Lymphopenia acted as an adverse factor for severity in patients with COVID-19: a single-centered, retrospective study

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Research Article

Keywords: SARS-CoV-2, COVID-19, lymphopenia, outcome

Posted Date: April 14th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-22849/v1

Abstract

Purpose: The outbreak of SARS-CoV-2 began in December and rapidly caused a pandemic. To investigate the significance of lymphopenia for the severity of the disease, this study was performed.

Methods: 115 patients confirmed COVID-19 from a tertiary hospital in Changsha, China were enrolled. The clinical, laboratory, treatment and outcome data were collected and compared between patients with lymphopenia or not.

Results: The median age was 42 years (1-75). 54 patients (47.0%) of the patients had lymphopenia on admission. In the group of lymphopenia, more patients had hypertension (30.8% vs 10.0%, P=0.006) and coronary heart disease (3.6% vs 0%, P=0.029) and more patients with leucopenia (48.1% vs 14.8%, P<0.001) and eosinophilia (92.6% vs 54.1%, P<0.001) were observed. Lymphopenia was also correlated with severity grades of pneumonia (P<0.001) and C-reactive protein (CRP) level (P=0.0014). Lymphopenia was associated with a prolonged duration of hospitalization (17.0 days vs 14.0 days, P=0.002). Moreover, the recovery of lymphocyte appeared the earliest before CRP and chest radiographs in severe cases, suggesting its predictive value for disease improvement.

Conclusion: Our results showed the clinical significance of lymphopenia for predicting the severity of COVID-19 and the recovery of the disease, emphasizing the need to monitor the lymphocyte count dynamically.

Background

Coronaviruses (CoV) are a large family of viruses that cause illness ranging from the common cold to more severe diseases such as Middle East Respiratory Syndrome (MERS-CoV)[1] and Severe Acute Respiratory Syndrome (SARS-CoV).[2] Recently, a novel coronavirus (SARS-CoV–2) has been first detected in Wuhan, China and caused pandemic spread of Coronavirus disease (COVID–19) all over the globe. The control of spreading, early diagnosis of COVID–19 and effective treatment still remains a great challenge.

SARS-CoV–2 is a highly infectious zoonotic coronavirus, which has been reported to be 96% identical at the whole-genome level to a bat coronavirus.[3] Common signs of infection include respiratory symptoms, fever, dry cough, shortness of breath and breathing difficulties. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome, septic shock, multiorgan failure and even death.[4, 5] Although COVID–19 shares much in common with SARS and MERS, its epidemiologic and clinical features has not been fully illustrated.

Among the laboratory abnormalities reported in patients with SARS, lymphopenia is prominent, which is more frequent in those patients with severe disease compared to those with non-severe disease.[6] Similarly, Lymphopenia is also observed in about 60% of patients with SARS-CoV–2 infection at the initial presentation.[4] However, the clinical significance and underlying mechanism are still not clear.

Here, we have retrospectively analyzed the frequency and association of lymphocytopenia and the severity of COVID–19.

Material And Methods

Study design and participants

For this single-centered, retrospective study, a total of 115 patients diagnosed of COVID–19 from The First Hospital of Changsha were included. All patients were admitted from January 17, 2020 to February 14, 2020. Written consent was obtained from all patients and the study was approved by the Ethical Committee of The First Hospital of Changsha. The diagnosis was based on clinical criteria and laboratory features according to the WHO interim guidance.[7] The final date of follow-up was March 13, 2020.

