and loss of lung tissue elasticity. The tissue gets deformed, arteriovenous anastomoses open; oxygen-poor blood starts to prevail in pulmonary vessels. Impaired gas exchange caused by interstitial edema, fluid buildup in the alveoli, increasingly more unventilated alveoli, and open arteriovenous anastomoses

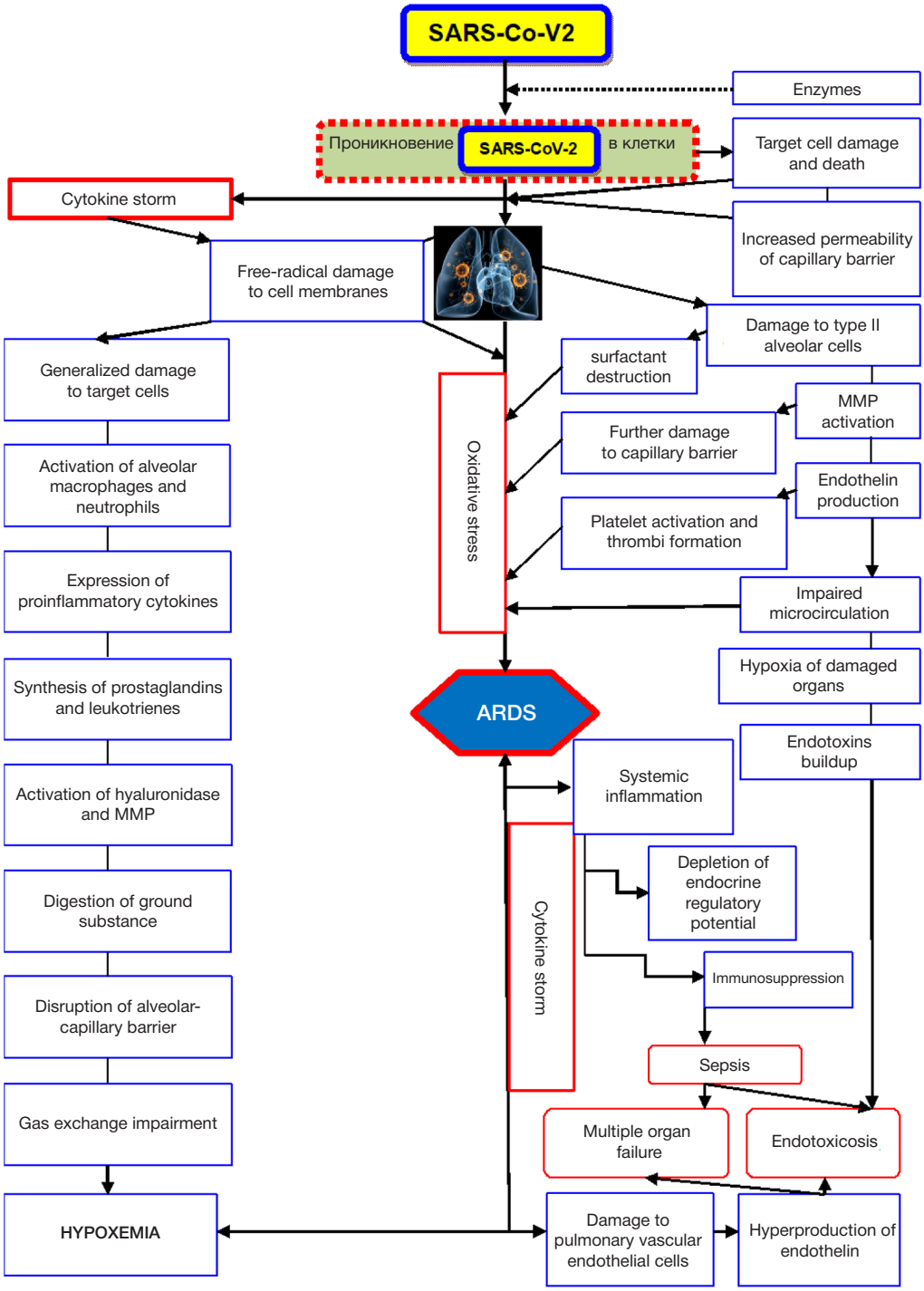

Fig. 2. The schematic representation of complications caused by the cascade of pathological responses to the novel coronavirus infection

leads to hypoxemia. Under hypoxemic conditions, plasminogen activators (urokinase) are inhibited and fibrinolysis is suppressed.

Progressing endothelin secretion precipitates pulmonary vasoconstriction, platelet activation and intravascular thrombosis. This process is intensified by fibrinolysis inhibition and affects microcirculation. In turn, hypoxemia and poor microcirculation lead to hypoxia in the affected organs, accumulation of metabolic waste, endotoxins, and low to moderate molecular weight molecules, which further stimulates production of proinflammatory cytokines (tumor necrosis factor, IL-1, IL-6, IL-8) and promotes mononuclear cell migration.

Activated MMP exiting into the intercellular space from infected cells aggravate damage to the alveolar-capillary barrier and stimulate migration of neutrophils, macrophages, monocytes, and T killers to the affected site. These events activate immune mechanisms involved in tissue damage and extracellular matrix destruction. Procollagen enters damaged alveoli and the interstitium, facilitating hyaline membrane formation in alveolar walls and causing interstitial compression. This further compromises the gas exchange function of the lungs. Neutrophil infiltration at the affected sites gives way to lymphocyte infiltration, which stimulates proliferation of fibroblasts, fibrin accumulation in the lungs and pulmonary tissue remodeling followed by the development of interstitial and intra-alveolar fibrosis.

Induced by respiratory failure, progressing hypoxemia and tissue hypoxia, coupled with direct viral damage to renal, pancreatic, cardiac and brain cells, drive progression of multiple organ failure, which signals a critical, life-threatening situation. Comorbidities only further aggravate a patient's condition. The ongoing cytokine storm maintains systemic inflammation, resulting in the inevitable depletion of endocrine regulatory potential, immunosuppression, opportunistic infections, sepsis, and endotoxic shock [36].

Being a trigger of multiple organ failure, hypoxemia necessitates prescription of coordinated zinc complexes capable of increasing hemoglobin affinity for oxygen. Coordinated zinc complexes hold promise for COVID-19 management as they can prevent death of infected cells.

Patients with COVID-19 complicated by pneumonia and the cytokine storm require intensive care that should include drugs inhibiting proinflammatory cytokines, such as recombinant analogs of endogenous cytokine receptor antagonists and therapeutic monoclonal antibodies. The Russian Ministry of Health has approved IL-6 receptor blockers for treating patients with COVID-19; among the approved drugs are tocilizumab, sarilumab, and olokizumab (monoclonal antibodies against IL-6), IL-1β inhibitors (canakinumab) and inhibitors of JAK kinase, which is the common signal pathway for many cytokines (ruxolitinib phosphate, baricitinib or tofacitinib). The therapeutic regimen can be enhanced with secukinumab (anti-IL-17A) recommended by the protocol for the management of hospitalized COVID-19 patients developed by the Medical Research and Education Center of Moscow State University [37].

To reduce inflammation, combination therapy for viral pneumonias often includes synthetic inhibitory peptides (analogs of peripheral enkephalins) and T-cell inhibitors. MMP inhibitors can be effective against interstitial edema. So far, the only approved MMP inhibitor is doxycycline. Immune response can be downregulated with free-radical binding aminoquinolines and glucocorticoids implicated in suppressing initiators of tissue damage. For example, dexamethasone is now recommended by the UK Department of Health for treating ARDS in severely ill patients with COVID-19. This recommendation was based on the preliminary results of a large-scale British clinical study RECOVERY, which was conducted in 11,000 patients with COVID-19 and looked into the efficacy and safety of monotherapies with lopinavir+ritonavir, low doses of dexamethasone, hydroxychloroquine, azithromycin, tocilizumab, and hyperimmune donor plasma [38]. Glucocorticoids are also capable of suppressing hyperimmune response and enhance surfactant production. Pathological immune response induced by histamine release from the mast cells of the respiratory mucosa and pulmonary mesenchyme can be curbed by H1-histamine blockers.

Bradykinin receptor blockers might be effective in countering inflammation and blocking cascades initiated by bradykinin release from damaged macrophages, epithelial and endothelial cells, thereby reducing interstitial pulmonary edema. However, these drugs are not available in Russia and hence cannot be tested in a clinical trial.

Respiratory failure following ARDS requires emergency care involving oxygen therapy, mechanical ventilation and extracorporeal membrane oxygenation (ECMO). Perfluorochemicals (perftoran) are a promising therapeutic approach to treating hypoxemia, ARDS and multiorgan dysfunction syndrome. Perftoran possesses rheological, hemodynamic, diuretic, membrane-stabilizing, cardioprotective and sorption properties [39, 40].

Correction of endothelial dysfunction directly induced by SARS-CoV-2 or mediated by oxidative stress is a separate line of therapy. Endothelial dysfunction is a key element in the pathogenesis of many diseases [41]. So far, it has been shown to have a role in atherosclerosis, arterial hypertension, chronic heart failure, chronic obstructive pulmonary disease, urinary tract disorders, inflammatory bowel disorders, and other conditions [42,43]. The cascade of pathologic events triggered by COVID-19 complications can be viewed through the lens of endothelial dysfunction [44], whose severity largely determines the clinical outcome. In patients with COVID-19, endothelial dysfunction develops in 4 stages. Stage I is the onset of viral pneumonia, stage II is generalized pulmonary damage, stage III includes respiratory and cardiac failure, stage IV is characterized by progressing toxemia [41].

The primary cause underlying stage I is hypercytokinemia. Generalized pulmonary tissue damage (to types I and II alveolar cells, pulmonary macrophages) induced by the virus is followed by the aggressive activation of alveolar macrophages and neutrophils, expression of proinflammatory cytokines (IL-1, IL-2, IL-6, IL-10, TNF) and activation of prostaglandin/leukotriene synthesis resulting in increased hyaluronidase activity. In turn, hyaluronidase digests the ground substance of the pulmonary interstitium and undermines the stability of the alveolar-capillary barrier. Proinflammatory cytokines and prostaglandins drive overexpression of selectins and adhesion molecules (ICAM-1, VCAM-1), which by interacting with leukocyte ligands foster their adhesion to the vascular endothelium and alveolar epithelium. This process is accompanied by a decline in endothelial NO-synthase expression resulting in reduced nitrogen oxide production and reduced vasodilating, anticoagulatory and anti-inflammatory endothelial function. Increased adhesion capacity of the endothelium and uncontrolled leukocyte adhesion have a significant role in the pathogenesis of local inflammatory response in ARDS and are implicated in renal damage, peripheral vasculitis and capillary purpura in later stages of the disease.

