Biomarkers of clinical course in covid-19 patients with cardiovascular comorbidity

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Abstract. A new coronavirus infection (COVID-19) tends to have severe course in patients with cardiovascular disease, with routine laboratory tests predicting adverse outcomes in such patients. The results of studies of interplaying factors are contradictory and require further investigation. The aim was to analyze the parameters of general blood analysis, inflammatory response, cholesterol and hemostasis in the groups of patients who underwent COVID-19-associated pneumonia with cardiovascular comorbidity. The study was conducted in Veliky Novgorod from December 2020 to April 2022 during inpatient treatment of patients diagnosed with COVID-19-associated novel coronavirus infection. We analyzed 108 case histories of patients. The patient's data was divided into 2 groups. Group I consisted of 86 patients with cardiovascular diseases at the time of admission. The control group consisted of 22 patients without concomitant cardiovascular diseases. The data of general blood analysis, biochemistry and hemostasis were assessed on the day of admission and on the day of discharge. Results. Average bed-days of patients with cardiovascular pathology were longer than in the control group; in addition, there was a correlation of the duration of hospitalization and CRP level with the initial level of total cholesterol. Also, positive correlation of CRP level with blood fibrinogen content was found, which was more expressed in patients with cardiovascular comorbidity. Conclusions. The results of our study, in general, do not contradict the results accumulated in the world. Those findings should be compared with other studies and to monitor COVID-19 disease trends.

Keywords: CRP WBC lymphocytes cholesterol fibrinogen

1. Introduction

Acute respiratory viral infection COVID-19 has spread around the globe, an infectious disease caused by coronavirus type 2, which is typified by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As such, there is no etiotropic treatment, which is a serious problem, since the disease has a high clinical and social significance [1].

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Older age (over 65 years), male gender, diabetes mellitus, obesity, the presence of cardiovascular disease, are known to increase the risk of hospitalization and the severe course of COVID-19. Patients with cardiovascular disease (CVD) are identified as a separate risk group. Based on statistical data, the presence of cardiovascular disease increases the possibility of hospitalization by 6 times. It has been recorded that patients with comorbidities have a 12-fold higher mortality rate compared with patients without comorbidities [2]. The results of other studies conducted in 5,000 inpatients with COVID-19 showed that, at the time of admission, changes in many blood parameters, such as troponin over 1 ng/mL, C-reactive protein over 200 mg/L, fibrin-fibrinogen degradation product D-dimer above 2,500 μg/L, and a saturation rate of less than 88% were more dependent on disease severity than on age and comorbidity [3].

In COVID-19 patients, endotheliocyte damage has been found in many organs due to exposure to the virus, a systemic inflammatory response, sometimes transient into a "cytokine storm" [4]. SARS-CoV-2 virus attachment and penetration into patient cells is directly dependent on lipids [5]. Binding of the virus to the ACE2 receptor occurs in lipid rafts on the surface of the cytoplasmic membrane, in which there is a high concentration of cholesterol [6,7].

Endothelial dysfunction in various CVDs, imbalance of oxygen demand and delivery, cardiotoxic effect of antiviral therapy - these pathogenetic mechanisms lead to myocardial damage [8]. At the same time, a study of acute MI hospitalizations in Northern California found that, between January 1 and April 14 both 2019 and 2020, the number of acute MI hospitalizations decreased as COVID-19 incidence increased [9]. The specific reason for such effect is unknown.

Therefore, the criteria for identifying patients at high risk of adverse outcomes, peculiarities of the course and treatment of new coronavirus infection in patients with comorbid pathology continue to be studied. [10,11].

During pandemics economic costs in the health care system increase dramatically, so the study of routine laboratory indicators, allows to predict the risk of adverse outcomes on the basis of biomarkers.

Aim of the study. To perform a prospective analysis of general blood count, inflammatory response, cholesterol levels, and hemostasis in patients with COVID-19-associated pneumonia and the presence of cardiovascular comorbidity; to identify possible interplay of those indices.

