SHORT COMMUNICATION

Elements of Th1/Th2 response and disease severity in COVID-19 patients: A short report

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Abstract

The presence of a complex immune dysregulation syndrome has been established in COVID-19 patients. We aimed to assess Th1/Th2 response in COVID-19 patients and its association with disease severity by performing a prospective cohort study in a tertiary hospital COVID-19 referral center. We report no difference between Th1/Th2 responses between patients with severe and mild disease, except for levels of interleukin-6 (IL-6) and IL-10. Future larger studies should examine lung-specific versus systemic inflammatory responses, as well as, diverse immunotypes driving poor clinical outcomes.

KEYWORDS
COVID-19, inflammation, SARS-CoV-2, Th1/Th2 response

SARS-CoV-2 infection is asymptomatic in most children and 40% of adults. However, approximately 20% of patients present with severe disease and require admission to the intensive care unit (ICU). In these patients, mortality can reach as high as 25%, with most deaths attributed to severe inflammation, mirrored in underlying cytokine storm and embolic complications. It is imperative to promptly identify patients with increased risk of severe disease and poor outcomes to ensure timely interventions.

Recent data has shown a complex immune dysregulation syndrome in COVID-19 patients-involving a number of cytokines that determines outcomes but also provides the chance of future immunomodulatory efforts.[1] However, data remains scarce and contradictory regarding Th1/Th2 response in these patients, as this mirrored in the levels of pro and anti-inflammatory cytokines.[1–3] We hypothesize that, Th1/Th2 responses at the time of admission could predict the outcome, hence could prove a useful tool for timely and specific immunomodulatory interventions. We aimed to assess whether Th1/Th2 response differed in patients with severe disease from mild disease, hence could represent a useful marker of severity and guide further treatment.

This was a prospective study carried out in the COVID-19 ward of a referral tertiary hospital in Greece. The study protocol was approved by the Regional Research Ethical Committee (164/27.04.2020) and conducted in accordance with the Helsinki Declaration. Following informed consent, patients admitted in the COVID-19 ward of University Hospital of Patras within a 4-month period, with confirmed COVID-19 disease, were included in this study. Patients were excluded if pregnant, had an autoimmune or neoplastic disease, administered immune-modulatory therapies, including corticosteroids up to the time of serum sampling, HIV, or refused to participate. This was chosen to avoid bias of subclinical chronic inflammatory response at baseline, due to systemic underlying disease. Epidemiologic, clinical, and laboratory characteristics were extracted from patients' records, whereas serum sampling for cytokine measurement was performed upon time of admission. Patients were classified into those with disease severity as per WHO ordinal scale for clinical improvement of 3–4 (mild) and those with 5 or above (severe), depending on the degree of need for oxygen supply.[4] Primary study endpoint was to assess the underlying Th1/Th2 inflammatory response, as this is reflected in pro- and anti-inflammatory mediator serum levels, in relation to disease severity and patients' outcome. Fisher exact test was used for comparison of categorical data. The Mann–Whitney U test was used for comparison of skewed continuous data and Kruskal–Wallis was used to detect differences in non-normally distributed data. Data normality was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests using a p value of 0.05.

