sciendo

10.2478/AMB-2023-0036

ORIGINAL ARTICLE

CHANGES IN THE CYTOKINE PROFILE IN PATIENTS DURING COVID-19 INFECTION

N. Ivanov[1], S. Mihailova[1], R. Bilyukov[2], C. Popov[3], T. Kundurzhiev[4], E. Naumova[1]

[1]Department of Clinical Immunology with Stem Cell Bank, University Hospital "Alexandrovska", National Expert Center for PID, Medical University – Sofia, Bulgaria
[2]Department of Propaedeutics of Internal Diseases, University Hospital "Alexandrovska", Medical University – Sofia, Bulgaria
[3]Department of Surgery, University Hospital "Alexandrovska", Medical University – Sofia, Bulgaria
[4]Department of Occupational Medicine, Faculty of Public Health, Medical University – Sofia, Bulgaria

Abstract. *COVID-19 has proven to be a disease that affects not only the respiratory tract but also leads to a state of generalized systemic hyperinflammation and overall immune dysregulation. An important role in its pathogenesis is the disturbance of many cytokines – a condition which, in its most pronounced form, is also called a „cytokine storm".* ***Objective:*** *To evaluate the serum cytokine levels during COVID-19 infection as potential biomarkers for the severity and course of infection.* ***Materials and methods:*** *By design, the study is a retrospective cross-sectional, in which the serum concentrations of 10 pro- and anti-inflammatory cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNF-α, and GM-CSF) were investigated in 36 individuals (divided into 3 groups by severity – with a mild form of the infection/presymptomatic, moderately severe and severe/critical) within two periods – before and after the second week from the onset of symptoms of the disease.* ***Results:*** *In the period up to the 2nd week, the serum concentrations of IFN-γ (p = 0.029), IL-1β (p = 0.017), and IL-5 (p = 0.014) showed a statistically significant correlation with the disease severity, however in the later stage of the disease the cytokine levels did not show any clinical value.* ***Conclusion:*** *Cytokine testing could be used to predict the severity of COVID-19 infection which could support individual therapeutic decisions. Analysis of a larger group of patients is needed to unfold the full potential of such testing.*

Key words: *COVID-19, cytokines, hyperinflammation, dysregulation, biomarkers, severity*

Corresponding author: *Nedelcho Ivanov, Department of Clinical Immunology with Stem Cell Bank, University Hospital "Alexandrovska", National Expert Center for PID, Medical University, 1 Sv. Georgi Sofiyski Str., Bg – 1431 Sofia, e-mail: nedelcho.ivanov93@gmail.com*

Received: *29 May 2023;* ***Revised/Accepted:*** *12 September 2023*

INTRODUCTION

To date, coronavirus disease 2019 (COVID-19), which originated in Wuhan, China, has spread to over 200 countries, including Bulgaria [1] infecting nearly 700,000,000 people of all ages, of whom approximately 1% have succumbed to the infection. Studying the immune response both during natural infection and after vaccination provides important information about the pathogenesis of the disease as well as about the immune response to the virus [2, 3]. To date, there is a body of evidence supporting the

important role of various cytokines in the pathogenesis of COVID-19. Studies have shown pathologically altered levels of several cytokines and chemokines, some of which are: interleukin (IL)-1b, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12, IL-17A, IL-18, IL-23, TNFα, IFNg, G-CSF, GM-CSF, MCP-1, etc. [4, 5]. For this reason, the expression "cytokine storm" is often used to describe the immunopathogenesis of the disease. The term was first used in the medical literature for graft-versus-host reaction in 1993 [6] and describes a state of uncontrolled over-release of multiple, mostly pro-inflammatory cytokines, which in turn trigger the release of other cytokines. A vicious cycle of positive feedback is generated, resulting in dysregulation and dysfunction of the immune mechanisms [4]. The term "cytokine storm" partially overlaps with the concepts of "hyperinflammation" and "cytokine release syndrome". However, more specific criteria – which are the cytokines involved and what should be their levels in order to observe this phenomenon are not standardized currently. The term „cytokine storm" is also mentioned in the literature when describing the pathogenesis of many infectious diseases, including cytomegalovirus (CMV), Epstein-Barr virus (EBV)-associated hemophagocytic lymphohistiocytosis (HLH), group A streptococcal infections, influenza, SARS-CoV-1 (severe acute respiratory syndrome – coronavirus 1), MERS-CoV (Middle East respiratory syndrome – coronavirus) and others. The term gained serious popularity in 2005 with the spread of bird flu. Apart from infectious diseases, however, a „cytokine storm" is also observed in diseases proceeding with a process of intense inflammation ("hyperinflammation") and massive cytolysis such as acute pancreatitis, acute respiratory distress syndrome (ARDS), macrophage activation syndrome (MAS), as an adverse reaction in CAR-T-cell (chimeric antigen receptor T-cell) therapy and others [6].

