



Analysis of the dynamic relationship between immune profiles and the clinical features of patients with COVID-19

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Background: The novel coronavirus disease (COVID-19) has been declared a global pandemic, with the cumulative number of confirmed cases and deaths exceeding 150 million and 3 million, respectively. Here, we examined the dynamic changes in the immune and clinical features of patients with COVID-19.

Methods: Clinical data of 98 patients with confirmed COVID-19 diagnosis were acquired from electronic medical records and curated. The data were analyzed based on the stage of the admission, deterioration, and convalescence, which included age, sex, severity, disease stages, biochemical indicators, immune cells, inflammatory cytokines, and immunoglobulins. Additionally, temporal changes in the immune response in patients undergoing continuous renal replacement therapy (CRRT) were also examined.

Results: Compared to mild stage patients, severe stage patients with COVID-19 exhibited a significant reduction in lymphocyte [23.10 (17.58–33.55) *vs.* 4.80 (2.95–6.50), $P < 0.001$], monocyte [8.65 (7.28–10.00) *vs.* 3.45 (2.53–4.58), $P < 0.001$], and NK cell levels [244.00 (150.50–335.00) *vs.* 59.00 (40.00–101.00), $P < 0.001$] but showed elevated levels of neutrophils [64.90 (56.30–73.70) *vs.* 90.95 (87.60–93.68), $P < 0.001$], inflammatory cytokines [Interleukin-10, 3.05 (1.37–3.86) *vs.* 5.94 (3.84–8.35), $P = 0.001$; and tumor necrosis factor- α , 11.50 (6.55–26.45) *vs.* 12.96 (12.22–36.80), $P = 0.029$], which improved during convalescence. Besides, the number of immune cells—T lymphocytes, B lymphocytes, helper T cells, suppressor T cells, NK cells, and monocytes, except neutrophils—slowly increased in critically ill patients receiving CRRT from 0 to 3 weeks.

Conclusions: Our results indicate that the surveillance of immune cells may contribute to monitoring COVID-19 disease progression, and CRRT is a potential therapeutic strategy to regulate the immune balance in critically ill patients.

Keywords: COVID-19; immune cells; cytokines; continuous renal replacement therapy (CRRT)

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Introduction

The outbreak of coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has rapidly swept worldwide (1-3). Hitherto, the global coronavirus case count has exceeded 150 million, with the cumulative number of deaths surpassing 3 million, leading to a profound and constantly evolving impact on global public health security and the world economy. This novel coronavirus could infect people via droplets and aerosols (4-7); furthermore, Moghadas *et al.* found that most transmissions were attributable to presymptomatic and asymptomatic individuals and probably caused an unexpected outbreak, which deeply threatened the prevention and treatment of infectious diseases (8). Several studies have shown that the elderly are more prone to COVID-19, presenting with initial manifestations of fever, cough, and myalgia or fatigue (9,10). Approximately 20–30% of cases need further intervention in intensive care units (ICU) due to serious complications, including acute respiratory distress syndrome, acute cardiac/renal injury, and septic shock (10-12). Currently, clinical treatment is empirically administered, consisting of symptomatic management, oxygen therapy, and antiviral drugs, with some critically ill patients needing continuous renal replacement therapy (CRRT) to help remove potential toxins and stabilize their metabolic statuses (13). Notably, the sequelae of patients with severe COVID-19 were much worse, accompanied by more severe impaired pulmonary diffusion capacities and abnormal chest imaging manifestations (14).

Evidence is mounting that the aberrant immune response was crucial in the pathogenesis of SARS-CoV-2. Several studies have reported that patients with severe COVID-19 exhibited sharply decreased counts of immune cells, including T cells, B cells, natural killer (NK) cells, monocytes, basophils, and eosinophils, but not neutrophils (15-19). In contrast, these patients displayed continuously increasing levels of pro-inflammatory cytokines such as interleukin (IL)-10, IL-2, IL-6, and tumor necrosis factor- α (TNF- α) (10,17). Moreover, Zhang *et al.* (18) found that the severity of COVID-19 correlated with increased immunoglobulin (Ig) G response. Besides, the single-cell landscape has revealed that inflammatory monocyte-derived macrophages increased in SARS-CoV-2-infected lungs, especially from patients with severe COVID-19 (20). Meanwhile, a specific subset of macrophages identified in patients with COVID-19 were probably associated with tissue fibrosis (21). Eventually, the disturbance of immunity by SARS-CoV-2

will result in damaged tissues, even causing shock or multiple organ dysfunction. From an epidemiological perspective, the coronavirus vaccine is the best method to prevent infection and restrict the COVID-19 pandemic; thus, many coronavirus vaccines have been developed and reported to be effective (22,23). However, the progress and effectiveness of the vaccine seem inadequate especially considering the recently mutated coronavirus.

Given the above complications, clinicians should comprehend the status of a disease at any time, promptly prevent the deterioration of the condition, and optimize the management and treatment for patients with COVID-19. Therefore, we analyzed the dynamic temporal alterations in immune cells and multiple cytokines in the peripheral blood of patients with COVID-19 during the hospital admission, initial deterioration, and convalescent stages and examined the effect of CRRT on immune homeostasis in critically ill patients. Here, we discuss the findings of this study, elaborating the role of immune cells and factors in the progression of COVID-19 and the potential therapeutic benefits of CRRT. We present the following article in accordance with the STROBE reporting checklist (available at <https://dx.doi.org/10.21037/atm-21-812>).

Methods

Study population

Ninety-eight patients diagnosed with COVID-19 were recruited for this study from the First Affiliated Hospital of the College of Medicine at Zhejiang University from January 02, 2020 to March 16, 2020. The flow diagram of selecting patients with COVID-19 is shown in *Figure 1*. All patients enrolled in the study signed written informed consents personally or via designated surrogates. The study was conducted following the Declaration of Helsinki (as revised in 2013). The study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University (No. 2020IIT195), and individual consents for this retrospective analysis were waived.

Procedures

Patient data, including clinical features (sex, age, symptoms, signs, body temperature, length of stay, chronic medical illness, comorbidities, dialysis modality), laboratory results (biochemical indicators, blood routine examination,

