VER
Vascular & Endovascular Review

Studying The Role Of Cytokines In Patients With Covid-19 As A Predictor Of Disease Severity

Kurbanova Z.Ch., Shamsutdinova M.I., Sabitkhodzhaeva S.U.

Tashkent State Medical University

RESUME

The aim of the study was to examine cytokine production, examining cytokines as predictors of disease severity in patients with COVID-19.

Study materials and methods. Sixty patients with COVID-19 (30 with moderate and 30 with severe disease) hospitalized at the Zangiota 1 Specialized Hospital for the Treatment of Patients with Coronavirus Infection and 25 apparently healthy individuals were examined. The COVID-19 diagnosis was based on current protocols of the Ministry of Health of the Republic of Uzbekistan. All patients tested positive for SARS-CoV-2 using real-time PCR on a throat swab. Immunological studies were performed using the ELISA method (IL-1β, IL-6, IFN-gamma).

Results and discussion. Measurement of total cytokine levels of IL-1 beta, IL-6, and IFN-gamma revealed a moderate and statistically significant increase in three cytokines ($p<0.0001$) in severe patients, indicating active inflammation. IFN-γ levels showed a slight increase in the severe group compared to the moderate group. Thus, total IL-1 beta and IL-6 levels showed significant heterogeneity. Early studies of COVID-19 indicated the role of proinflammatory cytokines such as IL-1 beta, IL-6, and IFN-gamma released by activated mast cells in the respiratory submucosa in exacerbating inflammation and pathogenesis. Despite the uncertainty of the therapeutic potential of IL-6 and IL-10, the observed levels of their elevation in patients with severe COVID-19 have prompted clinical researchers to explore their use as prognostic factors.

Conclusions. 1. A significant increase in the cytokines IL-1 beta, IL-6, and IFN-gamma was demonstrated in both groups of patients, with a significant increase in these cytokines observed in the group of severely ill patients.

2. It was shown that the proposed simple panel of three cytokines can be used as predictors for the rapid diagnosis of patients at higher risk of worsening the course of COVID-19 disease.

3. IFN-γ levels showed a slight increase in the severe group compared to the moderate group. IL-1 beta and IFN-gamma levels were shown to be age-dependent, while the age-dependent relationship for IL-6 was not significant. No gender-dependent relationship was found for IL-6 levels.

4. These studies will be key to identifying patients who are more likely to progress to severe disease and thus taking the necessary precautions.

KEYWORDS: Cytokine regulation of immunity, cytokines, interleukins, cytokine storm, immunocompetent cells, COVID-19, severity of coronavirus infection.

How to Cite: Kurbanova Z.Ch., Shamsutdinova M.I., Sabitkhodzhaeva S.U., (2025) Studying The Role Of Cytokines In Patients With Covid-19 As A Predictor Of Disease Severity, Vascular and Endovascular Review, Vol.8, No.20s, 142-146

INTRODUCTION

The COVID-19 pandemic, caused by SARS-CoV-2, has become a serious threat to humanity, affecting virtually all of humanity on earth and resulting in the deaths of more than 10 million people [1] worldwide. Intense inflammation, manifested by elevated cytokine levels, commonly referred to as a "cytokine storm," often leads to critical conditions such as ARDS (acute respiratory distress syndrome) and death due to multiple organ failure [1, 2, 4, 5, 13, 16].

The innate immune response is the first step in the defense mechanism against viral infection. Pattern recognition receptors in host dendritic cells recognize viral genomic DNA or RNA to initiate the production of cytokines and chemokines [3,4,9,11,12,16], which in turn attract immune cells such as macrophages, neutrophils, and T cells to the site of infection depending on their source and target cells [2,6,7,14]. Proinflammatory cytokines, including interleukins (ILs) such as IL-1, IL-6, and others, play a major role in the initial response, whereas anti-inflammatory molecules such as IL-10 are produced during the sustained response to infection to control inflammation and maintain immune homeostasis [5,6,9,15]. Increased acute lung injury caused by cytokine storm leading to death [1,2,6,7,11] is a signature of the coronavirus family previously reported for MERS-CoV and SARS-CoV infections [7,8,17,19].