Due to the complex interactions, pleiotropicity, and cascading cytokine interactions, the search for causal relationships has been extremely complex and has so far not led to an unequivocal categorical model of the exact sequence of events. Often, a parallel study of a large set of biological molecules is applied to discover different clinically relevant and prognostic markers for the course and severity of the infection in patients, including disease-related complications and post-infectious effects on the body.

OBJECTIVE

We hypothesized that serum cytokine levels monitored during the COVID-19 infection have the potential to prove to be useful biomarkers bearing information on the immunological phenotype, severity, and long-term consequences of the disease. Therefore, in the present study, we aimed to evaluate the serum concentrations of selected key pro- and anti-inflammatory cytokines in patients with different severity of COVID-19 infection in order to highlight those that would have prognostic significance for the course of the disease.

MATERIALS AND METHODS

Clinical material

Patients with COVID-19 infection diagnosed and treated at the University Hospital "Alexandrovska" and University Hospital "Sv. Anna" for the period April – November 2020 were included.

In the testing period of 2 weeks after the onset of the first symptoms of COVID-19 infection (or 8 days after a positive RT-PCR test), we evaluated 36 individuals (9 with a mild form of infection/asymptomatic; 16 with the moderately severe course; 11 – with the severe/critical course; men – 20; women – 16). Of them 24 patients were followed up for 2 weeks after initial disease presentation, (7 mild/asymptomatic, 10 moderate; 7 severe/critical). The severity of infection was categorized based on the CDC COVID-19 treatment guidelines [8], as follows:

- *mild/asymptomatic infection*: Individuals who test positive for SARS-CoV-2 using a virologic test (i.e., a nucleic acid amplification test (NAAT) or an antigen test) but who have no symptoms that are consistent with COVID-19 and individuals who have any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who do not have shortness of breath, dyspnea, or abnormal chest imaging.
- *moderate illness*: Individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation measured by pulse oximetry (SpO_2) ≥ 94% on room air at sea level.
- *severe/critical illness*: Individuals who have SpO_2 < 94% on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen (PaO_2/FiO_2) < 300 mm Hg, a respiratory rate > 30 breaths/min, or lung infiltrates > 50% and individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction.

Test procedure

To measure the serum levels of cytokines, we used a multiplex method based on magnetic-sphere fluo-

rimetric technology (Invitrogen ProcartaPlexTM Human Cytokine Magnetic10-plex Panel) and a detection system Luminex®100™. Individual serum concentrations of 10 pro- and anti-inflammatory cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, TNF-α and GM-CSF) was analysed. For initial results interpretation and calculation, we applied programs xPONENT® for detection and ProcartaPlex Analyst 1.0 for software analysis.

Statistical methods

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 (IBM Corp., Armonk, NY, USA). Categorical variables are presented as numbers and percentages, continuous variables with median, first, and third quartile (Q1 and Q3). The distribution of continuous variables was tested using the Kolmogorov-Smirnov test. The relationship between disease severity and categorical variables was assessed by Chi-square test or Fisher's exact test. For continuous variables, the association with disease severity was examined with a Kruskal-Wallis test. Comparisons between groups were performed with the Mann-Whitney test. Binary logistic regression (univariate and multivariate) was applied to estimate the dependence of the severity of the disease on the various factors. For statistically significant we accepted p values < 0.05.