Generalized pulmonary damage is associated with direct damage to endothelial cells induced by SARS-CoV-2 circulating in the bloodstream. There are a few entry points for the virus on the surface of endothelial cells. This promotes endothelial

dysfunction in pulmonary vessels, renal glomeruli, coronary and cerebral vessels. Damaged pulmonary vascular endothelial cells produce massive amounts of endothelin, provoking a local microvascular spasm and elevated arterial pressure [45]. Vascular fluid leaks into the interstitial space, causing interstitial edema, which impairs gas exchange between the capillaries and alveoli. This is followed by a dramatic loss of the gas exchange function, intensified hypoxemia, respiratory acidosis, and accumulation of carbon dioxide in the blood, which triggers hyperstimulation of respiratory and other brainstem centers controlling autonomic functions of the body. Respiratory failure progresses. In the kidneys, production of vasoconstricting H2 prostaglandins is more significant; it impairs glomerular blood flow and reduces excretion and reabsorption in distal nephron compartments.

During stage III (respiratory and circulatory failure), reduced blood flow, acidosis, hypoxemia and circulatory hypoxia exert their detrimental effects on the endothelium. Here, endothelial dysfunction is largely compensatory and aimed at improving microcirculation, lowering the increased vascular tone/spasm of regional vessels. However, at this stage secretion of vasodilatory factors (nitrogen oxide, endothelium-derived relaxing factor, endothelium-derived hyperpolarizing factor) and procoagulants, especially plasminogen activator inhibitor and von von Willebrand factor. Fibrinolysis suppression and activation of a coagulation cascade allow intravascular microthrombi to persist and therefore are important pathogenetic factors for multiorgan dysfunction syndrome.

During stage IV, endothelial damage is linked to endotoxicosis ensuing from disruption of the intestinal capillary barrier and absorption of intestinal and microbial toxins, impaired detoxifying liver function (specifically, impaired ammonia detoxification during the urea cycle) and poor excretion of metabolic waste products by kidneys as a result of the acute renal failure onset. Exposed to endotoxins, endothelial cells are unable to maintain sufficient nutrient and energy supply, the negative charge on their surface, hemorheological and coagulation balance Fibronectin, a platelet activation factor, becomes overexpressed. Altogether, these factors condition intravascular thrombi, compromise microcirculation, suppress the healthy function of the affected organs. Activated platelets increasingly secrete the platelet-derived growth factor, a fibroblast mitogen; this leads to vigorous procollagen and collagen production, formation of hyaline membranes in the lungs and eventually to fibrotic transformation of lung tissue. Stages of endothelial dysfunction and their effects are shown in Fig. 3.

There is a broad spectrum of medications for treating endothelial dysfunction [42]. Apart from the drugs used to block cytokine activation of endothelial dysfunction, endothelin receptor antagonists, relaxin-2 receptor activators, synthetic prostaglandins and polysulfated glycosaminoglycans (sulodexide, fractionated and unfractionated heparin capable of restoring the negative charge on the endothelial surface) have already demonstrated their efficacy. Microcirculation can be improved by adenosine derivatives (dipyridamole), methylxanthines and nicotine acid. Therapy with angioprotective agents may benefit patients with COVID-19, since their mechanism of action is associated with dampening oxidative stress in the vascular wall and reducing vascular inflammation. In patients experiencing severe complications, including acute myocardial infarction, pulmonary embolism, ischemic stroke, retinal or renal thrombosis, fibrinolysis activators are recommended; however, contraindications to fibrinolytic therapy should be heeded.

Essential phospholipids are indicated for treating endothelial dysfunction [46]. They exert direct effects on cell membranes by improving membrane elasticity and fluidity, reducing the density of phospholipid structures, restoring membrane permeability, activating phospholipid-dependent enzymes and transport proteins. Phospholipids reduce damage to endothelial cells, help to restore normal metabolism and increase cell secretory

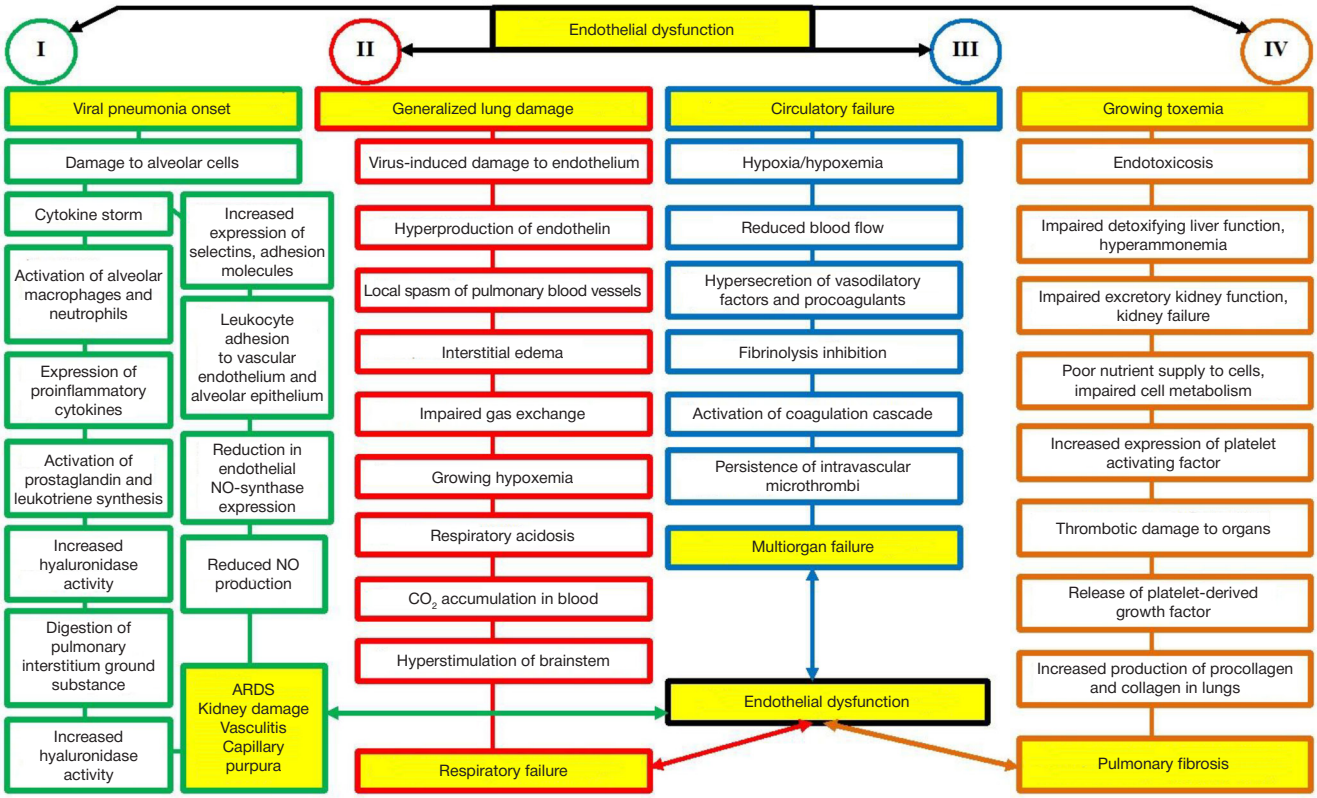

Fig. 3. Stages of endothelial dysfunction and their effects on the progression of pathology in patients with COVID-19

(endocrine-regulatory) potential. Additionally, phospholipids are capable of inhibiting lipid peroxidation, lowering prostaglandin concentrations, downregulating Reticuloendothelial cells and curbing their collagen production. This allows considering phospholipids as candidates for treating COVID-19 consequences. On the whole, the convalescence period involves using a wide range of therapeutic interventions, including medication and physiotherapy. This stage largely determines the quality of a COVID-19 patient's recovery; therefore, rehabilitation regimens for patients with COVID-19 should be regularly updated and refined.

CONCLUSION

Regular updates on the mechanisms underlying pathological processes caused by SARS-CoV-2 allowed us to hypothesize the most probable pattern of the disease progression from the first moments of infection to ARDS, multiple organ failure and endotoxicosis. The article does not cover other types of interventions for treating viral pneumonias. Information presented here is not exhaustive and should be amended and augmented by experts in pathophysiology, pathomorphology, infectious diseases, immunology, pulmonology, and anesthesiology, as more clinical and laboratory data are collected. However, our analysis exposes the key principles in treating COVID-19. Those include a comprehensive strategy involving pharmacotherapy for the main pathogenesis components and accounting for the severity and stage of the disease and personalized treatment based on the thorough evaluation of a COVID-19 patient's health and assessment of chronic exogenous and endogenous risk factors for possible complications.

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HYPERBARIC OXYGENATION THERAPY FOR TREATING COMPLICATED COVID-19: FIRST EXPERIENCE

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Highly virulent SARS-CoV-2 emerged in Wuhan, China, and rapidly spread across the globe afflicting 14.5 million and killing over 600,000 people. The key factors affecting the severity of COVID-19 include advanced age and respiratory failure requiring mechanical ventilation (MV). Mortality rates estimated for mechanically ventilated patients with SARS-CoV-2-induced respiratory failure are 76.4% in the 18–65 age group and 97.2% in individuals over 65 years. At present, extracorporeal membrane oxygenation (ECMO) remains a life-saving method of choice. It is essentially a lung bypass system for direct oxygenation of the blood. It is an invasive and costly procedure performed only at specialized medical care facilities. China, USA, Germany, France and Israel have already launched large-scale research and clinical studies of non-invasive approaches to improving the efficacy of oxygen therapy in patients with complicated viral pneumonia, such as hyperbaric oxygen therapy (HBOT). HBOT is a well-established treatment for anaerobic and aerobic infections accompanied by soft tissue necrosis, carbon monoxide poisoning, stubborn wounds, including non-healing diabetic ulcers, complications of radiation therapy, stroke sequelae, brain injuries, decompression sickness, and other conditions. The use of HBTO in patients with viral infection, pulmonary edema and pneumonia is supported by the laws of physics and clinical/physiological effects in response to the exposure of elevated air pressure and hyperoxic environment. This review provides rationale for using hyperbaric oxygenation therapy in patients with SARS-CoV-2-induced viral pneumonia and presents the first data on the beneficial effects of HBTO in Chinese patients with COVID-19 complications.

