Circulating biomarkers and their implications in COVID-19 pathogenesis

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Abstract. COVID-19 is an infectious disease caused by a new SARS-CoV-2 coronavirus. The coronavirus infection emerged in China in 2019 and quickly disseminated globally in a short time. According to WHO about 100 million people were infected in 2021, rising to 500 million in 2022. In most cases, the trajectory of COVID-19 concludes with a positive result. However, the likelihood of experiencing a serious form of the disease is quite high. A severe form of coronavirus infection causes a storm of reactions in the body. The increased release of cytokines into the blood in response to viral infection is called cytokine release syndrome and causes acute respiratory distress syndrome and multi-organ failure. Timely control of cytokine storm with drugs such as immunomodulators and cytokine antagonists, as well as reduction of lung inflammatory cell infiltration is the main way to improve treatment effectiveness and decrease the mortality rate in individuals suffering from COVID-19.

1 Introduction

In 2019, a novel coronavirus outbreak originating from the new SARS-CoV-2 (COVID-19) virus emerged in Wuhan, Hubei Province, China. The illness has impacted numerous nations across every continent, prompting the World Health Organization (WHO) to classify it as a pandemic [1].

According to WHO, about 100 million people were infected in 2021, and this number increased till 500 million in 2022. Despite the end of the pandemic in our time, there is no definitive cure for COVID-19 infection and research is still ongoing [2].

The severe course of COVID-19 infection is characterized by reactions such as systemic hyperinflammation, acute respiratory distress syndrome, and multiorgan failure. Cytokine storm syndrome (CSS), which causes excessive immune responses, is the main cause of the severe form of SARS-CoV-2 coronavirus infection. Due to the accumulation of modern information about the immunopathogenesis of the novel coronavirus infection SARS-COV-2 (COVID-19), several approaches are currently used in real medical practice to treat the observed cytokine storm associated with COVID-19.

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In addition, finding new ways of treatment aimed at the use of cytokine blockers, namely, timely control of COVID-19 with drugs such as immunomodulators and cytokine antagonists, reducing the infiltration of inflammatory cells in the lungs, is one of the urgent issues.

The aim of the study: to make a comparative analysis of the features of blood characteristics of individuals afflicted with COVID-19 and patients after treatment.

2 Materials and Methods

2.1 Objects

45-85 year old people, who received medical care at the infectious diseases unit of the municipal hospital No. 12 served the object of the study. 48 people were recruited. They were divided into 3 groups: 1 – group of people of different ages in normal condition; 2 – group of individuals diagnosed with COVID-19; 3 – group of patients receiving treatment. Informed consent for analysis and participation in the study was obtained from all subjects. The Local Ethics Committee of Al-Farabi Kazakh National University approved the study by the protocol № IRB-A268, dated February 18, 2021.

2.2 Blood analysis

Venous blood samples from people participating in the study were analyzed. For COVID-19, blood samples were collected between 3 and 7 post-infection days, and for patients receiving treatment blood samples were collected at the 7th week of treatment. The patients received drug therapy according to COVID-19 infection treatment guidelines No. 106 (from July 15, 2020) [3].

Diagnostic criteria for “cytokine storm syndrome” include immune parameters of interleukin-6 (IL-6), lymphocytes, biochemical and hematological parameters of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, creatinine, urea, leukocytes, erythrocyte sedimentation rate (ESR), prothrombin time (PTT), C-reactive protein (CRP), procalcitonin (PCT), D-dimer, ferritin.

Blood samples obtained from the individuals enrolled in the study were examined in analyzers. The following analyzers located in the laboratory of City Hospital No.12 were used to analyze blood parameters of the subjects: Cobas e 411 (Roche Diagnostics International Ltd., Switzerland), Architect plus c4000 (Abbott, USA), iFlash 1800 (Shenzhen YHLO Biotech Co., Ltd., China), Siemens CS-5100 (Siemens Healthineers AG, Germany), Vision V-Counter (West Medica Produktions- und Handels- GmbH, Austria).

ELISA (enzyme-linked immunosorbent assay) was conducted to ascertain the immunologic index of IL-6.

2.3 Statistical analysis

Statistical analysis was performed using the SPSS program. The average value and standard error of the indicators were calculated. One-way ANOVA analysis was performed to determine the significance of differences between groups. Changes between the groups were studied by interval *-p<0.05, **-p<0.01, ***-p<0.001.
3 Results and discussion

The single-factor analysis of variance proved that the blood indices of COVID-19 patients and treated patients were changed compared with normal conditions. Table 1 shows the comparative blood indices of normal patients, COVID-19 patients and patients receiving treatment after COVID-19.