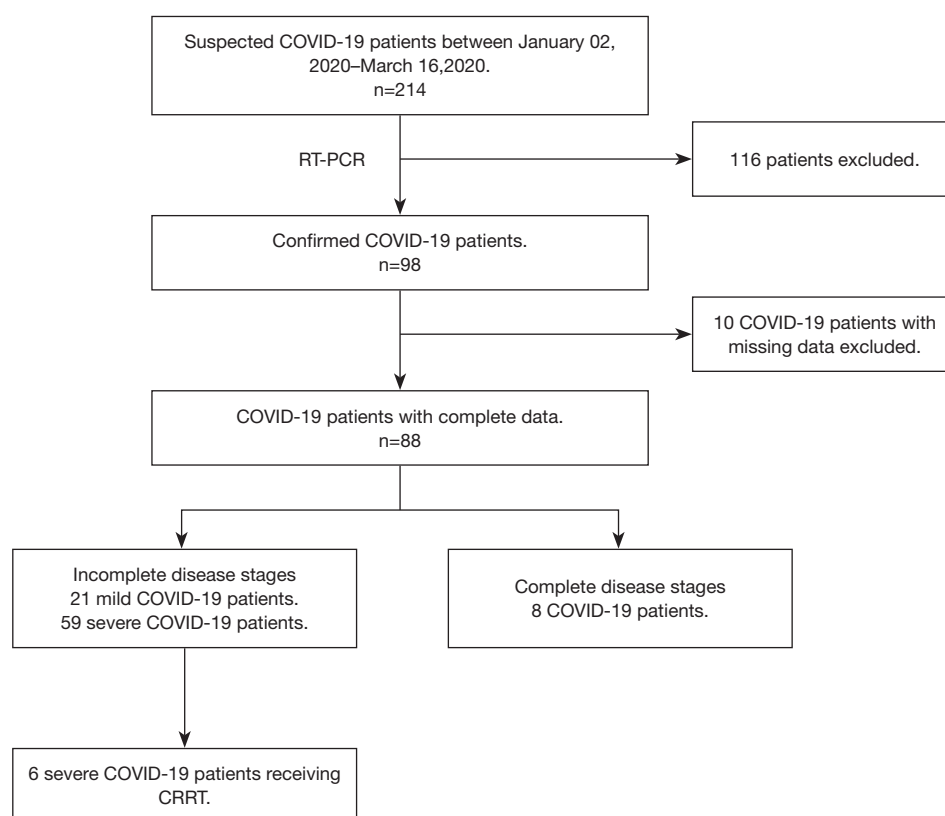


Figure 1 Selection procedure of COVID-19 patients. The diagnosis of COVID-19 was confirmed by RT-PCR assay. Incomplete disease stages represented that patients were lack of admission or deterioration stages; complete disease stages represented that patients progressed through admission, deterioration and convalescence stages during hospitalization. RT-PCR, reverse transcriptase-polymerase chain reaction.

infection-related biomarkers, inflammatory cytokines, immunoglobulins, complement proteins, immune cell subsets), treatment and outcomes were obtained from the hospital's electronic medical records. All patients underwent chest radiography or computed tomography and laboratory confirmation of SARS-CoV-2 at admission according to the WHO guidance as positive nasal and pharyngeal swab specimens by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay. Two researchers independently reviewed the data for accuracy and communicated with patients or their family members to ascertain the epidemiological and symptom data.

Classification of diseases

Patients with COVID-19 who met any of the following criteria were diagnosed as severe patients: (I) respiratory distress with the respiratory rate ≥ 30 breaths per minute; (II) oxygen saturation $\leq 93\%$ at rest; (III) arterial blood

oxygen partial pressure (PaO_2)/oxygen concentration (FiO_2) ≤ 300 mmHg (1 mmHg = 0.133 kPa); (IV) significant lesion progression $>50\%$ within 24–48 h on chest imaging; (V) respiratory failure requiring mechanical ventilation, shock, and ICU admission required for restoring combined organ function.

Statistical analysis

All variables were expressed as median with interquartile range (IQR). Individuals whose data could not be found were excluded. *T*-tests were used to compare means for normally distributed continuous variables; otherwise, the Mann-Whitney U test was applied. Line charts were drawn to display the dynamic change of variables. Proportions of categorical variables were compared using the χ^2 test or Fisher's exact test. All statistical analyses were performed using SPSS (Statistical Package for the Social Sciences) version 25.0 (SPSS Inc.). Two-sided *P* values of less than

0.05 were considered statistically significant.

Results

Demographic and clinical characteristics of patients with mild and severe COVID-19

Among the 98 patients with COVID-19, 10 were rejected for lack of integrity regarding their clinical data, and 8 were enrolled to be dynamically analyzed. Ultimately, 21 patients whose clinical data were collected on admission were diagnosed with mild conditions and did not deteriorate during hospitalization. Conversely, 59 patients were diagnosed with severe conditions requiring hospitalization. For these patients, additional clinical data were collected during convalescence.

Compared to patients with mild conditions, patients with severe conditions were remarkably older [median age, 61 (IQR, 51–70) years *vs.* 50 (IQR, 35–61) years; $P=0.002$]. The proportion of men among patients was not significantly different between severe (66.1% men) and mild (47.6%, $P=0.135$) cases. As shown in [Table S1](#), the severe group had a higher percentage of chronic disease, especially hypertension and diabetes, than the mild group (59.32% *vs.* 33.33%, $P=0.041$). The most common symptoms were fever (87.50%), dry cough (48.75%), shortness of breath (15.00%), myalgia (12.50%), fatigue (11.25%), and expectoration (10.00%).

Significant differences in immune profiles between patients with mild and severe COVID-19 on admission

Remarkably, significant differences in blood immune cells and infection-related biomarkers were observed between the mild and severe groups ([Table 1](#)). Patients with severe conditions exhibited a higher level of neutrophils (90.95% *vs.* 64.9%; $P<0.001$) and white blood cell counts (WBC, 9.80 *vs.* 4.60; $P<0.001$), and a lower percentage of lymphocytes (4.80% *vs.* 23.10%; $P<0.001$), monocytes (3.45% *vs.* 8.65%; $P<0.001$), and eosinophils (0.00% *vs.* 0.10%; $P<0.001$). Compared with patients in the mild group, those with severe cases displayed elevated levels of infection-related biomarkers, including high sensitivity C-reactive protein (hs-CRP, 33.94 *vs.* 7.62; $P=0.002$), and erythrocyte sedimentation rate (ESR, 49.00 *vs.* 16.00; $P<0.001$). TNF- α (5.94 *vs.* 3.05, $P=0.001$) and IL-10 (5.94 *vs.* 3.05; $P=0.001$) levels were higher in patients with severe conditions than in those with mild cases. Although IL-2, IL-4, IL-6 also

had an upward trend, the differences between the mild and severe groups were not statistically significant. Notably, patients with severe conditions exhibited slightly elevated aspartate aminotransferase (AST, 24.00 *vs.* 20.00, $P=0.043$) and blood lactic acid (1.95 *vs.* 1.50, $P=0.003$) but mildly decreased calcium (2.06 *vs.* 2.17; $P=0.001$) and albumin (34.55 *vs.* 44.10; $P<0.001$) levels ([Table S2](#)).

To explore the role of immune cells in coronavirus disease, immune cell subsets were analyzed in 56 patients (10 mild and 46 severe cases with complete data available, [Table 2](#)). The absolute lymphocyte count per microliter was dramatically decreased in severe patients (462.00 *vs.* 1,597.50/ μ L; $P<0.001$). The three main lymphocyte subset numbers—B cells, T cells, and NK cells—were also generally reduced in severe patients compared to mild patients. T cells and NK cell counts were less than half of the lower reference limit while B cells were slightly above the normal range, suggesting that NK and T cells may be more affected by SARS-CoV-2 in severe patients than mild patients. Among T cells, both helper T (Th) cells (CD3+, CD4+) and suppressor T (Ts) cells (CD3+, CD8+) were remarkably reduced in patients with severe COVID-19 compared to those with mild cases. Finally, abnormal clinical indicators observed in severe patients reverted to the normal range during convalescence.

The dynamic progression of 8 patients through admission, deteriorated, and convalescent stages

We retrospectively analyzed all patients with COVID-19 and found that 8 patients had subsequently progressed through the stages of admission, deterioration, and convalescence. To do this, we collected clinical data of these patients as they were admitted, deteriorated, and recovered, and analyzed laboratory data available only ([Figure 2](#)). The median duration from admission to deterioration was 5 (4–6) days. As the disease progressed, a dynamic variation in lymphocyte, monocyte, basophil, and eosinophil levels was observed; first declining and then rising. However, neutrophils and hs-CRP showed the opposite trend, consistent with previous findings ([Figure 2](#)). Other laboratory data of three consecutive stages are listed in [Table S3](#).