RESULTS

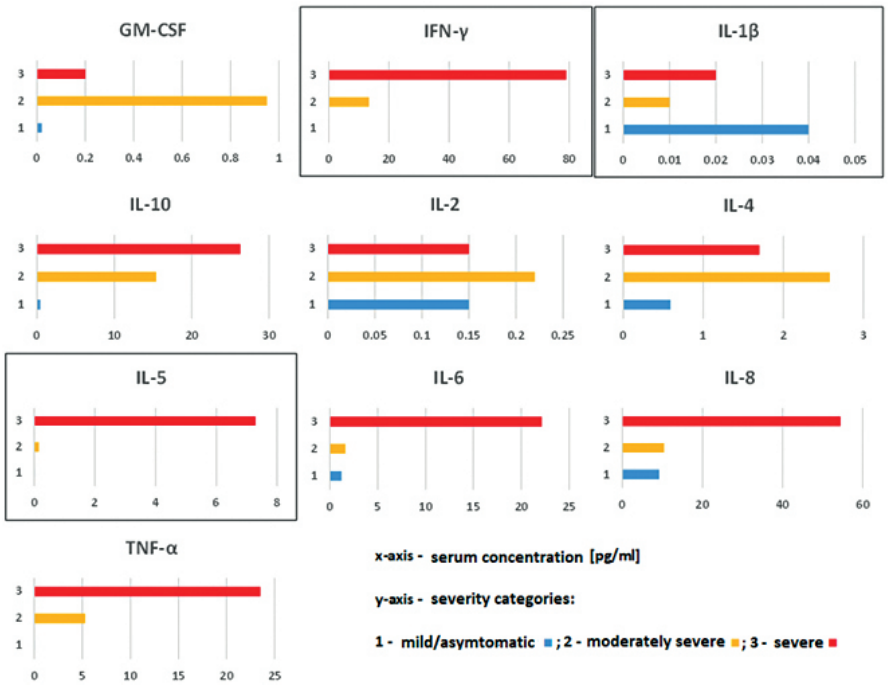
Determination of cytokine levels in the early infectious period (up to the 2nd week from the onset of symptoms)

The results for the individual cytokines depending on the severity of the infection are presented in Table 1 and Figure 1.

Table 1. Serum concentratons of cytokines up to the 2nd week from the onset of symptoms with regards to the disease severity

Indicator (up to 2 weeks)	Severity	N	Mean	Median	SD	Min	Max	Q1	Q3	p-value
GM-CSF	mild/asymptomatic	9	0,02	0,00	0,05	0,00	0,16	0,00	0,01	0,520
	moderately severe	16	0,95	0,00	3,49	0,00	14,01	0,00	0,04	
	severe/critical	11	0,20	0,00	0,45	0,00	1,47	0,00	0,16	
IFN-γ	mild/asymptomatic	9	0,01	0,00	0,03	0,00	0,06	0,00	0,03	**0,029**
	moderately severe	16	13,36	0,10	52,61	0,00	210,65	0,00	0,44	
	severe/critical	11	78,97	0,00	261,77	0,00	868,24	0,00	0,16	
IL-1β	mild/asymptomatic	9	0,04	0,04	0,04	0,00	0,10	0,01	0,07	**0,017**
	moderately severe	16	0,01	0,00	0,02	0,00	0,07	0,00	0,01	
	severe/critical	11	0,02	0,01	0,02	0,00	0,07	0,00	0,04	
IL-10	mild/asymptomatic	9	0,41	0,07	0,81	0,00	2,48	0,00	0,50	0,176
	moderately severe	16	15,39	0,45	31,38	0,00	105,47	0,02	14,56	
	severe/critical	10	26,27	2,77	54,39	0,00	174,68	0,02	38,44	
IL-2	mild/asymptomatic	9	0,15	0,05	0,30	0,00	0,94	0,00	0,14	0,542
	moderately severe	16	0,22	0,00	0,36	0,00	0,94	0,00	0,33	
	severe/critical	11	0,15	0,00	0,45	0,00	1,49	0,00	0,09	
IL-4	mild/asymptomatic	9	0,59	0,00	1,19	0,00	3,66	0,00	0,65	0,439
	moderately severe	16	2,58	0,26	6,53	0,00	24,85	0,00	1,18	
	severe/critical	11	1,70	0,00	5,33	0,00	17,75	0,00	0,26	
IL-5	mild/asymptomatic	9	0,01	0,00	0,02	0,00	0,07	0,00	0,01	**0,014**
	moderately severe	16	0,15	0,12	0,22	0,00	0,89	0,02	0,12	
	severe/critical	11	7,29	0,02	16,04	0,00	49,90	0,00	4,45	
IL-6	mild/asymptomatic	9	1,21	0,04	2,85	0,00	8,72	0,00	0,96	0,163
	moderately severe	16	1,63	0,59	3,23	0,00	13,24	0,21	1,45	
	severe/critical	10	22,13	1,93	55,21	0,00	178,39	0,07	13,12	
IL-8 (CXCL8)	mild/asymptomatic	9	9,19	3,35	15,57	1,01	48,90	1,45	9,97	0,075
	moderately severe	16	10,38	4,61	10,89	0,49	34,71	1,72	17,65	
	severe/critical	11	54,44	23,27	76,49	1,88	259,18	3,15	77,70	
TNF-α	mild/asymptomatic	9	0,02	0,00	0,03	0,00	0,08	0,00	0,03	0,109
	moderately severe	16	5,29	2,06	12,75	0,00	51,86	0,00	3,78	
	severe/critical	11	23,51	0,20	59,64	0,00	197,32	0,00	4,12	

