Dynamic changes in the immune response correlate with disease severity and outcomes during infection with SARS-CoV-2

Fang Zheng
First Hospital of Changsha
Ruochan Chen
Xiangya Hospital Central South University
Run Yao
Xiangya Hospital Central South University
Yaxiong Huang
First Hospital of Changsha
Ning Li (liningxy@csu.edu.cn)
Xiangya Hospital Central South University
Jiyang Liu (702801924@qq.com)
First Hospital of Changsha
Yuanlin Xie (xieyuanlin99@163.com)
first hospital of changsha

Research article

Keywords: Immune response, COVID-19, Dynamic change, Disease severity, Disease outcome

Posted Date: December 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-48550/v2

Version of Record: A version of this preprint was published at Infectious Diseases and Therapy on June 10th, 2021. See the published version at https://doi.org/10.1007/s40121-021-00458-y.

Abstract

Background:

The coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly spread throughout China and all over the world. Little is known about the dynamic changes in the patient immune responses to SARS-CoV-2, and how different responses are correlated with disease severity and outcomes.

Method:

74 patients with confirmed COVID-19 were enrolled in this prospective research. The demographic information, medical history, symptoms, signs and laboratory results were analyzed and compared between severe and non-severe patients. The leukocytes, lymphocyte subsets and inflammatory cytokines were longitudinally collected.

Results:

Of the 74 patients included, 17 suffered from severe disease. The severe patients tended be older (65.29 ± 12.33 years vs. 45.37 ± 18.66 years), and had a greater degree of underlying disease (41.18% vs. 24.56%) , lower baseline lymphocytes counts (0.69 ± 0.36 × 10⁹ vs. 1.46 ± 0.75 × 10⁹) , higher neutrophil-lymphocyte-ratios (NLRs; 3.76 (3.15–5.51) vs. 2.07 (1.48–2.93)) and lower baseline eosinophil counts (0.01 ± 0.01 × 10⁹ vs. 0.05 ± 0.07 × 10⁹), than that in non-severe patients. The baseline helper T (Th) cells (335.47 vs. 666.46/mL), suppressor T(Ts) cells (158 vs. 334/mL), B cells (95 vs. 210/mL), and natural killer (NK) cells (52 vs. 122/mL) were significantly decreased in severe cases compared to that in non-severe cases. In addition, the baseline neutrophils and B cells were positively correlated with the severity of COVID-19 and the baseline lymphocytes and Th cells were negatively correlated with the severity of COVID-19. The dynamic change of T cells, Th cells and IFN-γ in the severe cases were parallel to the amelioration of the disease.

Conclusions:

Collectively, our study provides novel information on the kinetics of the immune responses in a cohort of COVID-19 patients with different disease severities. Furthermore, our study indicates that both innate and adaptive immune responses correlate with better clinical outcomes.

1 Introduction

First reported in Wuhan in December 2019, coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly spread throughout China and all over the world [1-3]. Viral genome sequencing revealed SARS-CoV-2 to be a member of the β-coronavirus family, which also includes the Middle East syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV) [4, 5]. As a result of its rapid global spread and high

infectiousness, the World Health Organization (WHO) declared the COVID-19 outbreak a “Public Health Emergency of International Concern” (PHEIC) on 30 January 2020. According to the official report released by the WHO, as of Mar 30, 2020, a total of 693224 laboratory-confirmed cases have been found worldwide, with 33106 of these cases being fatal.

The major symptoms of COVID-19 are acute viral pneumonia as well as extrapulmonary manifestations[1, 3, 6]. Patients with SARS-CoV-2 infections present a wide range of disease severity, varying from asymptomatic to critical pneumonia with respiratory failure. The immune response is an important defense against viral infections and is often found to correlate with disease severity and prognosis [7, 8]. Currently, the pathogenesis of the pulmonary and extrapulmonary manifestations of COVID-19 remains poorly understood, and our understanding of the factors that affect disease severity is limited, although older age, underlying illness, lymphopenia and “cytokine storm” have been reported, in line with SARS and MERS [1, 2, 9, 10]. However, little is known about the dynamic changes in the immune response and inflammatory cytokines and their correlation with disease severity and outcomes during infection by SARS-CoV-2.

We sought to investigate the kinetics of the immune response and how this is correlated with disease severity and outcomes in patients with COVID-19. In this observational, single-center study, we analyzed clinical data from 74 hospitalized COVID-19 patients who collectively represented different degrees of disease severity. Additionally, we continuously investigated the immunological features and proinflammatory cytokines in patients during the entire duration of their hospitalization. We hope that this comparative and kinetic analysis will provide a better understanding of host-pathogen interactions and host immune responses, and that it may help in uncovering the underlying mechanisms contributing to COVID-19 pathogenesis in order to design an immune intervention or preventive vaccine for COVID-19 in the foreseeable future.

2 Methods

2.1 Study design and participants

All 74 patients included in this study had been confirmed as having COVID-19 and were admitted to the First Hospital of Changsha, Changsha, China, from January 29th to February 15th 2020. The First Hospital of Changsha was designated as “the specific hospital for the treatment of severe patients with COVID-19 in Changsha" by the government during the epidemic. This study was approved by the ethics committee of the First Hospital of Changsha city (No. 2020SK3013). Written informed consent was obtained from all patients.

2.2 Definition of severe and non-severe infections

COVID-19 was confirmed by detecting the presence of SARS-CoV-2 RNA in the nasopharyngeal swab samples using a virus nucleic acid detection kit (Sheng Xiang Medical Biotechnology Co., Ltd, No. 20203400064), according to the manufacturer’s protocol. The disease severity in all the hospitalized

COVID-19 patients was assessed on admission, based on the Seventh Revised Trial Version of the Novel Coronavirus Pneumonia Diagnosis and Treatment Guidance. A severe case was defined according to the following criterion: 1. respiratory distress with a respiratory rate >30 per min; 2. pulse oximeter oxygen saturation ≤ 93% in the resting state while breathing ambient air; 3. arterial blood oxygen partial pressure (PaO_2)/oxygen concentration (FiO2) ≤ 300 mmHg (1 mmHg = 0.133 kPa). All other patients were categorized as non-severe.