The dynamic immune pattern changes in patients with severe COVID-19 undergoing CRRT

CRRT contributes to the stabilization of metabolic and

Table 1 Immune findings in patients with mild and severe COVID-19

Immune findings	Norma range	Mild (N=21)	Severe (N=59)	Convalescence (N=55)	P (M vs. S)	P (S vs. C)
Blood routine examination						
WBC counts, $\times 10^9/L$	4.00–10.00	4.60 (3.80–5.30)	9.80 (6.15–12.50)	7.35 (5.43–9.78)	<0.001	0.005
Neutrophil percentage, %	50.00–70.00	64.90 (56.30–73.70)	90.95 (87.60–93.68)	72.70 (66.40–79.40)	<0.001	<0.001
Lymphocyte percentage, %	20.00–40.00	23.10 (17.58–33.55)	4.80 (2.95–6.50)	17.05 (11.28–20.48)	<0.001	<0.001
Monocyte percentage, %	3.00–10.00	8.65 (7.28–10.00)	3.45 (2.53–4.58)	7.50 (6.68–9.43)	<0.001	<0.001
Basophil percentage, %	0.00–1.00	0.15 (0.00–0.30)	0.10 (0.00–0.10)	0.25 (0.10–0.48)	0.064	<0.001
Basophils, $\times 10^9/L$	0.00–0.10	0.01 (0.00–0.02)	0.01 (0.00–0.01)	0.02 (0.01–0.03)	0.273	<0.001
Eosinophil percentage, %	0.50–5.00	0.10 (0.00–0.35)	0.00 (0.00–0.00)	0.80 (0.23–2.45)	<0.001	<0.001
Eosinophils, $\times 10^9/L$	0.02–0.50	0.01 (0.00–0.02)	0.00 (0.00–0.00)	0.06 (0.02–0.15)	<0.001	<0.001
Infection-related biomarkers						
hs-CRP, mg/L	0.00–8.00	7.62 (2.82–16.63)	33.94 (12.32–51.04)	1.55 (0.52–3.76)	0.002	<0.001
Procalcitonin, ng/mL	0.00–0.05	0.05 (0.03–0.07)	0.06 (0.03–0.08)	0.04 (0.03–0.06)	0.299	0.016
ESR, mm/h	0.00–15.00	16.00 (12.00–29.50)	49.00 (28.00–74.00)	13.50 (7.75–16.50)	<0.001	<0.001
Inflammatory cytokines						
IFN- γ , pg/mL	0.00–20.06	5.88 (3.95–9.58)	7.90 (4.21–14.90)	7.56 (3.66–12.56)	0.150	0.377
IL-10, pg/mL	0.00–2.31	3.05 (1.37–3.86)	5.94 (3.84–8.35)	1.78 (1.34–2.42)	0.001	<0.001
IL-2, pg/mL	0.00–4.13	0.95 (0.78–0.95)	0.95 (0.86–1.37)	0.71 (0.71–0.95)	0.080	<0.001
IL-4, pg/mL	0.00–8.37	1.77 (1.40–1.77)	1.77 (1.40–1.77)	1.05 (1.03–1.40)	0.741	<0.001
IL-6, pg/mL	0.00–6.61	17.38 (5.55–53.97)	26.48 (11.48–49.54)	6.39 (3.46–12.63)	0.295	<0.001
TNF- α , pg/mL	0.00–33.27	11.50 (6.55–26.45)	12.96 (12.22–36.80)	6.71 (6.71–12.55)	0.029	<0.001
Immunoglobulins						
Ig A, mg/dL	100.00–420.00	205.00 (181.00–231.00)	220.00 (158.00–281.00)	163.50 (111.50–200.50)	0.306	<0.001
Ig G, mg/dL	860.00–1,740.00	1,177.00 (1,025.00–1,295.00)	1,354.00 (1,040.75–1,864.75)	931.50 (804.00–1,330.50)	0.028	<0.001
Ig M, mg/dL	30.00–220.00	60.00 (40.25–84.00)	70.00 (51.50–107.75)	66.00 (52.00–101.50)	0.285	0.775
Complement proteins						
C3, mg/dL	70.00–140.00	144.00 (126.75–155.00)	130.00 (108.00–151.00)	121.00 (107.25–141.75)	0.079	0.078
C4, mg/dL	10.00–40.00	38.00 (34.25–45.00)	36.50 (28.75–42.00)	22.50 (18.00–32.00)	0.137	<0.001

Data are presented with median (IQR). N is the total number of patients with available data. P values are obtained by *t* test, or Mann-Whitney U test. IQR, interquartile range; WBC, white blood cell. hs-CRP, high sensitivity C-reactive protein. ESR, erythrocyte sedimentation rate. IFN, interferon. IL, interleukin. TNF, tumor necrosis factor. Ig, immunoglobulin. C3, complement 3. C4, complement 4.

hemodynamic status, preventing organs from further damage (13). Previously, Dastan *et al.* (24) reported that abnormal inflammatory cytokines in a patient with COVID-19 were relieved after administering CRRT

therapy. However, research on immune homeostasis and CRRT is still limited. Among the 59 patients with severe COVID-19, 6 patients receiving CRRT were recruited and the dynamic changes in the immune pattern after receiving

Table 2 Immune cell analysis in patients with mild and severe COVID-19

Immune cell subset	Norma range	Mild (N=10)	Severe (N=46)	Convalescence (N=36)	P (M vs. S)	P (S vs. C)
Lymphocytes, / μ L	1,530.00–3,700.00	1,597.50 (1,274.25–1,975.25)	462.00 (301.00–608.00)	1,252.00 (850.50–1,775.25)	<0.001	<0.001
T lymphocyte percentage, %	50.00–87.00	71.14 (65.24–76.78)	53.23 (47.56–62.25)	72.82 (66.98–77.88)	<0.001	<0.001
T lymphocytes, / μ L	955.00–2,860.00	1,200.00 (1,006.75–1,218.00)	247.00 (161.00–305.00)	890.00 (586.00–1,253.00)	<0.001	<0.001
B lymphocyte percentage, %	3.00–19.00	11.94 (10.80–14.54)	19.18 (13.72–25.50)	11.87 (7.61–19.13)	0.004	<0.001
B lymphocytes, / μ L	90.00–560.00	238.50 (180.50–249.75)	78.00 (46.00–144.00)	151.50 (107.25–231.50)	<0.001	<0.001
NK cell percentage, %	3.00–37.00	18.47 (11.03–24.34)	15.64 (10.78–26.54)	11.46 (6.00–15.43)	0.919	0.005
NK cells, / μ L	150.00–1,100.00	244.00 (150.50–335.00)	59.00 (40.00–101.00)	101.00 (72.00–234.00)	<0.001	0.001
Th lymphocyte percentage, %	21.00–51.00	39.70 (35.63–40.69)	27.45 (22.74–33.92)	38.88 (35.17–48.85)	0.001	<0.001
Th lymphocyte, / μ L	550.00–1,440.00	595.50 (450.25–801.75)	115.00 (72.00–155.00)	520.00 (332.50–660.50)	<0.001	<0.001
Ts lymphocyte percentage, %	12.00–47.00	25.65 (22.86–27.26)	21.14 (15.53–28.20)	27.15 (21.87–32.06)	0.150	0.001
Ts lymphocyte, / μ L	320.00–1,250.00	412.00 (309.25–562.50)	99.00 (50.00–138.00)	322.00 (220.00–486.00)	<0.001	<0.001

Data are presented with median (IQR). N is the total number of patients with available data. P values are obtained by *t* test, or Mann-Whitney U test. T lymphocytes (CD3+CD19–). B lymphocytes (CD3–CD19+). NK, natural killer cells (CD3–CD16+ and/or CD56+). Th, helper T lymphocytes (CD3+CD4+). Ts, suppressor T lymphocytes (CD3+CD8+). IQR, interquartile range.