Keywords: coronavirus, COVID-19, SARS-CoV-2, SARS-COV-2, hyperbaric oxygenation

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ГИПЕРБАРИЧЕСКАЯ ОКСИГЕНАЦИЯ В ЛЕЧЕНИИ ОСЛОЖНЕННЫХ СЛУЧАЕВ COVID-19: ОБЗОР ПЕРВОГО ОПЫТА ПРИМЕНЕНИЯ

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Высоковирулентный вирус SARS-CoV-2, впервые появившись в Ухане (Китай), быстро распространился по всему земному шару, поразил к настоящему времени более 14,5 миллионов человек и привел к смерти более 600 тысяч человек. Ключевыми критериями, влияющими на степень тяжести течения заболевания COVID-19, являются возраст пациента и развитие дыхательной недостаточности, требующей перевода пациента на искусственную вентиляцию легких (ИВЛ). Согласно опубликованным данным, смертность пациентов на ИВЛ при дыхательной недостаточности, вызванной вирусом SARS-CoV-2, составляет 76,4% в возрастной группе 18-65 лет и 97,2% в возрастной группе 65+ лет [1]. В настоящее время методом выбора спасения жизни при развивающейся дыхательной недостаточности является экстракорпоральная мембранная оксигенация (ЭКМО, «искусственное легкое»), заключающаяся в прямой оксигенации крови в обход пораженной легочной ткани. Данный метод является инвазивным, дорогостоящим и доступным только в клиниках специализированной медицинской помощи. В КНР, США, Германии, Франции, Израиле приступили к полномасштабным научным и клиническим исследованиям неинвазивных методов повышения эффективности кислородной поддержки пациентов при осложненном течении вирусной пневмонии, в первую очередь гипербарической оксигенации (ГБО) [2], которая является всемирно признанным методом лечения анаэробной и аэробной инфекций с некрозом мягких тканей, отравлений продуктами горения, хронических незаживающих ран, в том числе диабетических язв, осложнений лучевой терапии, последствий инсультов и травм головного мозга, декомпрессионной болезни и ряда других заболеваний и состояний [3]. Применение ГБО у пациентов с вирусной инфекцией, отеком легких и пневмонией, основано на знании законов физики и клинико-физиологических эффектов, возникающих в человеческом организме в ответ на одномоментное воздействие сразу двух факторов: повышенного давления и гипероксической среды. В настоящем обзоре приведено обоснование применения гипербарической оксигенации при вирусной пневмонии SARS-CoV-2 и первые сравнительные данные о положительном эффекте лечения ГБО в клинической практике в Китае при лечении осложненных форм заболевания новой коронавирусной инфекцией COVID-19.

Ключевые слова: коронавирус, SARS-CoV-2, COVID-19, SARS-COV-2, гипербарическая оксигенация

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Highly virulent SARS-CoV-2 emerged in Wuhan, China, and rapidly spread across the globe afflicting 14.5 million and killing over 600,000 people.

Clinical presentations of the virus are varied, ranging from no symptoms to severe complications and life-threatening multiorgan failure. Factors predisposing to severe disease include advanced age and pre-existing conditions: elderly and comorbid patients are at a higher risk for severe acute respiratory symptom (SARS) and death. Another laboratory predictor of disease severity is elevated D-dimer, a product of fibrinogen degradation, indicating hypercoagulability [4, 5].

Progressive respiratory failure requiring mechanical ventilation (MV) is the most important prognostic factor of severe disease and death in patients with COVID-19. Mortality rates estimated

for mechanically ventilated patients with SARS-CoV-2-induced respiratory failure are 76.4% in the 18–65 age group and 97.2% in individuals over 65 years [1, 6].

Extracorporeal membrane oxygenation (ECMO, also known as artificial lung technology) is a potentially life-saving alternative for patients with progressive respiratory failure. It is essentially a lung bypass system for direct oxygenation of the blood. It is an invasive and costly procedure performed only at specialized medical care facilities.

Hyperbaric oxygen therapy is becoming increasingly important now that there are more hospital admissions for moderate and severe forms of COVID-19. It is a highly effective non-invasive treatment that saves lives and, in most cases, eliminate the need for MV or ECMO [7].

Physical and physiological principles of HBOT

Hyperbaric oxygen therapy (HBOT) is a well-established treatment for anaerobic and aerobic infections accompanied by soft tissue necrosis, carbon monoxide poisoning, stubborn wounds, including non-healing diabetic ulcers, complications of radiation therapy, stroke sequelae, brain injuries, decompression sickness, etc. [3].

The idea of using HBOT in COVID-19 patients was neither random nor empirical. The rationale for HBOT is supported by universal gas laws and specifically by Dalton-Henry's law. A patient placed into a hyperbaric oxygen chamber breathes a high-pressure gas mixture enriched in oxygen. This increases the amount of oxygen dissolved in tissue. Oxygen uptake and binding by hemoglobin depends on the diffusion of dissolved oxygen across the alveolar or capillary wall into the blood plasma and across the red cell membrane to hemoglobin. Reduced diffusion of oxygen molecules results in falling blood oxygenation.

A standard mask oxygen therapy is ineffective in patients with virus-induced pulmonary interstitial edema and progressive respiratory failure since it cannot modulate gas pressure in alveoli and therefore cannot compensate for oxygen deprivation or dampen pulmonary and systemic inflammation. According to Dalton-Henry's law, HBOT should improve oxygenation by increasing the rate of oxygen diffusion in the lungs, oxygen solubility in the blood plasma, oxygen uptake by hemoglobin and oxygen delivery to hypoxic tissue by microvessels, thereby reducing or eliminating oxygen debt [3].

For a clinician, the clinical outcomes of a treatment are more important than the physical principles behind it. Firstly, HBOT improves oxygen saturation in tissue and reverses hypoxia (most importantly in the central nervous system) caused by pulmonary inflammation. Secondly, HBOT has a metabolic effect consisting in the stimulation of glucose breakdown and elevation of the levels of macroergic compounds, which creates sufficient potential for better endurance and therefore makes it possible to proceed to physical therapy in shorter time. Thirdly, HBOT stimulates epithelization and functional angiogenesis of capillaries and reduces the risk for thrombotic complications by promoting platelet disaggregation and exerting a heparin-like effect on the coagulation system. HBOT also has a vasopressor effect, resulting in edema resolution. Finally, HBOT enhances the effects of antiviral and antimicrobial therapies and reduces their side effects [3].

At cellular and molecular levels, increased hydrostatic pressure and hyperoxia from HBOT epigenetically modulate the expression of human protein-coding genes. HBOT stimulates expression of genes involved in growth regulation, cell repair, production of cellular mediators and anti-inflammatory factors. It also suppresses genes involved in the production of proinflammatory factors and apoptosis. For example, high levels of tissue dissolved oxygen have an antiviral effect consisting in the increased production of reactive oxygen species [8] and hypoxia inducible factor (HIF), which, in turn, stimulates synthesis of antiviral peptides (defensins, cathelicidins) and suppresses secretion of proinflammatory cytokines, including IL-6 implicated in the cytokine storm [7, 9, 10].

Multiple studies have shown that HBOT has a prolonged systemic effect on the pathophysiology of various conditions, including acute pulmonary inflammation, impaired tissue perfusion, severe acute respiratory distress syndrome, and heart failure sequelae [3, 11, 12].

Thus, HBOT, which is based on the principles of physiology and exploits the laws of physics for increasing diffusion and solubility of oxygen in the blood, might be an effective noninvasive alternative to ECMO in patients with COVID-19-induced pneumonia.

HBOT in managing COVID-19 complications: China's experience

In April 2020, the Wuhan Yangtze River Shipping General Hospital, China, published 2 articles on the clinical application of HBOT in patients with COVID-19-induced pneumonia.

The article describes 5 clinical cases of severe and critical disease in patients with CT-confirmed bilateral pneumonia and failing standard oxygen support (without intubation). Prior to HBOT, all patients had been receiving standard mask oxygen therapy (average $SatO_2$ = 70%).

HBOT was delivered at 1.6 ATA (in one case, the pressure was 2 ATA); the first session lasted for 90 min, the rest were 60 min long [13]. After each session, $SatO_2$ values were growing until the following morning in all patients. The 24-h SatO2 monitoring showed that oxygen saturation reached its minimum at 8 am and demonstrated a steady positive dynamic after the beginning of therapy.

Clinical improvement (fever resolution, normal respiration rate, cough relief) and better results of laboratory tests for arterial blood gases, fibrinogen and D-dimer levels were observed after 3 to 8 HBOT sessions. The mean SatO2 value was growing steadily every day ($p < 0.01$); the mean daily SatO2 after an HBOT session exceeded 95%. When the treatment was completed, the patients had a chest CT scan, which also showed improvement; later, the patients were discharged [13, 14].

The authors of the article provided additional consolidated data on 29 patients with milder forms of COVID-19 who had undergone HBOT and achieved similar results [14].

The significance of the foregoing case reports is supported by historical facts. The medical personnel in Wuhan reproduced the experiment conducted by Dr. Cunningham in a Kansas-City clinic (MI, USA) in 1918 during the pandemic of Spanish influenza. Cunningham used a similar HBOT regimen (air pressure of 1.6 ATA, the same number of sessions) in an agonizing patient with severe respiratory failure [15]. The treatment brought immediate relief to his patient, just like in the reports of Chinese physicians.

International clinical trials of HBOT for COVID-19

Clinical trials of hyperbaric oxygenation and protocol development for this type of therapy have been already launched in US, Germany, France, and Israel in collaboration with other countries [2]. Among the trials registered at the National Library of Medicine of the National Institutes of Health

Table. The list of actively recruiting clinical studies of the efficacy of hyperbaric oxygenation in the therapy of COVID-19 by National Institutes of Health, USA.