2.3 Data collection

Primary data, including demographic information, medical history, symptoms, signs, laboratory results, radiological, and therapeutic characteristics were collected from electronic medical records. The day of admission was defined as Day 1. Laboratory tests included analysis of routine blood, lymphocyte subsets, infection-related biomarkers, and inflammatory cytokines which were analyzed at different time points (Day 1, Day 8, Day 15, Day 20, and Day 25). The total number of lymphocytes in peripheral blood was counted using a hemocytometer. Lymphocyte subset percentages were determined using FACSCalibur (Becton Dickinson Co., Ltd). The absolute numbers of different lymphocyte subsets were calculated by multiplying the percentages by the total lymphocyte count. The levels of inflammatory cytokines were also determined using FACSCalibur according to the manufacturer's instructions (Becton Dickinson Co., No. P010002, Tian Jin Kuang Bo Co., No. 20180072).

2.4 Flow cytometry

Immune cells quantitation and classification were determined using BD Multitest™ IMK kit (BD Co., Ltd) according to manufacturer's instructions, which was used with BD FACSCalibur™ flow cytometers to determine the percentages and absolute counts of the following mature human lymphocyte subsets in peripheral whole blood for immunophenotyping: T lymphocytes (CD3+), B lymphocytes (CD19+), Natural killer (NK) lymphocytes (CD3−CD16+ and/or CD56+), Helper/inducer T lymphocytes (CD3+CD4+), Suppressor/cytotoxic T lymphocytes (CD3+CD8+). BDMultitest™ CD3/CD8/CD45/CD4 contains FITC-labeled CD3, clone SK7; PE-labeled CD8, clone SK1; PerCP-labeledCD45, clone 2D1 (HLe-1); and APC-labeled CD4, clone SK3. BDMultitest™ CD3/CD16+CD56/CD45/CD19 contains FITC-labeled CD3, clone SK7; PE-labeled CD16, clone B73.1, and PE-labeled CD56, clone NCAM 16.2; PerCP-labeled CD45, clone 2D1 (HLe-1); and APC-labeled CD19, clone SJ25C1. When whole blood of the patient is collected and added to the reagent, the fluorochrome-labeled antibodies in the reagent bind specifically to leucocyte surface antigens. The stained samples are treated to lyse erythrocytes. During acquisition, the cells travel past the laser beam and scatter the laser light. The stained cells fluoresce. These scatter and fluorescence signals, detected by the instrument, provide information about the cell's size, internal complexity, and relative fluorescence intensity. BD Multitest reagents employ fluorescence triggering, allowing direct fluorescence gating of the lymphocyte population to reduce contamination of unlysed or nucleated red blood cells in the gate. When BD Trucount™ Tubes are used, a known volume of sample is stained directly in a BD Trucount Tube. The lyophilized pellet in the tube dissolves, releasing a known number of fluorescent beads. During analysis, the absolute number (cells/μL) of gated cells in the sample

can be determined by comparing cellular events to bead events. Then appropriate cytometer-specific BD software is used, and absolute counts are determined by the software. The absolute numbers of different lymphocyte subsets were calculated by multiplying the percentages by the total lymphocyte count. The limitation of this method is that absolute counts are not comparable between laboratories using different manufacturers' equipment.

2.5 Quantitation of cytokines

We used the BD™ CBA Human Th1/Th2/Th17 Cytokine Kit (BD Co., Ltd) to measure Interleukin-2 (IL-2), Interleukin-4(IL-4), Interleukin-6 (IL-6), Interleukin-10 (IL-10), Tumor Necrosis Factor-α (TNF-α), Interferon-γ (IFN-γ), and Interleukin-17A (IL-17A) protein levels in patients, according to manufacturer's instructions. Seven bead populations with distinct fluorescence intensities have been coated with capture antibodies specific for IL-2, IL-4, IL-6, IL-10, TNF-α, (IFN-γ, and IL-17A proteins. The seven bead populations are mixed together to form the bead array, which is resolved in a red channel of the flow cytometer. During the assay procedure, we mix the cytokine capture beads with serum samples and incubate them with the PE-conjugated detection antibodies to form sandwich complexes. The intensity of PE fluorescence of each sandwich complex reveals the concentration of that cytokine. After acquiring samples on the flow cytometer, we use FCAP Array™ software to generate results in graphical and tabular format. All the samples were measured in duplicate and the mean values were used for analysis. The BD CBA Kit is not recommended for use on stream-in-air instruments for which signal intensities may be reduced, adversely affecting assay sensitivity. Quantitative results or protein levels for the same sample or recombinant protein run in ELISA and BD CBA assays may differ. A spike recovery assay can be performed using an ELISA standard followed by BD CBA analysis to assess possible differences in quantitation.

2.6 Statistical Analysis

Continuous variables were expressed as means ± standard deviation (SD) or medians with ranges, and categorical variables were expressed as frequencies and percentages. Continuous variables were compared using a *t*-test (for a normal distribution) or a Mann—Whitney U test (for a skewed distribution). The chi-squared test or Fisher's exact test were used to compare categorical variables. A multivariate logistic regression analysis was performed by taking the severity of COVID-19 (yes or no) as dependent variable and variables which found significant during univariate analysis were selected as independent variables. The longitudinal data from repeated measures were compared by the generalized linear mixed model. $P < 0.05$ was considered statistically significant. SPSS statistical soft-ware (Macintosh version 26¢0, IBM, Armonk, NY, USA), and GraphPad PRISM 5.0 software (GraphPad Software, San Diego, CA, USA) and EmpowerStats (X&Y Solutions, Inc, Boston, MA, USA). were used for statistical analysis.

3 Results

3.1 Demographic and clinical characteristics

As of February 15, 2020, 74 patients who were confirmed to have COVID-19 on admission to the First Hospital of Changsha, were included in our study (Table 1). Among these, 17 patients (23.0%) were clinically diagnosed with severe infections, with the remaining 57 patients being categorized as non-severe. The average patient age was 49.95 ± 19.28 years, and 35 patients (47.3%) were men. A total of 67 patients (90.54%) had a history of exposure to potential transmission sources (having a history of travel or residence in Wuhan and its surrounding areas, or contact with infected individuals). Of the 74 patients, 25 (33.78%) patients had underlying diseases, including hypertension, diabetes, liver cirrhosis, and cardiovascular diseases. A higher percentage of comorbidities was found in the severe patients (41.18%, n = 17) than that in the non-severe patients (31.58%, n = 57). Compared with non-severe patients, the severe patients were significantly older (65.29 ± 12.33 years vs. 45.37 ± 18.66 years; P < 0.001). There was no significant difference in sex between the severe and non-severe patients (P = 0.595). The most common symptoms revealed by our study were fever (54.05%), cough (51.35%), fatigue (32.43%), pharyngalgia (13.51%), shortness of breath (12.16%). Moreover, severe patients were significantly more likely to suffer from fever (76.47% vs. 47.37%) and shortness of breath (35.29% vs. 5.26%), compared to non-severe patients.