N – number of individuals tested, Mean – the average concentration of particular cytokine, SD – standard deviation, Min – the lowest value, Max – the highest value, Q1 – first quartile, Q3 – third quartile.



N – number of participants, Mean – average concentration, SD – standard deviation, Min – lowest value, Max – the highest value, Q1 – first quartile, Q3 – third quartile. *Note: "severe" – to be read as severe/critical*

Fig. 1. Mean serum concentrations of tested cytokines in the early infectious period (up to week 2 of symptom onset) with regards to the severity of the disease

Based on the statistical analysis we observed an association of serum concentrations of IL-1β, IL-5, IL-8, and IFN-γ with the severity of the disease. However, data for IFN-γ, IL-1β, and IL-5 reached statistical significance. IL-8 values remained close but outside the accepted limit for statistical significance. It is important to note here that a single sample with values of IL-6 1686.85 pg/ml and IL-10 1508.12 pg/ml, was excluded from the analysis due to its potential to generate serious statistical bias. Further elaboration of associations with established statistical significance ($p < 0.05$) by the Kruskal-Wallis test with the non-parametric Mann-Whitney test showed levels of significance between individual groups (mild/asymptomatic: moderate; mild/asymptomatic: severe/critical; moderate-severe: severe) in Table 2:

Table 2. Intergroup comparison of statistical significance levels with the Mann-Whitney test

Indicator	Compared groups		
	mild/asymptomatic	mild/asymptomatic	moderately severe
	moderately severe	severe/critical	severe/critical
	P	P	P
IFN-γ	**0,015**	0,583	0,079
IL-1β	**0,006**	0,060	0,316
IL-5	**0,002**	**0,041**	0,900

From the presented data it is clear that the serum concentrations of IFN-γ and IL-1β differ significantly only between the groups of patients with a mild/asymptomatic and moderately severe form of course, and IL-5 levels – both between mild/asymptomatic and moderate, and between mild/asymptomatic and severe.

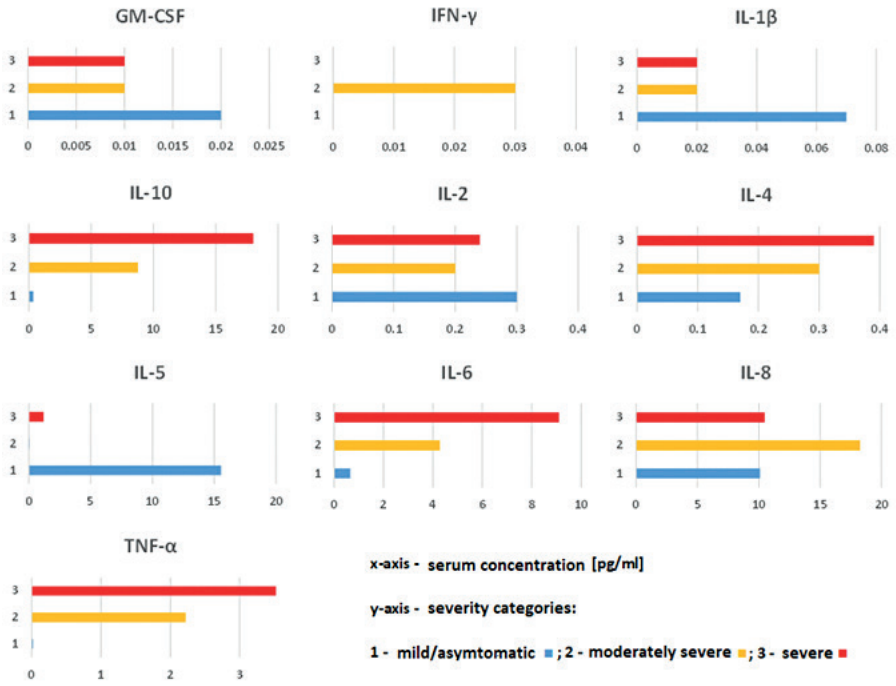
Determination of cytokine levels in the period after the 2nd week from the onset of symptoms