CRRT were examined. The details of the CRRT therapy are provided in [Table S4](#).

As indicated in [Figure 3](#), the number of immune cells, including T lymphocytes, B lymphocytes, helper T cells, suppressor T cells, NK cells, and monocytes slowly increased in 6 patients receiving CRRT from 0 to 3 weeks. Conversely, neutrophils, IL-6, and blood lactic acid displayed a declining trend in the 6 patients after CRRT intervention, indicating control of infection and improvement of hypoxia ([Tables S5,S6](#)).

Discussion

Infection by SARS-CoV-2 causes an immune system imbalance during the progression of pneumonia. Host immune responses, including the innate and adaptive immune responses, play a capital role in the defense against viral infections. The comparative analysis of

patients with mild and severe cases found that dysregulated immune system was a common feature among patients with laboratory-confirmed COVID-19. Therefore, retrospectively analyzed peripheral blood data to examine the dynamical changes in the immune response in patients with COVID-19.

Our results were consistent with those of previous studies (10,16) showing that most patients with severe illness had at least one preexisting chronic disease. These findings implied that elderly patients suffering from chronic conditions were more prone to COVID-19 probably owing to a weaker immunity.

Our analysis of laboratory data revealed that patients with severe COVID-19 presented with increased levels of neutrophils and lymphopenia compared with patients in the mild group. The opposite change in neutrophils and lymphocytes was consistent with the previous findings (1,12,16,17) and indicated a serious imbalance of immune

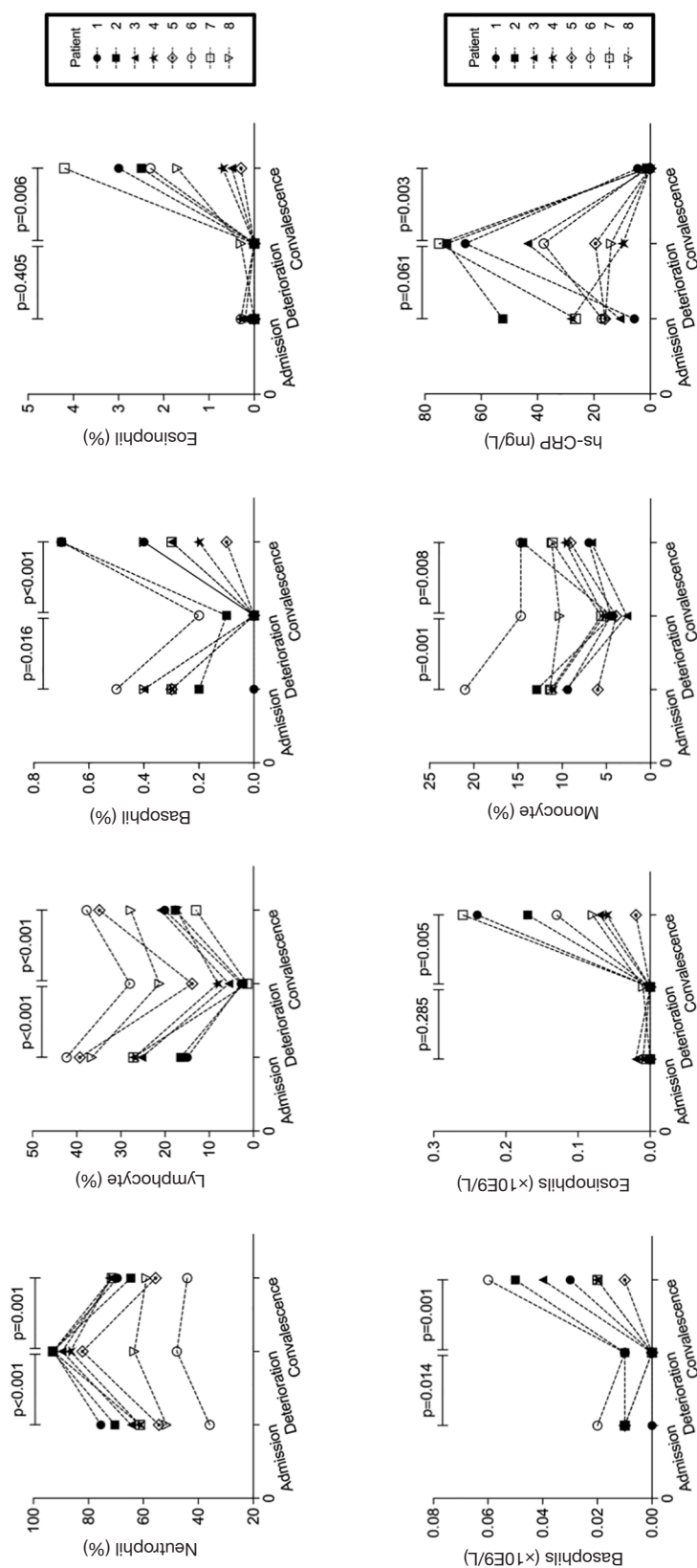


Figure 2 The dynamic changes in immune cells in COVID-19 patients. The percentage of neutrophils, lymphocytes, basophils, eosinophils and hs-CRP was tracked to monitor their changes in 8 patients with COVID-19. The 8 patients progressed through admission, deterioration and convalescence stages during hospitalization. hs-CRP, high sensitivity C-reactive protein.