The following objectives were set for this purpose:

1) To perform a systematic Russian and foreign literature search for articles reflecting current state of understanding of the severity of the course of COVID-19 in patients with cardiovascular comorbidity;

2) To conduct a retrospective analysis of case histories of patients with COVID-19 and concomitant cardiovascular pathology in Veliky Novgorod;

3) To analyze the interplay of inflammatory and coagulation biomarkers, cholesterol levels in COVID-19 patients with concomitant cardiovascular pathology;

2. Materials and Method.

The study was conducted in Veliky Novgorod from December 2020 to April 2022 during inpatient treatment of patients diagnosed with new coronavirus infection caused by COVID-19. Analysis of medical records of 108 patients undergoing inpatient treatment in one of the COVID hospitals of Novgorod region was performed. The patient data was divided into 2 groups. Group I consisted of 86 patients with cardiovascular diseases at the time of admission. Among them there were 33 men, mean age 64.2±12.2 years, and 53 women, mean age 67.7±13.4 years ($p>0.05$). The comorbidity structure was as follows: essential hypertension (EH) in 42 patients (48.84%), EH+ischemic heart disease (IHD) in 23 patients (26.74%), EH+IHD+ heart failure (HF) in 20 patients (23.26%), HF in 1 patient (1.16%). According to the severity of hypertension the distribution was as follows: Stage 1 was noted in 3 patients (3,53 %), Stage 2 - in 36 patients (42,35 %), Stage 3 - in 46 cases (54,12 %). Group II (control) consisted of 22 patients without concomitant cardiovascular diseases. Gender composition was 10 men, mean age 43.6±14.9 years, and 12 women, mean age 43.2±11.8 years ($p>0.05$).

Patients of both groups were admitted for treatment in a state classified as moderate or severe. X-ray (computed tomography) signs of pneumonia were the criteria of severity. Clinical diagnosis was established on the basis of complaints, life history data, objective examination data and laboratory diagnosis. Treatment was performed taking into account provisional guidelines of the Ministry of Health of the Russian Federation "Prevention, diagnosis and treatment of new coronavirus infection (COVID-19)" version 13.1 (09.11.2021) [12]. The diagnosis of COVID-19 was verified by polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP); the examined biomaterial was obtained from nasopharynx and oropharynx. All patients were discharged home under the supervision of a district therapist for further treatment once their condition had improved.

Data was obtained at two points: on the day of admission and at discharge. The following routine investigations were carried out: blood cell count, coagulation tests, biochemical analysis. C-reactive protein, leukocyte and lymphocyte counts were considered as immunoinflammatory indices, fibrinogen content – as blood clotting index. Total cholesterol (TC) blood level was also assessed. The following ranks were formed among Group I patients based on their TC blood levels: rank I - normal level less than 5 mmol/L; rank II - moderately elevated level 5-7 mmol/L; rank III - high level 7 mmol/L or more.

Statistical processing was performed using StatSoft Statistica 10 software package. When comparing normally distributed indices, Student's t-test was used; in case the sample did not follow the normal distribution law, nonparametric Mann-Whitney U test and Kruskal-Wallis test were used. Wilcoxon test was used to compare the indices before and after treatment, Spearman rank correlation coefficient was used to establish relations between the indices. P value less than 0.05 was considered statistically significant.

3. Results

The duration of hospital stay in patients with concomitant cardiovascular pathology averaged 4.6 bed days longer than in the control group, 18.4±7.2 days and 13.8±4.9 days, respectively ($t=1.681$, $p=0.002$). In the first group of patients, gender differences were noted in the analysis of the duration of hospital treatment. It was shown that men had a shorter median of 17 bed-days (interquartile range, IQR 11-19 bed-days) and women had 19 bed-days (IQR 15-22 bed-days, Mann-Whitney U test $Z= 2.17571585$ $p=0.029577$). In the control group, the duration of hospital stay was not dependent on gender ($p>0.05$). Considering that we formed subgroups of patients depending on blood TC levels, as well as the important role of lipids in the pathogenesis of COVID-19 and cardiovascular disease, it was interesting to analyze the possible relationships between its level and the duration of hospitalization in patients. An inverse correlation between serum TC content and was duration of hospitalization revealed. At normal TC levels, the median was 19 bed-days (IQR 16-22 bed-days), at moderately elevated TC levels the median was 12.5 bed-days (IQR 9.75-18 bed-days, at high TC levels - 13.5 bed-days, IQR 11.75-18.75 bed-days (Kruskal-Wallis test: $H=8,444199$ $p =0,0147$).