Data Collection

Epidemiological, clinical data were collected from the patients with COVID–19 upon hospitalization. The illness severity was defined according to the Chinese management guideline for COVID–19 (version 7.0).[8] Mild grade was defined as patients who had mild clinical manifestations and the imaging tests identified no sign of pneumonia. Severe grade was defined as cases who met any of the following: a. respiratory rate exceeded 30 times per minute; b. blood oxygen saturation less than 93%; c. oxygenation index (PaO_2/FiO_2 [pressure of oxygen in arterial blood/fraction of inspire oxygen]) less than 300. Any patients who needed mechanic ventilation because of respiratory failure, who presented as shock or who were monitored in intensive care unit (ICU) because of multiple organ failure were critical cases. The other cases were classified of general grade. Blood counts, blood biochemistry, chest radiographs and computed tomographic (CT) scans were performed on initial days after admission. Therapeutic measures and outcomes data were collected from the electronic medical network of The First Hospital of Changsha. All of the information was reviewed and checked by two independent doctors to ensure the accuracy.

Laboratory procedures

As previously reported,[4] throat-swap specimens were taken from upper respiratory tract and the confirmation experiments for SARS-CoV2 were performed by real-time RT-PCR following the recommendation of China National Center for Disease Control. A cycle threshold value (Ct value) less than 37 was defined as a positive test, and a Ct value over 40 was defined as a negative record.

Statistical analyses

Continous variables were presented as mean (SD) if they are normally distributed or median (IQR) if they are not. Categorical variables were described as count (%). Statistical analyses were performed using the Pearson χ2 test, Fischer's exact test, and Mann–Whitney *U*-test. Kaplan–Meier methods and log-rank tests were used to compare the time to recover (TTR) in patients with lymphocytopenia or not. TTR was defined as the length of time from admission to the date when lung image showed signs of improvement. Analyses were conducted using the SPSS statistical package (SPSS, Chicago, IL, USA) and GraphPad Prism (GraphPad Software, San Diego, CA, USA). P-values of <0.05 were considered significant.

Results

Clinical characteristics of patients

The clinical characteristics were summarized in Table 1 and the patients were divided into two groups depending on whether they had lymphopenia (lymphocyte count <1.0× 10⁹/L) or not. 54 patients (47.0%) of the patients enrolled had lymphopenia on admission to the hospital. The median age of all the patients was 42 years (IQR 1–75). Four patients (3.5%) were under 14 years old, and three of them had no pneumonia. In patients with lymphopenia on admission, the median age was 49 years (IQR 21–75), which was significantly older than patients with no lymphopenia. Most patients (73/93 [78.5%]) had specific exposure history, which was either a previous travel to Wuhan or an exposure to a patient diagnosed of COVID–19. Less than half (38/112 [33.9%]) had chronic complications, including hypertension (22/112 [19.6%]), diabetes (9/112 [8.0%]), cardiovascular disease (4/112 [3.6%]) and hepatitis B (6/112 [5.4%]). Of note, there are more patients with hypertension (P = 0.006) and coronary heart disease (P = 0.029) in the group of lymphopenia than patients with no lymphopenia. On the contrary, all six patients with chronic hepatitis B had no lymphopenia on admission (P = 0.019).

Table 1 Clinical and laboratory characteristics of patients with SARS-CoV2 infection.

