JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Coronavirus Pandemic

Comparative study of TNF-α and IL-10 levels at different times of the course of COVID-19

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Abstract

Introduction: SARS-CoV-2 infection (COVID-19) induces dysregulated production of pro- and anti-inflammatory cytokines, called the cytokine storm, leading to the development of severe pneumonia and ARDS. We aimed to examine the dynamic cytokine response on different days of the disease in adult COVID-19 patients.

Methodology: Our study included 142 patients (with SARS-CoV-2 PCR-positive nasopharyngeal samples) with varying disease severity and admitted on different days of the disease. We examined the presence and mean levels of TNF-α and IL-10 and did a correlation and logistic regression analysis.

Results: TNF-α levels were high in all patients, with mean levels being the highest on day 5 of the disease. IL-10 was high only in a quarter of the patients. The levels of IL-10 were also the highest on day 5, which was significantly different from the mean levels on the other days of the disease. Average IL-10 levels were not any higher than the normal range on the other days. We found a significant positive correlation between the levels of TNF-α and IL-10 during the first week of the infection. In the second week, the positive correlation was no longer significant, and starting from day 10, there was even a slight negative correlation. IL-10 level increase showed prognostic significance for severe, but not the critical forms of the disease.

Conclusions: The uncontrolled immune response to SARS-CoV-2 in the second week of the disease can be the result of dysregulated production of endogenous anti-inflammatory cytokines. This leads to a severe disease course and a possible unfavorable outcome.

Key words: COVID-19; TNF-α; IL-10; dysregulated immune response.

J Infect Dev Ctries 2023; 17(9):1207-1212. doi:10.3855/jidc.18067

(Received 12 February 2023 – Accepted 26 April 2023)

Introduction

The coronavirus disease 2019 (COVID-19, caused by the SARS-CoV-2 virus) caused a pandemic that precipitated a global healthcare and economic crisis. The virus causes complex pathologic changes in the body, including dysregulated production of pro- and anti-inflammatory cytokines. The extensive immune response to the virus has been shown to be a great risk factor for mortality from COVID-19. Specifically, the sudden increase in immune mediators, called the cytokine release syndrome or "the cytokine storm", leads to the development of severe disease manifestations such as severe pneumonia, acute lung injury, and acute respiratory distress syndrome (ARDS) [1]. Research evidence also shows that people with congenital defects in type 1 interferon response have a delayed interferon release, which opens ways for unrestricted replication of SARS-CoV-2 and the development of cytokine storm [2-4]. Moreover, the disease course often worsens during the second week of the infection, when the virus load has decreased, further promoting the role of immune-mediated injury as the main pathogenetic mechanism in the disease [5]. Furthermore, Peng *et al* have demonstrated that cytokine storm is the chief mortality factor in immunocompromised patients with COVID-19, as it plays a vital role in the progression of infection and the ensuing multi-organ failure [6].

The primary cytokines involved in a cytokine storm are interleukins, interferons, tumor necrosis factor-α, and colony-stimulating factors. Overproduction of proinflammatory cytokines (IL-6, IL-12, IL-1β, IFN, TNF-α) during COVID-19 causes alveolar and epithelial cell apoptosis in the lungs, leading to hypoxia and interstitial edema [7]. In particular, TNF-α levels were high in patients admitted to the ICU, demonstrating its link to worsened disease course in COVID-19 [8,9]. Moreover, several acute manifestations of the disease, such as vascular/endothelial and pulmonary injury, have also

been linked to excessive TNF-α production [10]. Several investigations have proposed that severe COVID-19 patients have a significant and steady rise in TNF-α levels compared to the non-severe patient population, yet others, on the other hand, showed that the rise is not significantly different. [11-16].

In addition to boosting pro-inflammatory cytokines, COVID-19 causes a rise in anti-inflammatory cytokines, namely IL-10. It regulates the activation and differentiation of various types of T and B lymphocytes, as well as the production of IFN-γ and TNF-α [17-19]. The anti-inflammatory effect of IL-10 occurs by inhibiting macrophage activation and infiltration into the site of injury, with the secondary effect of attenuating proinflammatory cytokine expression [20]. Whether a high level of IL-10 is a precipitating factor for the onset of a cytokine storm or low IL-10 is responsible for the persistence of such an exaggerated immune response still remains unclear. Current literature indicates that IL-10 levels increase in the second week of infection, suggesting that IL-10 typically contributes to the down-regulation of the cytokine storm [8]. Given its immune-modulating role over cytokines, some researchers have recommended the use of recombinant forms of IL-10 in the treatment of acute respiratory distress syndrome in COVID-19 patients [21]. Thus, the aggressive and uncontrolled immune response to SARS-CoV-2 could be related to low levels of endogenous anti-inflammatory cytokines [22]. On the other hand, many studies have indicated that high levels of IL-10 correlate with severe disease course and poor prognosis in COVID-19: hence, the cytokine has been credited with a possible prognostic significance [23-27].