3.2 Laboratory findings

The laboratory findings in patients with different degrees of disease severity are shown and compared in Table 2. Among the 74 patients who underwent laboratory examinations on admission, most tended to have lower lymphocyte counts, elevated enzyme marker (i.e. creatine kinase, lactate dehydrogenase, and aspartate aminotransferase) and infection-related biomarker (i.e. erythrocyte sedimentation rate and C-reactive protein) levels, compared to laboratory reference ranges. There were also numerous differences in blood cell counts, infection related biomarkers, enzymes, and other biochemical markers between the severe and non-severe patients. Severe patients tended to have a higher percentage of neutrophils (78.04% vs. 61.19%; P < 0.001), much lower lymphocytes counts (0.69 ± 0.36 × 10⁹ vs. 1.46 ± 0.75 × 10⁹; P < 0.001), higher neutrophil-to-lymphocyte ratios (NLRs) (3.76 (3.15–5.51) vs. 2.07 (1.48–2.93); P < 0.001), and lower eosinophil counts (0.01 ± 0.01 vs. 0.05 ± 0.07; P < 0.001). Compared to non-severe patients, severe patients presented higher C-reactive protein levels, erythrocyte sedimentation rates, lactate dehydrogenase levels, aspartate aminotransferase levels, D-dimer levels, creatine kinase levels, and lower albumin levels (P < 0.05, all).

3.3 Lymphocyte subset analysis

Limited by the detection ability of our hospital, we were only able to test several common subtypes of lymphocytes in all of the 74 patients (Table 3). The total number of T cells, B cells, and natural killer (NK) cells were significantly decreased in patients with COVID-19, and this was more evident in the severe group (675.0 vs. 1379.0/mL; P < 0.001) than in the non-severe group. In patients with COVID-19, NK cells were below normal levels, and T and B cells were both within the lower levels of the normal range. The levels of the three main subsets of lymphocytes were shown to be more suppressed in severe cases, as

their counts were nearly half of those in non-severe patients (500 vs. 1014/mL, P < 0.001; 95 vs. 210/mL, P < 0.001; 52 vs. 122/mL, P < 0.001).

Different subsets of T cells were further analyzed, including helper T cells (CD3+CD4+), suppressor T cells (CD3+CD8+), and regulatory T cells (CD3+CD4+CD25+CD127low+). The levels of both helper T cell (CD3+CD4+) and suppressor T cells (CD3+CD8+) were decreased in patients with COVID-19, and this was more pronounced in severe patients compared to that in non-severe patients (335.47 vs. 666.46/mL, P < 0.001; 158 vs 334 mL, P < 0.001). However, there was no significant difference in the percentage of regulatory T cells between severe and non-severe cases (P = 0.617). The helper T cell/suppressor T cell ratio (Th/Ts) remained in the normal range, and there was no difference between the two subgroups.

3.4 The immune cells factors related to the severity of COVID-19.

The result of univariable analysis demonstrated that immune cells including lymphocytes, neutrophils, eosinophils, T cells, NK cells, Th cells and Ts cells were related to the severity of COVID-19. In multivariate logistic regression analysis, we observed neutrophils (OR (95%CI): 3.79 (1.07, 13.46), P=0.039) and B cells (OR (95%CI): 1.01 (1.00, 1.02), P=0.049) were independent risk factors for assessing the severity of COVID-19 and lymphocytes (OR (95%CI): 0.01 (0.00, 0.57), P=0.027) and Th cells (OR (95%CI): 0.99 (0.99, 1.00), P=0.019) were protect factors of the severity of COVID-19 (Table 4).

3.5 The kinetics of immune response and correlation with disease severity and outcome

We analyzed the kinetics of white blood cells associated with disease severity and outcomes in patients with COVID-19. Significant increases in the neutrophil counts of the severe group were observed at day 8 and 15 compared to the non-severe group (Fig. 1A). With improved patient conditions, the number of neutrophils in severe patients decreased significantly after day 15. We took a generalized linear mixed model to find that the severity of disease (F = 0.719, P = 0.459) and curing time (F = 3.132, P = 0.136) were not related to neutrophil counts. The lymphocytes in the severe group was significantly lower than non-severe group at day 1,8,15,20 and 25 (Fig. 1B). With improved patient conditions, the number of lymphocytes in severe patients gradually increased. We found the severity of disease (F = 11.244, P = 0.044) was related to lymphocytes counts, but curing time (F = 3.228, P = 0.115) was not related to lymphocytes counts in generalized linear mixed model. At day 1, eosinophils of severe group were significantly decreased compared to the non-severe group (Fig. 1C). At other time points, we found no significant differences in eosinophils counts between the two groups.

We further analyzed the kinetics of lymphocyte subsets associated with disease severity and outcomes in patients with COVID-19. The similar trend was observed in T cells (Fig.2A), Ts cells (Fig.2B) and Th cells (Fig.2C). In severe patients, the numbers of T cells, Th cells and Ts cells increased from day 1 to day 20, and decreased after day 20. Before day 15, the numbers of T cells, Th cells and Ts cells in severe group were significantly lower than non-severe group. We found that the severity of disease (F = 6.208, P = 0.047) and curing time (F = 4.730, P = 0.017) were related to T cells and the severity of disease (F = 16.747, P = 0.009) and curing time (F =10.727, P = 0.002) were also related to Th cells. No significant

differences in NK cells were observed between the two groups during the whole observation period (Fig. 2D).

