Chapter

Alteration of Cytokines Level and Oxidative Stress Parameters in COVID-19

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Abstract

In addition to the proinflammatory state, cytokine production, and cell death, SARS-CoV-2 infection is also associated with oxidative stress as demonstrated by increase in reactive oxygen species (ROS) levels and an alteration of antioxidant defense during the infection. Proinflammatory cytokines and chemokines play an important role in respiratory infections caused by viruses including SARS-CoV-2 by activation of the adaptive immune response. In case when the response is not controlled, it can lead to lung tissue involvement in the course of acute respiratory distress syndrome (ARDS) or can result in multiple organ failure. Oxidative stress markers show good correlation with several cytokines, which can be measured at the beginning of the disease in a primary care setting to predict the course of COVID-19.

Keywords: oxidative stress, cytokines, COVID-19, NLR, PLR

1. Introduction

Even though the pathogenesis of COVID-19 is still unclear, during the past 2 years, we can say that in general the mechanisms involved in this disease depend on several modalities with the final aim of the virus to learn how to escape the immune system of the host [1, 2]. Virus pathogenicity, comorbidities of the infected individual, and the ability of the host immune system to respond to the induced cytopathic effect have significant impact on the progress and outcome of the disease. The virus SARS-CoV-2 has so far affected more than 400 million people globally, with over 5 million deaths. The largest number of deaths, approximately 73%, is in the population over 65 years of age [3].

Cytokine storm syndrome has been widely discussed and proposed as one of the underlying etiologies of respiratory failure in patients infected with SARS-CoV-2. Cytokines present a group of polypeptides signaling molecules responsible for regulation of number of biological processes by using cell surface receptors. Proinflammatory cytokines and chemokines play an important role in respiratory infections caused by viruses including SARS-CoV-2 by activation of the adaptive immune response. When infected with influenza, an excessive amount of reactive

oxygen species (ROS) is generated in several tissues including the alveolar endothelium and epithelium [4] where induced cytokines expression by activation of Toll-like receptors (TLRs) is reported to be responsible for the pathogenesis [5, 6]. Increased oxidative stress is also present during infections with human respiratory syncytial virus [7], rhinoviruses [8], and many other viruses, which has been a subject of discussion in many previously published reviews [9–16], and several other experimental studies propose that the so-called cytokine storm correlated with direct tissue injury, which afterward results in unfavorable prognosis in patients with severe COVID-19 [10]. In patients with severe COVID-19, increased levels of several cytokines, namely IL-6, IL-10, IL-2R, and TNF-α, have been reported in recently published articles on this subject [17, 18]. However, other authors suggest that more cytokines, such IL-1β, IL-1RA, IL-8, and IL-18, are also included in the pathogenesis of SARS-CoV-2 infection [10, 17, 18].

In general, patients infected with SARS-CoV-2 have either normal or reduced white blood cells (WBC) count and lymphocytopenia, and patients with severe form of the disease have additional presence of significant increase of elevated neutrophil levels, D-dimers, accompanied with continuous decrease of lymphocytes and increase of levels of certain cytokines and chemokines.

The infection with SARS-CoV-2 follows the same pathway as the innate immune response as suggested by several authors [4, 14]. That is to say, reactive oxygen species present a strong ligand and a direct mediator in the inflammasome (NLP3) trigger. Furthermore, the reactive oxygen species activate the NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), and this further elicits the transcriptional levels of NLPR3, which are additionally enhanced by TLR and NLR (nod-like receptor) ligands. As a final outcome, this means that the ROS can increase inflammasome either directly or indirectly [12, 16]. In addition of ROS, H_2O_2 activates the NF-κB, which contributes to additional production of inflammatory cytokines [19]. Increased levels of the following cytokines: IL-6, TNF-α, IL-1β, IP-10, GCSF, MCP-1, MIP1-α/CCL3, followed by elevated blood ferritin levels, are also observed in patients infected with SARS-CoV-2 [11, 20]. Literature data has also discussed that the imbalance in the T-helper cell subsets (Th1/Th2/Th7) and regulatory T-cells also contribute to the COVID-19 pathogenesis. CD4+ T cells are divided into different subtypes based on their cytokine production, namely Th1 is producing IFN-γ, IL-2, and TNF-α, Th2: IL-4, IL-5, IL-9, IL-13, whereas the regulatory T-cells are producing TGF-β and IL-10, among others.