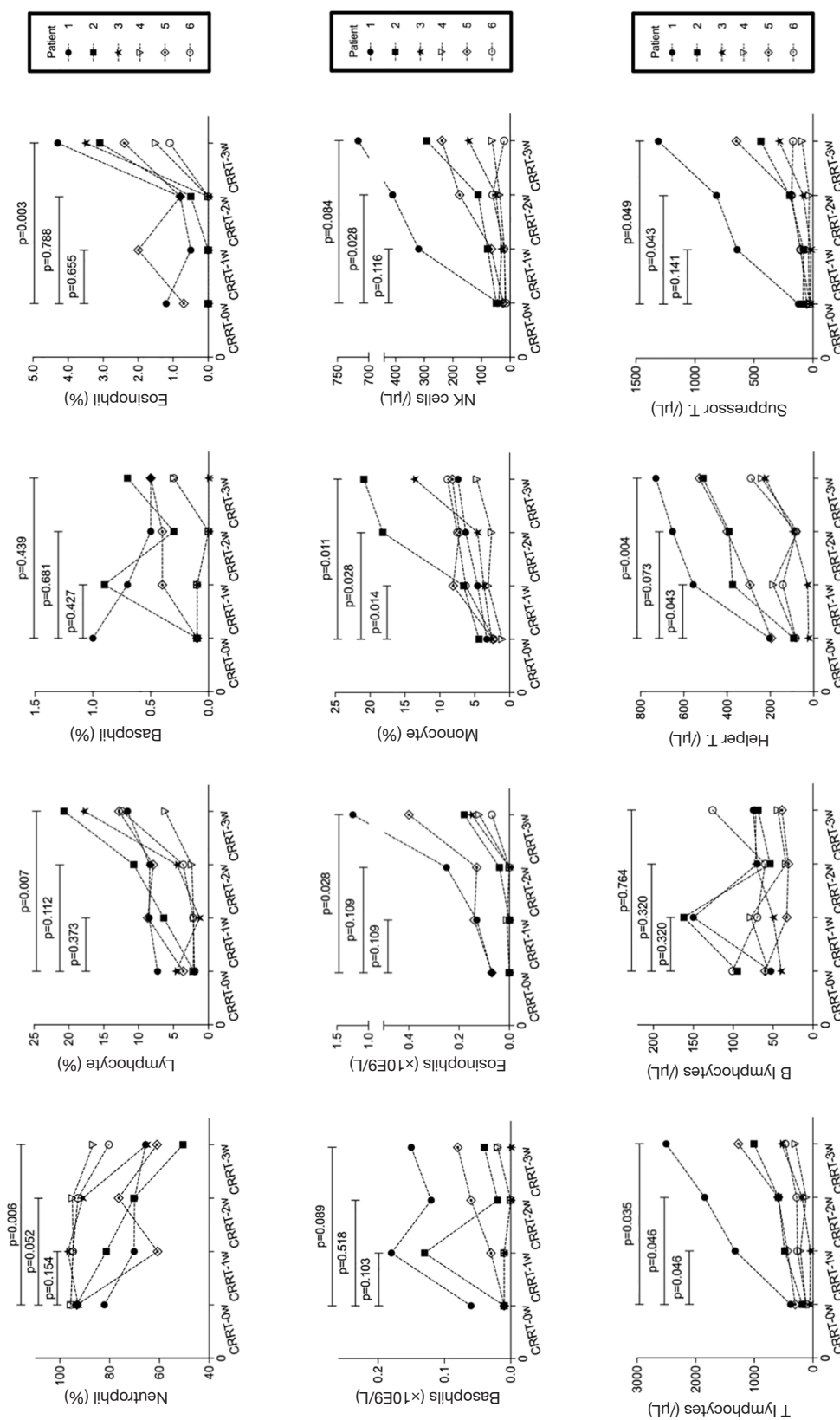


Figure 3 Changes in immune cell subsets after CRRT therapy. Changes in neutrophils, lymphocytes, basophils, eosinophil, monocytes, NK cells, T lymphocytes, B lymphocytes, helper T cells and suppressor T cells were tracked in 6 critically ill patients receiving CRRT therapy from 0 to 3 weeks. CRRT, continuous renal replacement therapy.

homeostasis in these patients, which was improved during convalescence. Moreover, we noticed a comprehensive reduction of all lymphocyte subsets, including CD3+CD4+ T cells, CD3+CD8+ T cells, B cells, NK cells, monocytes, basophils, and eosinophils. Two explanations for this phenomenon have been proposed. First, it is thought that blood immune cells are recruited into the infection site in the lung (15), and second, that those immune cells die due to hyperactivation of apoptosis and the P53 signaling pathway (25). The severe immune disturbance in infected patients could also be accounted for by the fact that although CD4+ or CD8+ T cells are inadequate (or scanty) in patients with COVID-19, these cells are hyperactivated, as proved by high proportions of HLA-DR and CD38 double-positive fractions (15). Monocytes also play a role in immune imbalance. Qin *et al.* (16) reported that regulatory T (Treg) cells were decreased in patients with COVID-19, and it has been suggested that Treg cells may restrain immune response by affecting monocyte-derived DC (MoDCs) (26,27). Moreover, Chen *et al.* (28) confirmed that IL-35-treated MoDCs exhibited potent inhibition of CD4+/CD8+ T cell immune responses. Therefore, a better understanding of the functions of immune cells after SARS-CoV-2 infection is essential.

High levels of a fatal cytokine storm have also been reported in patients with severe COVID-19 compared to patients with the mild form of the disease (16-18). In a cytokine storm, cytokines derived from innate and adaptive immune cells cause abnormalities which may result in tissue injuries and multiple organ failure. In our study, only IL-10 and TNF- α were mainly up-regulated in the severe group. However, we also found that other proinflammatory cytokines, including IL-2, IL-4, IL-6, and Interferon- γ , were slightly elevated. In a previous study, Shi *et al.* (17) reported that IL-2 and IL-6 were sharply enhanced in severe patients 15–20 days after inpatient admission. Therefore, we collected the clinical data where patients were probably in early deterioration. Our analysis showed that besides cytokines, infection-related markers (hs-CRP, D-dimer, and ESR) were also elevated when patients' status deteriorated.

Notable variations in immunoglobulins have also been observed. Currently, detection of SARS-CoV-2-specific IgM and IgG is used to diagnose patients with COVID-19. In our previous study, a higher IgG level was also found in patients with severe disease, consistent with previous research (18,29). As Tirado *et al.* (30,31) reported, virus-specific antibodies can enhance the entry of the

virus into the host cells, and in some cases, also enhance virus replication, through interaction with Fc and/or complement receptors, including monocytes, macrophages, and granulocytic cells, leading to the depletion of immune cells in peripheral blood. Furthermore, the presence of IgG before viral clearance abrogated wound-healing responses and accelerated the recruitment of proinflammatory monocytes/macrophages in pathological tissue (32). The aberrant immunoglobulin response was attenuated during convalescence. In this study, we analyzed the dynamic modifications in immune cell populations in patients with COVID-19 who underwent the admission, deterioration, and convalescent stages and obtained identical results to those of the comparative analysis of patients with mild and severe disease.

Notably, our results showed that D-dimer levels became elevated as the patients continued to deteriorate. Some reports (33,34) have indicated that D-dimer levels were significantly associated with acute kidney injury (AKI) and that D-dimer levels on admission were an independent predictor of contrast-induced acute kidney injury and long-term mortality (35). Therefore, D-dimer levels and the multiple organs that might be involved should be constantly monitored and the necessary preventive treatment measures implemented in time.