We analyzed the kinetic changes of inflammatory cytokine levels, including IL-2 (Fig. 3A), IL-4 (Fig. 3B), IL-6 (Fig. 3C), IL-10 (Fig. 3D), IL-17A (Fig. 3E), IFN-γ (Fig. 3F) and TNF−α (Fig. 3G). There were no significant differences in the levels of IL-2, IL-4, IL-17A, IFN-γ and TNF−α between non-severe group and severe group at day 1,8,15,20 and 25. And all of them showed a gradual downward trend. But in generalized linear mixed model, we found the severity of disease ($F = 10.535$, $P = 0.048$) and curing time ($F = 10.439$, $P = 0.023$) were related to IFN-γ levels and curing time ($F = 39.345$, $P < 0.001$) were related to TNF−α levels. IL-6 levels showed sustained increases in the severe group compared to the non-severe group until day 20. The IL-6 levels of the non-severe group remained basically unchanged. And at day 1, 8, 15 and 20, The IL-6 levels in severe patients were significantly higher than non-severe patients. We observed IL-10 levels showed sustained increases in the severe group from day 1 to day 8. Reductions in serum IL-10 levels in the severe group started at day 8. At day 1, 8 and 15, IL-10 levels in the severe group were significantly increased compared to the non-severe group.

4 Discussion

The rapid and wide spread of SARS-CoV-2 infection in China and in the world has resulted in a tremendous loss of safety in peoples' lives [11]. In this study, we systematically analyzed clinical characteristics, dynamic changes in the immune response including changes in proinflammatory cytokines, in 74 patients with different degrees of disease severity. Although the number of patients included in our study is limited, our study provides several novel findings, including the observations that SARS-CoV-2 might mainly act on lymphocytes, induces an inflammatory cytokine storm in the body, and generates a series of immune responses. In addition, the dynamic changes in multiple immune cells and cytokines that we have observed, as well as their association with disease severity and outcomes during hospitalization, might help us develop effective treatment strategies and a preventive vaccine to treat and control COVID-19 in the near future. Our research helps us more clearly delineate the progression of COVID-19 in humans, and also provide a scientific basis for a better understanding of its pathogenesis.

In total, old age and shortness of breath were more common in severe patients. Lymphopenia, including T cells, B cells, and NK cells and an increase in NLR were common among patients with COVID-19, and were more pronounced in the severe patients. These results are in accordance with other studies [12, 13, 14(Wu, 2020 #48(Wu, 2020 #48]) and the findings of limited autopsies and biopsies, which reported markedly shrunken spleens and a significant reduction in lymphocytes (Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment, 7th edition). Immune cells are important effectors of a host's immune system, and play crucial roles in anti-viral infections [15]. The protective response is T cell dependent, with CD4 helping B cells, geared toward the production of specific neutralizing antibodies, and cytotoxic CD8 cells capable of eliminating infected cells [16]. It is worth noting that 80% of the infiltrating cells in COVID-19 are CD8[17]. CD4+ T helper cells coordinate immunity by releasing various cytokines, while CD8 + suppressor T cells directly kill the target cells during viral infections [10]. B cells perform their

humoral immune function by releasing neutralizing antibodies, presenting antigens, and regulating immune function. NK cells can kill non-specific target cells infected by virus, and play an important role in the early anti-viral response [18]. The reduction in lymphocyte counts may allow SARS-CoV-2 to spread and progress in the early stages of an infection. Based on these data, we suggest that COVID-19 might damage immune cells, including T cells, B cells, as well as NK cells, and that the immune system is impaired to various degree and this correlates with disease severity.

We also noted that the most severe patients presented higher neutrophils counts and lower lymphocytes counts, i.e., there was an increase in NLR, compared to that in the non-severe patients. Transient or persistent leukocytosis, primarily due to increased levels of neutrophils, is a well-known phenomenon in systemic inflammation and infections [19, 20]. Our results are consistent with data from several studies [2, 12], suggesting that there is a serious disturbance in the internal environment, secondary bacterial infections, and potential critical condition in these severe patients. These significant changes in white blood cells prompted us to quantify inflammatory cytokines. Consistently, a wide range of inflammation-related biomarkers and cytokines, were elevated and this was more evident in severe patients, suggesting that an inflammatory cytokine storm may have a role in disease progression.

Eosinophils are generally considered as multifunctional cells that function as part of the innate immune system and are associated with allergic and parasitic inflammation responses. However, several studies have shown that there is an intricate correlation between eosinophils and severe infectious diseases, including bacterial and viral infections [21, 22]. The translocation of eosinophils from the lungs of mice infected with influenza virus has been shown to reduce morbidity and viral burden, improve lung function, and increase the levels of CD8(+) T cell in the airways [22]. Our study found that eosinopenia was common among patients with COVID-19, and this was more significant in the severe patients. Moreover, the number of eosinophils steadily increased as the patient's condition improved. These data imply that eosinopenia might be considered to be a potential marker of disease severity in COVID-19 patients, and that eosinophils might have a protective role in SARS-CoV-2 infections.

In order to investigate dynamic changes in the immune response, we further analyzed the kinetics of the immune response that were associated with clinical resolution of COVID-19. All of the recovered patients exhibited a gradual and persistent increase in lymphocyte counts, including helper T cells, suppressor T cells, and NK cells, strongly suggesting that both innate and adaptive immune system play a protective role in fighting the SARS-CoV-2 infection. Plasma levels of cytokines and chemokines are also increased in COVID-19, but are higher in severe infections, and includes IL-2, IL-2R, IL-6, IL-7, IL-8 IL-10, IP10, MIP1A, and TNF-α[2, 16]. High levels of plasmatic IL-6 have been consistently reported in COVID-19 and even appear to be associated with poor prognosis and risk of death[23]. Thus, its measurement has been proposed as a good biomarker to monitor these patients. Additionally, as the patients improved the levels of most of inflammatory cytokines examined, including IFN-γ, IL-10, IL-17A, IL-2, IL-4, IL-6, and TNF−α generally decreased, indicating their potential as biomarkers and indicators of disease severity and prognosis.

There are several limitations in our study. First, it is a single center study with a relatively small sample size. We propose that a larger cohort of patients with COVID-19 should be used to assess the dynamic changes in the immune response to avoid any potential bias. Second, all the patients studied here recovered from COVID-19, thus we do not have any information on the processes that occur in patients who do not recover from COVID-19. Third, as a result of our limited testing ability, only part of the immune response could be analyzed in our hospital. The immune response to SARS-CoV-2 in humans should be characterized in much more detail in the future.