In this chapter, authors would like to share some experimental data, which were obtained in the last 2 years since the beginning of the COVID-19 pandemic. First, we will present results obtained by a highly standardized cytokine assay where we have measured plasma levels of IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-γ, TNF-α, IL-1α, MCP-1, and EGF potentially associated as key factors with the cytokine storm syndrome in critically ill COVID-19 patients. Afterward, we have investigated which of these cytokines involved in the cytokine storm of COVID-19 show good association/correlation with the oxidative stress markers determined with fast and inexpensive photometric analytical method [20, 21]. Also we will present results in regard to the relation between the cytokines, oxidative stress markers, and the most commonly used inflammation-related biomarkers (CRP, D-dimers, PLR, NLR, and LDH) in severe form of the disease will be discussed. Additionally, the readers can obtain information for which we believe could enhance the knowledge of the altered lymphocyte subsets and their correlation with the oxidative stress markers as a tool with prognostic power to differentiate between moderate and severe COVID-19.

2. Clinical laboratory parameters abnormalities in moderate and severe COVID-19 patients

Our results obtained from 50 hospitalized COVID-19 patients who were hospitalized at the University Clinic for Infectious Diseases and Febrile Conditions, Skopje, Republic of North Macedonia, within a period of 2 months at the beginning of the pandemic were included in this study. All patients were confirmed to have SARS-CoV-2 infection by real-time reverse transcriptase–polymerase chain reaction assay from nasal and pharyngeal swab specimen. The diagnosis and classification of COVID-19 were based on the Interim Guidance for Clinical Management of COVID-19 issued by the World Health Organization. Patients with moderate form of the disease were adults from both sexes with clinical signs of pneumonia with no signs of severe pneumonia and $SpO_2 > 90\%$ on room air. In terms of the severe cases, they additionally had at least one of the following conditions: $SpO_2 < 90\%$ on room air, respiratory rate more than 30 breaths per minute, or presence of severe respiratory distress.

The work was performed by a multidisciplinary group, including clinical experts in COVID-19 management.

When admitted to hospital, patients classified with the moderate form of the disease demonstrated abnormal values for CRP, LDH, glucose, and NLR. Namely, on hospital discharge, these clinical laboratories were improved significantly, namely the mean value for C-reactive protein (CRP) was 44.1 mg/L versus 7.16 mg/L, LDH was 280.3 IU/L versus 283.4 IU/L, and neutrophil to leukocyte ratio (NLR) was 6.5 versus 5.1 at admission and discharge, respectively. Whereas patients with severe form of the disease had more prominent abnormal values of CRP, LDH, creatinine kinase (CK), ALT, AST, glucose, WBC, and NLR levels. It is worth noting that the mean values of CRP, LDH, CK, ALT, and NLR in the patients with severe COVID-19 were higher when compared with the patients with the moderate form of the disease upon admission and discharge. In general, our results are added value to data obtained by other research groups where lymphopenia can be used as reliable marker of the severity of the disease and the necessity for hospitalization. Meta-analyses by Lagunas-Rangel [22] demonstrated that patients with severe form of COVID-19 had increased NLR and decreased lymphocyte-CRP ratio when compared with moderate and mild form of the disease. Also, large number of studies, including our results, had showed that in the patients with severe form of the disease and the ones that did not survive had low platelet counts. Namely, the results from our hospitalized patients with severe COVID-19 had increased WBC, neutrophils, NLR, and platelet to lymphocyte ratio (PLR). The coagulation profile revealed elevated levels for D-dimer, prolonged PT with normal aPPT and TT. Patients with severe form of the disease that have recovered had a statistically significant difference for NLR and PLR ($p < 0.01$), and the values were decreased to 70% and 67%, respectively, when compared with the values of these parameters on admission. Additionally we have observed increase in the lymphocytes for 53%. Also, WBC decreased to 18%, neutrophils to 11%, platelets to 19%, PT to 22%, and D-dimers to 85%, but the difference was not statistically significant when compared between admission and hospital discharge ($p > 0.05$). Patients that had deterioration of their condition and died had continuous increase in WBC and neutrophils (34%), NLR (55%), decrease in lymphocytes (42%), PT (18%), and D-dimers (50%). We have only observed statistically significant difference only for the lymphocytes as parameter compared with the admission values. Reported results from meta-analysis [23] with 1779 patients demonstrated that thrombocytopenia is

associated with fivefold increased risk for disease complications and death. D-dimer has also been applied as predictor for developing complications of the disease such as requiring mechanical ventilation, developing of acute respiratory distress syndrome, etc. Experiences from different clinical centers worldwide suggest that patients who had significant increase in the D-dimers should be considered for hospitalization even if there is an absence of symptoms.