Thus, in COVID-19, numerous clinical studies have reported elevated levels of both pro-inflammatory and anti-inflammatory cytokines [9,10,11,19]. A recently published large meta-analysis summarized elevated levels of IL-2, IL-2R, IL-4, IL-6, IL-8, IL-10, TNF-α, and interferon-γ (IFN-γ) in the severe group of patients, whereas no significant increase in IL-1β and IL-17 levels was found [12,17]. A two-group meta-analysis synthesized based on individual patient data reported statistically significant odds

ratios ($p<0.05$) for the development of severe disease for only two cytokines, IL-6 and IL-10 [13,16,18]. Some meta-analyses reported differences in IL-6 levels between severe and non-severe COVID-19 patients in terms of standardized mean difference [14,17], mean difference [15,18], or mean ratio [6,7,9,16], which could potentially be used as a threshold to distinguish between severe and non-severe patients. All of these pooled meta-analytic results were associated with high levels of heterogeneity. Another synthesis of three clinical studies found an elevated IL-6/IFN-γ ratio in severe patients [17] with significant heterogeneity. A recent meta-analysis including 6242 patients in 24 studies found elevated IL-6 and IL-10 levels in severe COVID-19 patients, with little or no heterogeneity reported for IL-10 [18].

Clinical studies of COVID-19 patient cohorts have investigated the role of IL-6 alone [19] or IL-6 together with other cytokines including IL-10, IL-2, IL-4, TNF-α, and IFN-γ as a prognostic factor for severe disease [20]. Meta-analysis studies described previously [12,14,15,16] concluded that cytokine levels are elevated in patients with severe COVID-19 but did not attempt to establish their prognostic significance, with the exception of the study by Elshazli et al., who adopted tree curve and receiver operating characteristic (ROC) analysis to assess the prognostic potential of several laboratory parameters including IL-6 [13]. Another study with a cohort of 501 patients [3,7] attempted to create a mortality risk model using several clinical parameters and IL-6 level. However, despite numerous clinical studies and meta-analyses, a reliable prognostic method that could predict patient progression to severe disease based on cytokine levels at admission remains elusive. In this study, we attempt to develop a prognostic method through a meta-analysis of levels of 13 commonly used cytokine markers between groups of patients with severe and non-severe disease by constructing a classifier using a logistic regression model.

Therefore, the objective of this study was to establish that SARS-CoV-2, the cause of the ongoing COVID-19 pandemic, induces high levels of cytokines such as IL-1 beta, IL-6, IFN-γ, and others in infected patients with moderate to severe disease. However, despite the vast number of publications in this area, the role of cytokines in COVID-19 remains poorly understood. Determining the role of cytokines in COVID-19 is key to effective clinical management. This study conducted a comprehensive meta-analysis to establish the relationship between induced cytokines and COVID-19 disease severity to aid in prognosis and clinical treatment.

STUDY MATERIALS AND METHODS

Sixty hospitalized patients with confirmed COVID-19 (34 men and 26 women) were examined at the Zangiota-1 Specialized Hospital for treatment of patients with coronavirus infection. A comparison of immunophenotyping results was conducted with 25 apparently healthy controls of similar age and gender. The diagnosis of COVID-19 was based on current protocols of the Ministry of Health of the Republic of Uzbekistan, using a combination of clinical symptoms, disease severity assessment, computed tomography (CT) scans, and laboratory data. All patients were laboratory-confirmed to be positive for SARS-CoV-2 using real-time polymerase chain reaction (RT-PCR) of throat swab samples.