5 Conclusions

Collectively, our study provides novel information towards understanding the kinetics of the immune response in a cohort of COVID-19 patients with different degrees of disease severity. Furthermore, our study indicates that both innate and adaptive immune responses are correlated with clinical outcomes. We hope that this study has provided evidence that sets the stage for identifying the predictors of outcomes and also potential intervention strategies for COVID-19.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of First Hospital of Changsha (KL-2020006). The study was conducted in accordance with the guidelines of the Declaration of Helsinki and the principles of Good Clinical Practice. All patients provided written informed consent to include their clinical and biological data in the manuscript for scientific purposes. Data of the patients submitted were anonymized.

Consent to publish

All authors read and approve the manuscript and agree to publish.

Conflict of Interest

The authors declare no competing non-financial/financial interests.

Author Contributions

Fang Zheng, Yuanlin Xie, Ning Li and Jiyang Liu designed the study. Fang Zheng and Yaxiong Huang collected the data. Ruochan Chen and Run Yao analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Funding

The collection, analysis, and interpretation of data and writing the manuscript was supported by grants from Innovative Major Emergency Project Funding against the New Coronavirus Pneumonia in Hunan Province (NO.2020SK3013 and NO.2020SK3014), National Natural Sciences Foundation of Hunan province (NO. 2019JJ30041) and Innovation-Driven Project of Central South University (NO.2020CX044).

Data Availability Statement

More detailed data are available upon request; the interested scientific researchers could contact directly Dr. Fang Zheng, Dr. Ruochan Chen for further information.

Acknowledgments

We thank Brain for his critical reading of our manuscript.

References

1. Huang, C., et al., *Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China.* Lancet, 2020. **395**(10223): p. 497-506.
2. Qin, C., et al., *Dysregulation of immune response in patients with COVID-19 in Wuhan, China.* Clin Infect Dis, 2020.
3. Henry, B.M. and J. Vikse, *Clinical Characteristics of Covid-19 in China.* N Engl J Med, 2020. **382**.
4. Lu, R., et al., *Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding.* Lancet, 2020. **395**(10224): p. 565-574.
5. Malik, Y.S., et al., *Emerging novel coronavirus (2019-nCoV)-current scenario, evolutionary perspective based on genome analysis and recent developments.* Vet Q, 2020. **40**(1): p. 68-76.
6. To, K.K., et al., *Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study.* Lancet Infect Dis, 2020.
7. Channappanavar, R. and S. Perlman, *Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology.* Semin Immunopathol, 2017. **39**(5): p. 529-539.
8. Min, C.K., et al., *Comparative and kinetic analysis of viral shedding and immunological responses in MERS patients representing a broad spectrum of disease severity.* Sci Rep, 2016. **6**: p. 25359.
9. Chen, N., et al., *Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study.* Lancet, 2020. **395**(10223): p. 507-513.
10. Prompetchara, E., C. Ketloy, and T. Palaga, *Immune responses in COVID-19 and potential vaccines: Lessons learned from SARS and MERS epidemic.* Asian Pac J Allergy Immunol, 2020. **38**(1): p. 1-9.
11. Ding, Q., et al., *The clinical characteristics of pneumonia patients coinfected with 2019 novel coronavirus and influenza virus in Wuhan, China.* J Med Virol, 2020.

12. Wang, D., et al., *Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China.* JAMA, 2020.
13. Zhu, N., et al., *A Novel Coronavirus from Patients with Pneumonia in China, 2019.* N Engl J Med, 2020. **382**(8): p. 727-733.
14. Zhu, Y., et al., *Clinical and CT imaging features of 2019 novel coronavirus disease (COVID-19).* J Infect, 2020.
15. Li, C.K., et al., *T cell responses to whole SARS coronavirus in humans.* J Immunol, 2008. **181**(8): p. 5490-500.
16. Garcia, L.F., *Immune Response, Inflammation, and the Clinical Spectrum of COVID-19.* Front Immunol, 2020. **11**: p. 1441.
17. Li, G., et al., *Coronavirus infections and immune responses.* J Med Virol, 2020. **92**(4): p. 424-432.
18. Thornton, J., *Don't forget chronic lung and immune conditions during covid-19, says WHO.* BMJ, 2020. **368**: p. m1192.
19. Berhane, M., et al., *The Role of Neutrophil to Lymphocyte Count Ratio in the Differential Diagnosis of Pulmonary Tuberculosis and Bacterial Community-Acquired Pneumonia: a Cross-Sectional Study at Ayder and Mekelle Hospitals, Ethiopia.* Clin Lab, 2019. **65**(4).
20. Liu, X., et al., *Prognostic Significance of Neutrophil-to-Lymphocyte Ratio in Patients with Sepsis: A Prospective Observational Study.* Mediators Inflamm, 2016. **2016**: p. 8191254.
21. Percopo, C.M., et al., *Activated mouse eosinophils protect against lethal respiratory virus infection.* Blood, 2014. **123**(5): p. 743-52.
22. Samarasinghe, A.E., et al., *Eosinophils Promote Antiviral Immunity in Mice Infected with Influenza A Virus.* J Immunol, 2017. **198**(8): p. 3214-3226.
23. Wu, C., et al., *Risk Factors Associated With Acute Respiratory Distress Syndrome and Death in Patients With Coronavirus Disease 2019 Pneumonia in Wuhan, China.* JAMA Intern Med, 2020. **180**(7): p. 934-943.

Tables

Table 1 Demographics and clinical features of patients with COVID-19

	Total (n=74)	Non-severe (n=57)	Severe (n=17)	P-value
Age (years)	49.95 ± 19.28	45.37 ± 18.66	65.29 ± 12.33	<0.001
Sex				0.595
Male	35 (47.30%)	26 (45.61%)	9 (52.94%)	
Female	39 (52.70%)	31 (54.39%)	8 (47.06%)	
Epidemiology	67 (90.54%)	53 (92.98%)	14 (82.35%)	0.189
Underlying disease	21 (28.38%)	14 (24.56%)	7 (41.18%)	0.224
Fever	40 (54.05%)	27 (47.37%)	13 (76.47%)	0.035
Dry cough	31 (41.89%)	23 (40.35%)	8 (47.06%)	0.780
Expectoration	14 (18.92%)	9 (15.79%)	5 (29.41%)	0.289
Shortness of breath	9 (12.16%)	3 (5.26%)	6 (35.29%)	<0.001
Myalgia	6 (8.11%)	5 (8.77%)	1 (5.88%)	0.702
Headache	4 (5.41%)	2 (3.51%)	2 (11.76%)	0.186
Dizzy	4 (5.41%)	2 (3.51%)	2 (11.76%)	0.186
Fatigue	24 (32.43%)	15 (26.32%)	9 (52.94%)	0.04
Abdominal pain	1 (1.35%)	1 (1.75%)	0 (0%)	0.582
Diarrhea	4 (5.41%)	3 (5.26%)	1 (5.88%)	0.921
Nausea and vomiting	2 (2.70%)	1 (1.75%)	1 (5.88%)	0.357
Pharyngalgia	10 (13.51%)	10 (17.54%)	0 (0.00%)	0.063
Rhinorrhea	2 (2.70%)	1 (1.75%)	1 (5.88%)	0.357