	Total (N=115)	Patients with lymphopenia (n=54)	Patients with no lymphopenia (n=61)	*P* value
General characteristics and clinical manifestation				
Sex				0.322
Malea	61 (53.0%)	26 (48.1%)	35 (57.4%)	
Female	54 (47.0%)	28 (51.9%)	26 (42.6%)	
Age, years (range)	42 (1-75)	49 (21-75)	40 (1-72)	0.001
Exposure history	73/93 (78.5%)	23/37 (62.2%)	50/56 (89.3 %)	0.002
Comorbidity	38/112 (33.9%)	21/52 (40.4%)	17/60 (28.3%)	0.179
Hypertension	22/112 (19.6%)	16/52 (30.8%)	6/60 (10.0%)	0.006
Diabetes	9/112 (8.0%)	5/52 (9.6%)	4/60 (6.7%)	0.567
Coronary heart disease	4/112 (3.6%)	4/52 (7.7%)	0/60 (0%)	0.029
COPD	4/112 (3.6%)	3/52 (5.8%)	1/60 (1.7%)	0.243
Hepatitis B	6/112 (5.4%)	0/52 (0%)	6/60 (10.0%)	0.019
Others	8/112 (7.1%)	4/52 (7.7%)	4/60 (6.7%)	0.834
Onset symptoms				
Fever	73/97 (75.3%)	34/37 (91.9%)	39/60 (65.0%)	0.003
Cough	60/97 (61.9%)	26/37 (70.3%)	34/60 (56.7%)	0.18
chill	10/97 (10.3%)	4/37 (10.8%)	6/60 (10.0%)	0.898
Fatigue	44/97 (45.4%)	21/37 (56.8%)	23/60 (38.3%)	0.077
Muscle soreness	10/97 (10.3%)	5/37 (13.5%)	5/60 (8.3%)	0.415
Nausea or vomiting	4/97 (4.1%)	2/37 (5.4%)	2/60 (3.3%)	0.618
Diarrhea	5/97 (5.2%)	3/37 (8.1%)	2/60 (3.3%)	0.302
Time from illness onset to hospital admission, days	5.5 (1-40)	5.0 (1-25)	6.0 (1-40)	0.05
Laboratory test and imaging data				
WBC count, × 10⁹/L	4.87 (1.75-17.11)	4.08 (1.75-14.71)	5.72 (2.63-17.11)	0.008
<4.0	35 (30.4%)	26 (48.1%)	9 (14.8%)	<0.001
Lymphocyte count, × 10⁹/L	1.06 (0.17-9.54)	0.76 (0.17-1.82)	1.51 (1.02-9.54)	<0.001
Eosinophil count, × 10⁹/L	0.02 (0.00-0.42)	0.00 (0.00-0.42)	0.05 (0.00-0.35)	0.002
<0.02	83 (72.2%)	50 (92.6%)	33 (54.1%)	<0.001
Hemoglobin, g/L	129 (77-174)	126 (77-168)	133 (89-174)	0.071
Anemia	17 (14.8%)	11 (20.4%)	6 (9.8%)	0.112
Platelet count, × 10⁹/L	181 (35-685)	163 (78-334)	217 (35-685)	0.015
<100				
ALT, U/L	19.70 (5.40-79.37)	20.02 (10.88-55.30)	19.70 (5.40-79.37)	0.795
>40	11/105 (10.5%)	4/44 (9.1%)	7/61 (11.5%)	0.694
Globulin, g/L	25.32 (18.20-34.67)	25.46 (20.38-32.9)	25.20 (18.20-34.67)	0.792
Albumin, g/L	37.86 (27.91-47.35)	36.13 (28.12-44.52)	38.87 (27.91-47.35)	0.001
Creatinine, umol/L	48.29 (5.17-255.71)	48.46 (20.58-255.71)	48.29 (5.17-229.8)	0.773
LDH, U/L	161.5 (88.2-463.8)	187.7 (88.2-463.8)	152.4 (107.1-379.7)	0.022
C-reactive protein	13.07 (0.1-101.94)	24.53 (0.7-91.6)	8.92 (0.1-101.94)	0.005
ESR	35.5 (3.0-552.0)	43.5 (0.3-552.0)	28.5 (4.0-115.0)	0.044
Bilateral lung involvement in lung CT scan	64/92 (69.6%)	26/33 (78.8%)	38/59 (64.4%)	0.15
Disease severity status				<0.001