All hospitalized patients whether classified with the moderate or the severe form of the disease had low partial pressure of oxygen, although it did not show statistically significant difference between the two groups. However, this observation had clinical importance indicating the need for supplemental oxygen therapy in both groups with moderate and with severe disease. The measured oxygen saturation was lower in the severe group of patients (86.26%), and this parameter had strong statistical significance, supporting the clinical indication for application of oxygen therapy. The analysis showed that in both groups, the partial pressure of carbon dioxide and pH values were within the reference values, the levels of bicarbonates were slightly increased, but without a significant difference between the patients with moderate and severe form of the disease. The base excesses were increased in the group of patients with moderate disease, and it was statistically significant, but no disturbances in acid balance ratio occurred within any of the group. Similar results were reported by Mumoli et al. [24] at hospital admission of 88 COVID-19 patients, as well as by Doaei et al. [25], who measured similar values of the blood gas parameters in critically ill patients with COVID-19 to those reported in our study. On the other hand, Deniz et al. [26] detected a mild increase of pH and bicarbonate and relatively low pCO_2 in COVID-19 patients compared with non-COVID-19 individuals.

3. Cytokine alteration and oxidative stress in patients with severe COVID-19

Cytokines as well as chemokines and growth factors together with the lipid metabolites present one of the key players in the immune cell function and their differentiation, which on the other hand, means that following a dysregulation in the process, various diseases can arise [11–13, 16]. In this chapter, we present some of our recently obtained results as an add-on to the clinical and scientific evidences in terms of oxidative stress, which is increased in patients with severe COVID-19. We have also obtained results that the measured oxidative stress parameters in these patients show a good correlation with the level of cytokines and with some of the commonly used laboratory biomarkers. This was a case–control pilot study focused primarily on the possibility to apply the oxidative stress parameters (d-ROM, PAT, and OS index) measured on a spectrophotometric system as a fast and low-cost prognostic tool for disease progression and potentially predict the outcome of COVID-19 in patients. Abnormal levels of several cytokines involved in the adaptive immunity (IL-2, IL-4) or proinflammatory cytokines and interleukins (IFNs, IL-1, IL-6, IL-10 IL-17, and TNF-α) were reported [11, 17, 27, 28] mainly as results from retrospective studies and reviews.

In our case–control pilot study, we have measured the levels of 11 cytokines (IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-γ, TNF-α, IL-1α, MCP-1, and EGF) in 14 critically ill patients. Afterward, these values were compared with the levels of the same cytokines in individuals that were not infected with the SARS-CoV-2 virus. By using t-test, we have obtained statistically significant increase ($p < 0.05$), which was

observed in regard to the levels of IL-6, IL-8, IL-10, VEGF, MCP-1, and EGF in the SARS-CoV-2 patients. Additionally, the levels of IL-2, IFN-γ, TNF-α, and IL-1α were also increased, but, however, this difference was not considered to be significant when compared with the noninfected individuals ($p > 0.05$) [20, 21]. Important finding of our case–control pilot study was that the oxidative stress parameters, d-ROM (448.8 U.Carr), OS index (107.7), and PAT (3048 U.Corr), were significantly higher ($p < 0.05$) in the infected patients when compared with those not infected. Additionally, we have also explored the correlation among the abovementioned cytokines (IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-γ, TNF-α, IL-1α, MCP-1, and EGF), the oxidative stress parameters (d-ROM, PAT, OSI), and some of the commonly used laboratory parameters (CRP, LDH, PLR, D-dimer, and NLR) by using the Spearman r calculation. These calculations were generated as heat map, and we have obtained a significant positive correlation between all investigated cytokines and the parameters of the oxidative stress (d-ROM, PAT, and OSI), except between IL-10 and the total antioxidant capacity, PAT, for which a negative a correlation was obtained. According to the performed analysis, we have obtained a nonsignificant correlation between OS index and the IL-8 ($p = 0.8552$) and also between d-ROM and VEGF ($p = 0.999$). The strongest correlation was demonstrated in the case of IL-6, which was estimated as significant with all of the markers of the oxidative stress, d-ROM ($r = 0.9725$, $p < 0.01$), PAT ($r = 0.5000$, $p < 0.01$), and the oxidative stress index ($r = 0.9593$, $p < 0.05$). Also, our results present evidence for similar behavior between IFN-γ and d-ROM ($r = 0.4006$, $p < 0.01$), PAT ($r = 0.6030$, $p < 0.01$) and the oxidative stress index ($r = 0.4298$, $p < 0.05$). Additionally, statistically we have investigated the correlation between the commonly used inflammation biomarker, CRP, and the levels of the investigated cytokines. In this case we have observed a strongest correlation with several of the investigated cytokines, namely with IL-6, IL-8, MCP-1, and IFN-γ. Furthermore, in terms of correlation, a strong correlation was obtained between the investigated inflammatory cytokines IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-γ, TNF-α, IL-1α, and MCP-1, except between IL-6 and EGF where negative correlation was obtained. It has been demonstrated that the high expression of IL-6 in COVID-19 patients can hasten the inflammatory process, which can on the other hand contribute to the cytokine storm and can contribute to disease worsening. The importance of the IL-6 has been potentiated by using tociluzimab, a monoclonal antibody, which can block the receptor of IL-6 for which it has been reported that the cytokine hyperproduction has been reversed [10].