Table 2 Laboratory data of patients with COVID-19

	Normal Range	Total (n=74)	Non-severe (n=57)	Severe (n=17)	P-value
Blood routine					
Leucocytes, 10▯/L	3.5–9.5	4.78 ± 1.72	4.73 ± 1.47	4.93 ± 2.41	0.913
Hemoglobin, g/L	110-150	131.22 ± 16.67	131.40 ± 15.57	130.59 ± 20.48	0.877
Platelet, 10^6/L	100-300	180.16 ± 64.43	189.39 ± 63.24	149.24 ± 60.18	0.027
Lymphocytes, 10▯/L	1.1–3.2	1.17 (0.76-1.62)	1.27 (0.95-1.70)	0.64 (0.46-0.95)	<0.001
Neutrophils, 10▯/L	1.8–6.3	2.77 (2.05-3.60)	2.74 (2.03-3.47)	3.14 (2.37-5.86)	0.008
Neutrophil-to-lymphocyte ratio		2.31(1.60-3.32)	2.07(1.48-2.93)	3.76(3.15-5.51)	<0.001
Eosinophils, 10▯/L	0.02-0.52	0.01 (0-0.05)	0.03 (0.01-0.06)	0 (0-0)	<0.001
Lymphocytes , %	20.0-50.0	26.52 ± 10.70	29.68 ± 9.36	15.94 ± 7.82	<0.001
Neutrophils , %	40.0-75.0	65.06 ± 12.11	61.19 ± 9.97	78.04 ± 9.44	<0.001
Eosinophils , %	0.4-8.0	0.20 (0-1.20)	0.70 (0.10-1.40)	0 (0-0.10)	<0.001
Biochemical index					
Creatine kinase isoenzyme, U/L	0-16	10.50 (7.70-14.88)	10.30 (7.50-13.70)	11.20 (9.30-16.70)	0.092
Creatine kinase, U/L	25-170	64.00 (44.45-92.22)	60.40 (43.40-90.10)	67.90 (61.00-108.90)	0.007
Triglyceride, mmol/L	0.56-1.77	1.11 (0.73-1.46)	1.23 (0.73-1.48)	1.08 (0.76-1.19)	0.512
Total cholesterol, mmol/L	2.84-5.69	3.98 ± 0.85	4.03 ± 0.81	3.80 ± 1.00	0.453
High density lipoprotein, mmol/L	1.14-1.91	0.90 ± 0.26	0.91 ± 0.24	0.86 ± 0.31	0.625
Low density lipoprotein, mmol/L	1.0-3.0	2.78 (2.25-3.14)	2.78 (2.29-3.14)	2.78 (1.78-3.10)	0.504
Alanine aminotransferase, U/L	0-40	18.43 (13.95-23.07)	18.19 (14.20-22.10)	18.95 (13.86-23.80)	0.542
Aspartate aminotransferase, U/L	0-45	24.95 (20.29-33.08)	23.22 (19.50-28.10)	33.24 (27.13-41.34)	<0.001
Total bilirubin, μmol/L	1.7-17.1	10.21 (7.60-16.24)	10.10 (7.70-15.60)	10.54 (7.23-16.45)	0.979
Albumin, g/L	60-80	38.54 ± 4.60	39.90 ± 3.66	33.98 ± 4.56	<0.001
A/G	3.5/5.5	1.53 ± 0.33	1.62 ± 0.30	1.24 ± 0.26	<0.001
Creatinine, μmol/L	44-133	58.20 (43.47-67.50)	58.50 (44.34-68.60)	49.99 (43.30-64.40)	0.634
Urea nitrogen, mmol/L	1.8-7.1	5.12 ± 2.50	4.88 ± 2.48	5.92 ± 2.49	0.057
Chloride ion, mmol/L	96-108	103.02 ± 3.54	103.38 ± 3.43	101.83 ± 3.72	0.047
Potassium ion, mmol/L	3.5-5.5	4.13 ± 0.48	4.19 ± 0.47	3.95 ± 0.46	0.048
D-Dimer, mg/L	0-0.5	0.26 (0.14-0.44)	0.21 (0.14-0.42)	0.39 (0.18-0.66)	0.007
Erythrocyte sedimentation rate, mm/h	0-40	40.50 (14.25-66.50)	33.00 (13.00-55.00)	68.00 (46.00-86.00)	0.007
High-sensitivity C-reactive protein, mg/L	0-8	11.10 (3.04-32.82)	5.95 (2.28-15.34)	54.53 (31.60-70.67)	<0.001
Lactate dehydrogenase, U/L	0-252	185.29 ± 78.52	158.40 ± 46.25	275.44 ± 97.12	<0.001