Exclusion criteria for the healthy control group included active respiratory infection, infection with other infectious agents (HIV, syphilis, tuberculosis, influenza, adenovirus infection, and other respiratory viral infections), severe systemic diseases, malignancies, and other chronic diseases, including hematological disorders, cachexia, active bleeding, malnutrition, cardiovascular, renal, pulmonary, and liver dysfunction. Written informed consent was obtained from all healthy individuals. The study included 30 patients with moderate and 30 patients with severe forms of the disease, based on clinical protocols for the diagnosis and treatment of coronavirus infection published by the Ministry of Health of the Republic of Uzbekistan.

Severe patients were defined according to the following criteria: respiratory rate ≥24 times/min; pulse oximeter oxygen saturation (SpO_2) ≤85% at rest; partial pressure of oxygen (PaO_2) <60 mmHg. Significant differences were observed in laboratory parameters of COVID-19 patients infected at the moderate and severe stages, including platelet count ($p<0.0001$), total lymphocyte percentage ($p<0.0001$), neutrophil percentage ($p<0.0001$), monocyte percentage ($p=0.0003$), prothrombin time ($p=0.0004$), albumin ($p=0.0008$), total bilirubin ($p<0.0001$), lactate dehydrogenase ($p=0.006$), blood urea ($p=0.03$), alanine aminotransferase ($p=0.01$), aspartate aminotransferase ($p=0.05$), C-reactive protein ($p<0.0001$), and erythrocyte sedimentation rate ($p=0.02$).

Immunological studies were conducted in the Fundamental Immunology Laboratory of the Institute of Human Immunology and Genomics of the Academy of Sciences of the Republic of Uzbekistan under a scientific agreement between the Institute and the clinic of the State Institution "Specialized Hospital "Zangiota 1" for the treatment of patients with coronavirus infection. The studies included measuring the levels of key immune system cytokines to assess the immunoreactivity of patients with severe forms of the disease. Serum production of the key cytokines IL-6, IL-1 beta, and IFN-gamma in the peripheral blood of patients was assessed. Vector-Best ELISA kits from Novosibirsk were used for the study.

All statistical analyses and graph preparation were performed using GraphPad Prism version 8.0 software (GraphPad Software Inc.). Categorical variables were presented as frequencies or percentages, and continuous variables were shown as means ± standard deviations or medians with interquartile ranges (IQR). The parametric two-tailed Student's t-test and the nonparametric Mann-Whitney U-test were used to calculate mean differences between groups, as appropriate. Categorical variables were compared using Fisher's exact test. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

A literature search was conducted for 13 cytokines from 18 clinical studies. The standardized mean difference for the selected 6 cytokines, IL-1 beta, IL-6, and IFN-gamma, between the groups of patients with severe and non-severe forms of COVID-19 were summarized using a random-effects model. A classifier was built using a logistic regression model with cytokines having a

significant SMD as covariates. It was found that IL-6, IL-1 beta, and IFN-gamma showed a statistically significant SMD in all synthesized studies. The classifier with the mean values of both IL-6 and IL-1 beta as covariates performed well with an accuracy of ~92%, which was significantly higher than the accuracy reported in the literature with IL-6 and IL-1 beta as separate covariates. Thus, it was demonstrated that the simple panel of only two cytokine markers proposed by the authors can be used as predictors for the rapid diagnosis of patients at higher risk of worsening the course of COVID-19 disease and, thus, can be well managed for a favorable prognosis. A meta-analysis was conducted in accordance with PRISMA guidelines [2,4,7,11,17]. A literature search was conducted in Pubmed, Google Scholar, and preprint archives such as medRxiv, bioRxiv, and the SSRN library for English-language articles published in 2020 up to May 31, 2020. The search terms included COVID-19-related terms in the article title ("2019-nCov" or "nCoV-2019" or "novel coronavirus" or "SARS-CoV-2" or "COVID-19" or COVID19 or "novel coronavirus") along with terms such as "cytokine levels" and combinations of common cytokine names and gene symbols. The search strategy was reviewed by all authors, and it was decided to retain articles that had not undergone peer review given the current situation. Identified articles were screened and shortlisted with inclusion criteria as clinical studies with laboratory data on at least two cytokines in patient groups with severe and non-severe COVID-19. Shortlisting exclusion criteria included review articles, opinion pieces and commentaries, studies including other pathological conditions or complications associated with COVID-19, and studies without mean or median data on cytokines and their variances for each group.