The research performed by our study group has contributed to the evidences that more than a few cytokines and other clinically relevant biomarkers were significantly increased in patients with severe form COVID-19 in comparison to the individuals that were not infected by the virus, which was followed with coagulopathy as determined by worsening of the platelet-related parameters (PLR, D-dimers, and IL-6) and the increased levels of MCP-1 as thrombosis-related indicator. MCP-1 levels were estimated to be much higher in critical hospitalized patients in the ICU accompanied by decrease of platelet count in patients that do not survive, which was reported also by Huang et al. [27]. In our case–control pilot study, unfortunately all patients had severe form of COVID-19 and all of them had died during hospitalization. Besides, in our study we have witnessed that the levels of several of the investigated cytokines had been increased more than 10 times above the levels of the noninfected, which we have considered as a baseline. It is worth noticing that the significant increase of the vascular endothelial growth factor (VEGF) levels more than 10 times can be related to the essential VEGF role in the endothelial cell activation by binding to cell surface

VEGF receptors. This increase was considered to be statistically significant ($p < 0.01$). The upregulation of VEGF was also observed in several other viral infections, and also it has been investigated as a potential targeted therapy in viral diseases [28]. Higher levels of VEGF in hospitalized COVID-19 patients were also reported in a published study by Huang et al. [27]. The cytokine profile in COVID-19 pregnant women was investigated in a published study by Tanacan et al. [29], in which this research group has reported significantly higher values for IFN-γ and IL-6 with lower values of IL-2, IL-10, and IL-17. This situation was especially pronounced in those patients who had complications such as miscarriage and preterm delivery [29].

From the above presented results and the available literature data, we can conclude that the strong correlation between the investigated cytokines including chemokines and growth factors (IL-2, IL-4, IL-6, IL-8, IL-10, VEGF, IFN-γ, TNF-α, IL-1α, MCP-1, and EGF), the oxidative stress parameters (d-ROM, OSI, and PAT), and some of the commonly used clinical markers (CRP, D-dimers, NLR, PLR) is in agreement with the proposed "cytokine storm" as potential mechanism of the SARS-CoV-2 infection. In general, the so-called "cytokine storm" occurs when large numbers of white blood cells (leukocytes) are activated followed by release of a high concentration of proinflammatory cytokines, primarily IL-6, IL-10, IFN, MPC-1, IL-1, IL-2, and IL-8. Mostly, SARS-CoV-2 infection is related with increased oxidative stress, the proinflammatory state, cytokine production, and cell death confirmed by increase in the levels of the ROS and an alteration of antioxidant defense during the infection [15, 21, 30]. This interplay is depicted in **Figure 1**.

As proposed previously [11, 27, 30], RNA viruses trigger the oxidative stress by disturbing the pro-antioxidant–antioxidant balance for which we believe that the same mechanism exists also during the infection with SARS-CoV-2. During the last 2 years, we have performed several investigations with COVID-19 patients and we have managed to demonstrate increased oxidative stress and increased cytokines levels in these patients. Actually, we have observed significantly higher level of the markers of the oxidative stress (d-ROM and OS index values) and decreased total antioxidant capacity (PAT) in the COVID-19 patients when compared with disease-free individuals, supporting the theory that viral infection will increase the oxidative stress in the organism and further complicate the course of the disease (**Figure 1**).