TABLE 1 Patient characteristics and outcomes

	Mild disease (n = 18)	Severe disease (n = 9)	Statistical significance
Male (%)	9 (50)	6 (67)	0.6
Age (years) (median, IQR)	63.0 (19)	61.5 (28)	0.6
Days following symptom initiation	9 (3)	8 (3)	0.2
CCI score (median, IQR)	2.0 (1)	2.5 (3.3)	0.5
SOFA score on presentation (median, IQR)	1.0 (1)	2.0 (1)	**0.002**
WBC (median, IQR)	6.2 (3.2)	5.9 (3.3)	0.07
CRP (mg/L) (median, IQR)	7.6 (7.2)	12.1 (5.5)	0.06
Ferritin (ng/ml) (median, IQR)	197 (249)	726 (437.5)	0.06
Outcome			
Death (%)	0 (0)	2 (22)	0.1
Length of stay (days) (median, IQR)	10 (1)	8 (3)	0.2
Cytokine levels (pg/ml) (median, IQR)			
IL-6	25.0 (17.3)	91.0 (85.6)	**0.004**
IFN-γ	5.7 (24)	11.8 (19.9)	0.9
IL-2	0.0 (8.2)	5.1 (7.9)	0.6
IL-4	0.0 (17.8)	4.9 (24.9)	0.6
IL-10	9.7 (15.7)	22.4 (10.2)	**0.04**
IL-17	21.5 (162.9)	47.2 (112.6)	0.9
TNF-α	20.1 (47.8)	31.5 (43.6)	0.9
Th1/Th2 response (median, IQR)			
IFN-γ/IL-4	0.8 (0.9)	0.8 (0.9)	0.8
IFN-γ/IL-10	0.6 (1.1)	0.5 (0.9)	0.5
TNF-α/IL-4	1.6 (1.8)	1.7 (1.8)	0.9
TNF-α/IL-10	2.0 (2.5)	1.4 (1.9)	0.2
IL-2/IL-4	0.0 (0.3)	0.3 (0.3)	0.8
IL-2/IL-10	0.0 (0.5)	0.2 (0.4)	0.8
IL-6/IL-10	1.82 (1.4)	4.65 (22.6)	**0.02**

Abbreviations: CCI, Charlson's Co-morbidity Index; CRP, C-reactive protein; IFN, interferon; IL, interleukin; IQR, interquartile range; SOFA, Sequential Organ Assessment Score; TNF, tumor necrosis factor; WBC, white blood cells.

From 65 Caucasian patients screened, in total, 27 patients were included in this study following implementation of exclusion criteria. Nine patients presented severe disease, hence required intensive care. Patients' characteristics and associated parameters are shown in Table 1. Groups were comparable in age, gender, and time, following initiation of symptoms and Charlson's Comorbidity Index. Sequential Organ Assessment Score significantly differed between groups, but not values of white blood cells, C-reactive protein, or ferritin (Table 1). No difference was noted in cytokine levels or Th1/Th2 response as this is reflected in respective cytokine ratios except for interleukin-6 (IL-6) and IL-10 levels and IL-6/IL-10 ratio (Table 1 and Figure 1). No difference in outcome was observed between groups.