Mild	13 (11.3%)	2 (3.7%)	11 (18.0%)	
General	74 (64.3%)	30 (55.6%)	44 (72.1%)	
Severe	22 (19.1%)	17 (31.5%)	5 (8.2%)	
Critical	6 (5.2%)	5 (9.3%)	1 (1.6%)	
Treatment				
Antiviral treatment				
Lopinavir and ritonavir	78/95 (82.1%)	34/37 (91.9%)	44/58 (75.9%)	0.047
Interferon beta-2b	37/95 (38.9%)	14/37 (37.8%)	23/58 (75.9%)	0.859
Recombinant human cytokine derived protein	48/95 (38.9%)	21/37 (56.8%)	27/58 (46.6%)	0.332
Antibacterial treatment	48/94 (51.1%)	27/36 (75.0%)	21/58 (36.2%)	<0.001
Systemic corticosteroid treatment	33/94 (35.1%)	22/36 (61.1%)	11/58 (19.0%)	<0.001
Human γ-immunoglobulin	32/94 (34.0%)	19/36 (52.8%)	13/58 (22.4%)	0.003
Respiratory support				0.013
Nasal cannula	67/91 (73.6%)	22/35 (62.9%)	45/56 (80.4%)	
High-flow nasal cannula	17/91 (18.7%)	9/35 (25.7%)	8/56 (14.3%)	
Non-invasive ventilation	1/91 (1.1%)	1/35 (2.9%)	0/56 (0%)	
Invasive ventilation	3/91 (3.3%)	3/35 (8.6%)	0/56 (0%)	
Prognosis				
Improved and discharged	113(98.3%)	52 (96.3%)	61 (100%)	0.317
Inpatient treatment	1 (0.9%)	1 (1.9%)	0 (0%)	
Death	1 (0.9%)	1 (1.9%)	0 (0%)	

Data are median (IQR), n (%), or n/N (%), where N is the total number of patients with available data. P values comparing patients with lymphopenia or not are from χ^2 test, Fisher's exact test, or Mann-Whitney U test. COPD=chronic obstructive pulmonary disease; WBC=white blood cell; ALT=alanine aminotransferase; LDH=lactate dehydrogenase; ESR=erythrocyte sedimentation rate.

The most common onset symptoms were fever (75.3%), dry cough (61.9%), fatigue (45.4%), chill (10.3%) and muscle soreness (10.3%). In the group of lymphpenia, more patients had fever as initial symptom (91.9% vs 65.0%, P = 0.003). The median time from the appearance of symptoms to admission was 5.5 days (IQR 1–40).

More leucopenia (white blood cell count <4.0× 10⁹/L), eosinophilia (eosinophil count <0.02× 10⁹/L) and thrombocytopenia (platelet count <100× 10⁹/L) were observed in patients with lymphopenia (P = 0.008, 0.002 and 0.015, respectively) than patients with normal lymphocyte aount. The median albumin level was 36.13 g/L (IQR [28.12–44.52]) in patients with lymphopenia, which was significantly less than that in patients with no lymphopenia (P = 0.001). More patients in the group of lymphopenia had elevated lactate dehydrogenase (LDH) level (187.7 U/L [IQR 88.2–463.8] vs 152.4 U/L [IQR 107.1–379.7], P = 0.002). Inflammatory indicators were elevated, such as c-reactive protein (CRP, median 13.07 mg/L [IQR 88.2–463.8]) and erythrocyte sedimentation rate (median 35.5 mm/h [IQR 3.0–552.0]). However, in patients with lymphopenia, both indicators were even higher (P = 0.005 and 0.044, respectively).

The association of lymphocytopenia with the severity of pneumonia

According to the Chinese management guideline for COVID–19, the severity of this disease was classified as four grades: mild, general, severe and critical. Among 115 cases of COVID–19, most patients were classified as general cases (74 [64.3%], Table 1). Mild cases, i.e. patients who had no evidence of pneumonia, were only 13 patients (11.3%). And the median age of this category was 29 years (IQR 8–67), which was significantly younger than other categories (P<0.001). In patients with lymphopenia, 22 patients (40.8%) were in severe or critical category, which was significantly more than patients with no lymphopenia (9.8%, P<0.001). Meanwhile, the more severe the illness was, the higher the incidence of lymphopenia was. As shown in Figure 1A, the median lymphocyte count of mild cases was 2.45 (0.98–3.81) × 10⁹/L, which was significantly higher than general cases (1.12 [0.43–9.54] × 10⁹/L, P<0.0001), severe cases (0.77 [0.37–2.26] × 10⁹/L, P<0.0001) and critical cases (0.74 [0.17–1.56] × 10⁹/L, P = 0.0018). We further compared the association of eosinophil count and the severity. As shown in Figure 1B, the median eosinophil count of mild cases was 0.09 (0.03–0.3) × 10⁹/L, which was significantly higher than general cases (0.01 [0.00–0.35] × 10⁹/L, P = 0.0003), severe cases (0.01 [0.00–0.42] × 10⁹/L, P = 0.0006) and critical cases (0.00 [0.00–0.09] × 10⁹/L, P = 0.0375).