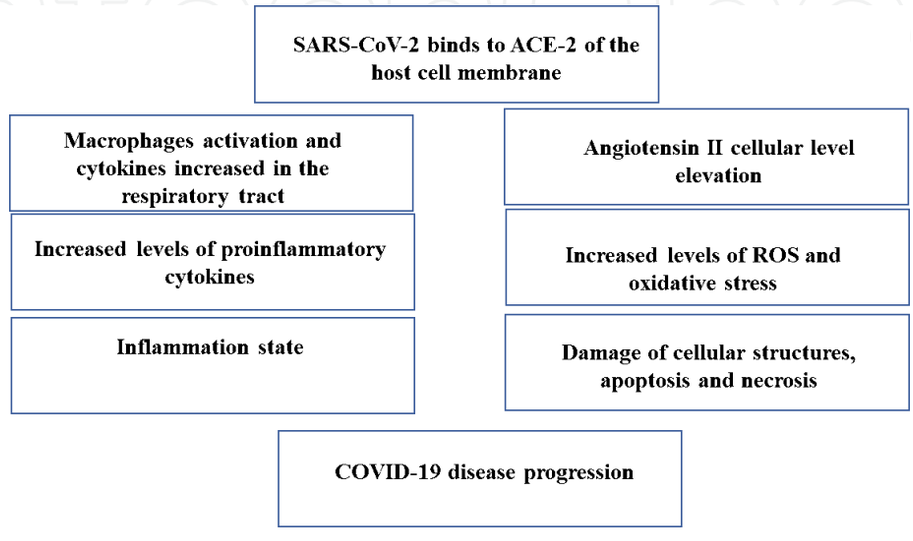

Figure 1.
Interplay of factors in COVID-19.

While we consider that the oxidative stress index value presents a significant parameter, which can be used in clinical practice for patient classification, we can also try in future activities, to implement a certain principle of supplementation with antioxidants especially when there is relevant knowledge for more than a few vitamins (vitamin C, vitamin D, selenium, quercetin, and other polyphenols) with proven antioxidant, anti-inflammatory, and even antiviral capacity [31]. Also, information on several clinical trials for potential therapy in COVID-19, such as tocilizumab, a monoclonal antibody IL-6 receptor antagonist; sarilumab – IL-6 antagonist; and anakinra, a recombinant IL-1 receptor antagonist against selected cytokines is in progress, some of which have already been published [31–38]. Even though, data on their efficacy are evolving and additional studies are needed for those treatments to be administered routinely in COVID-19.

3.1 Alteration of oxidative stress markers, inflammatory cytokines, and lymphocyte subsets in moderate and severe COVID-19

In regard to the pathophysiology of the virus, SARS-CoV-2 enters into the cell through the angiotensin-converting enzyme-2 (ACE-2), mainly through the Toll-like receptor-7 (TLR-7) that is present in the endosomes (**Figure 1**). The activation of this receptor requires production of IL-6, IL-12, and TNF-a enabling cytotoxic CD8+ T cells generation. This process results in further formation of antigen-specific B- cells and antibodies production through CD4+ helper T cells [39]. The suppression of the immune response can occur as negative reaction from immune activation and that is one of the reasons we have additionally performed analysis on several lymphocyte subsets in one of our studies. Th1 cells, natural killer cells (NK cells), and CD8+ T cells are main sources of INF-γ, whose production has been increased in COVID-19 patients. The increase of this cytokine (INF-γ) triggers Th1 response to eradicate the viral infect as one of the human immune strategies. Results reported since the beginning of the COVID-19 pandemic stated that more than a half of the patients had low lymphocytes number, and moreover pathological findings suggested that the patients who died of COVID-19 had significant increase of the concentration of proinflammatory CCR6+ Th17 within the CD4+ cells [2]. This point toward that overactivation of T-cells in combination with high cytotoxicity of CD8 T cells is partially responsible for severe immune injuries in COVID-19 patients. Several reported clinical studies indicate that low CD8+ T cells counts and high neutrophil-to-lymphocyte ratios (NLR) are associated with increased risk for disease severity and mortality in critically ill COVID-19 patients [40–43]. Namely, the high ratio between the neutrophils and leukocytes in COVID-19 patients leads to redox imbalance as a result of increased reactive oxygen species (ROS) production. In addition, the activation of macrophages and the polymorphonuclear cells also has effect on the oxidative damage to the tissues, and they can lead to organ failure. Patients with moderate form of COVID-19 had lower values of the measured concentration of free radicals (d-ROMs) and hence lower oxidative stress index (OSI) when compared with the patients classified with the severe form of the disease ($p < 0.01$). Additionally, the moderate group had increased total antioxidant capacity (PAT) in comparison with the severe group of patients, but however, this difference did not reach a statistical significance ($p > 0.05$). In our previous study, d-ROM (concentration of free radicals) and OSI demonstrated a good correlation with IL-6 and VEGF out of the 11 screened cytokines as predictors of disease worsening in severe COVID-19 patients [21]. In this study we have used them as parameters to potentially predict the so-called "cytokine

storm," and herein we report a statistically significant difference of IL-6 and VEGF levels between the two groups of patients (moderate vs. severe) ($p < 0.01$).