We aimed to examine Th1/Th2 response in COVID-19 patients and their association with disease severity. We report no significant difference, except for IL-6 and IL-10 levels between groups. Previous authors have reported increased levels of IL-2, IL-10, TNF-α, IL-7, monocyte chemoattractant protein-1, granulocyte colony-stimulating factor, inducible protein-1, and macrophage inflammatory protein 1-alpha in patients with SARS-CoV-2, admitted in the ICU, than those who did not need intensive care.[3] Those patients suffered increased complications, including secondary infections, acute respiratory distress syndrome (ARDS), and cardiovascular adverse events, and hence increased mortality. On the contrary, similar to our findings, a previous report did not observe significant differences between patients with mild and severe disease.[5] As also shown in our study, both IL-6 and IL-10 levels' concentrations and the respective ratios were significantly higher in patients developing more severe symptoms of COVID-19 and eventually requiring aggressive ventilation compared to those who did not.[5,6] This comes in line with past observations, showing that their combined use exhibits nearly 100% specificity and 83.3% sensitivity for classification of patients in severe and nonsevere categories,[6] whereas IL-6/IL-10 ratio can be predictive of clinical outcome.[7] These findings suggest an enhanced Th2 response in these patients and a respective Th1 counterpart in the lockdown mode. This imbalance has been previously reported in SARS infections and is similar to that observed in influenza-infected elderly patients who represent a high-risk patient group for poor outcomes.[8] This supports the concept of "cytokine storm," in line with data from larger cohorts identifying TNF-a and IL-6 as independent predictors of disease severity.[1] However, consequent studies called this hypothesis into question, showing that inflammatory cytokine levels in the plasma of patients with COVID-19 are similar or even lower than patients with ARDS and sepsis.[9] Whether this is a result of increased viral load, rather than a dysregulated response that requires correction remains to be seen. In our study, we found no significant difference in Th1/Th2 response, as reflected in the majority of serum levels of major pro- and anti-inflammatory cytokines and their ratio, except for the IL-6/IL-10 ratio. It is possible that injurious host response may be more compartmentalized to the lung or gradually progress its way extrapulmonary in some individuals, rather than reflect an ab initio systemic cytokine storm, mirrored in cytokine plasma levels. This could explain the fact that in SARS-CoV-2 patients who do not require supplemental oxygen, dexamethasone may be harmful and the fact that in our study, irrespective of the severity of presentation, outcomes were not found to differ significantly.
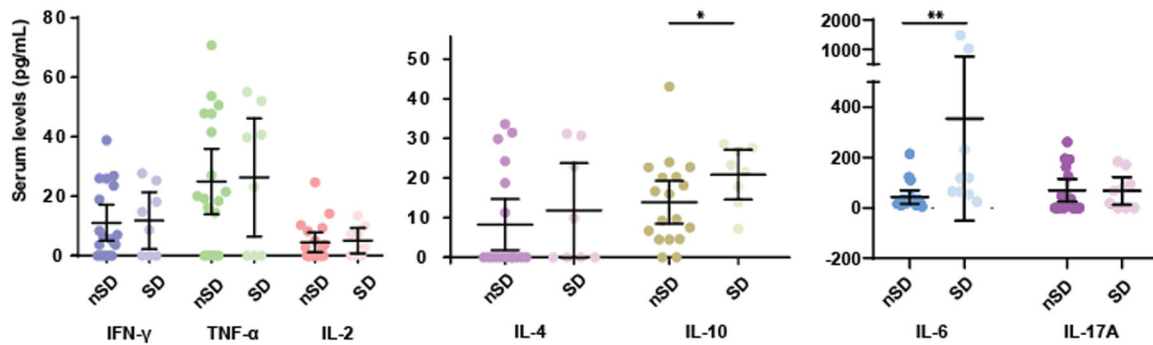
FIGURE 1 Scatter dot blot depicting cytokine serum levels of patients with mild and severe disease. A significant difference is noted between groups in IL-6 and IL-10 levels. IFN, interferon; IL, interleukin; TNF, tumor necrosis factor; Lines and error bars showing mean with 95% confidence interval, respectively. * and **Statistical significance of <0.05 and <0.01, respectively

Deeper and sequential temporal assessment of lung-specific versus systemic inflammatory responses, as well as, studies examining diverse immunotypes driving poor clinical outcomes in COVID-19, can increase our understanding and optimize therapeutic interventions.

This study has a number of limitations. First, it represents a single-center small-scale study, which may limit generalizability, even though our hospital represents a referral tertiary health care facility for SARS-CoV-2. Moreover, to minimize interindividual variation, we excluded patients with systemic diseases or immunomodulatory therapies. This could have affected the number of patients finally included and inevitably resulted in a small sample size; however, this size was powerful enough to detect differences among groups. Even though, our study was designed to assess biomarkers and immunologic profiling at the time of admission, dynamic development of viral replication and inflammatory response merits consideration. Sequential measurement of respective markers as the disease progresses and specimen collection from various sites is useful, hence could determine the exact timing and site that immunomodulatory intervention could provide, that is, more benefit than harm. Future design of larger prospective clinical trials using multivariate model analysis could overcome these issues and build on our findings, and pave the way for better understanding and promptly identifying patients with a high risk of poor outcomes.

AUTHOR CONTRIBUTIONS

Karolina Akinosoglou was responsible for patient management, analyzed the data, wrote the manuscript, and drew figures; Karolina Akinosoglou and Vasilina Dimakopoulou collected data and respective samples; Anne-Lise Delastic performed immunologic profile assessment; Markos Marangos and Charalambos Gogos advised patient management; and Charalambos Gogos critically corrected the manuscript. All authors contributed to the study's perception and design, and have seen and approved the manuscript.

DATA AVAILABILITY STATEMENT

Data can be made available upon request, according to GDPR.

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