	Medical facility, city (state), country	Number of participants	ClinicalTrials.gov ID
1.	NYU Winthrop Hospital (New-York, USA)	40	NCT04332081
2.	Ochsner Medical Center, (Louisiana, USA)	48	NCT04343183
3.	White River Wound Healing Center (Arkansas, USA), Community Hospital (California, USA), Innovative Healing Systems (Florida, USA), Decatur Memorial Hospital (Indiana, USA), Providence Medical Wound Care Center (Kansas, USA), West Jefferson Medical Center (Louisiana, USA), Ascension Providence Rochester Hospital Wound Care Center (Michigan, USA), CHI Health Center (Tennessee, USA), Klinika Baromedical (Poznan, Poland)	100	NCT04386265
4.	Sainte Anne Military Teaching Hospital (Toulon, France)	100	NCT04344431
5.	Shamir Medical Center (Zerifin, Israel)	30	NCT04358926
6.	Bergmannsheil und Kinderklinik Buer GmbH (Gelsenkirchen, Germany), Krankenhaus St. Joesf (Regensburg, Germany), Blekingesjukhuset (Karlskrona, Sweden), Karolinska Institutet (Stockholm, Sweden), University of California (California, USA)	200	NCT04327505

(USA; see Table), there are 6 ongoing studies, of which 2 are international multicenter trials.

Importantly, information on the clinical trials conducted by countries not listed in the NLM registry is available only at WHO's International Clinical Trials Registry Platform [16]; the platform allows conducting a detailed search in national registries. For example, the Chinese Clinical Trial registry has published information about the trial of HBOT for treating COVID-19-induced pneumonia (ID ChiCTR2000032011) conducted by the Sixth Medical Center of PLA General Hospital (Beijing, China); the trial is now recruiting 45 patients [17].

Unfortunately, the Russian medical science has lost is leadership in hyperbaric medicine over the recent decades. The managerial approach to public health has caused stagnation in this field of research. Lack of clinical and research facilities for basic and applied research has forced many Russian specialists to take guidance in the data provide by their foreign colleagues.

In light of this, the first reports of positive clinical effects of HBOT observed in patients with severe COVID-19 receiving medical care in the ICU of Sklifosovsky Research Institute of Emergency Care and Burnazyan Federal Medical and Biophysical Center of FMBA, Russia, in June 2020, pose a significant value [18].

The data published by Burnazyan Federal Medical and Biophysical Center demonstrate a remarkable beneficial effect of HBOT sessions conducted as part of the combination therapy for patients with mild and severe COVID-19. After the very first session, there was a considerable improvement in patient condition manifested as a significant increase in oxygen saturation in the capillary blood. HBOT mitigates hypoxia and positively affects patient condition before the administered medication therapy can have its effect, stabilizes blood gas composition and helps to avoid mechanical ventilation in some cases, which is, undoubtedly, a considerable treatment success. The obtained clinical data encouraged the authors to recommend hyperbaric oxygenation as part of the combination therapy outlined in the interim guidance of the Russian Ministry of Healthcare [19].

Some aspects of using HBOT in COVID-19 patients

There should be strict adherence to safety and infection control measures aimed at preventing cross-contamination in hospital areas designated for COVID-19 patients and inside hyperbaric chambers. In Wuhan, patients flows arriving for the procedure and leaving the "red" zone were separated; hyperbaric chambers, other equipment and ventilation/gas exhaust systems underwent disinfection on a regular basis. None of the healthcare workers delivering HBOT to 35 COVID-19 patients contracted the infection; by contrast, cross-contamination rates reported by other hospital units were significant. Adherence to infection control and prevention measures is critical; otherwise, a hyperbaric chamber can become the source of contamination for both medical personnel and patients.

The majority of Russian clinics are equipped with monoplace hyperbaric chambers. This allows medical personnel to implement a personalized approach to treatment and disinfection. The patient can remain in the prone position for the entire session length. Prone positioning ensures good pulmonary blood flow, complete lung expansion and improved ventilation of areas that would be hypoventilated in a patient lying in the supine position.

HBOT technique used in patients with COVID-19 does not differ from a regular HBOT technique. Patients are eligible for this treatment if they do not have contraindications, their hemodynamics are stable and they breathe unassisted. The respiratory rate, blood pressure and SatO2 must be monitored before, during and after the session in order to ensure there is no oxygen overdose and to prevent oxygen poisoning.

CONCLUSION

There is first encouraging evidence of using hyperbaric oxygen therapy for treating life-threatening complications of the novel coronavirus infection. Many healthcare facilities have already launched large-scale clinical and research studies to investigate the potential of hyperbaric oxygen therapy [2, 13, 14, 18].

As part of a combination therapy for the complications of viral pneumonia, HBOT prevents critical hypoxemia and thereby eliminates the need for mechanical ventilation.

Provided by Russian and international teams, research and clinical data on using HBOT in patients with pneumonia and respiratory failure caused by COVID-19 are crucial for re-introducing this method into clinical practice and employing it for managing patients with SARS-CoV-2 or other viral infections.

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COUNTERMEASURES AGAINST THE INTRODUCTION AND SPREAD OF CORONAVIRUS INFECTION COVID-19 IN MEDICAL ORGANIZATIONS

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The article presents a brief epidemiological characteristic of a new coronavirus infection. The risks of infection of medical workers and measures to counter the drift and spread of COVID-19 in medical organizations are considered.

Keywords: COVID-19, coronavirus infection, medical organizations, sanitary and anti-epidemic measures, infectious safety

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МЕРЫ ПРОТИВОДЕЙСТВИЯ ЗАНОСУ И РАСПРОСТРАНЕНИЮ КОРОНАВИРУСНОЙ ИНФЕКЦИИ COVID-19 В МЕДИЦИНСКИХ ОРГАНИЗАЦИЯХ

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В статье представлена краткая эпидемиологическая характеристика новой коронавирусной инфекции. Рассмотрены риски инфицирования медицинских работников и меры противодействия заносу и распространению COVID-19 в медицинских организациях.

Ключевые слова: COVID-19, коронавирусная инфекция, медицинские организации, санитарно-противоэпидемические мероприятия, меры инфекционной безопасности

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Viruses stand out from other dangerous pathogens as capable of causing global outbreaks of deadly infections posing a threat to mankind. Emerging high-consequence infectious diseases of the 21st century include severe acute respiratory syndrome (SARS, 2002), Middle East respiratory syndrome (MERS, 2012), H5N1 avian influenza (2007), A (H1N1) pdm swine flu (2009), Zika, and the list can be continued. In 2014–2015, Ebola spread to new territories in West Africa; brought to Europe, it exposed how ill-prepared European public health systems were for a biological threat [1].

However, the novel coronavirus has surpassed the foregoing infections in scale and escalated into a pandemic in a matter of months. The first cases of previously unknown pneumonia were reported at the end of December, 2019 in Wuhan (Hubei Province, Central China). On January 30, WHO defined the outbreak of the novel coronavirus infection as a public health emergency of international concern. On February 11, 2020 WHO announced a name for the novel coronavirus disease (COVID-19), following the notification of the National Health Commission of the People's Republic of China on the provisional name for the novel coronavirus pneumonia (February 9, 2020). On March 11, 2020 WHO declared a pandemic.

Over the past decade, 4 new coronaviruses have been discovered. Viral mutations are the primary cause underlying the emergence of novel viruses and viral strains. Conspiracy theories about their laboratory origin are not discussed in this paper for obvious reasons.

Mutations make viruses more contagious, pathogenic and capable of crossing the species barrier and invading new hosts. Infections caused by emerging pathogens and mutant strains of previously known microorganisms have a more severe course because the host lacks defense mechanisms against the unknown pathogen.

The novel coronavirus has rapidly spread across the globe. This suggests that we have limited knowledge about the potential of zoonotic viruses; human encroachment on wildlife habitat, expansion of transport networks, increasing migration, climate changes, advances in biotechnology and other factors will significantly increase the risk of such plagues in the future [2].

The COVID-19 pandemic exposed vulnerabilities of public healthcare systems. Many countries failed to organize public

health surveillance, promptly respond to the increasing need for protective personal equipment (PPE), sufficient bed capacity and mechanical ventilators, coordinate the work of auxiliary services, etc. [3].

Countries that managed to create centers for coordinated response against COVID-19 are the most successful in fighting the infection by reinforcing sanitary control, providing medical care, developing diagnostic methods, therapies and vaccines.

The turn of year 2019/20 has taken its place in history as the time of the COVID-19 pandemic. The novel coronavirus infection is an acute infection that predominantly affects the respiratory tract and is caused by SARS-CoV-2, an RNA virus from the Betacoronavirus genus of the Coronaviridae family. SARS-CoV-2 is putatively derived from a recombination of a bat coronavirus and an unidentified coronavirus. SARS-CoV-2 has over 80% sequence homology with SARS-CoV.

The causative agent of COVID-19 is a Risk Group II pathogen. COVID-19 was included in the list of diseases posing a community threat [Executive order № 66 of the Government of the Russian Federation dated January 31, 2020].

The COVID-19 pandemic is characterized by a high transmission rate: the virus spreads via respiratory droplets, has a long incubation period and can be asymptomatic in contagious individuals; at the moment, there is no vaccine and etiotropic treatment against the virus.

Close-knit communities with actively interacting members, including rotational shift workers, healthcare workers or people living in social care facilities, are a cauldron for COVID-19. Herd immunity and specifically postvaccination immunity against COVID-19 will slow down the spread of the disease.

At the Member State Briefing on the COVID-19 pandemic evaluation held on July 9, 2020, WHO Director-General Tedros Adhanom Ghebreyesus said that “the pandemic is still accelerating”.

Epidemiological situation

As on July 18, 2020, about 14 million COVID-19 cases were reported worldwide and approximately 600,000 people died. So far, 800,000 COVID-19 cases and 12,000 deaths have been reported in Russia [1] Hundreds of COVID-19 hotspots have been identified, including healthcare facilities.

Preventing the spread of the novel coronavirus in healthcare settings is of critical importance. In the absence of vaccines and effective etiotropic treatments against COVID-19, infection prevention and control measures have become the mainstay of fighting the disease. Guidelines have been developed for healthcare personnel working with patients who seek medical care during the pandemic [4].

Healthcare workers are at very high risk for COVID-19. Thousands of health workers worldwide contracted the virus when providing medical care to infected patients. One of the underlying causes is lack of training: frontline health workers, except for infectious disease specialists, are not trained in infection prevention and control (including infections with droplet transmission)[5].

Infected patients are an increasingly common source of nosocomial COVID-19. However, there is mounting evidence that physicians, nurses, technicians, hospital elevator operators, cleaners, and security guards can also be the source of the virus. There are reports of patients infected by their attending physicians who were on vacation abroad but did not self-isolate for 14 days on return. Some patients admitted to non-COVID hospitals concealed the fact that they had travelled to disease-stricken countries or had physical contact with infected family members.