Table 3 Lymphocyte Subsets of patients with COVID-19

	Normal Range	Total (n=74)	Non-severe (n=57)	Severe (n=17)	P-value
Lymphocyte Subsets					
T cells+B cells+NK cells /ul	1100.0-3200.0	1322.00(876.25-1717.25)	1379.00(1086.00-1886.00)	675.00(376.00-1314.00)	<0.001
T cells (CD3+CD19-) /ul	955.0-2860.0	905 (622.25-1231.75)	1014 (751-1414)	500 (202-716)	<0.001
T cells (CD3+CD19-) /ul %	50.0-84.0	69.35 ± 9.89	70.88 ± 8.54	64.24 ± 12.45	0.014
B cells (CD3-CD19+) /ul	90.0-560.0	199 (131-348.50)	210 (172-364)	95 (80-243)	0.005
B cells (CD3-CD19+)%	5.0-18.0	19.05 ± 8.44	17.35 ± 6.44	24.76 ± 11.61	0.001
NK cells (CD3-/CD16+CD56+) /ul	150.0-1100.0	98 (52.50-164)	122 (60-167)	52 (35-85)	0.007
NK cells (CD3-/CD16+CD56+)%	7.0-40.0	10.00 (5.00-13.75)	9.00 (5.00-13.00)	11.00 (9.00-14.00)	0.912
T cells Subsets					
Th cells (CD3+CD4+) /ul	550.0-1440.0	590.42 ± 324.34	666.46 ± 309.16	335.47 ± 236.18	<0.001
Ts cells (CD3+CD8+)/ul	320.0-1250.0	314.50 (204.25-480.75)	334 (266-505)	158 (66-209)	<0.001
Th cells (CD3+CD4+) %	27.0-51.0	42.96 ± 12.32	43.47 ± 12.88	41.24 ± 10.39	0.515
Ts cells (CD3+CD8+)%	15.0-44.0	25.59 ± 9.13	26.11 ± 8.31	23.88 ± 11.60	0.382
Th/Ts	0.71-2.78	1.79 (1.28-2.64)	2.05 ± 1.17	2.55 ± 2.60	0.266
Regulatory T cells (CD3+CD4+CD25+CD127low+) /ul	5.36-6.30	3.75 (2.82-4.83)	3.81 (2.82-5.01)	3.45 (3.12-3.88)	0.617

Table 4 Logistic regression analysis of immune cells associated with the severity of COVID-19.

Variables	Univariate analysis		Multivariate analysis	
	OR (95%CI)	P value	OR (95%CI)	P value
Lymphocytes, 10□/L	0.02 (0.00, 0.16)	<0.001	0.01 (0.00, 0.57)	0.027
Neutrophils, 10□/L	1.56 (1.09, 2.25)	0.015	3.79 (1.07, 13.46)	0.039
Eosinophils, 10□/L	0.00 (0.00, 0.00)	0.014		
T cells+B cells+NK cells /ul	1.00 (1.00, 1.00)	<0.001		
T cells /ul	1.00 (0.99, 1.00)	<0.001		
NK cells /ul	0.99 (0.98, 1.00)	0.035		
Th cells /ul	1.00 (0.99, 1.00)	0.001	0.99 (0.99, 1.00)	0.019
B cells /ul	1.00 (0.99, 1.00)	0.145	1.01 (1.00, 1.02)	0.049
Ts cells /ul	0.99 (0.98, 1.00)	0.001		

OR: odd ratio; CI: confidence interval

Figures

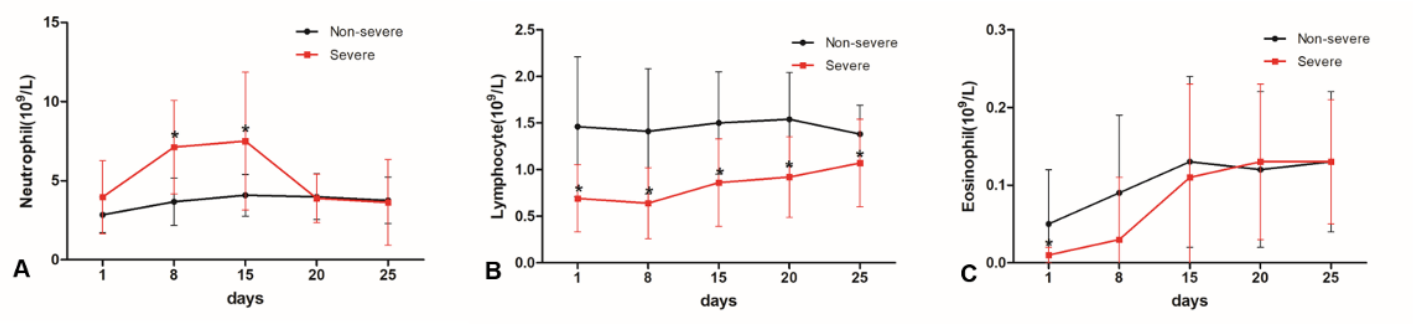

Figure 1

Kinetic analysis of white blood cells in COVID-19 patients. (A) Neutrophil, (B) Lymphocyte, (C) Eosinophil. Error bars, mean± SD. * indicated that there was a statistical difference between Non-severe and Severe at the same time point ($p<0.05$).