Lymphopenia was highly correlated with laboratory manifestations in SARS-CoV–2 infected patients (Figure 1), specifically, lymphocyte count was inversely related to CRP level (Figure 2A, P = 0.0014) and neutrophil count (Figure 2D, P<0.0001) and positively associated with serum albumin level (Figure 1B, P<0.0001). But there was no significant correlation between lymphocyte count and Ct value (Figure 2C).

As levels of CRP and LDH have been used in the management of infection, we next analyzed the value of lymphopenia for assessing the severity of COVID–19 in comparison with these inflammatory markers. The receiver-operator curve (ROC) plots was used to express the prognostic value of illness severity of these parameters in terms of grades of pneumonia, bilateral lung involvement in lung CT scan and presence of abnormal lung image on discharge (see Figure 3). For prediction of severe or critical disease, the area under the curve (AUC) of lymphopenia was 0.854; in comparison, with CRP, it was 0.870 and with LDH, it was 0.810 (Figure 3A). For prediction of bilateral lung involvement, the AUC of lymphopenia was 0.714; in comparison, with CRP and LDH, it was 0.782 and 0.672 respectively (Figure 3B). For prediction of abnormal lung image on discharge, the AUC of lymphopenia was 0.792, in comparison, with CRP and LDH, it was 0.856 and 0.782 respectively (Figure 3C).

Risk factors for prolonged time of hospitalization

Furthermore, risk factors for the duration time of hospitalization were analyzed. As shown in Figure 4, severity grades of COVID–19, the presentation of respiratory failure, the requirement of ICU care and lymphopenia are indicators for prolonged time of hospitalization. The median duration of hospitalization in patients of critical grade was 29.0 days (95% confidence interval [CI] 26.581–31.260), which was

significantly longer than patients of other grades (severe grade: median 20.0 days [95% CI 17.188–22.812]; general grade: median 12.0 days [95% CI 9.477–14.523]; mild grade: median 13.0 days [95% CI 11.281–14.719]; P<0.001; see Figure 4A). Patients presented with respiratory failure had a significantly longer hospitalization time of 29.0 days (95% CI 20.320–37.680) compared with patients with no respiratory failure (median 13.0 days [95% CI 11.160–14.480], P<0.001; see Figure 4B). Apparently, ICU care was also associated with significantly longer duration of hospitalization (ICU care: median 25.0 days [95% CI 16.086–33.914]; no ICU care: median 13.0 days [95% CI 11.170–14.830]; P<0.001; see Figure 4C). And lymphopenia had an adverse impact on duration of hospitalization (patients with lymphopenia: median 17.0 days [95% CI 13.007–20.993]; no lymphopenia: median 14.0 days [95% CI 12.093–15.907]; P = 0.002; see Figure 4D). However, eosinophilia, elevated CRP and comorbidity had no impact on the duration of hospitalization (Figure S1).