Measurement of total cytokines IL-1 beta, IL-6, and IFN-gamma was performed using a flow cytometry-based immunoassay. Analysis of the mean value of each marker revealed a moderate and statistically significant increase in three cytokines ($p<0.0001$) in severe patients. IFN-γ, a type II interferon, showed a slight increase in the severe group compared with the moderate group. Thus, total IL-1 beta and IL-6 values showed significant heterogeneity. The results showed that IL-1 beta and IFN-gamma levels were dependent on age differences, while the age dependence of IL-6 was not significant. No gender-dependent relationship was found for IL-6 levels, expressed as a difference in the percentage of men between the two groups. These results attribute some of the observed heterogeneity in the mean IL-6 and IL-1 beta values to the difference in mean age between the severe and non-severe groups. Our results show that of the cytokines analyzed, only the levels of proinflammatory IL-6 and IL-1 beta are significantly elevated in the severe group of patients, as reported in other studies [2,10]. Therefore, with the persistent spread of COVID-19 in most societies, a suitable prognostic test that can predict the progression of the disease to severe disease in patients with sufficient accuracy is needed for effective management and care. Early studies on COVID-19 have pointed to the role of proinflammatory cytokines such as IL-1 beta, IL-6, and IFN-gamma released by activated mast cells in the airway submucosa in aggravating the inflammatory state and pathogenesis [4,9,10,14,16] and the potential of inhibiting some of these cytokines as a possible supportive therapy. Conti and colleagues have extensively studied the respiratory dysfunction caused by the induction of the IL-1 beta cytokine family in pathogenic viral infections and proposed anti-inflammatory cytokines such as IL-37 or IL-38 as potential therapeutic agents for severe cases of COVID-19. However, subsequent clinical trials and meta-analyses, including the present study, have failed to definitively establish a significant increase in IL-1 levels in patients [5,14].

Elevated levels of IL-6, a pro-inflammatory molecule, are known to reduce NK cell activity and are also associated with decreased granzyme and perforin levels, causing impaired lytic activity [12,15,18]. In patients with COVID-19, exacerbation symptoms such as fever, elevated inflammatory markers such as CRP and serum ferritin, and progressive chest CT imaging were associated with elevated IL-6 levels, which decreased during recovery [5,15]. This association of IL-6 with pulmonary disease has been previously reported in patients with pneumonia [3,4,7,9] or severe radiotherapy-induced pneumonitis [17].

Although cytokine levels are elevated in patients with severe COVID-19, the therapeutic significance remains unclear. Corticosteroids, which can potentially suppress cytokines by inhibiting the transcription factor NF-κB, have been used in patients with COVID-19. The RECOVERY study, which included 2104 patients receiving dexamethasone compared with 4321 patients receiving standard care, showed that dexamethasone reduced mortality in severe patients with invasive ventilation or oxygen support, but had no effect on patients with mild symptoms [2,15]. However, a meta-analysis of studies related to SARS-CoV, SARS-CoV-2, and MERS-CoV infection showed an increased hazard ratio for mortality (HR 2.11, 95% CI: 1.13–3.94) in patients receiving corticosteroids [7]. Theoharides and Conti [16] argued that the use of dexamethasone as an immunosuppressant may be beneficial in the short term for severe COVID-19 patients, but it will negatively impact recovery in the long term due to the damaging effects of dexamethasone on T cell defense and B cell antibody production. Although the therapeutic potential of IL-6 and IL-10 remains unclear, their observed elevated levels in patients with severe COVID-19 have prompted clinical researchers to explore their use as prognostic factors. In an earlier study in children with pneumonia, the IL-6/IL-10 ratio at admission was an indicator of severe disease with a sensitivity and specificity of 76.5% and 83.3%, respectively [5,6].