The results we obtained in the study for the patients with mild infection/asymptomatic, moderately severe, and severe course, during this later period are presented in Table 3 and Figure 3. No statistically significant associations were found. Close to statistical significance ($p = 0.07$) was only the level of TNF-α between studied groups. When comparing the results from the two testing periods (up to and after the 2nd week), there was a tendency for higher levels of IL-6, and IL-10 in the group with the severe course and increased concentration of IL-5 in the group of mild/asymptomatic subjects during the period after two weeks.

DISCUSSION

In this study, we tested the concentrations of 10 cytokines in 36 patients with different severity of COVID-19 infection and found associations of some cytokines with disease severity in the early stage of the illness (up to week 2 of symptom onset).

It is now well known that the delayed response to type I interferons in coronavirus infections is one of the main factors associated with a more severe course of the disease [9, 10]. Due to the early manifestation of



N – number of participants, Mean – average concentration, SD – standard deviation, Min – lowest value, Max – the highest value, Q1 – first quartile, Q3 – third quartile. Note: "severe" – to be read as severe/critical

Fig. 3. Serum concentrations in the period after the 2nd week from the onset of symptoms in the different groups depending on the severity of the disease

Table 3. Serum concentrations of cytokines after 2nd week from the onset of symptoms with regards to the disease severity

Indicator (after 2 weeks)	Severity	N	Mean	Median	SD	Min	Max	Q1	Q3	p-value
GM-CSF	mild/asymptomatic	7	0,02	0,00	0,04	0,00	0,11	0,00	0,02	0,900
	moderately severe	10	0,01	0,00	0,02	0,00	0,07	0,00	0,01	
	severe/critical	7	0,01	0,00	0,01	0,00	0,02	0,00	0,02	
IFN-γ	mild/asymptomatic	7	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,232
	moderately severe	10	0,03	0,00	0,08	0,00	0,25	0,00	0,01	
	severe/critical	7	0,00	0,00	0,00	0,00	0,00	0,00	0,00	
IL-1β	mild/asymptomatic	7	0,07	0,04	0,08	0,00	0,24	0,01	0,10	0,194
	moderately severe	10	0,02	0,00	0,03	0,00	0,07	0,00	0,06	
	severe/critical	7	0,02	0,00	0,04	0,00	0,10	0,00	0,04	
IL-10	mild/asymptomatic	7	0,36	0,00	0,80	0,00	2,14	0,00	0,35	0,376
	moderately severe	10	8,75	0,49	19,16	0,00	61,74	0,00	8,58	
	severe/critical	7	17,99	0,02	28,32	0,00	63,18	0,00	55,01	
IL-2	mild/asymptomatic	7	0,30	0,09	0,55	0,00	1,51	0,00	0,28	0,374
	moderately severe	10	0,20	0,00	0,58	0,00	1,84	0,00	0,05	
	severe/critical	7	0,24	0,00	0,55	0,00	1,49	0,00	0,09	
IL-4	mild/asymptomatic	7	0,17	0,00	0,31	0,00	0,78	0,00	0,39	0,613
	moderately severe	10	0,30	0,00	0,46	0,00	1,31	0,00	0,59	
	severe/critical	7	0,39	0,00	1,04	0,00	2,76	0,00	0,00	