It is known that viral shedding occurs as early as 48 h before the onset of symptoms, peaks at days 1–3 from onset and continues through day 12 in mild/moderate cases and through day 14 in severe cases.

Transmission through respiratory droplets occurs during close physical contact (> 15 min, at < 2m distance) with an infected individual who has respiratory symptoms. Walking past an infected patient in a lobby is not so dangerous. Coughing and sneezing patients expel virus-containing aerosols from their respiratory tract. If these particles land on the oral/nasal mucosa or the conjunctiva of a susceptible individual, they cause infection.

Another route of transmission is through airborne dust particles. SARS-CoV-2 has been found to retain its viability for up to 3 days in large drying mucus droplets that fall on fomites, from where the virus can travel further on dust [6].

In healthcare settings, the virus can be transmitted through medical equipment, such as a pulse oximeter, thermometer, other devices near the infected individual, fomites (door handles, smartphone screens), food or water. The virus can spread through hand-to-eye, hand-to-nose or hand-to-mouth contact.

There is evidence of fecal-oral transmission. It is reported that viral RNA is detected in stools of convalescent individuals for 4 weeks. The nucleocapsid protein of SARS-CoV-2 was detected in the cytoplasm of the epithelial cells in salivary glands, the stomach, the duodenum, the rectum, and the urinary system. The virus might replicate in the liver and the intestine [6].

The risk of infection increases if a person does not adhere to infection prevention measures.

Measures for protecting healthcare workers against COVID-19 include using engineering and administrative controls and PPE. Control of infection sources is of fundamental importance. Each patient should be viewed as potentially infected with COVID-19. Measures for controlling the source of infection include early diagnosis, case detection, identification of asymptomatic patients, isolation of infected patients and individuals with suspected COVID-19.

In order to prevent the spread of COVID-19 in healthcare facilities, it is imperative that:

- patients suspected to have COVID-19 be accommodated in isolation rooms or rooms with an airlock lobby and a dedicated bathroom; patients with different COVID-19 severity and at different stages of the disease should not be cohorted in the same ward;
- patients suspected to have COVID-19 be housed in single rooms; patients with confirmed COVID-19 admitted to an infectious disease hospital can be housed together (2–4 persons in the room) if their beds are at least 1.5–2 m apart and the minimum space per person is 8 m2, as required by the sanitary regulations;
- patients wear face masks in the presence of healthcare workers or other patients; hand hygiene should be performed;
- patients do not leave their wards; mobile phones are allowed (also in intensive care units) but must be disinfected with an alcohol-based sanitizer [7].

Administrative and engineering controls include limiting the number of health workers who have direct contact with patients, minimizing the number of visits to the ward, using telehealth technologies to consult patients. Single-use or dedicated devices (phonendoscopes, blood pressure cuffs, pulse oximeters and thermometers) should be preferred.

Disinfection is one of the central measures for infection prevention and control. SARS-CoV-2 has been proved to be

sensitive to UV light and high temperatures. Exposure to 56 °C for 39 min or to 70 °C for 5 min kills the virus. Disinfectants, including chlorine-based, active-oxygen base, and others, can effectively inactivate the virus. Routine indoor disinfection with disinfectants approved for use near people can be performed in the presence of a patient. Used dishes/utensils, linen, items of care should be soaked in disinfecting solutions.

Hand hygiene using antiseptics for skin disinfection should be performed by medical personnel after each contact with the skin of the infected patient (or suspected to have COVID-19), their mucous membranes, secretions, dressings and items of care near the patient. In the presence of people, indoor air can be decontaminated using UV recirculators and filtration systems (including electric-powered systems).

Medical waste, including patients' secretions (feces, urine, sputum, etc.), is highly hazardous class B waste and should be subject to physical decontamination by exposure to thermal energy, microwaves or radiation; this means that a medical facility should be adequately outfitted with specialized equipment. Disposal of non-decontaminated class B medical waste outside the premises is prohibited. After applying physical methods of decontamination, this waste can be temporarily stored, accumulated, transported, destroyed and buried together with class A waste. Chemical disinfection can be used for decontaminating food and patients' secretions only.

In COVID-19 hotspots, terminal disinfection must be performed with chlorine-based and active oxygen-based agents since they are the most reliable and effective against enveloped viruses, including the novel coronavirus. In the absence of people, indoor air should be decontaminated using open UV irradiators and disinfecting aerosols.

In a non-COVID medical facility with exposures to COVID-19 patients, terminal disinfection should be performed by companies authorized to provide disinfection services or by trained staff. Bedding items should be disinfected in a sterilization chamber. Ventilation ducts are decontaminated using aerosol disinfectants or mist generating systems following the established protocols.

Measure for protecting medical personnel and patients include:

- irrigation therapy (nasal irrigation and gargling using hypertonic saline)
- topical medications that form a protective barrier;
- medication prophylaxis (recombinant IFNα, etc.) [6].

Establishing timely and accurate diagnosis of COVID-19 is critical for containing the spread of the virus in a healthcare facility.

According to the Sanitary Rules 3.1.3597-20 on the prophylaxis of the novel coronavirus infection (COVID-19), symptomatic healthcare workers at risk of occupational exposure to COVID-19 should be prioritized for laboratory testing. As a second-order priority, asymptomatic health care workers at risk for COVID-19 should be tested for the virus once a week until the first detection of IgG antibodies.

A few groups at risk for occupational exposure to COVID-19 can be identified among healthcare workers depending on the type of medical care they provide:

- very high occupational risk (must wear type I protective clothing): medical personnel of hospitals for infectious diseases exposed to patients with confirmed COVID-19; pathologists involved in performing autopsies of COVID-19 patients;
- high risk (must wear type II protective clothing): EMS teams involved in transportation of infected patients; medical personnel of COVID-19 hospitals following up patients suspected to have the infection; ER personnel; health workers delivering care to patients with respiratory infections, intensive care or specialized care (dentists, ophthalmologists, otorhinolaryngologists, pulmonologists); personnel of outpatient clinics, paramedical and midwifery stations who visit patients suspected to have COVID-19 in their homes; staff involved in performing routine and terminal disinfection;
- in addition, the high-risk group includes members of surgical teams performing urological, eye, thoracic, and septic emergency surgery in COVID-19 hospitals;
- medium occupational risk (must wear type III-IV protective clothing): all healthcare workers, including isolation facility staff and those who visit seemingly healthy individuals isolated in their homes (type III) [7].

The risk of COVID-19 spread in a healthcare facility increases during aerosol-generating diagnostic or therapeutic interventions, which include:

- endotracheal intubation;
- bronchoscopy;
- open suctioning of airways;
- nebulizer treatment;
- manual ventilation prior to intubation; putting the patient in the supine position; disconnecting the patient from the ventilator;
- non-invasive ventilation, including bilevel positive airway pressure ventilation and continuous positive pressure ventilation; high-frequency oscillatory ventilation;
- tracheostomy and cardiopulmonary resuscitation;
- upper GI endoscopy;
- surgery involving use of high-speed cutters;
- emergency dental care (using high-speed burrs);
- sputum induction;
- high-flow O_2 delivery, including nasal canula

Medical personnel performing aerosol-generating procedures must wear protection, as prescribed by the Sanitary Rules 3.1.3597-20 on the "Prophylaxis of the novel coronavirus infection (COVID-19):

- single-use respirators (filtration masks) filtering 99% of solid and liquid particles or higher-level devices (respiratory helmet);
- eye protection (goggles or face shield);
- a biohazard gown (or another type or protective clothing), gloves, a water-proof apron.

Respirator donning and doffing, decontamination and disposal procedures must be strictly adhered to. An adequate seal of a respirator to the face cannot be achieved if the user has a beard or moustache [7].

At the beginning of the epidemic, healthcare workers contributed to the spread of COVID-19 due to inadequate protection, insufficient knowledge about the virus and PPE deficit. Due to high patient burden and atypical symptoms, many patients were misdiagnosed. Besides, on-site laboratory tests were unavailable.

The analysis of the accumulated data reveals that the primary cause of the nosocomial COVID-19 spread was poor compliance with infection prevention and control measures while delivering medical care to patients with suspected COVID-19, delayed diagnosis and nonadherence to disinfection standards.

Amendments introduced to Federal Law № 100 (April 1, 2020) address responsibility for non-compliance with infection prevention and control measures (Article 236 of the Criminal Code). A healthcare worker found guilty of negligent transmission of infection to colleagues or patients will be fined 700,00 roubles or an equivalent of 18 monthly salaries. Non-compliant healthcare workers can lose their post or be banned

from working in public health for 1–3 years or sentenced to 2 years in prison (Clause 1 of Article 236 of the Criminal Code).

The head of a medical facility can be held liable for non-compliance with infection prevention and control measures even if no individuals have been infected. The head of a medical institution is responsible for providing the medical personnel with respirators and disinfectants, organizing routine temperature checks and medical examination of the personnel prior to shift start aimed at identifying individuals with symptoms of respiratory infection.

Points to consider by heads of healthcare facilities are provided in the checklist below:

• Collect and compile normative documents on organizing medical care during the COVID-19 pandemic;

• Assign members of the medical personnel to specific tasks or issue a protocol explaining workflow during the pandemic;

• Implement prevention measures on the premises and among personnel (facemasks, hand hygiene with sanitizers, airing rooms, disinfection);

• Make sure you have sufficient supply of disinfectants, PPE, other medical products; check contracts, consignment bills, etc.

• Arrange personnel trainings on infection prevention and control (https://edu.rosminzdrav.ru/); familiarize them with normative documentation.

In order to be ready to respond to a COVID-19 threat, a healthcare facility must have an emergency plan specifying procedure for infection prevention and control in cases when a person (a patient or a staff member) is suspected to have an infectious disease. Annual training sessions are the most effective tool for testing the preparedness of a healthcare facility to an infection threat [8].

CONCLUSION

Measures for preventing and controlling the spread of COVID-19 in healthcare facilities are specified in the updated Interim Guidance on the Prophylaxis, diagnosis and treatment of the novel coronavirus infection (COVID-19) issued by the Russian Ministry of Healthcare; Order No. 198n dated March 19, 2020 on the Provisional workflow in a healthcare facility aimed at implementing preventive measures and reducing risks of spread of the novel coronavirus infection COVID-19 issued by the Russian Ministry of Healthcare; normative documents by Rospotrebnadzor (Sanitary Rules 3.1.3597-20 on the Prevention of the novel coronavirus infection (COVID-19), etc.