Table 1.

Comparative characteristics of proinflammatory cytokines in moderate and severe cases of Covid-19, M± m

Patient groups	Serum IL-1β production	Serum IL-6 production	Serum IFN-gamma products
Moderately ill patients	20.4 ±1.74*	16.57 ±1.40 *	12.52 ±1.40 *
Severely ill patients	33.48 ±2.16*	28.55 ±1.62 *	6.55 ±1.42 *
Control	6.53±1.80	4.82±1.33	4.11±1.24

Note: * - differences with control values

Therefore, our results indicate a possible dysregulation of the immune response against COVID-19, characterized by three

cytokines: IL-1 beta, IL-6, and IFN-gamma, shifting the balance between non-severe and severe patient categories. Therefore, measurement of both markers is necessary to delineate the boundary. Measuring serum IL-6 and IL-1 beta levels is inexpensive and can be performed upon admission to a clinic or care center with minimal equipment. Such measurement will be key to identifying patients at higher risk of progression to severe disease and thus, implementing necessary precautions. We believe that further trials with multiple cohorts of COVID-19 patients or the assimilation of patient-level data from existing cohorts will be necessary to develop and validate the test. In addition to diagnostic potential, the analysis of such data will likely reveal the possibility of a potential therapeutic strategy targeting either IL-6, IL-1 beta, or both.

We studied serum and spontaneous concentrations of proinflammatory cytokines, such as IL-1β and IL-6, in patients with moderate to severe forms of COVID-19. A comparative analysis of serum production of proinflammatory cytokines in patients with moderate to severe forms of coronavirus infection revealed a significant increase in serum IL-1 beta, IL-6, and IFN-gamma in the moderate group, as shown in Table 1. However, the studied values in the severe group of patients showed a significant increase in all serum and spontaneous IL-1 beta and IL-6 levels compared to the control data. This is shown in Table 1. A closer look at the values between the study groups reveals a significant difference in IL-1 beta production between the groups, with a significant 1.6-fold increase in IL-1 beta observed in the severe group. Based on spontaneous IL-1 beta production, this cytokine was also significantly elevated in the severe group by 2.3 times. Regarding IL-6 production, a significant difference in serum and spontaneous production was also observed in both patient groups. For example, in the severe group, IL-6 levels in peripheral blood serum increased almost twofold.

CONCLUSIONS

1. A significant increase in the cytokines IL-1 beta, IL-6, and IFN-gamma was demonstrated in both groups of patients, with a significant increase in these cytokines observed in the group of severely ill patients.
2. It was shown that the proposed simple panel of three cytokines can be used as predictors for the rapid diagnosis of patients at higher risk of worsening the course of COVID-19 disease.
3. IFN-γ levels showed a slight increase in the severe group compared to the moderate group. IL-1 beta and IFN-gamma levels were shown to be age-dependent, while the age-dependent relationship for IL-6 was not significant. No gender-dependent relationship was found for IL-6 levels.
4. These studies will be key to identifying patients who are more likely to progress to severe disease and thus taking the necessary precautions.