Continuation of Table 3

IL-5	mild/asymptomatic	7	15,55	0,00	41,11	0,00	108,78	0,00	0,07	0,958
	moderately severe	10	0,06	0,00	0,12	0,00	0,37	0,00	0,12	
	severe/critical	7	1,18	0,00	3,01	0,00	8,01	0,00	0,25	
IL-6	mild/asymptomatic	7	0,66	0,00	1,41	0,00	3,80	0,00	0,81	0,204
	moderately severe	10	4,28	0,76	7,86	0,00	24,62	0,32	5,18	
	severe/critical	7	9,09	1,23	20,42	0,00	55,16	0,00	5,88	
IL-8 (CXCL8)	mild/asymptomatic	7	10,09	3,22	12,51	1,28	28,53	2,83	28,21	0,918
	moderately severe	10	18,25	5,08	38,50	1,92	127,12	2,67	12,40	
	severe/critical	7	10,47	8,01	9,20	1,01	24,33	1,85	20,79	
TNF-α	mild/asymptomatic	7	0,02	0,03	0,02	0,00	0,03	0,00	0,03	0,070
	moderately severe	10	2,22	2,74	2,30	0,00	7,07	0,03	3,09	
	severe/critical	7	3,60	0,03	8,01	0,00	21,61	0,00	2,74	

this phenomenon – even before the development of a pronounced clinical picture, before the patient seeks medical care, and because enough data has already accumulated in the world literature, we chose not to include type I IFN in the investigated cytokine set.

The overall picture shows a simultaneous increase of cytokines with extremely divergent functions: IFN-γ as a classical Th1 mediator associated with enhanced cell-mediated immunity; IL-5 – representative of Th2 immune response and with a key role in the development and mobilization of eosinophils and the generation of humoral immunity; IL-1β, representative of the classic pro-inflammatory triad – IL-1β, IL-6, and TNFα, obtained as a result of activation of the NF-κB-pathway associated with the acute inflammatory phase of innate immunity.

The differences in the rest of the investigated cytokines did not show statistical significance, but some showed trends. The other two pro-inflammatory cytokines – IL-6 and TNFα, and IL-10, mainly recognized for its anti-inflammatory properties, were also elevated. Usually, the mobilization of a Th1 or Th2 immune response is associated with the activation of counter-regulatory mechanisms that lead to an inhibition in the production and effect of cytokines of the "opposite" class. The fact that high levels of cytokines were simultaneously detected in both groups suggests the presence of an over-stimulus that overcomes the threshold of this inhibition. Ultimately, the multidirectional "unfocused" cytokine background leads to an overall dysregulation in the immunological response and an increased "braking distance" of the overactivated immune system.

IFN-γ levels showed a clear statistically significant relationship with disease severity. But what is the immunological logic behind this? Contrary to expectations that in the first phase of the disease, elevated Th1 cytokines would have a beneficial effect on viral clearance and overall survival of patients with COVID-19, the exact opposite trend was observed. Luo et al. found that despite increased total plasma concentration of IFN-γ, the proportion of IFN-γ-producing CD4+ T-lymphocytes decreased and that of CD8+ increased accordingly in the severe course [11]. Coppock et al. described decreased T-cell production of IFN-γ in response to mitogen stimulation, which they suggested was due to exhaustion as a consequence of the initial overactivation [12]. A study by Gadottie et al. [13] also found a relationship between serum IFN-γ concentrations and the severity of the course of COVID-19.

IL-1β as one of the major pro-inflammatory cytokines already mentioned shows surprisingly low concentrations (0.00-0.24 pg/mL) and a little albeit statistically significant difference between groups. However, we could speculate that this could be due to its short half-life of 21 minutes. Data in support of this finding are also provided by Del Valle et al. [14].

Increased levels of IL-5 in severe/critical and moderate forms are supported by the publications of Zhang et al. [15] and Liu et al. [16], while the team of Ghazvai et al. [17] did not find a statistically significant difference in serum IL-5 concentrations in patients with COVID-19 and healthy controls. IL-5 is one of the main type 2 cytokines. Its increase in the early phases of infection may cause impaired viral clear-

ance due to clonal/immune deviation. The phenomenon has been described in several infectious diseases such as leprosy, tuberculosis, leishmaniasis, HIV/AIDS, etc. [18] and represents a kind of "delusion" of the immune system, in which it deviates in the direction of developing an immune response that is suboptimal or generally unsuitable for fighting the specific pathogen.

