Dynamic Changes in the Immune Response Correlate with Disease Severity and Outcomes During Infection with SARS-CoV-2

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Abstract

Background:

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:

A total of 74 patients with confirmed COVID-19 were included from January 29th to February 15th 2020. Clinical characteristics and dynamic changes in immune response were analyzed and compared between severe and non-severe patients.

Results

Of the 74 patients, 17 suffered from severe disease and 57 from non-severe disease. Patients with severe disease 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 lymphocytes counts (0.69 ± 0.36 × 10^10 vs. 1.46 ± 0.75 × 10^10), higher neutrophil-lymphocyte-ratios (NLRs; 3.76 (3.15–5.51) vs. 2.07 (1.48–2.93)) and lower eosinophil counts (0.01 ± 0.01 × 10^10 vs. 0.05 ± 0.07 × 10^10), than that in non-severe patients. The number of immune cell subtypes, including helper T cells, suppressor T cells, B cells, and natural killer cells was significantly decreased in severe cases compared to that in non-severe cases (335.47 vs. 666.46/mL; 158 vs. 334/mL; 95 vs. 210/mL; 52 vs. 122/mL, respectively). As the condition of the patients improved, the number of neutrophils decreased significantly in the severe patients. All patients who recovered exhibited a gradual and persistent increase in eosinophil counts and lymphocyte counts, including helper T cells, suppressor T cells, and natural killer cells. In addition, the levels of most of inflammatory cytokines, including IFN-γ, IL-2, IL-4, IL-6, IL-10, IL-17A, and TNF-α generally decreased as the patients gradually recovered.

Conclusions

Collectively, our study provides novel information on the kinetics of the immune responses to COVID-19. 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” (PEHIC) 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 (PaO2)/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 Statistical Analysis

Continuous variables were expressed as means ± standard deviation, 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.

All analyses were performed with the statistical software package R (http://www.R-project.org, The R Foundation) and EmpowerStats (http://www.empowerstats.com, X&Y Solutions, Inc, Boston, MA, USA). A two-tailed P-value below 0.05 was considered significant.

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 (30.0%) 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.

| 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 |

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 that in normal people. 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).
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, 10⁶/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, 10⁶/L      | 100–300      | 180.16 ± 64.43| 189.39 ± 63.24      | 149.24 ± 60.18  | 0.027   |
| Lymphocytes, 10⁶/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, 10⁶/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, 10⁶/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 |
|                          | Normal Range | Total (n = 74) | Non-severe (n = 57) | Severe (n = 17) | P-value |
|--------------------------|--------------|---------------|---------------------|----------------|---------|
| 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 |

### 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/µL; P < 0.001) than in the non-severe group. In patients with COVID-19, the numbers of the three main subsets of lymphocytes were generally decreased, with NK cells having below normal levels, and T and B cells both being 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/µL, P < 0.001; 95 vs. 210/µL, P < 0.001; 52 vs. 122/µL, P < 0.001).
Table 3  
Lymphocyte Subsets of patients with COVID-19

| Lymphocyte Subsets | Normal Range | Total (n = 74) | Non-severe (n = 57) | Severe (n = 17) | P-value |
|--------------------|--------------|---------------|---------------------|-----------------|---------|
| 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**

| T 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 |
| T 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 |

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 cells (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/µL, P < 0.001; 158 vs 334 µL, 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 kinetics of immune response and correlation with disease severity and outcome

We further analyzed the kinetics of the immune response associated with disease severity and outcomes in patients with COVID-19 (Fig. 1). All of the 74 patients included in our study eventually recovered. With improved patient conditions, the number of neutrophils decreased significantly in severe patients. All of the recovered patients exhibited a gradual and persistent increase in lymphocyte counts, including Th cells, Ts cells, and NK cells, as well as eosinophils, and this trend was more evident in severe patients. Additionally, the levels of most inflammatory cytokines, including IFN - γ, IL-2, IL-4, IL-6, IL-10, IL-17A, and TNF–α generally decreased as the patients gradually recovered. Regulatory T cells showed a downward trend in non-severe patients and an upward trend in severe patients (data not shown). The CD4/CD8 ratio decreased in both patient types, but this was more evident in severe patients (data not shown).

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]) 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]. 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 [16]. 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 [17, 18]. 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 [19, 20]. 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 [20]. 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. 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.

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. However, there are several limitations in our study. First, it is a retrospective, 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. 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

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.

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.

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**Figures**

**Figure 1**

The kinetics of immune response and correlation with disease severity and outcome (A) The number of neutrophil, lymphocyte and eosinophil in severe and non-severe patients with COVID-19 at different time points. (B) The number of T cells, Th cells, Ts cells and NK cells in severe and non-severe patients with COVID-19 at different time points. (C) The serum level of IL-2, IL-4, IL-6 and IL-10 in severe and non-severe patients with COVID-19 at different time points. (D) The serum level of IL-17A, INF-γ and TNF-α in severe and non-severe patients with COVID-19 at different time points. Note: *P < 0.05