White blood cell (WBC) count in the blood of Group I patients increased at discharge compared with admission: median change 1.5*10^9/L, IQR -1.2-6*10^9/L, Wilcoxon test $Z=3.03710827$ $p<0.0024$, the number of lymphocytes also increased: median change 0.8*10^9/L, IQR 0.1-1.3 Wilcoxon test $Z=5.08322939$ $p<0.001$. Cell count count in group II also increased: WBC median by 5.5*10^9/L IQR 1.075-8.6*10^9/L, Wilcoxon test $Z=3.32772915$ $p<0.001$, lymphocytes by 0.9*10^9/L, IQR 0.2-1.6*10^9/L Wilcoxon test $Z=3.52252305$ $p<0.001$. We also analyzed the dependence of changes in leukocytes on the TC blood levels. Compared with admission, in Group I there was a decrease in leukocyte levels at discharge only in patients with high TC levels: in patients with normal TC the WBC increase median was +2.2*10^9/L, IQR 0.1-6.1*10^9/L, with moderately elevated TC - 0.0, IQR -7.3-2.4*10^9/L, with high TC -4.8*10^9/L, IQR -8.5--0.5*10^9/L (Kruskal-Wallis test: $H=9.038257$ $p =0.0109$). Note the negative dynamic of WBC count in high TC patients. Lymphocyte count in Group I also decreased in patients with high TC levels: while normal TC levels were accompanied with the median change +0.9*10^9/L, IQR 0.4-1.3*10^9/L, in moderately elevated TC patients it was +0.1*10^9/L, IQR -0.6-0.9*10^9/L, and with high TC –

lymphocyte count decreased -0.7*10^9/L, IQR -1.1-0.0*10^9/L (Kruskal-Wallis test: H=14.20225 p=0.0008).

WBC blood count of Group I patients at discharge increased compared with admission: median change +1.5*10^9/L, IQR -1.2-6*10^9/L, Wilcoxon test Z=3.03710827 p<0.0024, lymphocyte count also increased: median change +0.8*10^9/L, IQR 0.1-1.3 Wilcoxon test Z=5.08322939 p<0.001. WBC count in group II also increased: median by 5.5*10^9/L IQR 1.075-8.6*10^9/L, Wilcoxon test Z=3.32772915 p<0.001, and lymphocytes by 0.9*10^9/L, IQR 0.2-1.6*10^9/L Wilcoxon test Z=3.52252305 p<0.001. We also analyzed the dependence of changes in leukocytes on the TC levels. Compared with admission, in Group I there was a decrease in WBC blood count at discharge, but only in patients with high TC: in normal TC patients the median of 2.2*10^9/L, IQR 0.1-6.1*10^9/L was observed, in moderately elevated TC patients there was 0 median change, IQR -7.3-2.4*10^9/L, in patients with high TC – median decreased -4.8*10^9/L, IQR -8.5--0.5*10^9/L (Kruskal-Wallis test: H=9.038257 p =0.0109). Lymphocyte count at discharge in Group I patients also decreased only in high TC level patients: with normal TC levels, the median change was +0.9*10^9/L, IQR 0.4-1.3*10^9/L, with moderately elevated TC it was +0.1*10^9/L, IQR -0.6-0.9*10^9/L, with high TC there was a decrease -0.7*10^9/L, IQR -1.1-0.0*10^9/L noted (Kruskal-Wallis test: H=14.20225 p=0.0008).

Median blood fibrinogen content at admission in group I patients was 3.90 g/L, IQR 3.37-4.63 g/L, in group II - 3.95 g/L, IQR 3.69-4.50 g/L. The results of biochemical blood tests taken from patients before discharge confirmed the reliability of the decrease in this biomarker (Wilcoxon test Z=6.08534418 p<0.001 in group I, Wilcoxon test Z=3.29985891 p<0.001 in group II). In the course of treatment, patients with cardiovascular comorbidity showed a decrease in fibrinogen levels, but the effect was weaker the higher the baseline TC level was: with normal TC, the median change was -1.50 g/L, IQR -2.68--0.53 g/L; with moderately elevated TC, -0.70 g/L, IQR -1.35-0.06; with high TC, -0.40, IQR -1.17-1.43 (Kruskal-Wallis test: H=8.688106 p=0.0130).

CRP is an acute phase protein, associated with inflammation. At admission, patients in both groups had elevated levels of CRP, with patients with cardiovascular comorbidity having higher levels compared with control: median 82.7 mg/L, IQR 34.8-119.7 mg/L versus 31.9 mg/L IQR 17.65-83.35 mg/L (Mann-Whitney U test Z= 2.17571585 p = 0.029577). CRP levels at discharge normalized in patients in both groups (median 4.4 mg/L, IQR 1.2-10.35 mg/L and 2.4 mg/L, IQR 0.8-5.4 mg/L, respectively, Mann-Whitney U test Z=2.03935682 p=0.0414152412).