Considering the obscure role that pro- and anti-inflammatory cytokines play in the pathogenesis and progression of COVID-19, we decided to examine the dynamic changes of the cytokine levels during the infection and understand their role in the progression of the disease. Our initial hypothesis was that low levels of IL-10 in the second week of SARS-CoV-2 infection led to a rampant rise in TNF-α, which in turn leads to severe disease course and the development of an acute respiratory distress syndrome.

We aim to examine and compare the presence and mean levels of TNF-α and IL-10 in adult patients infected with SARS-CoV-2 with varying disease severity and on different days of the disease.

Methodology

As part of the study, we included 142 oropharyngeal swab PCR-positive patients (61 males and 81 females, with an average age of 63.627 ± 11.693 years): All participants were hospitalized at the National Center for Infectious Diseases of Armenia, on various days of the disease onset, as marked by symptom onset, in the time period from January to October, 2021. At the time, the prevalent SARS-CoV-2 strain was the so-called delta variant. Sixty-two patients (with an average age of 62.758 ± 11.467) came to the clinic during the first week of the infection, whereas 80 (with an average age of 64.3 ± 11.892) were admitted in the second week of the disease. The majority of the patients (n = 42) were admitted on the 5th day after the onset of symptoms (i.e., day 5 of the disease). The others were admitted on the 7th (n = 37), 10th (n = 25), 8th (n = 15), and 4th day (n = 13) of the disease.

We assessed COVID-19 severity by using the WHO Clinical Management Living guideline [28]. Based on WHO guidelines, patients with moderate disease had a persistent fever, and bilateral pneumonia on a computerized tomography (CT) scan, but had a pulse oxygen saturation (SpO_2) of > 93%. Those with a severe disease course again had bilateral pneumonia on CT, but either had an acute respiratory distress syndrome (ARDS), or a respiratory rate (RR) of ≥ 30/min and SpO_2 of ≤ 93%; critical disease patients required the use of a ventilator device, had some form of shock or end-organ damage. Most patients had only hypertension as a comorbid condition. Patients who received Vitamin D or any steroid (dexamethasone) or antiviral (remdesivir, umifenovir) medications before hospitalization were excluded from the study. According to our patient cohort selection strategy, we excluded any patient who showed signs and symptoms of other acute or chronic infectious conditions. Patients gave their written informed consent. The study was reviewed and approved by the Ethics committee of Yerevan State Medical University.

COVID-19 was diagnosed based on the findings from the clinical (history, physical exam), laboratory (complete blood counts and blood biochemistry, oropharyngeal swab PCR), and imaging (lung CT, ECG, and ultrasound) examinations. Blood was collected in the mornings on the day of admission, according to the venipuncture instructions. TNF-α and IL-10 levels were quantified using a widely accepted sandwich ELISA method and MR-96A microplate reader.

We analyzed the data using the Python programming language and used the Wilcoxon Rank Sum test to assess and compare the distribution of variables among the study groups. To measure the degree of association between the variables, we used

Spearman's rank correlation. We also used logistic regression analysis to assess the multicategorical variables. A value of $p < 0.05$ was considered statistically significant.

Results

On admission, 68.3% of patients had severe COVID-19, while 15.5% had critical and only 16.2% had moderate COVID-19. However, based on laboratory tests, the severity distribution of the patients changed, and all patients with moderate severity were classified as severe. Therefore, the study group consisted of severe (84.5%) and critical (15.5%) patients.

TNF-α levels were high in all 142 patients on various days of the disease. People in the first week of the disease had a higher mean TNF-α level (32.30 ± 46.50 pg/mL) than those in the second week of the disease (25.94 ± 25.52 pg/mL) ($p > 0.05$). IL-10 was high in 29% of patients during the first week of COVID-19, and 24% during the second week, with respective values of 27.84 ± 24.26 pg/mL and 25.58 ± 19.14 pg/mL ($p < 0.05$).

Afterward, we compared the levels of TNF-α and IL-10 on different days of the disease: TNF-α levels were higher on all days, and the mean levels are presented in Table 1. According to the results, the levels of TNF-α on days 5, 8, and 10 were significantly higher compared to those on day 4. Furthermore, TNF-α levels on day 5 of the disease were significantly higher than those on days 8 or 10 ($p < 0.05$). Therefore, we can state that the levels of TNF-α were high in all patients, and the mean levels were highest on day 5 of the disease. By the end of the first week (i.e., on day 7), TNF-α levels decreased but didn't reach the upper limit of the normal range. However, it started increasing again in the second week, and on days 8 and 10 the levels were significantly higher compared to days 4 and 7 ($p < 0.05$).