We further compared the recovery time of lymphocyte, eosinophil, CRP level and chest radiograph before clearance of SARS-CoV2 RNA. Since many patients still presented abnormal lung image when they were discharged, the recovery time of chest radiograph was defined as the length of time from admission to the date when lung image showed signs of improvement. Of note, Among 71 patients presented with an elevated CRP level, only 45 of them (63.4%) had a normalized CRP before discharge. And among 80 patients whose chest radiographs showed lung lesion, only 6 patients' CT scan were normal on discharge. There were still 6 patients (7.5%) showed no sign of improvement of CT scan before discharge, despite recovery of symptoms and clearance of SARS-CoV2 RNA. Compare with CT scan (median recovery time 12.5 days [4.0–32.0]) and CRP (median recovery time 12.0 days [4.0–23.0]), the recovery of lymphocyte was faster (median recovery time 9.0 days [3.0–23.0], see Figure 5) in severe and critical cases, which indicated that the normalizing of lymphocyte count predicted disease improvement. In mild and general cases, the normalizing of CRP was the most sensitive indicator for disease improvement.

Treatment and outcome

Of the 115 patients, 95 patients' treatment records were available, which were summarized in Table 1. As for respiratory support, more patients in the group of lymphopenia acquired higher levels of supports, such as high-flow nasal cannula (25.7% vs 14.3%), non-invasive ventilation (2.9% vs 0%) and invasive ventilation (8.6% vs 0%, P = 0.013). All 95 patients received antiviral treatment. The most common used drugs were lopinavir and ritonavir (82.1%), which was more frequently used in patients with lymphopenia (91.9% vs 75.9%, P = 0.047). Antibacterial treatment was used among 48 (51.1%) patients. There were more patients received antibiotics in lymphopenia group than patients with no lymphopenia (75.0% vs 36.2%, P<0.001). In 61.1% of the patients with lymphopenia, systemic corticosteroid was used, which was significantly more than the proportion in patients with no lymphopenia (19.0%, P<0.001). And human γ-immunoglobulin(IVIG) was used in about half of the patients with lymphopenia (52.8%), which was also significantly more than that in patients with no lymphopenia (22.4%, P<0.001).

To investigate whether patients would benefit from the treatment of IVIG or corticosteroid, Kaplan-Meier curves were conducted according to the therapeutic choice and lymphocyte count (Figure 6). In the group of patients with lymphopenia, the median TTR of patients treated with IVIG was 11.0 days (95% CI 7.801–14.199), which was even significantly longer than patients without IVIG treatment (median 7.0 days [95% CI 5.533–8.467], P = 0.001, Figure 6A). So we compared the patients composition and found out that there were more severe or critical cases in IVIG group than that in patients without IVIG treatment (73.7% vs 0%, P<0.001). But in patients without lymphopenia, IVIG treatment had no benefit for the disease (median TTR 7.0 days [95% CI 6.223–7.777] vs 8.0 days [95% CI 5.737–10.263], P = 0.190, Figure 6B). Among all patients who received IVIG treatment, lymphopenia still acted as an adverse factor on TTR (median TTR 8.0 days [95% CI 5.737–10.263] vs 11.0 days [95% CI 7.801–14.199], P = 0.049, Figure 6C). Likewise, no significant benefit from the treatment of corticosteroid was observed, no matter in the group of lymphopenia (Figure 6D) or in patients without lymphopenia (Figure 6E). However, among all patients who received corticosteroid treatment, there was no difference of TTR between patients with and without lymphopenia (median TTR 10.0 days [95% CI 7.711–12.289] vs 9.0 days [95% CI 6.842–11.158], P = 0.201, Figure 6F).

Discussion

This study retrospectively analyzed the characteristics of patients infected with SARS-CoV–2 and identified that lymphopenia acted as a good predictor for severity of COVID–19. In particular, more patients had hypertension and coronary heart disease in the group of lymphpenia. Lymphopenia was associated with inflammatory markers, grades of pneumonia severity and prolonged hospitalization. Additionally, normalization of lymphocyte count indicated the recovery of COVID–19. Especially in severe and critical cases, lymphocyte count normalizing was the first appeared predictor for disease improvement. In mild and general cases, the normalizing of CRP was the most sensitive indicator for disease improvement.