In our study, critically ill patients additionally received CRRT, which slowly improved their overall immune state. Pohl *et al.* (36) were the first to report that CRRT therapy could reduce the high circulating macrophage migration inhibitory factor, which amplified the immune response, to reduce mortality due to severe sepsis. Subsequently, several studies showed that CRRT modulated the immune imbalance and improved prognosis in most critically ill patients with septic shock or severe pancreatitis by increasing cellular immunity and restoring monocyte functions (37-41). Moreover, CRRT was also suggested for inclusion in the early stage of bundle therapy for severe pneumonia as an effective way to clear inflammatory mediators, improve immune function, reduce complications and delay the rapid progression of severe pneumonia (42). Currently, extracorporeal membrane oxygenation (ECMO) is used for treating critically ill patients with COVID-19 (43). However, Hu *et al.* (44) showed that ECMO promoted the release of inflammatory cytokines, induced immune disorders, and impaired renal function, and ECMO combined with CRRT treatment could maintain immune balance and alleviate renal injuries *in vivo* studies. Further research to explore the effect of CRRT on the immune

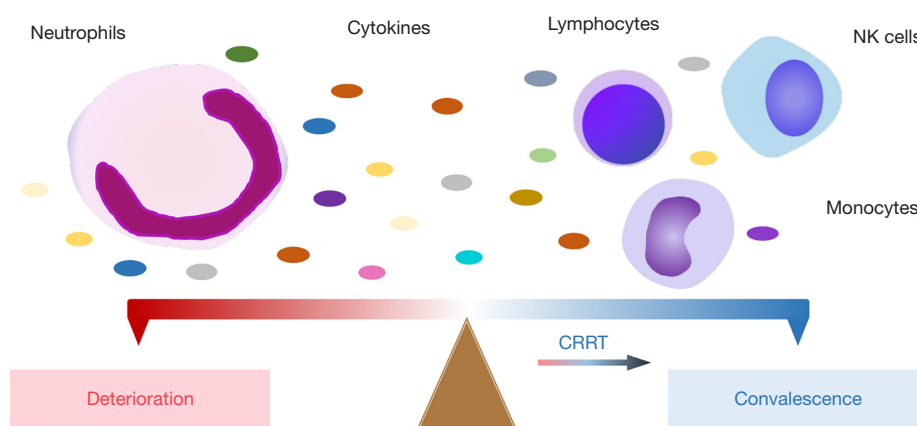


Figure 4 A schematic of the proposed mechanism of disease severity of COVID-19. Patients' conditions deteriorated accompanying an increase in neutrophils and a corresponding decrease in lymphocytes/monocytes/NK cells. Otherwise, patients gradually improved.

system is needed.

We acknowledge some limitations in our retrospective study. Firstly, this study was a single-center, small-sample study of patients with COVID-19; specifically, patients who progressed through admission, deterioration, and convalescence, and received the CRRT therapy. Secondly, repeated infection or secondary infection might affect the findings. Thirdly, the CRRT group lacked a comparable control group. Nevertheless, our findings highlighted that patients with severe COVID-19 were associated with lymphopenia, neutrophilia, abnormalities of cytokines, and immunoglobulin and that CRRT therapy contributed to the restoration of immune homeostasis (Figure 4). The immunopathological mechanism of SARS-CoV-2 in innate and adaptive immune cells warrants further exploration.

Conclusions

Overall, the surveillance of immune cells can be used to monitor COVID-19 disease progression and have direct implications for symptomatic treatment and patient care. Besides, CRRT intervention alleviated the aberrant immune response in critically ill patients, which could be considered as a potential therapeutic strategy. Finally, we believe that our study makes a significant contribution to the literature, chiefly in the context of the rapidly evolving COVID-19 pandemic, and provides a foundational analysis of clinical data to build on by other researchers in the field of immunology.

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Footnote

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Ethics Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted following the Declaration of Helsinki (as revised in 2013). The study was approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, College

of Medicine, Zhejiang University (No. 2020IIT195), and individual consents for this retrospective analysis were waived.

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Table S1 Basic characteristics in patients with mild and severe COVID-19

Clinical features	All patients (N=80)	Mild (N=21)	Severe (N=59)	P (M vs. S)
Characteristics				
Age, median (IQR), yr	57 (48-66)	50 (35-61)	61 (51-70)	0.002
Sex, male/female	49:31	10:11	39:20	0.135
Chronic medical illness				
Any	42 (52.50)	7 (33.33)	35 (59.32)	0.041
Cardiovascular disease	7 (8.75)	1 (4.76)	6 (10.17)	0.762
Cerebrovascular disease	1 (1.25)	0 (0.00)	1 (1.69)	1.000
Chronic kidney disease	1 (1.25)	0 (0.00)	1 (1.69)	1.000
Chronic liver disease	6 (7.50)	2 (9.52)	4 (6.78)	1.000
Chronic obstructive pulmonary disease	4 (5.00)	1 (4.76)	3 (5.08)	1.000
Diabetes	12 (15.00)	1 (4.76)	11 (18.64)	0.240
Hypertension	34 (42.50)	3 (14.29)	31 (52.54)	0.002
Tuberculosis	1 (1.25)	0 (0.00)	1 (1.69)	1.000
Symptoms and signs				
Abdominal pain	1 (1.25)	0 (0.00)	1 (1.69)	1.000
Chest stuffiness	7 (8.75)	1 (4.76)	6 (10.17)	0.762
Chills	1 (1.25)	0 (0.00)	1 (1.69)	1.000
Diarrhea	4 (5.00)	0 (0.00)	4 (6.78)	0.521
Dizziness	2 (2.50)	0 (0.00)	2 (3.39)	1.000
Dry cough	39 (48.75)	8 (38.10)	31 (52.54)	0.255
Dry throat	1 (1.25)	0 (0.00)	1 (1.69)	1.000
Expectoration	8 (10.00)	1 (4.76)	7 (11.86)	0.611
Fatigue	9 (11.25)	1 (4.76)	8 (13.56)	0.488
Fever	70 (87.50)	17 (80.95)	53 (89.83)	0.501
Headache	3 (3.75)	0 (0.00)	3 (5.08)	0.563
Myalgia	10 (12.50)	6 (28.57)	4 (6.78)	0.027
Nausea and vomiting	3 (3.75)	0 (0.00)	3 (5.08)	0.563
Pharyngalgia	1 (1.25)	0 (0.00)	1 (1.69)	1.000
Shortness of breath	12 (15.00)	0 (0.00)	12 (20.34)	0.059

Data are presented with median (IQR), Ratio, n (%), in which N is the total number of patients with available data. IQR, interquartile range. P values comparing mild and severe group are obtained by χ^2 test, Fisher' exact test, or Mann-Whitney U test. yr, year (s).