Algorithms for providing healthcare to a patient suspected to have COVID-19 have been developed, including safety precautions. For healthcare facilities, infection prevention and control includes strict adherence to sanitary precautions while delivering medical care to patients with suspected COVID-19 and timely detection of such patients. Routine temperature checks and medical examinations should be performed on healthcare workers, aiming to detect individuals with ARVI symptoms. Healthcare workers and support personnel must undergo regular trainings on infection prevention and control. Competence of healthcare workers, their ability to use PPE will determine the efficacy of measures for preventing the spread of COVID-19 in healthcare facilities.

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MEDICAL EVACUATION OF PATIENTS COVID-19

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The aim of this article was to summarize the experience of the National Center for Disaster Medicine "Zashchita" in organizing and performing medical evacuations (including those by air) of patients with COVID-19. Materials and methods used in the study included legal, normative and guidance documents, emergency call forms, methods for preparing for and performing medical transport of patients with COVID-19, EMS safety guidelines. The article lists basic normative documents regulating medical evacuation of patients with infections, including COVID-19, and describes the missions carried out by Zashchita and their outcomes. So fat, the Center has successfully completed 555 medical evacuations, including 64 aeromedical missions. Biosafety of EMS teams involved in medical evacuations was ensured following the existing safety guidelines. For long journeys over 1 h, PPE should be donned upon arrival at the scene before leaving the EMS vehicle. Using patient isolation transport units is mandatory during medical evacuations of COVID-19 patients by air. Prior to starting a mass medical evacuation of patients with COVID-19, their number and condition should be assessed to determine the priority sequence for evacuation and the required oxygen supply.

Keywords: COVID-19, coronavirus infection, PPE, medical evacuation, biosafety, aeromedical mission, patient isolation transport unit

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МЕДИЦИНСКАЯ ЭВАКУАЦИЯ БОЛЬНЫХ COVID-19

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Цель исследования — обобщение опыта специалистов ВЦМК «Защита» по организации и проведению медицинской эвакуации больных COVID-19 в том числе авиационным транспортом. Материал и методы исследования: нормативные, методические документы, карты вызовов, методики подготовки и проведения медицинской эвакуации больных COVID-19, обеспечения инфекционной безопасности медицинской бригады, санитарного транспорта. Рассмотрены основные нормативные документы, регламентирующие организацию и проведение медицинской эвакуации больных инфекционного профиля, в том числе с новой короновирусной инфекцией COVID-19. Представлены основные результаты деятельности специалистов Всероссийского центра медицины катастроф "Защита" ФМБА России по проведению медицинской эвакуации больных COVID-19. Всего выполнено 555 эвакуаций, в том числе 64 авиационным транспортом. Обеспечение инфекционной безопасности специалистов медицинских бригад выполнялось в соответствии с действующими нормативными документами. Их применение было достаточным для защиты бригады. При существенном увеличении времени доезда до инфекционного больного (1 час и более) СИЗ целесообразно одевать по прибытии на место, в санитарном транспорте. Применение транспортировочного изолирующего бокса при проведении санитарно-авиационной эвакуации обязательно для больных COVID-19. Организации массовой медицинской эвакуации больных COVID-19 следует начинать с предварительной оценки их количества и тяжести состояния, на основании которых определяется состав бригад СМП, очередность транспортировки, объем неснижаемого запаса кислорода в санитарном транспорте. Приведены примеры из практики организации и проведения санитарно-авиационных медицинских эвакуаций больных COVID-19.

Ключевые слова: средства индивидуальной защиты, COVID-19, коронавирусная инфекция, медицинская эвакуация, инфекционная безопасность, санитарно-авиационная эвакуация, транспортировочный изолирующий бокс

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Prompt transport to a receiving facility is an essential part of delivering specialized or hi-tech medical care to patients infected with the novel coronavirus infection (COVID-19). In Russia, medical evacuation and transport of patients, including those with high-consequence infectious disease, are regulated by Order 69n of the Russian Ministry of Healthcare and Social Development (dated 01/31/2012) and the Chief Medical Officer's recommendations [1, 2]. As more and more people contracted the disease, a need arose for clarifying the procedure of medical evacuation/transportation and improving EMS personnel safety. This spurred an upadte (ver.7) of the Interim recommendations providing detailed information on the stages of medical transportation/evacuation developed and revised by the experts of the National Center for Disaster Medicine "Zashchita" of FMBA (NCDM Zashchita), Russia, based on the experience accumulated to date [3].

There are a few important normative documents clarifying and amending currently existing guidelines on medical evacuation/transport that were developed to regulate the evacuation procedure in patients suspected, probable or confirmed to have COVID-19. The EMS patient transport protocol for suspected/confirmed COVID-19 cases is provided in FMBA Order 112 dated 04/18/2020 and Order 126 dated 04/24/2020 [4, 5]. The protocol requires a responding EMS team cooperating with an infectious disease specialist to determine the number and order of evacuations for patients with confirmed or suspected COVID-19 and coordinate the transportation route. FMBA Order 112 dated 04/18/2020 describes the PPE donning and doffing procedure and requires the EMS personnel to put on their protective gowns under the supervision of the EMS team leader upon arriving at the scene prior to entering the patient's home or isolation area. The EMS personnel involved in medical evacuation are not required to self-isolate for 14 days as before but instead are closely monitored for the entire length of the COVID-19 incubation period.

NCDM Zashchita has developed a guidance on the medical evacuation and transportation of patients with infectious diseases, including high-consequence infectious diseases. The

guidance is open for criticism and improvements at the website of NCDM Zashchita [6].

The letter of Rospotrebnadzor[1] (The Russian Federal Service for Surveillance on Consumer Rights Protection and Human Well-being) has authorized the air medical services (AMS) of NCDM Zashchita involved in the medical evacuation of patients and disaster victims to proceed with the assigned tasks without having to self-isolate for 14 days after the mission given that the medical personnel strictly complies with all biosafety rules. This decision has significantly improved the efficacy of medical evacuation and transportation.

Our search for the international literature on the medical evacuation of COVID-19 patients did not yield any results. So, the aim of this study was to summarize the experience of the Center for Emergency Air Evacuation (CEAE) and the Multipurpose Field Hospital (MFH) of NCDM Zashchita in organizing and performing medical evacuations (including those by air) of patients with COVID-19.

Materials and methods used in the study include legal, normative and guidance documents, emergency call forms, methods for preparing for and performing medical transport of patients with COVID-19, EMS safety guidelines.

Study results and analysis

Medical evacuation of patients with COVID-19 was performed by the medical personnel of NCDM Zashchita.

The first aeromedical evacuation mission of COVID-19 patients (3 confirmed and 5 suspected cases) was conducted by CEAE personnel on February 21-24, 2020. The patients were transferred from Tokyo, Japan, to a receiving facility in Kazan on board of a specialized aircraft. Of 8 patients, 5 had a history of face-to-face contacts with infected individuals and 3 were positive for COVID-19. Visual examination of the patients, change of respirators and non-contact temperature assessment were performed in a vehicle prior to boarding the plane. On examination, all patients had normal temperature readings and no health complaints. During the flight, which lasted for 18 h including refueling stops, the patients' condition was closely monitored. At refueling stops, the patients and the medical crew stayed on board. The EMS crew consisted of an anesthesiologist and a paramedic. The EMS crew were wearing PPE, including Tychem 2000 C hooded coveralls (DuPont), FFP3-standard 6800 full respirator masks (3M), high shoe covers with straps, surgical gloves, and QUARTZ-1M protective gowns [7].

The EMS team were wearing PPE over medical scrub pants and a V-neck top. The escorting Rospotrebnadzor and EMERCOM personnel and the aircraft crew members were wearing similar PPE. The patients were wearing FFP3 respirators without exhalation valves. Upon landing, the patients were transported to the specialized receiving facility; after the flight, the aircraft was cleaned and disinfected; the aircraft personnel also underwent the decontamination procedure [8].

Most aeromedical missions tasked by the Russian government were conducted using medically equipped aircrafts.

At the time of the performed evacuations, 40.0% of the patients were positive for COVID-19; other patients, including those with a past history of contacts with infected individuals, did not take a COVID-19 test or their test results were unavailable. However, all patients had clinical signs of pneumonia, including a high respiration rate, labored breathing, fever, and some other symptoms suspicious of COVID-19.

Indications and contraindications to air transport were determined based on the severity of the patients' condition. Among direct contraindications were refractory bleeding, hemodynamic instability, pneumothorax, pneumocephalus, and other conditions that could not be corrected or stabilized on board of an aircraft or an EMS car. None of the patients had contraindications to transport.

During short-term (>1 h) evacuation missions, patients were continuously monitored for their heart rate, oxygen saturation and body temperature. Blood pressure was measured once every 15 minutes. Other parameters were measured depending on a patient's condition. In most cases, there was no need for pathogenic therapy, but all patients received oxygen. During long-term missions (including air evacuations), the amount of diagnostic and therapeutic interventions was determined by a patient's condition.

In all cases, after CEAE received a request for medical evacuation, information about the time of the diagnosis and the involved specialist, severity of the condition and COVID-19 test results was further clarified.

According to the interim guidance (ver.7) on the prophylaxis, diagnosis and treatment of the novel coronavirus infection released by the Russian Ministry of Healthcare, an EMS team involved in medical evacuation must include 1 physician, 1 paramedic and 1 nurse [3]. On our missions, there was no nurse, whose responsibilities were distributed between the 2 remaining members of the team.

Protecting EMS personnel against the infection is an important priority. The personnel involved in the transport of patients suspected to have COVID-19 must wear class 4B disposable protective clothing and FFP2 or higher-class respirators.

In our experience, donning all PPE prior to leaving the EMS station is not well-reasoned. Considering conflicting recommendations on PPE donning (before leaving the EMS station or at the scene) provided in the normative documents, we follow the guidelines provided by FMBA Order 112 dated 04/18/2020. EMS personnel engaged in patient transportation in the city of Moscow put on their protective gowns before leaving the EMS station; eye protection and respirators are put on upon arrival at the scene before leaving the EMS vehicle. For emergencies outside Moscow or longer journeys (>1 h), coveralls, eye protection and respirators are donned upon arriving at the scene before leaving the car.