There was an inverse correlation between the decrease of CRP and TC content in the blood of Group I patients: with normal levels of TC, the median change in CRP level at discharge relative to admission was -83.7 mg/L, IQR -123.8-33.8 mg/L, with moderately elevated TC change was -49.4 mg/L, IQR -56.6-14.2 mg/L, with high TC -52.2, IQR -74.7-21.9 (Kruskal-Wallis test: H=9.266486 p=0.0097).

The study of correlations between biochemical blood parameters of patients revealed a strong positive correlation between CRP and fibrinogen content on admission in both groups, with values of 0.62 and 0.33, respectively (r-Spearman correlation p<0.05), while on discharge, there was a strong positive relationship between CRP and fibrinogen content only in group I, with a value of 0.70 (r-Spearman correlation p<0.05), but not in the control group.

4. Discussion

There were men and women in the experimental and control groups. It is known that standardized CVD mortality rates of men in economically developed European countries and Russia are higher, but the absolute number of CVD deaths is significantly higher in women [13]. Estrogens and progesterone have a protective effect in women during the reproductive period. According to modern concepts, they reduce the formation of cholesterol, low-density lipoproteins (LDL); contribute to an increase in high-density lipoprotein (HDL) levels. However, after menopause arterial hypertension and cardiovascular diseases associated with hypertension incidence increases sharply [14].

The association of greater COVID-19 severity (in our observation, by duration of hospital stay) with low serum TC levels on admission has also been described by other researchers [15,16]. However, there are reports of a more severe course of COVID-19 in patients with elevated cholesterol levels [17].

Laboratory parameters to monitor disease progression include a highly sensitive indicator, C-reactive protein, [18]. In our observations CRP as an indicator of the severity of systemic inflammation decreases as a result of treatment, however, in patients with high levels of cholesterol it is accompanied by a less significant decrease in CRP. It is known that endothelial dysfunction and high cholesterol levels are interrelated [19]. And in COVID-19, endotheliocyte damage has been found in many organs due to exposure to the virus, a systemic inflammatory response, sometimes transiently turning into a "cytokine storm" [4]. This can probably explain the slower decrease of CRP values in this case. Clinically, the patients demonstrated stable positive dynamics, which was reflected in the length of their hospital stay.

It was noted that in the course of treatment the lymphocyte count in both groups increased, which is a reflection of the positive dynamics in the course of the inflammatory process. It was proved that steady decrease of lymphocyte count in peripheral blood is an early sign of severe/critical condition of patients with COVID-19 [20]. The most reliable predictors for assessing the severity of the disease according to the results of various studies include increased levels of CRP, LDH, D-dimer, as well as decreased blood platelet and lymphocyte count [21].

In our study the increase of leukocyte and lymphocyte count occurred in patients with normal TC, but in patients with high serum cholesterol content there was their decrease observed, which may indicate the emerged immune system dysregulation on the background of pronounced endothelial dysfunction and extensive pharmacotherapy. Lymphopenia, T-cell depletion, impaired cytotoxic lymphocyte activity, urgent granulopoiesis and increased neutrophil activation are just some of the examples of immune dysregulation reported in patients with severe COVID-19 [22].

Hyperfibrinogenemia as a manifestation of systemic inflammation is a characteristic feature of COVID-associated coagulopathy. Its level usually correlates with the severity of the disease [23]. Elevated fibrinogen level increases cardiovascular risk due to adverse effects on plasma viscosity, coagulation, platelet activity, inflammation and atherogenesis Analyzing our data, it was noted that during treatment there was a decrease in fibrinogen level, which is probably associated with a decrease in activity of the inflammatory process, and the use of anticoagulant therapy. Analysis of the relationship of fibrinogen disorders revealed a statistically significant correlation ($p < 0{,}001$) with the content of C-reactive protein at fibrinogen values over 5.0 g/L according to Clauss [24]. In this study, we also noted a significant correlation between CRP level and blood fibrinpgen content.

5. Conclusion

Existing COVID-19 incidence of remains a serious problem, especially as it poses high risk of severe disease course especially to patients with comorbidities. Therefore, further analysis of case data is necessary, especially among groups of people with cardiovascular diseases, as this pathology is still has increasing trend of prevalence and mortality in the world. The results of our study, in general, do not contradict the results accumulated in the world. These data should be used to monitor and respond to COVID-19 morbidity trends.

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