Table 1. Comparative blood values of normal patients, patients with COVID-19 and patients after COVID-19 treatment

<table>
<thead>
<tr>
<th>No.</th>
<th>Blood parameters</th>
<th>Normal conditions (n=21)</th>
<th>COVID-19 (n=27)</th>
<th>Post-treatment patients (n=27)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alanine aminotransferase IU/L</td>
<td>16.15±1.67</td>
<td>64.31±9.05</td>
<td>33.67±0.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2</td>
<td>Aspartataminotransferase IU/L</td>
<td>16.15±1.67</td>
<td>38.86±3.27</td>
<td>38.00±0.48</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>3</td>
<td>Bilirubin, µmol/L</td>
<td>10.11±1.50</td>
<td>11.28±1.01</td>
<td>109.68±4.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>4</td>
<td>Creatinine, µmol/L</td>
<td>65.95±4.01</td>
<td>80.15±8.64</td>
<td>99.04±2.17</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>5</td>
<td>Urea, mmol/L</td>
<td>4.69±0.41</td>
<td>8.93±0.99</td>
<td>9.17±0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6</td>
<td>Leukocytes, 10³/µL</td>
<td>5.96±0.36</td>
<td>10.24±0.69</td>
<td>5.74±0.01</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>7</td>
<td>Erythrocyte sedimentation rate, mm/h</td>
<td>5.30±0.80</td>
<td>36.35±1.61</td>
<td>30.25±0.03</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>8</td>
<td>Prothrombin time, seconds</td>
<td>11.81±0.25</td>
<td>12.98±0.45</td>
<td>11.77±0.03</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>9</td>
<td>C-reactive protein, mg/L</td>
<td>3.95±0.18</td>
<td>79.88±4.68</td>
<td>4.19±0.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>10</td>
<td>Procalcitonin, ng/mL</td>
<td>0.37±0.03</td>
<td>7.33±4.46</td>
<td>0.11±0.002</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>11</td>
<td>D-dimer, mg/L</td>
<td>0.34±0.02</td>
<td>1.59±0.33</td>
<td>0.61±0.0009</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>12</td>
<td>Ferritin, ng/mL</td>
<td>53.76±11.33</td>
<td>1113.55±87.46</td>
<td>283.33±24.46</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>13</td>
<td>Lymphocytes, 10³/µL</td>
<td>2.67±0.33</td>
<td>12.45±124</td>
<td>27.33±1.09</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

According to the study results, ALT, AST, and bilirubin levels were different in each group. For instance, if we compare patients with normal condition and patients with COVID-19, then during COVID-19 ALT increased 4 times, AST increased 2 times, and bilirubin increased 1.11 times. If we compare the values of ALT, AST and bilirubin after treatment with normal conditions, then ALT, AST increased 2 times, and bilirubin – 10 times. ALT, AST, and bilirubin levels changed after treatment for COVID-19. ALT decreased up to 2 times compared with COVID-19 treatment, AST remained unchanged after COVID-19 treatment, and bilirubin increased up to 10 times compared with COVID-19 treatment.

During COVID-19 creatinine index increased 1.21 times compared to normal, and urea index was 8.93±0.99 in COVID-19. This index shows that the amount of urea increased by 50% compared to normal conditions. Creatinine level in treated patients was 99.04±2.17. This index increased up to 1.5 times compared to normal conditions, and creatinine increased up to 1.23 times compared to the index during COVID-19.

As the results of study demonstrate, the number of leukocytes was 5.96±0.36 normally, 10.24±0.69 with COVID-19 and 5.74±0.01 after treatment. White blood cells increased 2 times during COVID-19 in compare to normal conditions. After treatment, this indicator returned to normal.

ESR was 5.30±0.80 normally, 36.35±1.61 with COVID-19 and 30.25±0.03 after treatment. This figure increased 6.8 times during COVID-19 and decreased 1.2 times after treatment.

According to the study results, prothrombin time under COVID-19 increased 1.09 times compared to normal conditions. The prothrombin time index after treatment was 11.77±0.03. This index normalized after drug therapy. The CRP index was 3.95±0.18 in normal
conditions. During COVID-19, the index of CRP increased 20 times and amounted to 79.88±4.68. It returned to norm after treatment.

As the results of study demonstrate, procalcitonin levels were 0.37±0.03 in normal, 7.33±4.46 during COVID-19 and 0.11±0.01 after treatment. Procalcitonin increased 19.81 times during COVID-19. After treatment, procalcitonin decreased up to 7 times compared to the level during COVID-19.

D-dimer levels increased 4.67 times during COVID-19. After treatment, this decreased 4 times compared to levels during COVID-19 and returned to normal.

Normal ferritin was 53.76±1.33. During COVID-19, this figure increased by 20.71 times. After treatment, ferritin levels in patients with COVID-19 decreased 5 times in comparison to the level with COVID-19, but were slightly higher than in normal. After the treatment, ferritin was 283.33±2.5.

During the study, it was noticed that lymphocytes gradually changed. In normal, the number of lymphocytes was 2.67±0.33. During COVID-19, the number of lymphocytes increased by 4.66 times. In our study, the lymphocyte level increased 2.5 times after treatment in compare to the level during COVID-19. The rate increased 10.23 times more than normal after treatment.

The normal level of IL-6 was 2.63±0.51. During COVID-19, the amount of IL-6 increased up to 53.88 times. After the treatment, the level of IL-6 in patients with COVID-19 decreased by 30.81 times compared to the level with COVID-19 and increased by 2 times compared to the normal state.