Table S2 Biochemical findings in patients with mild and severe COVID-19

Biochemical Indicators	Norma Range	Mild (N=21)	Severe (N=59)	Convalescence (N=55)	P (M vs. S)	P (S vs. C)
Blood Lactic Acid, mmol/L	0.50-1.60	1.50 (1.20-1.70)	1.95 (1.60-2.53)	1.80 (1.20-2.40)	0.003	0.070
D-dimer, µg/L FEU	0.00-700.00	262.50 (175.75-596.00)	813.00 (363.00-1549.75)	568.50 (346.00-1222.25)	<0.001	0.213
Urea, mmol/L	3.60-9.50	4.29 (3.35-4.76)	6.12 (4.61-8.40)	5.57 (4.75-7.88)	<0.001	0.635
Uric acid, µmol/L	208.00-428.00	237.50 (195.50-334.50)	220.00 (170.00-282.50)	215.00 (166.00-278.25)	0.412	0.384
Creatinine, µmol/L	57.00-111.00	66.00 (55.00-89.00)	72.00 (64.00-88.00)	70.00 (56.25-77.75)	0.248	0.043
eGFR (EPI-Cr)		94.41 (77.83-102.23)	92.01 (72.32-105.67)	98.16 (91.63-105.39)	0.724	0.055
Globulin, g/L	20.00-40.00	26.25 (23.35-28.45)	28.70 (25.80-33.60)	24.90 (21.45-29.80)	0.010	<0.001
Albumin, g/L	40.00-55.00	44.10 (40.40-46.60)	34.55 (31.80-39.10)	38.10 (33.60-40.85)	<0.001	0.011
ALT, U/L	9.00-50.00	20.00 (13.00-26.00)	21.50 (14.00-37.25)	40.50 (26.25-61.00)	0.342	<0.001
AST, U/L	15.00-40.00	20.00 (18.00-22.00)	24.00 (17.00-36.00)	21.00 (15.00-25.50)	0.043	0.003
Total Bilirubin, µmol/L	0.00-26.00	10.45 (5.58-12.28)	10.75 (7.90-17.70)	9.80 (6.60-12.40)	0.157	0.062
Hemoglobin, g/L	131.00-172.00	135.00 (122.50-147.75)	133.00 (121.00-146.75)	121.00 (104.00-132.50)	0.376	<0.001
Hematocrit, %	38.00-50.80	40.70 (36.33-43.10)	38.60 (34.55-43.10)	36.55 (31.33-40.85)	0.296	0.009
Blood platelet, x10E9/L	83.00-303.00	187.00 (141.50-271.00)	189.50 (156.00-232.75)	171.50 (118.25-224.75)	0.668	0.152
Natrium, mmol/L	137.00-147.00	140.00 (138.00-141.00)	138.00 (136.00-140.00)	140.00 (139.00-142.00)	0.015	0.001
Potassium, mmol/L	3.50-5.30	3.69 (3.53-4.15)	3.90 (3.59-4.07)	3.98 (3.69-4.20)	0.459	0.197
Total Calcium, mmol/L	2.11-2.52	2.17 (2.10-2.28)	2.06 (1.99-2.17)	2.16 (2.06-2.27)	0.001	<0.001

Data are presented with median (IQR). N is the total number of patients with available data. IQR, interquartile range. P values are obtained by t test, or Mann-Whitney U test. ALT, alanine aminotransferase. AST, aspartate aminotransferase.

Table S3 Biochemical findings in dynamic patients with COVID-19

Biochemical Indicators	Norma Range	Hospitalization (N=8)	Deterioration (N=8)	Convalescence (N=8)	P (H vs. D)	P (D vs. C)
Urea, mmol/L	3.60-9.50	4.03 (3.03-5.53)	7.35 (4.41-8.35)	4.78 (4.00-6.23)	0.029	0.094
Uric acid, µmol/L	208.00-428.00	265.50 (235.25-348.00)	224.00 (215.75-235.50)	254.00 (211.25-328.00)	0.104	0.091
Creatinine, µmol/L	57.00-111.00	82.50 (76.00-92.50)	71.50 (62.50-76.75)	67.50 (58.50-69.75)	0.007	0.178
eGFR (EPI-Cr)		95.22 (87.03-102.95)	105.60 (98.71-121.74)	114.19 (99.42-120.92)	0.033	0.574
Globulin, g/L	20.00-40.00	27.30 (26.73-31.28)	38.35 (26.33-41.80)	25.50 (21.45-33.25)	0.093	0.013
Albumin, g/L	40.00-55.00	42.05 (38.60-44.98)	35.30 (31.00-36.95)	39.75 (37.75-41.75)	0.001	0.006
ALT, U/L	9.00-50.00	18.50 (12.00-27.75)	15.50 (11.00-18.25)	48.50 (21.50-71.75)	0.325	0.161
AST, U/L	15.00-40.00	20.50 (16.50-27.75)	16.50 (13.75-19.00)	19.00 (13.25-34.00)	0.310	0.398
Total Bilirubin, umol/L	0.00-26.00	10.90 (9.70-14.28)	12.75 (9.33-22.90)	10.70 (8.75-13.28)	0.272	0.098
Hemoglobin, g/L	131.00-172.00	150.50 (137.50-153.75)	133.00 (120.00-136.00)	128.50 (118.50-135.50)	0.003	0.575
Hematocrit, %	38.00-50.80	43.30 (41.15-44.93)	38.80 (36.38-40.78)	38.15 (36.25-40.03)	0.004	0.662
Blood platelet, x10E9/L	83.00-303.00	151.50 (131.75-184.00)	243.50 (201.75-271.50)	223.50 (173.75-284.75)	<0.001	0.570
Natrium, mmol/L	137.00-147.00	139.50 (138.00-140.75)	136.00 (136.00-138.75)	140.00 (135.50-141.75)	0.120	0.065
Potassium, mmol/L	3.50-5.30	3.74 (3.49-3.90)	4.26 (3.75-4.75)	3.94 (3.77-4.34)	0.070	0.396
Total Calcium, mmol/L	2.11-2.52	2.15 (2.06-2.26)	2.09 (1.92-2.16)	2.20 (2.10-2.28)	0.063	0.026

Data are presented with median (IQR). N is the total number of patients with available data. IQR, interquartile range. P values are obtained by t test, or Wilcoxon test. ALT, alanine aminotransferase. AST, aspartate aminotransferase.

Table S4 The detailed information about CRRT therapy

Patients	Age	Sex	Life support	Comorbidities	Model	Duration (h)	Vascular Access Site	Blood Flow (ml/min)	Anticoagulation	Replacement fluid (ml/h)	Dialysate (ml/h)	Ultrafiltration rate (ml/h)
Patient 1	75	Male	Mechanical Ventilation+ECMO	Hypertension, diabetes, tuberculosis	CVVH	24	Right femoral vein	250	Heparin-free/citrate	3000	0	200
Patient 2	91	Male	Mechanical Ventilation	Hypertension, cardiovascular disease	CVVH	24	Right femoral vein	230	Low molecular weight heparin	2000	0	150-200
Patient 3	62	Male	High-frequency Ventilation	Hypertension, dabetes	CVVH	8-12	Right femoral vein	220	Low molecular weight heparin	3000	0	150-200
Patient 4	82	Female	Mechanical Ventilation+ECMO	Hypertension, cardiovascular disease	CVVH	24	Right femoral vein	230	Citrate	3000	0	150-200
Patient 5	91	Male	Mechanical Ventilation+ECMO	Hypertension	CVVHD	24	Right femoral vein	160	Citrate	1000	1000	150
Patient 6	73	Male	Mechanical Ventilation+ECMO	Hypertension	CVVHDF	12-24	Left femoral vein	150	Heparin-free/citrate	2000	0	150

CVVH, continuous veno-venous hemofiltration. CVVH, continuous veno-venous hemodialysis. CVVHDF, continuous veno-venous hemodiafiltration.