Although the association with IL-6 was not among those found to be statistically significant in our study, we could speculate that it would have reached significance in a larger number of patients. The major role of IL-6 was presented in a meta-analysis by Mulchandani et al. [4]. The publication included 23 studies with a total of 2477 subjects (mild to moderate = 993 and severe/critical 1484). The data indicated the overall statistical significance of the association between serum IL-6 concentration and severity ($p = 0.00001$). Chang et al. [19] found that the IL-6 levels in non-severe forms of COVID-19 were not significantly different from the levels in healthy controls, in contrast to severe cases where there was a statistically significant difference. Other cytokines with marked differences in the three studied patient' groups but without statistical significance were IL-8, TNFα, and IL-10. IL-8 (CXCL8) is a cardinal chemokine in the chemotaxis of neutrophils and other immune cells to the site of inflammation. Several publications have shown its correlation with leukocytosis, neutrophilia, severity, and mortality in COVID-19 [20, 21, 22]. It was demonstrated that together, TNF-α and IL-8 trigger neutrophil NETosis [23, 24] and directly correlate with the risk of developing ARDS [22]. IL-10 is one of the main anti-inflammatory and immunoregulatory cytokines, and its increased levels are usually associated with a state of homeostatic immune tolerance or with a counterregulatory response, being negative feedback of increased pro-inflammatory cytokines. In cases with severe COVID-19, increased IL-6 induces the expression of IL-10, which, however, is unable to limit the unfolding systemic inflammatory process, and even impairs its resolution [25].

Of note, limitation of our study is the small study sample. The studied patients were a small group, therefore it is difficult to draw cardinal conclusions.

In conclusion, the cytokine correlation observed in the present study could be used to stratify patients into subgroups by severity/risk of complications, and type of disease course. Thus cytokine testing in selected patients could have prognostic and therapeutic value. However, a larger group of patients needs to be studied to support these observations.

Financing: *The study was supported by the Bulgarian National Science Fund (grant # KP-06-DK1/13/29.03.2021).*

Disclosure Summary: *The authors have nothing to disclose.*

REFERENCES

1. Rangachev A, Marinov GK, Mladenov M. The demographic and geographic impact of the COVID pandemic in Bulgaria and Eastern Europe in 2020. Sci Rep, 2022, 12(1), 6333. doi.org/10.1038/s41598-022-11420-4.
2. Nikolova M, Todorova Y, Emilova R, et al. Induction of humoral and cellular immune responses to COVID-19 mRNA and vector vaccines: A prospective cohort study in Bulgarian healthcare workers. J Med Virol, 2022, 94(5), 2008-2018. doi.org/10.1002/jmv.27572.
3. Mihaylova A, Lesichkova S, Baleva M, et al. Durability of humoral and cell-mediated immune response after SARS-CoV-2 mRNA vaccine administration. J Med Virol, 2023, 95(1), e28360. doi.org/10.1002/jmv.28360.
4. Mulchandani R, Lyngdoh T, Kakkar AK. Deciphering the COVID-19 cytokine storm: Systematic review and meta-analysis. Eur J Clin Invest. 2021 Jan;51(1):e13429. doi: 10.1111/eci.13429.
5. Yang L, Xie X, Tu Z. et al. The signal pathways and treatment of cytokine storm in COVID-19. Sig Transduct Target Ther, 2021, 6, 255. doi.org/10.1038/s41392-021-00679-0.
6. Ferrara JL, Abhyankar S, Gilliland DG. Cytokine storm of graft-versus-host disease: a critical effector role for interleukin-1. Transpl Proceed. 1993 Feb;25(1 Pt 2):1216-1217. doi.org/10.1016/0952-7915(93)90139-J.
7. Tisoncik JR, Korth MJ, Simmons CP, et al. Into the eye of the cytokine storm. Microbiol Mol Biol Rev. 2012 Mar;76(1):16-32. doi: 10.1128/MMBR.05015-11. doi.org/10.3390/microorganisms9102159.
8. Centers for Disease Control and Prevention. Clinical Spectrum of SARS-CoV-2 Infection Last Updated: March 6, 2023.
9. Channappanavar R, Fehr AR, Vijay R, et al. Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. Cell Host Microb. 2016;19(2):181-193. doi: 10.1016/j.chom.2016.01.007.
10. Blanco-Melo D, Nilsson-Payant BE, Liu W-C, et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. Cell. 2020;181(5):1036-1045.e9. doi: 10.1016/j.cell.2020.04.026.
11. Luo, Xiao, Hua, et al. T cell immunobiology and cytokine storm of COVID-19. Scand J Immunol 93.3 (2021): e12989. doi: 10.1111/sji.12989.
12. Coppock D, Zurlo CE, Meloni JM, et al. Interferon gamma release assay mitogen responses in COVID-19. Infect Dis Clin Pract, 2022, 30(1), p.e1085. doi: 10.1097/IPC.0000000000001085.
13. Gadotti AC, de Castro Deus M, Telles JP, et al. IFN-γ is an independent risk factor associated with mortality in patients with moderate and severe COVID-19 infection. Virus Res. 2020 Nov;289:198171. doi: 10.1016/j.virusres.2020.198171.
14. Del Valle DM, Kim-Schulze S, Huang HH et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat Med, 2020, 26, 1636–1643. doi: 10.1038/s41591-020-1051-9.
15. Zhang Z, Ai G, Chen L, et al. Associations of immunological features with COVID-19 severity: a systematic review and meta-analysis. BMC infectious diseases, 2021, 21, 1-9. doi: 10.1186/s12879-021-06457-1.