The findings from the research demonstrate that the blood parameters of patients who had recovered from COVID-19 and undergone treatment exhibited alterations compared to baseline conditions. Our results align with prior research with highlights presented below [4-17]. In the study, levels of ALT, AST, bilirubin, creatinine and urea were slightly increased during COVID-19 compared with normal conditions. According to the results of scientists’ research, SARS-CoV-2 directly damages liver and kidney tissue through its S protein and ATP2 protein, so these indicators indicate liver and kidney dysfunction. In addition, drug therapy can cause liver and kidney dysfunction. Increased levels of liver transaminases have been demonstrated to correlate directly with the severity of COVID-19 illness [4]. The existence of comorbidities such as diabetes and metabolic syndrome is also linked to raised levels of liver enzyme [5-6]. The increase in white blood cells in COVID-19 can be explained by the excessive activation of the body's immune system. In severe COVID-19, cytokine storm syndrome (CSS) occurs, causing multi-organ inflammation. Activation of cells of the innate immune system – dendritic cells, alveolar macrophages and neutrophils – stimulates the release of pro-inflammatory mediators (IL-1β, IL-6, IL-18, INF) and the differentiation of naive T lymphocytes into Th1. and cytotoxic lymphocytes (CTL or CD8+) [7]. Changes in ESR can be an indirect sign of current inflammation or other pathological process. Another reason for increased ESR during COVID-19 is CSS, which develops during a severe form of infection. As a result of inflammation, destruction of cells and tissues occurs, erythrocytes become heavy with immunoglobulins and other substances. The increase in ESR persists until the virus is in the body [8]. Elevated PTT has been noted in COVID-19 patients necessitating intensive care [9]. An elevated concentration of CRP may serve as a predictive biochemical indicator for the onset of chronic obstructive pulmonary disease and pre-eclampsia. Utilizing the CRP index can help anticipate the severity of the condition [10]. The study of PCT levels has become widespread due to the results of studies showing a very rapid increase in the content of this protein in response to infection. Thus, it has been studied that PCT is more effective in controlling the course of the disease than CRP [11]. Experts of the International Society of Thrombosis and Haemostasis Specialists consider increasing by 3-4-times in D-dimer level in a patient with COVID-19 as an independent indicator of hospitalization. The mechanism of hypercoagulability in patients with COVID-19 may be
associated with marked endothelial dysfunction and induction of platelet aggregation (endothelium carries ATP2 receptors and is a target of SARS-COV-2 virus). A series of separate papers have also been published in which increased titers of antibodies to phospholipids have been found in patients with COVID-19 and massive thrombosis; however, since they are often defined by a marked inflammatory response, such transient changes may be nonspecific [12]. Based on some studies, the lymphocyte level often decreases after COVID-19. And as a result of our study, the level of lymphocytes after therapy increased by 2.5 times compared to the level during COVID-19. After treatment, the rate increased 10.23 times compared to normal [13]. One of the key mediators of hyperinflammation in COVID-19 is IL-6. Multiple research investigations have indicated that COVID-19 patients exhibit heightened serum IL-6 levels, and these levels are directly linked to the severity of the illness. Consequently, elevated serum IL-6 levels have been suggested as an indicator of disease severity. The cytokine network is a sophisticated and diverse mechanism that regulates the immune response to antigenic stimulation. Among the most potent stimulators of cytokines are SARS-COV-2 viruses. Throughout the inflammatory process, immune system cells initiate the production of numerous anti-inflammatory cytokines (IL-1, IL-6, etc.) [14-15]. High concentration of IL-6 in the serum of patients with severe COVID-19 is associated with severe fever, development of bilateral widespread (>50%) lung damage, progression of ARDS, need for mechanical ventilation, development of respiratory failure and high risk of fatal outcome of the disease [16]. A recent retrospective study across multiple centers involving 150 confirmed Chinese COVID-19 patients revealed heightened IL-6 and ferritin levels (with an average of 1297.6 ng/mL in non-survivors and 614.0 ng/mL in survivors) as predictive factors for mortality. These findings suggest that increased levels of these markers may indicate a link between hyperinflammation and disease fatalities [17].

4 Conclusion

Changes in blood parameters of patients with critical COVID-19 and patients receiving treatment were determined. During COVID-19, ALT, AST, bilirubin, creatinine, urea, leukocytes, ESR, PTT, CRP, PCT, d-dimer, and ferritin increased slightly due to CSS. It is recommended that these indicators be used as early predictors of CSS. After the drug therapy, it was known that leukocyte counts, PTT, CRP, PCT, D-dimer normalized, and ferritin did not change significantly compared with those in COVID-19. Changes in immune system parameters in COVID-19 patients and post-treatment patients were revealed. During COVID-19, IL-6 and lymphocyte counts had been increased due to CSS compared to normal conditions. As an IL-6 inhibitor was used during therapy, IL-6 normalized and lymphocyte counts were increased compared to normal.

References

11. A. Zubarev, Procalcitonin is a new marker for the diagnosis of severe infection (review) [Electronic resource]: Intensive care, /2017 // URL: http://criticare.chat.ru/004.html (Data accessed February 21, 2024)