EMS vehicle drivers also wear protective gowns, respirators and medical gloves. Goggles are not used because they might affect the driver's reaction to an unexpected situation on the road. A patient compartment is separated from the driver's cabin by a polyethylene screen. During medical evacuations, the EMS team stays in the patient compartment and does not have any physical contact with the driver.

Table 1. Number of patients with COVID-19 evacuated by the Center for Emergency Air Evacuation and the Multipurpose Field Hospital of NCDM Zashchita between February 23, 2020 and June 20, 2020

Patients	All types of transport	Aeromedical transport			EMS vehicles
		Total	In Russia	From abroad	
With confirmed or suspected COVID-19	555	63	52	11	492

During transport, the air in the vehicle is decontaminated by a bactericidal UV air recirculatory. Traces of biological materials in the vehicle are decontaminated using liquid disinfectants, collected into containers and disposed of as class B medical waste. Inside the vehicle, PPE and shoes are decontaminated with disinfectants.

NCDM Zashchita has developed and continues to implement the following protocol for aircraft disinfection:

– Once the patient is transferred to a receiving facility, all internal surfaces of the EMS vehicle, door handles and medical equipment are decontaminated with a disinfectant;

– EMS personnel returns to the headquarters still wearing PPE;

– At the headquarters, the vehicle, PPE and shoes are additionally decontaminated with disinfectants in a special decontamination area; the patient compartment of the vehicle is irradiated using an UV air recirculator. PPE are treated with a disinfectant (exposure time is controlled) and then are disposed of as class B medical waste;

– A record of the decontamination procedure is made in the log book, specifying the disinfectant used, exposure time and time for air decontamination [7].

Medical supplies inventory is restocked after the EMS team returns to the vehicle. This reduces the risk of accidental contamination of the headquarters building. Documentation, including emergency call forms, is decontaminated in a dry-air sterilizer.

Patient isolation transport units (PITU) are mainly used during aeromedical missions because:

– decontamination and setup of PITU for the next patient is a time- and labor-intense procedure, meaning they cannot be used in a series of emergency transportations;

– total decontamination of the entire aircraft with an infected patient on board transported without PITU is impossible.

A PITU envelope can be positively or negatively pressurized as per its manufacturer's protocol. Based on our experience of transporting COVID-19 patients, there are a few downsides to maintaining negative pressure inside PITU. First, despite the sufficient number of filters, the PITU envelope "shrinks", making it harder to perform medical manipulations and inciting anxiety and psychomotor agitation in the patient because of small confined space and "closing" capsule walls. During all aeromedical missions performed by Zashchita, mild positive pressure was maintained inside PITU. The inlet pump was not operated at full capacity, which allowed the battery to hold a charge for 1.5–2 more hours.

While preparing for a medical evacuation, we followed the minimum weight — maximum functionality rule. Therefore, we did not consider using portable hyperbaric chambers as an alternative to PITU. However, we believe that in the future it will be possible to transport infectious patients in intensive and isolated care units based on the Afalina module developed by Lomonosov MSU [8].

Below, we describe the medical evacuation of as severely ill COVID-19 patient inside a PITU on board of a specialized aircraft.

A patient with suspected COVID-19 progressed to severe bilateral multisegmental pneumonia, as suggested by a CT scan, was transferred on board of an AN-148 aircraft from Grozny to Moscow on May 22–23, 2020; the patient was accompanied by the medical personnel from NCDM Zashchita. To ensure medical personnel and cabin crew safety and prevent contamination of aircraft surfaces and the ventilation system, the patient was placed in a PITU (emergency bag BIO-BAG EBV-30/40). Upon arriving at Grozny airport, the EMS team donned their PPE and were taken to the hospital by an ambulance vehicle. At the hospital, the EMS team evaluated the patient's condition and did the paperwork. The patient was placed in an emergency bag; cables and sensors for vital signs monitoring (ECG, SpO_2, blood pressure) were passed through the side ports. In order to compensate for respiratory failure, oxygen was delivered to the patient via a Venturi mask. Negative pressure was created and maintained in PITU further on so as to achieve the highest level of biological safety (BSL-4). Before loading the patient into an EMS vehicle, the EMS crew, PITU and the equipment (an electrocardiographic monitor and an oxygen balloon) underwent a decontamination procedure with a disinfectant. No accidents occurred during the flight. On board, the patient was receiving symptomatic treatment. The patient's condition was stable; no need arose for opening PITU en route to Moscow.

In Moscow, 2 critical care transport vehicles had been waiting for the patient. One vehicle with 2 EMS responders wearing PPE took the patient to the receiving facility. The patient was loaded into the car and taken out of the capsule. The medical crew accompanying the patient on board of the aircraft, the equipment and PITU were taken by the second vehicle to Zachshita headquarters for decontamination and cleaning. The total evacuation time was 4.5 h; flight duration was 2 h.

This example illustrates the protocol followed by our EMS team, which aimed at maintaining a high biosafety level during medical evacuation of patients with COVID-19 inside a PITU.

Normally, aeromedical evacuations of patients, including patients with COVID-19, are carried out in several stages. In the first stage, the EMS team prepares the patient for the flight: the patient should be stabilized, provided with oxygen or put on a ventilator if necessary. In the second stage, the patient is transported from the medical facility to the aircraft. The next stage is the flight itself. Finally, the patient is unloaded from the aircraft and taken to the receiving facility. Thus, one EMS team performs a multistage evacuation, which raises the competence bar for the team and stiffens the requirements for the equipment used during the mission.

Below, we provide another example of a multistage medical evacuation of a critically ill patient.

Patient B., 78 years, was transferred from Cherkessk to Moscow on board of a specialized aircraft; the mission was carried out by the ESM team from NCDM Zachshita. This was a 4-stage medical evacuation. Measures taken at each stage were aimed at ensuring safety at the following stage.

The first stage was preparatory. Because it was impossible to obtain exhaustive information about the patient's condition during the preceding video call and the emergency was pressing, the EMS team had to take the patient's full medical history upon arrival in Cherkessk. After the obtained data were analyzed, the patient's condition was assessed as critical. Adjustments were made to the therapy and parameters of respiratory support. After the patient was stabilized, a decision was made to transfer him to Moscow on board of a specialized aircraft.

In order to prevent the spread of COVID-19, the patient was placed in PITU. The ventilator circuit, IV lines, cables and sensors for vital signs monitoring, and drainages were passed through the service ports. All medical equipment was outside the capsule and could be accessed by the medical personnel. Before leaving the hospital, the EMS crew, PITU with the patient, and the medical equipment were decontaminated with disinfectants.

In the second stage, the patient was taken to Cherkessk airport in a class C critical care transport vehicle outfitted with

monitoring and therapy equipment. Importantly, the number of maneuvers with a critically ill patient should be minimized. This means that the EMS vehicle transporting the patient should be as close as possible to the aircraft. Boarding should be performed using an ambulift, which can lift a person on a stretcher to the aircraft door. The airport in Mineralnye Vody did not have an ambulift, so the medical personnel had to load the patient in on their own, which might have had a negative effect on the patient's condition.

In the third stage, the patient was transported to Moscow on board of an An-148 aircraft outfitted with medical equipment. During the flight, the patient was on a ventilator and receiving IV therapy, his vital signs were continuously monitored. Because the aircraft was properly equipped, the EMS team were able to perform interventions safely and effectively.

In the final stage, a receiving vehicle should come as close as possible to the aircraft to pick up the patient. After the patient was loaded into a critical care transport vehicle, he was transported to the Intensive care unit of Pirogov National Medical and Surgical Center, without deterioration. PITU and the medical equipment were decontaminated. All medical materials used during the mission were single-use items and were disposed of according to the current regulations.

Although the number of COVID-19 patients is falling in Russia, CEAE and MFH medical teams still have to carry out medical evacuations.

Recently, FMBA hospitals repurposed for COVID-19 patients have started to go back to their normal routine. Executive order № 1470 of the Russian Government dated 06/03/2020 and FMBA Order № 172 dated 06/10/2020 have authorized NCDM Zachshita to perform transfers of critically ill patients from FMBA hospitals to specialized facilities. On June 16-17, EMS crews from Zashchita transported 7 patients with COVID-19 from the clinic at the Federal Center of Brain Research and Neurotechnology to specialized medical facilities for further medical care.

Below, we provide an example of a mass medical evacuation of patients with COVID-19.

Two moderately severe and 5 severe patients including one individual on mechanical ventilation were transferred from the clinic at the Federal Center of Brain Research and Neurotechnology to specialized medical facilities.

Transportation was performed by 2 critical care teams consisting of an intensivist and a paramedic and a regular EMS team consisting of a physician and a paramedic. PPE was worn by all members of the involved teams.

The first-response team (the physician and the paramedic) was tasked to assess the situation and decide on the sequence of medical evacuations [9]. Upon arrival at the clinic, the team evaluated the condition of the patients, their eligibility for transport and their need for oxygen. The team then reported to the headquarters, and the following decisions were made:

– moderately severe patients should be evacuated by the first-responders;

– another paramedic should join the critical care team because one of the patients was morbidly obese;

- the most severely ill patient on a vent should be evacuated first;

– oxygen supply should be 1.5 times higher than required for one journey to the receiving facility.

The CCT vehicle transported one patient at a time. During transport, all patients were stable. After each evacuation, the crews underwent decontamination, restocked on oxygen and proceeded to the next evacuation.

At the time of manuscript preparation, 15 mass evacuations had been carried out. The following steps should be taken to ensure successful medical evacuation of multiple patients between hospitals:

– dispatch an EMS crew to location to assess the condition of all patients eligible for transport;

– analyze the obtained data and determine the sequence of evacuations (triage);

– ensure that oxygen supply is sufficient for each journey.

Unfortunately, the number of COVID-infected patients is still high in some Russian regions. At the moment, 2 EMS teams from NCMD Zashchita are carrying out medical evacuations of patients with COVID-19 from Blagoveshchensk to the specialized facilities of Komsomolsk-on-Amur and Aldan. Between 13 and 20 of June, 2020, a total of 44 patients were transferred to the receiving facilities; the patients had confirmed or suspected COVID-19, moderately severe double pneumonia, stage I/II respiratory failure. The patients were evacuated in groups of 22 individuals. While boarding and disembarking the plane in the order or priority, a 1.5 m distance was kept between the patients. This distance was maintained on board; all patients were wearing face masks and medical gloves. Severely ill patients were accommodated in the forebody area to enable unobstructed access for the medical team.