In contrast to TNF-α, IL-10 was high only in a quarter of the patients at most: only 7.7% of patients admitted on day 4 of the disease had higher than normal levels of the cytokine, and the highest frequency was seen on day 5, with 36.6% of patients having high levels of IL-10, which was significantly higher compared to days 4 and 7 ($p < 0.05$). Afterward, a decrease in this indicator is observed on the 7th and 8th day (high in only 22.6% and 10% of patients, respectively), but again increases on day 10, with high levels observed in 31.3% of patients (significant increase from days 4 and 8, $p < 0.05$). It is noteworthy that in our study, all severe patients with high IL-10 levels recovered from COVID-19.

Mean levels of IL-10 among the entire patient population were higher than normal only in patients admitted on day 5 of the disease, and the levels were significantly different from those admitted on other days (Table 1). It is worth pointing out that average IL-10 levels were not any higher than the normal range on the other days of the disease. Therefore, the highest levels of IL-10 were documented only on day 5 of COVID-19, and those levels were significantly higher compared to all other days of the disease.

We then examined the correlation between the levels of IL-10 and TNF-α. We revealed a statistically significant positive correlation between the levels of IL-10 and TNF-α during the first week of the disease (r = 35.27%, $p < 0.01$). Interestingly, this correlation was no longer significant during the second week of COVID-19 (r = 12.21%, $p = 0.28$). In addition, TNF-α and IL-

Figure 1. ROC curve of IL-10 and TNF-α for predicting severe form of COVID-19.

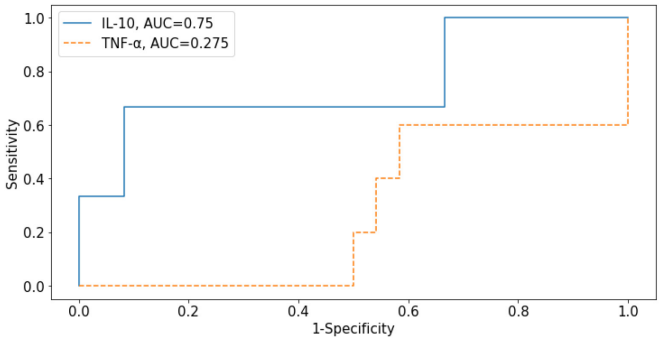


Table 1. Mean plasma levels of TNF-α and IL-10 in severe and critical COVID-19 patients on different days of the disease.

Days	n	TNF-α (N-0-6pg/mL)				IL-10 (N-0-31pg/mL)			
		mean	SD	Lowest value	Highest value	mean	SD	Lowest value	Highest value
Day 4	13	18.01	7.03	11.40	32.66	20.10	6.99	9.02	36.31
Day 5	42	38.24*°	55.53	7.39	273.10	30.95^{+}	28.53	9.12	180.86
Day 7	37	21.87°	25.19	6.93	147.07	27.73	26.80	8.65	158.04
Day 8	15	35.43*	44.36	11.14	157.09	20.32	9.21	8.39	42.90
Day 10	25	30.48*	23.56	7.69	80.616	24.24	10.27	11.94	43.47

* there is a significant difference compared to day 4 ($p < 0.05$); ° there is a significant difference compared to days 8 and 10 ($p < 0.05$); $^{+}$ there is a significant difference compared to all other days ($p < 0.05$).

10 had a significant positive correlation on days 4 and 5 of the disease (r = 20%, $p = 0.017$ and r = 30.61% $p = 0.042$, respectively), and already on day 10, they started correlating negatively (r = -12.35%, $p > 0.05$).

Considering the aforementioned, we decided to test the prognostic value of the changes in TNF-α and IL-10 levels in different severity degrees of COVID-19. By using logistic regression analysis, we designed ROC curves of TNF-α and IL-10 for predicting severe and critical forms of COVID-19 (Figures 1 and 2).

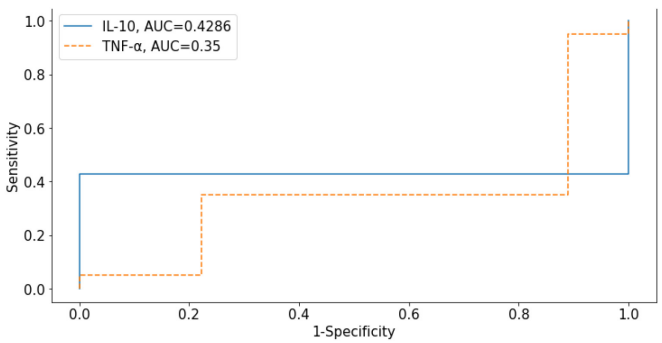
In severe COVID-19, the area under the ROC curve of IL-10 is high (AUC = 0.75), which means that increasing levels of IL-10 can predict the severe course of the disease. However, similar results were not observed for the critical form of the disease (AUC of IL-10 = 0.4286), diminishing the role of the cytokine in predicting the critical course of COVID-19.