Unlike the previous report that SARS-CoV–2 may infect more men than women,[5] the sex ratio was balanced in the present study. The patients with chronic underlying disease were about one third and the severe or critical cases accounted for about one fourth, which were less than other cohorts reported in Wuhan.[5, 4, 9] The differences could be attributed to the early insufficiency of medical resources in Wuhan, which gave priority of admission to those patients who were weak and had comorbidities. Interestingly, in patients with lymphopenia, there were more patients having hypertension and coronary heart disease (Table 1). On the contrary, more cases with hepatitis B were observed in the group of no lymphopenia.

Angiotensin-converting enzyme 2 (ACE2), the receptor for SARS-CoV[10] and SARS-CoV2,[11] is a surface molecule localized on arterial and venous endothelial cells, arterial smooth muscle cells and respiratory tract,[12] which is a homologue of ACE. ACE and ACE2 play different roles in the rennin-angiotensin system (RAS). ACE generates angiotensin II, the increase of which was reported to be correlated to pathogenesis of heart failure and hypertension,[13] whereas ACE2 negatively regulates the level of

angiotensin II.[14] ACE inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) decrease the level of ACE and increase the level of ACE2,[15] which could increase the risk of SARS-CoV–2 infection. We infer that the imbalance of ACE/ACE2 axis contributed to the severity of disease, which could be the reason why more patients had hypertension and coronary heart disease were in the group of lymphopenia, since ACEIs and ARBs are commonly used drugs under these circumstances.

The characteristics and pathogenesis of COVID–19 are both similar and different to the prior severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). The major clinical symptoms resulting from these coronavirus-infected diseases include fever, cough, fatigue, myalgia and gastrointestinal symptoms.[4, 16, 2] However, the clinical manifestation of COVID–19 could be more asymptomatic. Of this cohort of patients, about 25% had no fever on admission (Table 1), which implies that it is more challenging to identify COVID–19 patients and control the pandemic over the globe.

Risk factors for adverse outcomes in patients with SARS and MERS have been investigated in previous studies.[17, 1, 16, 18, 19] Specifically, older age, comorbidity, low serum albumin, and concomitant infectious were identified as factors associated with poor outcomes. Lymphopenia was common in both SARS and MERS patients and has also been reported as an important predictor for severe disease in SARS[20] and MERS[21]. In the current study, about half of the patients presented with lymphopenia on admission, which was comparable with the frequency in SARS as reported,[20] but it was lower than the frequency of lymphopenia reported in COVID–19 patients in Hubei Province.[22] Our results revealed that lymphopenia was associated with disease severity, which is consistent with the study by Liu Y, et al[23] that lymphopenia was positively correlated with the severity of acute lung injury in patients with COVID–19. As shown in Figure 5, the recovery of lymphocyte count was the first sign to show up in severe and critical cases before they improved and discharged, suggesting that normalizing of lymphocyte was a more sensitive indicator than CRP or CT scan for prediction of disease recovery in patients of severe and critical grades.

Lymphopenia was reported in varies types of virus-infected diseases, such as SARS[20, 6, 24], MERS[18, 21] and respiratory syncytial virus[25]. As for the underlying mechanism of lymphopenia, previous study reported that lymphopenia in SARS may be caused by enhanced vascular sequestration associated with increased soluble vascular cell adhesion molecule–1 levels,[26] but it remains unclear in patients with COVID–19. It has been well known that the treatment of glucocorticoid results in lymphopenia by causing the migration of lymphocytes from the peripheral blood.[27] Meanwhile, viral infections would inevitably lead to the activation of hypothalamic-pituitary-adrenal axis under stress, resulting the up-regulation of endogenous corticosteroids[28], which might involve in the immunopathogenesis of lymphopenia of COVID–19.