An intensive care unit was deployed in Svobodny by the specialists of NCMD Zashchita to prepare severely ill patients for medical evacuation. Over 130 patients were consulted on

Fig. 1. Patient isolation transport unit (PITU)

Fig. 2. Transport of patient with COVID-19 in PITU

the possibility of medical evacuation and received supportive treatment.

Special attention was paid to the medical evacuation of the infected veterans — participants of the Victory Parade. Before the parade, about 30 veterans aged over 94 years were accommodated in a Moscow region rehab center. In case of acute respiratory or other symptoms, CEAE and MFH crews transferred the patients to the Clinical hospital № 123 for further care. On the day of the Parade, medical personnel accompanied the veterans to the Red Square and back to the facility.

FMBA will provide medical care to the participants of the Tavrida Youth Arts Forum held in Crimea in June–July 2020. In case of emergency, medical evacuations will be performed by the teams of NCMD Zashchita using the accumulated knowledge and experience.

CONCLUSIONS

1. At the beginning of the pandemic, medical evacuations of COVID-19 patients were carried out following the existing guidelines, which was sufficient to ensure biosafety of the involved EMS crews.

2. NCMD Zashchita ensures biosafety of its personnel by following the official guidelines specified in normative documents reinforced with additional techniques.

3. For long journeys over 1 h, PPE should be donned upon arrival at the scene before leaving the EMS vehicle.

4. Prior to starting a mass medical evacuation of patients with COVID-19, their number and condition should be assessed to determine the priority sequence for evacuation and the required oxygen supply.

5. There are a few specific aspects to the aeromedical evacuation of COVID-19 patients distinguishing it from the medical evacuation of patients with other conditions. The analysis of past evacuations allowed us to significantly improve our evacuation strategies.

6. Compliance with the current guidelines and regulations at all stages of medical evacuation ensures a high level of biosafety, protects the EMS team involved, prevents the spread of infection and patient deterioration.

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INOBVIOUS PATHOGENETIC LINKS OF MECHANISMS EFFECTS ON THE HUMAN ORGANISM OF THE SARS-COV-2 VIRUS

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The authors formulated a hypothesis about an important link in the pathogenesis of COVID-19, in which the increasing hypoxia and an acute response of the body like a general adaptation syndrome, accompanied by systemic pathological changes, including dangerous disorders of rheology and blood coagulation, play a key role.

Keywords: coronavirus, COVID-19, hypoxia general adaptation syndrome, cortisol immunity, neutrophils, monocytes, lymphocytes, cytokines

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НЕОЧЕВИДНЫЕ ПАТОГЕНЕТИЧЕСКИЕ ЗВЕНЬЯ МЕХАНИЗМОВ ВОЗДЕЙСТВИЯ НА ОРГАНИЗМ ЧЕЛОВЕКА ВИРУСА SARS-COV-2

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Авторами сформулирована гипотеза о важном звене патогенеза COVID-19, в котором ключевую роль играют нарастающая гипоксия и острая ответная реакция организма по типу общего адаптационного синдрома, сопровождающиеся системными патологическими сдвигами, в том числе опасными нарушениями реологии и коагуляции крови.

Ключевые слова: коронавирус, COVID-19, гипоксия, общий адаптационный синдром, кортизол, иммунитет нейтрофилы, моноциты, лимфоциты, цитокины

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Today, specialists in various fields of knowledge pay much attention to the problems associated with COVID-19, a new disease caused by the SARS-CoV-2 coronavirus that has grown into a developing pandemic. The volume of scientific information on COVID-19 is growing exponentially, but for obvious reasons there are still few works summarizing these scattered pieces of data.

This report puts up for discussion some theses of the hypotheses about one of the likely significant links in the pathogenesis of the disease caused by coronavirus. These theses and hypotheses were formulated based on the published information describing properties of SARS-CoV-2. Intentionally, their presentation is as succinct as the format of this brief report allows; sequentially, they are formulated as follows:

A. In many COVID-19 cases, coronavirus infection causes development of severe mixed hypoxia resulting from primary respiratory failure with damage to the lungs (respiratory hypoxia), violations of structure of hemoglobin of erythrocytes and the associated inability of the latter to transport oxygen (hemic hypoxia), a result of viral myocarditis (circulatory hypoxia) and the fading activity of respiratory enzymes in mitochondria (primary tissue hypoxia).

1. SARS-CoV-2 virus enters cells as a complementary attachment to the receptors of angiotensin-converting enzyme-2 (ACE-2) expressed by lung tissue cells [14, 19, 21, 31].

2. When COVID-19 patients take antihypertensive drugs systematically, the cells of their bodies can increase expression of ACE-2 3–5 times, since antihypertensive drugs block conversion of angiotensin-1 into angiotensin-2 or block angiotensin-2 receptors [14, 15], which promotes penetration of virions into cells, accelerates development of the disease and, quite likely, ultimately makes its course more severe [28].

3. With virions blocking ACE-2 receptors, the normal metabolism of angiotensin-2 (AT-2) is disrupted, which apparently increases AT-2 level locally, inside the lungs, and causes local intrapulmonary vascular hypertension. Further, the blocking probably disrupts the body's arterial regulation system. It is possible that levels of AT-2 expression and virus load make this violation of AT-2 metabolism more pronounced.

4. Vascular hypertension develops as an intrapulmonary symptom against the background of the body's inflammatory response to virus infection; this hypertension leads to the development of pulmonary edema and increased hydration of interstitial tissue, which CT scans visualize as "ground glass opacities". In many COVID-19 patients, respiratory function disruptions and growing hypoxia manifest as a pronounced drop in blood oxygenation. As hypoxia spreads to circulatory, hematic systems and tissue, the body suffers total oxygen deficiency and launches hypoxia mitigation and compensation mechanisms.

B. The first response of the body to acute hypoxia is the genetically determined general adaptation syndrome (GAS), which implies a sharp and significant increase of the cortisol level.

1. Further drop in oxygenation translates into buildup of acute hypoxia and dysfunction of many tissues and organs, including those critical to the body's vital functions [5, 12].

2. The body perceives acute hypoxia as a dangerous violation of homeostasis [10, 11].

3. Same as other life-threatening disorders of homeostasis, acute hypoxia triggers a set of GAS reactions [9, 11] and, consequently, a significant increase of the cortisol level [2, 7].

Nota bene! Currently, there are no routines to control cortisol level in COVID-19 pneumonia patients adopted in clinical practice, therefore there is but a small chance of finding published papers describing hypoxia and cortisol level correlation in COVID-19 patients.

C. Increased level of cortisol significantly changes functioning of the immune system, hematopoiesis, rheological and coagulation properties of blood (maintenance thereof), and disrupts production of eicosanoids [4].

1. The release of cortisol boosts the number of neutrophils [4, 6, 22, 23], which makes them attack not only virions but also pulmonary epithelium, vascular endothelium and other cells. Massive damage to the lung vessels' endothelium cells stimulates formation of parietal blood clots that disrupt hemodynamics, with further development of circulatory hypoxia being the result thereof.

2. The release of cortisol suppresses functional activity of lymphocytes and negatively affects their number [4, 18, 24], which bereaves the immune system of the majority of its antiviral competences that enable selective and targeted response to viral infection (blocking virions from entering cells and their subsequent elimination, as well as selective destruction of infected cells).

Nota bene! The fact that blood plasma transfusions from people that recovered form COVID-19 to current COVID-19 patients in severe conditions proves a very effective therapy (such blood plasma contains antibodies to SARS-CoV-2) highlights the importance of this selective and targeted immune response.

3. The release of cortisol degrades the number of monocyte macrophages, which normally eliminate virions tagged by antibodies [4, 13].

4. The release of cortisol increases the number of erythrocytes and platelets and enhances the vasoconstrictor effect of other hormones [4], which worsens blood fluidity, increases its viscosity and coagulability, thus promoting thrombosis (including disseminated intravascular coagulation). Jointly, these changes further aggravate hypoxia through the development of its circulatory component [13].

5. The release of cortisol suppresses production of the whole range of eicosanoids, including prostacyclins and thromboxanes [4]. In patients whose blood is prone to grow highly viscous and clot (which is typical for patients with increased basal level of cortisol), the disappearance of this pair of "operational control moderators" of rheological properties of blood can lead to thrombosis (including disseminated intravascular coagulation syndrome, acute respiratory distress-syndrome) and significantly boost circulatory hypoxia [13].

6. The release of cortisol, as can be expected, contributes greatly to the development of cytokine storm. Inflammatory cytokines, produced on the mass scale at the initial stage of response to inflammation by mast cells and then by neutrophils, which are abundant in the inflammation zone, should normally trigger activation of the adaptive immunity system (lymphocyte system) and migration of monocyte macrophages to the inflammatory zone. At the final stages of inflammation response, anti-inflammatory factors produced by macrophages (transforming TGF-β growth factor) and partly by lymphocytes [29] block migration of new neutrophils to the inflammation zone and production of inflammatory cytokines by those neutrophiles and mast cells [20, 25, 26]. Against the background of increased basal level of cortisol, when the function of macrophages and lymphocytes is suppressed, inflammatory response cannot complete and inflammation can continue in a self-sustaining mode or develop further, turning into a cytokine storm. Eicosanoid mediators (lipoxins, resolvins, prostaglandin D2, etc.) normally play an equally important role in the completion of inflammatory response [14, 17], but in the considered case their production is also repressed by cortisol [1, 3, 4].

At the end of this sequence of theoretical considerations, we considered it appropriate to present for discussion several generalizations related to the hypothesis, which, in our opinion, are of practical importance.

1. Decreased blood oxygenation and increased cortisol level precede changes in the rheological and coagulation properties of the blood.

If this assumption finds laboratory-backed confirmation in COVID-19 pneumonia patients, the time-conjugated moments of onset of the blood oxygenation level decrease and cortisol level increase should trigger a mandatory blood coagulation properties monitoring routine and an appropriate anticoagulation course.

2. Acute hypoxia developing in COVID-19 pneumonia patients harms those with malfunctioning organs more severely when such malfunctioning is caused by chronic diseases. Organs working at the top of their capabilities will fail in the event of insufficient supply of oxygen, even if its level is extremely low but sufficient to maintain the vitality of the body. Pathological process quickly acquires properties of an avalanche.

3. The search for means and ways to prevent the negative effect cortisol has on the physiological processes in the bodies of severe covid pneumonia patients (and, probably, in cases of pneumonia of different etiologies) can be considered a promising direction of research aimed at increasing the efficacy of treatment of inflammatory lung diseases.

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