Discussion

Only one study has shown that the levels of TNF-α do not increase throughout the entire course of COVID-19 [29]. On the other hand, many have demonstrated that the levels of the cytokine increase in the first days of the disease and stay elevated during the entire disease course [8,27]. We also obtained similar results. Therefore, high TNF-α levels can be regarded as an independent predictor for disease severity and prognosis [9,27,30,31].

Concerning IL-10, several research papers have noted that high levels of the cytokine are linked to a severe course of COVID-19 and that it might have a prognostic significance [23-27]. At the same time, high levels of IL-10 during the second week of the disease outline the latent immune capabilities of the body responsible for controlling the cytokine storm [8]. However, we could observe a significant rise in mean IL-10 levels only on day 5 of the disease, with the cytokine levels not crossing the upper limit of the normal range on any of the other days. The results more likely suggest that aggressive and uncontrolled immune response to SARS-CoV-2 can be caused by the low levels of endogenous anti-inflammatory cytokines. The low or total lack of expression of IL-10 as a consequence of immunity problems has great importance especially in age-related diseases as well as in endothelial dysfunction typical of COVID-19 disease [22].

Figure 2. ROC curve of IL-10 and TNF-α for predicting critical form of COVID-19.



It is more compelling to examine whether there was any significant correlation between the changing levels of TNF-α and IL-10. During the literature review, we could only find one study, which displayed high second-week levels of TNF-α along with high levels of IL-10 [8].

The subsequent correlation analysis illustrated that the positive correlation between TNF-α and IL-10 levels was significant only in the first week of the infection: therefore, the uncontrolled immune response during the second week of the disease course could be related to low levels of IL-10 cytokine. A retrospective analysis of the cases included in our study showed that patients who had a greater imbalance of these cytokines became critical or finally died.

The correlation analysis based on days of the disease further demonstrated that on day 10, TNF-α and IL-10 levels correlated negatively. Moreover, even though logistic regression analysis showed that IL-10 rise is a reliable prognostic sign for a severe course of the disease, all our patients with severe COVID-19 and high levels of IL-10 recovered from the disease. At the same time, IL-10 does not show prognostic significance in critically ill patients.

Therefore, emphasizing the key role of endogenous anti-inflammatory cytokines in the pathogenesis of COVID-19, it should be noted that the renin-angiotensin-aldosterone system, besides dysregulated cytokine production, plays an important role in the progression of cytokine storm. The replicating virus decreases the expression of the gene for angiotensin-converting enzyme (ACE 2), thereby decreasing the degradation of angiotensin. The ensuing high levels of angiotensin lead to an increase in vascular permeability in the lung tissue, further exacerbating lung injury and precipitating an acute respiratory distress syndrome [32].

Mudd *et al* have further indicated that despite the variety of immune-related dysregulations during COVID-19, only a handful of people (3-4%) develop the classical phenotype of cytokine storm, and even so, the serum levels of cytokines are significantly lower than those in other pathologies [33]. Furthermore, others suppose that the excessive production of

cytokines is only "the tip of the iceberg" in the progression of COVID-19, and does not play a role in clinical deterioration [34]. This led to other researchers suggesting even replacing the term "cytokine storm" with alternative pathogenetic mechanisms like endovasculitis, direct cytopathic effect of the viral particles, lymphodepletion, and others [35].

Conclusions

In summary, we can state that our results confirm the hypothesis that low levels of IL-10 during the second week of SARS-CoV-2 infection can result in the uncontrolled production of TNF-α, and lead to severe forms of the disease and to other possible unfavorable outcomes. Nevertheless, the dysfunction in cytokine response is one of the main mechanisms in COVID-19 progression and subsequent end-organ failure. Even so, since many patients still have an excessive cytokine response, alternative pathogenetic mechanisms should be sought to explain the severe manifestations of COVID-19.

Acknowledgements

We thank the National Center for Infectious Diseases for providing access to the database, Yerevan State Medical University for the support, Alisa Davtyan (Teaching Associate at the American University of Armenia) for assistance in data analysis. The authors did not receive any financial support for the research, authorship, or publication of this article.

Authors' Contributions

All authors have significantly contributed to the work reported in the article. Arshak Ghazaryan: conceptualization, methodology, data curation, investigation, software, formal analysis, writing – original draft. Naira Gyulazyan: conceptualization, writing – review and editing, project administration, supervision. Vigen Asoyan, Alvard Hovhannisyan, Melanya Kozmoyan, Arevhat Karapetyan, Armine Minasyan: contribution to data curation and resources.

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Conflict of interests: No conflict of interests is declared.