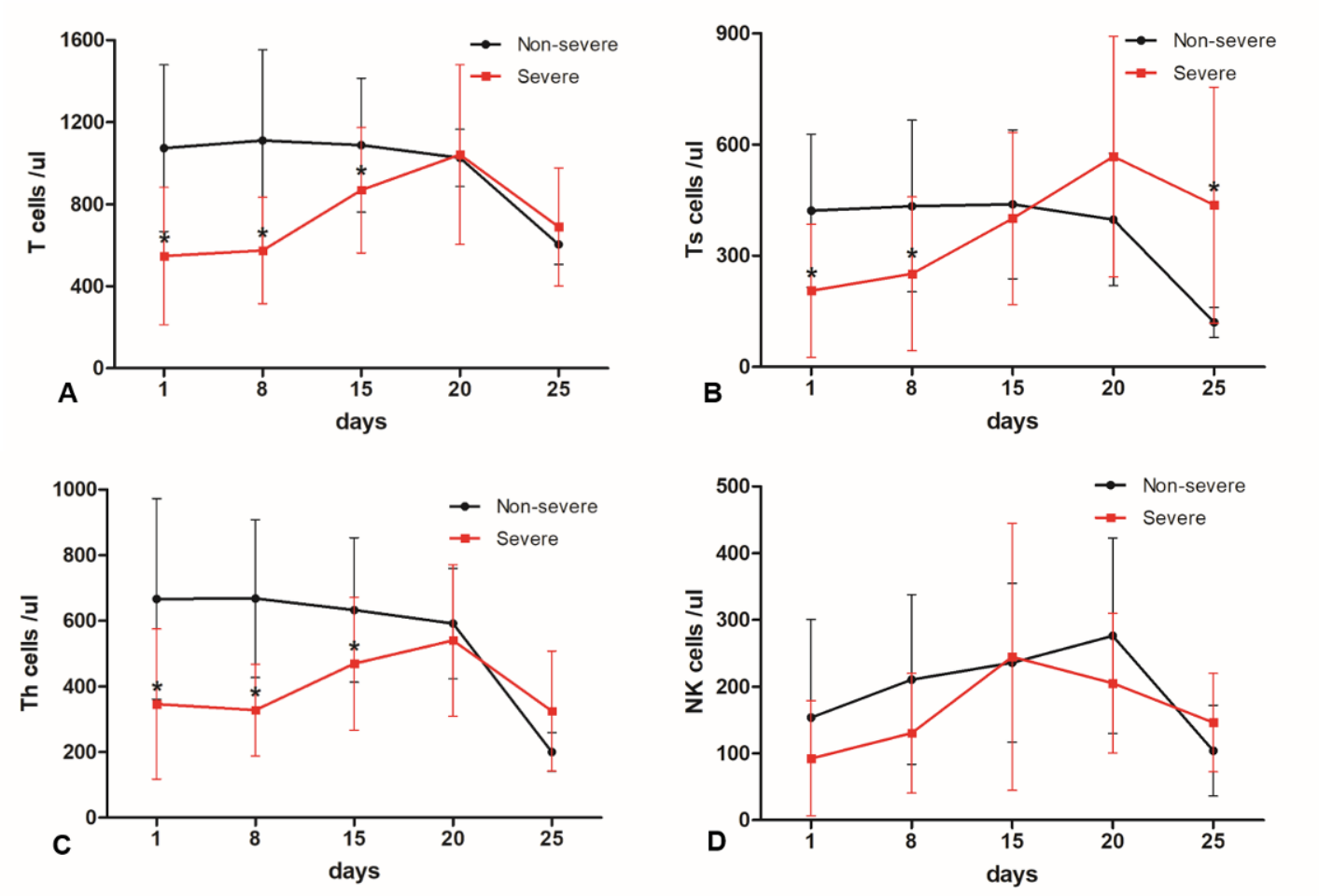

Figure 2

Kinetic analysis of different lymphocyte subsets in COVID-19 patients. (A) T cells, (B) Ts cells, (C) Th cells, (D) NK cells. Error bars, mean± SD. * indicated that there was a statistical difference between Non-severe and Severe at the same time point (p<0.05).

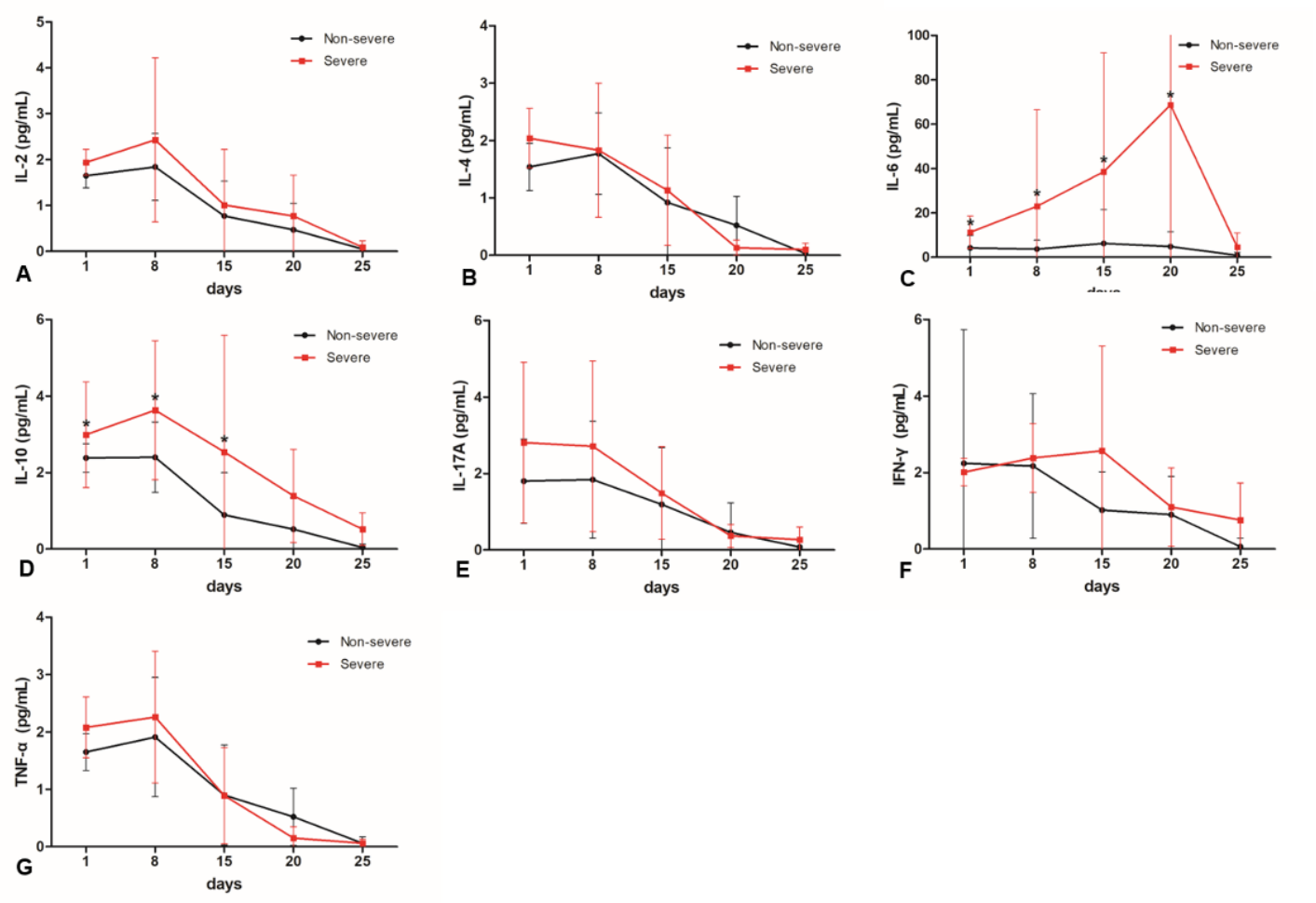

Figure 3

Kinetic analysis of inflammatory cytokines levels in COVID-19 patients. (A) IL-2, (B) IL-4, (C) IL-6, (D) IL-10, (E) IL-17A, (F) IFN-γ, (G) TNF−α. Error bars, mean± SD. * indicated that there was a statistical difference between Non-severe and Severe at the same time point (p<0.05).