REFERENCE

1. Hanaei S, Rezaei N. COVID-19: developing from an outbreak to a pandemic. Archives of Medical Research. 2020. doi:10.1016/j.arcmed.2020.04.021.
2. Saghazadeh A, Rezaei N. Immune-epidemiological parameters of the novel coronavirus–a perspective. Expert Review of Clinical Immunology. 2020;16:465–70. doi:10.1080/1744666X.2020.1750954.
3. Lotfi M, Hamblin MR, Rezaei N. COVID-19: transmission, prevention, and potential therapeutic opportunities. Clinica Chimica Acta. 2020;508:254–66. doi:10.1016/j.cca.2020.05.044.
4. Yazdanpanah F, Hamblin MR, Rezaei N. The immune system and COVID-19: friend or foe? Life Sciences. 2020;256:117900. doi:10.1016/j.lfs.2020.117900.
5. Liu WJ, Zhao M, Liu K, Xu K, Wong G, Tan W, Gao GF. T-cell immunity of SARS-CoV: implications for vaccine development against MERS-CoV. Antiviral Research. 2017;137:82–92. doi:10.1016/j.antiviral.2016.11.006.
6. Xu H, Zhong L, Deng J, Peng J, Dan H, Zeng X, Li T, Chen Q. High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. International Journal of Oral Science. 2020;12:1–5. doi:10.1038/s41368-020-0074-x.
7. Rokni M, Ghasemi V, Tavakoli Z. Immune responses and pathogenesis of SARS - CoV - 2 during an outbreak in Iran: comparison with SARS and MERS. Reviews in Medical Virology. 2020. doi:10.1002/rmv.2107.
8. He F, Deng Y, Li W. Coronavirus disease 2019 (COVID - 19): what do we know? Journal of Medical Virology. 2020;92:719–25. doi:10.1002/jmv.25766.
9. Chen J, Lau YF, Lamirande EW, Paddock CD, Bartlett JH, Zaki SR, Subbarao K. Cellular immune responses to severe acute respiratory syndrome coronavirus (SARS-CoV) infection in senescent BALB/c mice: CD4+ T cells are important in control of SARS-CoV infection. Journal of Virology. 2010;84:1289–301. doi:10.1128/JVI.01281-09.
10. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. The Lancet Respiratory Medicine. 2020;8(4):420–22. doi:10.1016/S2213-2600(20)30076-X.
11. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. Intensive Care Medicine. 2020;1–3.
12. Williams AE, Chambers RC. The mercurial nature of neutrophils: still an enigma in ARDS? American Journal of Physiology-Lung Cellular and Molecular Physiology. 2014;306:L217–L30. doi:10.1152/ajplung.00311.2013.
13. Cameron MJ, Bermejo-Martin JF, Danesh A, Muller MP, Kelvin DJ. Human immunopathogenesis of severe acute respiratory syndrome (SARS). Virus Research. 2008;133:13–19. doi:10.1016/j.virusres.2007.02.014.
14. Min CK, Cheon S, Ha NY, Sohn KM, Kim Y, Aigerim A, Shin HM, Choi JY, Inn KS, Kim JH. Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity. Scientific Reports. 2016;6:1–12. doi:10.1038/srep25359.
15. Golshani M, Saghazadeh A, Rezaei N. SARS-CoV-2–a tough opponent for the immune system. Archives of Medical Research. 2020.

16. Shakoory B, Carcillo JA, Chatham WW, Amdur RL, Zhao H, Dinarello CA, Cron RQ, Opal SM. Interleukin-1 receptor blockade is associated with reduced mortality in sepsis patients with features of the macrophage activation syndrome: re-analysis of a prior Phase III trial. Critical Care Medicine. 2016;44:275. doi:10.1097/CCM.0000000000001402.
17. Saghazadeh A, Rezaei N. Towards treatment planning of COVID-19: rationale and hypothesis for the use of multiple immunosuppressive agents: anti-antibodies, immunoglobulins, and corticosteroids. International Immunopharmacology. 2020;84:1–6. doi:10.1016/j.intimp.2020.106560.
18. Coomes EA, Haghbayan H. Interleukin-6 in COVID-19: a systematic review and meta-analysis. medRxiv. 2020.
19. Richardson P, Griffin I, Tucker C, Smith D, Oechsle O, Phelan A, Stebbing J. Baricitinib as potential treatment for 2019-nCoV acute respiratory disease. The Lancet. 2020;395:e30–e1. doi:10.1016/S0140-6736(20)30304-4.
20. Fardet L, Galicier L, Lambotte O, Marzac C, Aumont C, Chahwan D, Coppo P, Hejblum G. Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. Arthritis & Rheumatology. 2014;66:2613–20. doi:10.1002/art.38690.
21. Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nature Immunology. 2014;15:1009. doi:10.1038/ni.3002.