Table S5 Immune findings in patients with COVID-19 who received CRRT therapy

Immune Findings	Norma Range	CRRT_0w (N=6)	CRRT_1w (N=6)	CRRT_2w (N=6)	CRRT_3w (N=6)	P (0 vs. 1w)	P (0 vs. 2w)	P (0 vs. 3w)
Infection-related Biomarkers								
hs-CRP, mg/L	0.00-8.00	100.88 (51.59-155.74)	83.42 (42.81-183.37)	78.51 (26.99-135.28)	70.82 (39.16-166.54)	0.940	0.151	0.529
Procalcitonin, ng/mL	0.00-0.05	0.70 (0.35-4.45)	0.47 (0.22-1.02)	0.39 (0.20-1.75)	1.18 (0.48-1.48)	0.249	0.500	0.917
Inflammatory Cytokines								
IFN-γ, pg/mL	0.00-20.06	11.72 (2.41-29.36)	10.36 (7.10-20.96)	6.77 (3.52-15.76)	9.48 (6.84-33.18)	0.549	0.394	0.811
IL-10, pg/ml	0.00-2.31	8.21 (7.12-11.05)	8.69 (7.12-14.07)	5.49 (1.79-7.60)	6.59 (4.08-12.27)	0.753	0.052	0.463
IL-2, pg/ml	0.00-4.13	0.83 (0.71-1.08)	1.15 (0.71-1.95)	1.05 (0.71-1.67)	1.41 (0.65-1.68)	0.685	0.598	0.194
IL-4, pg/ml	0.00-8.37	1.22 (1.03-1.46)	1.45 (1.03-3.79)	1.44 (1.03-8.74)	0.68 (0.33-1.05)	0.225	0.343	0.026
IL-6, pg/ml	0.00-6.61	384.41 (229.40-812.99)	233.54 (68.59-979.16)	225.03 (56.15-751.05)	218.79 (131.25-516.12)	0.463	0.081	0.146
TNF-α, pg/ml	0.00-33.27	9.44 (6.71-48.58)	56.38 (1.34-96.63)	41.30 (11.09-107.78)	13.95 (1.45-36.21)	0.063	0.173	0.829
Immunoglobulins								
Ig A, mg/dL	100.00-420.00	152.50 (115.75-206.25)	202.50 (158.25-286.50)	234.00 (172.25-378.75)	255.50 (199.25-281.75)	0.083	0.047	0.000
Ig G, mg/dL	860.00-1740.00	1883.50 (1400.50-2536.50)	1837.00 (1439.75-2305.75)	2481.00 (1746.00-3246.50)	2247.00 (1289.00-2909.25)	0.989	0.134	0.531
Ig M, mg/dL	30.00-220.00	35.50 (29.75-60.75)	61.00 (42.50-76.00)	64.50 (52.75-85.50)	63.50 (25.25-74.75)	0.025	0.023	0.223
Complement Proteins								
C3, mg/dL	70.00-140.00	90.00 (58.50-101.75)	107.50 (86.50-119.50)	110.00 (90.25-132.75)	96.00 (77.50-108.00)	0.154	0.056	0.540
C4, mg/dL	10.00-40.00	18.00 (13.75-25.50)	17.00 (12.75-24.25)	16.50 (14.00-25.25)	13.50 (11.25-24.50)	0.438	0.476	0.262

Data are presented with median (IQR). N is the total number of patients with available data. IQR, interquartile range.

P values are obtained by t test, or Wilcoxon test.

hs-CRP, high sensitivity C-reactive protein. IFN, interferon. IL, interleukin. TNF, tumor necrosis factor. Ig, immunoglobulin. C3, complement 3. C4, complement 4.

Data are presented with median (IQR). N is the total number of patients with available data. IQR, interquartile range. P values are obtained by t test, or Wilcoxon test. hs-CRP, high sensitivity C-reactive protein. IFN, interferon. IL, interleukin. TNF, tumor necrosis factor. Ig, immunoglobulin. C3, complement 3. C4, complement 4.

Table S6 Biochemical findings in patients with COVID-19 who received CRRT therapy

Biochemical Indicators	Norma Range	CRRT_0w (N=6)	CRRT_1w (N=6)	CRRT_2w (N=6)	CRRT_3w (N=6)	P (0 vs. 1w)	P (0 vs. 2w)	P (0 vs. 3w)
Blood Lactic Acid, mmol/L	0.50-1.60	3.30 (2.85-4.25)	2.15 (1.83-2.63)	1.60 (1.48-2.33)	1.50 (1.00-1.95)	0.006	0.004	0.001
D-dimer, ug/L FEU	0.00-700.00	7656.50 (2077.00-18670.50)	10680.00 (3124.00-15579.25)	8329.00 (2847.50-16773.75)	5481.50 (3830.75-17970.00)	0.345	0.978	0.778
Urea, mmol/L	3.60-9.50	18.73 (10.33-31.30)	6.47 (4.33-19.33)	9.37 (6.44-16.85)	8.96 (5.50-13.17)	0.006	0.015	0.048
Uric acid, umol/L	208.00-428.00	252.00 (137.25-453.50)	100.00 (58.00-161.50)	80.50 (63.50-135.25)	125.00 (62.75-167.25)	0.018	0.020	0.050
Creatinine, umol/L	57.00-111.00	163.50 (53.00-544.25)	59.50 (47.25-208.75)	77.00 (51.75-187.75)	112.00 (42.50-241.25)	0.028	0.075	0.229
eGFR (EPI-Cr)		31.38 (9.40-102.13)	83.12 (27.86-105.47)	73.80 (32.25-100.88)	52.98 (17.16-101.49)	0.050	0.113	0.678
Globulin, g/L	20.00-40.00	33.60 (28.45-39.28)	31.95 (27.45-39.93)	37.55 (34.33-45.70)	35.70 (31.78-41.55)	0.857	0.039	0.591
Albumin, g/L	40.00-55.00	35.30 (31.50-37.35)	36.80 (33.45-39.18)	36.30 (34.88-40.80)	36.90 (33.43-40.68)	0.456	0.241	0.249
ALT, U/L	9.00-50.00	17.00 (13.50-27.75)	19.00 (14.25-41.75)	17.50 (14.00-31.50)	24.00 (14.75-50.75)	0.525	0.884	0.387
AST, U/L	15.00-40.00	32.00 (25.75-34.25)	32.50 (15.75-97.00)	36.50 (26.00-70.25)	36.50 (29.25-105.25)	0.600	0.132	0.178
Total Bilirubin, umol/L	0.00-26.00	17.70 (5.28-42.38)	36.15 (14.88-61.23)	23.05 (10.58-61.23)	33.25 (12.18-80.88)	0.017	0.338	0.096
Hemoglobin, g/L	131.00-172.00	92.00 (85.50-101.50)	91.50 (83.75-101.00)	88.00 (80.50-91.75)	78.50 (70.75-85.25)	0.704	0.116	0.029
Hematocrit, %	38.00-50.80	27.35 (24.70-31.00)	27.70 (24.58-31.88)	25.70 (23.73-29.18)	22.95 (21.23-26.40)	0.960	0.454	0.051
Blood platelet, x10E9/L	83.00-303.00	90.00 (58.50-120.25)	76.50 (64.25-110.00)	77.50 (65.75-106.50)	80.00 (33.25-133.75)	0.682	0.542	0.680
Natrium, mmol/L	137.00-147.00	140.00 (137.75-149.50)	139.50 (135.00-140.25)	137.50 (133.75-139.25)	138.50 (136.75-139.25)	0.221	0.081	0.224
Potassium, mmol/L	3.50-5.30	4.28 (3.99-4.76)	4.40 (3.93-4.62)	4.28 (4.05-4.43)	4.16 (4.09-4.28)	0.907	0.562	0.493
Total Calcium, mmol/L	2.11-2.52	2.00 (1.84-2.20)	2.15 (2.04-2.32)	2.30 (2.17-2.39)	2.30 (2.20-2.36)	0.069	0.006	0.007

Data are presented with median (IQR). N is the total number of patients with available data. IQR, interquartile range. P values are obtained by t test, or Wilcoxon test. ALT, alanine aminotransferase. AST, aspartate aminotransferase.