In terms of the alteration of the lymphocyte subsets, we have observed decreased levels of leukocytes and its subsets, namely CD4+, CD8+, CD3+, NK cells, and the absolute number of CD8 ($p < 0.05$). Also, CD19+ and CD45+ were decreased in the severe group in comparison to the moderate group of patients, but the difference did not reach a statistical significance ($p > 0.05$), presumably due to small sample size. These results are shown in **Table 1**.

Moreover, we have also investigated the correlation by calculation of the Spearman r among all investigated parameters in both groups separately. We have demonstrated that in the moderate group, a good correlation between the levels of IL-6 and VEGF and NK cells was obtained (for IL-6, $r = 0.6973$, $p < 0.05$; and for VEGF, $r = 0.6498$, $p < 0.05$), whereas in the severe group only these cytokines correlated with CD45+ (for IL-6, $r = 0.5610$, $p < 0.05$; and for VEGF, $r = 0.5462$, $p < 0.05$). The results from our investigation had shown that both parameters CD45+ and the oxidative stress index can be considered as an applicable diagnostic standard in distinguishing severe form of the disease and disease complications.

The oxidative stress index can be used as potential marker with a diagnostic value since we have obtained very high values in patients with severe COVID-19 as well as OSI demonstrated a good correlation with IL-6, CD45+, CD4+, and absolute number of CD8 cells. In the severe group, we have observed a high level of the proinflammatory cytokine IL-6, which probably contributes to the T lymphocyte deficiency (CD4+, CD8+, CD3+) [17, 28]. Several authors have reported that during viral infections with the virus, CD4+ T lymphocytes are activated into T helper cells, and then they secret proinflammatory cytokines as IL-6. The activated immune cells enter into pulmonary circulation and can lead to serious lung injury [17, 21, 28]. In a study of Mudd et al. [44], gene expressions differences between influenza and COVID-19 patients were investigated. Namely, in comparison to the influenza state, INF-γ and IFN-α response pathways were downregulated within the patients with COVID-19 in terms of B-cells, CD8+ T cells, regulatory T cells, plasma blasts, and monocyte subsets. Hence, because of this interplay between the defect in the host immunity and the increased cytokines level, the evaluation of the adaptive and the innate immunity of the COVID-19 patient might be useful in terms of immunomodulatory therapies.

Subset	**Severe COVID-19 patients mean ± SEM (n = 16)**	**Moderate COVID-19 patients mean ± SEM (n = 19)**	**p (t-test)**
Leukocytes	12.28 ± 1.055	7.637 ± 0.8581	**0.0016**
CD4+	0.1711 ± 0.0184	0.6765 ± 0.0653	**0.0001**
CD8+	0.1034 ± 0.0191	0.3157 ± 0.0332	**0.0001**
NK	0.06225 ± 0.01656	0.1498 ± 0.02519	**0.0087**
Absolute CD8	99.63 ± 10.78	317.4 ± 32.42	**0.0001**
CD45+	0.7382 ± 0.1188	1.266 ± 0.1428	0.7594
CD19+	0.1852 ± 0.0495	0.2686 ± 0.0861	0.4296
CD3+	0.4038 ± 0.0629	0.9057 ± 0.0114	**0.0009**

Table 1.
Alteration in lymphocytes subsets in severe and moderate COVID-19 patients on hospital admission. Results are expressed as mean ± SEM.

4. Conclusion

Upon available data and the results from our clinical experience in the last 2 years of the pandemic, we can conclude that most probably the interplay between the defect in the host immunity and the cytokines hyperproduction is an important factor in the immunopathology of the SARS-CoV-2 infection. Our experience demonstrated that the levels of certain cytokines, namely IL-6, IL-8, IL-10, VEGF, MCP-1, and EGF, were significantly increased in the critically ill COVID-19 patients. Moreover, we have proved a good correlation of the increased level of IL-6 with the oxidative stress index, which can be considered as evidence that the cytokine storm syndrome lies as an immune-pathogenesis during SARS-CoV-2 infection.

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Conflict of interest

The authors declare no conflict of interest.

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