16. Liu Y, Chen D, Hou J et al. An inter-correlated cytokine network identified at the center of cytokine storm predicted COVID-19 prognosis. Cytokine, 2021, 138, 155365. doi: 10.1016/j.cyto.2020.155365.
17. Ghazavi A, Ganji A, Keshavarzian N et al. Cytokine profile and disease severity in patients with COVID-19. Cytokine, 2021, 137, 155323. doi: 10.1016/j.cyto.2020.155323.
18. Bretscher PA. A strategy to improve the efficacy of vaccination against tuberculosis and leprosy. Immunol Today. 1992 Sep;13(9):342-5. doi: 10.1016/0167-5699(92)90168-7.
19. Chang Y, Bai M, You Q. Associations between Serum Interleukins (IL-1β, IL-2, IL-4, IL-6, IL-8, and IL-10) and Disease Severity of COVID-19: A Systematic Review and Meta-Analysis. BioMed Res Int, 2022. doi: 10.1155/2022/2755246.
20. Masso-Silva JA, Moshensky A, Lam MTY, et al. Increased Peripheral Blood Neutrophil Activation Phenotypes and Neutrophil Extracellular Trap Formation in Critically Ill Coronavirus Disease 2019 (COVID-19) Patients: A Case Series and Review of the Literature. Clin Infect Dis. 2022 Feb 11;74(3):479-489. doi: 10.1093/cid/ciab437.
21. Kaiser R, Leunig A, Pekayvaz K, et al. Self-sustaining IL-8 loops drive a prothrombotic neutrophil phenotype in severe COVID-19. JCI Insight. 2021 Sep 22;6(18):e150862. doi: 10.1172/jci.insight.
22. Borges L, Pithon-Curi TC, Curi R, Hatanaka E. COVID-19 and Neutrophils: The Relationship between Hyperinflammation and Neutrophil Extracellular Traps, Mediators of Inflammation, vol. 2020, Article ID 8829674, 7 pages, 2020. doi. org/10.1155/2020/8829674.
23. Cheng OZ, Palaniyar N. NET balancing: a problem in inflammatory lung diseases. Frontiers Immunol, 2013, 4, 1. doi. org/10.3389/fimmu.2013.00001.
24. Saitoh T, Komano J, Saitoh Y, et al. Neutrophil extracellular traps mediate a host defense response to human immunodeficiency virus-1. Cell Host Microbe. 2012 Jul 19;12(1):109-16. doi: 10.1016/j.chom.2012.05.015.
25. Lu L, Zhang H, Dauphars DJ, He YW. A potential role of interleukin 10 in COVID-19 pathogenesis. Trends in Immunology, 2021, 42(1), 3-5. doi: 10.1016/j.it.2020.10.012