Eosinophils only count for 0.4%–8% of leucocytes in the peripheral blood, but act as rather important inflammatory mediators and involve in innate immunity, allergies, parasitic infection and virus infection.[29] In the current study, we found that the frequency of eosinophilia (72.2%) was higher than lymphopenia. Eosinophilia was associated with lymphopenia and its recovery also acted as an indicator

for the improvement of the disease. The median recovery time of eosinophils was shorter than CRP and CT scan in severe and critical cases. Thrombocytopenia was rarely observed in our study and had no significant impact on the outcome.

Currently, no specific antiviral treatment is available for SARS, MERS and COVID–19. A range of treatments including lopinavir/ritonavir, interferon-β2b and recombinant human cytokine derived protein was used in this cohort of patients, but no improvement for outcome by any of them was observed. As a previous therapeutic measure for SARS, IVIG has not been confirmed effective for outcome improvement. [30] In our study, no benefit from the approach of IVIG was observed, no matter patients were with lymphopenia or not. Given the high cost of IVIG and economic burden for public health systems, more investigations of this approach are needed to provide evidence for a large-scale use.

Our study has some limitations. For example, some of the laboratory examination records and treatment records were not available since this is a retrospective study. In condition, an examination of lymphocyte subsets is unavailable in most of the patients. So there was still no evidence for which subset contributed the profound lymphopenia in COVID–19 patients. On this basis, a more comprehensive and thorough investigation is necessary in the future.

In summary, by analyzing the clinical data of 115 patients with COVID–19, our results showed that lymphopenia was common and correlated with the severity of COVID–19. To the best of our knowledge, this is the first retrospective study revealing the significance of the normalizing of lymphocyte count for predicting disease improvement, putting emphasis on the need to monitor the blood cell count dynamically in the management of COVID–19.

Declarations

Funding: This research was supported in part by National Natural Science Foundation of China (No. 81800198 to LH; No. 81670160 to PH), the Innovative Major Emergency Project Funding against the Novel Coronavirus Pneumonia in Hunan Province (No. 2020SK3014 to Jiyang Liu), and Scientific Research Project of Hunan Provincial Health Commission (No. 20200216 to Jiheng Liu).

Competing interests: The authors declare no competing interests.

Ethics approval and consent to participate: Written consent was obtained from all patients and the study was approved by the Ethical Committee of The First Hospital of Changsha.

Consent for publication: Not applicable.

Availability of data and material: Not applicable.

Code availability: Not applicable.

Authors' contributions: Conceptualization, Zhiguo Zhou and Hongling Peng; Data curation, Jiheng Liu, Ming Luo, Ruijuan Li, Yan Zhou, Dixuan Jiang and Xin Tan; Formal analysis, Heng Li and Lingzhen Wu; Funding acquisition, Heng Li and Jiyang Liu; Methodology, Ming Luo and Zhihua Wang; Project administration, Hongling Peng; Resources, Jiheng Liu, Lingzhen Wu, Yan Zhou, Dixuan Jiang and Xin Tan; Software, Ruijuan Li; Supervision, Jiyang Liu and Guangsen Zhang; Validation, Jiheng Liu; Writing – original draft, Heng Li; Writing – review & editing, Xianfeng Lin, Zhihua Wang, Haiying Zhong and Wenli Zheng.

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Supplemental Figure

Figure S1. Kaplan-Meier curves for duration of hospitalization in patients within different categories. (A) Kaplan-Meier curves for duration of hospitalization according to eosinophil count; The blue line indicates patients with eosinophil count≥0.02× 10⁹/L; The green line indicates patients with eosinophil count<0.02× 10⁹/L; P = 0.793; (B) Kaplan-Meier curves for duration of hospitalization according to the level of CRP; The blue line indicates patients with normal CRP level; The green line indicates patients with elevated CRP; P = 0.094; (C) Kaplan-Meier curves for duration of hospitalization according to comorbidity; The blue line indicates patients with no comorbidity; The green line indicates patients with comorbidities; P = 0.782. CRP, C-reactive protein.

Figures

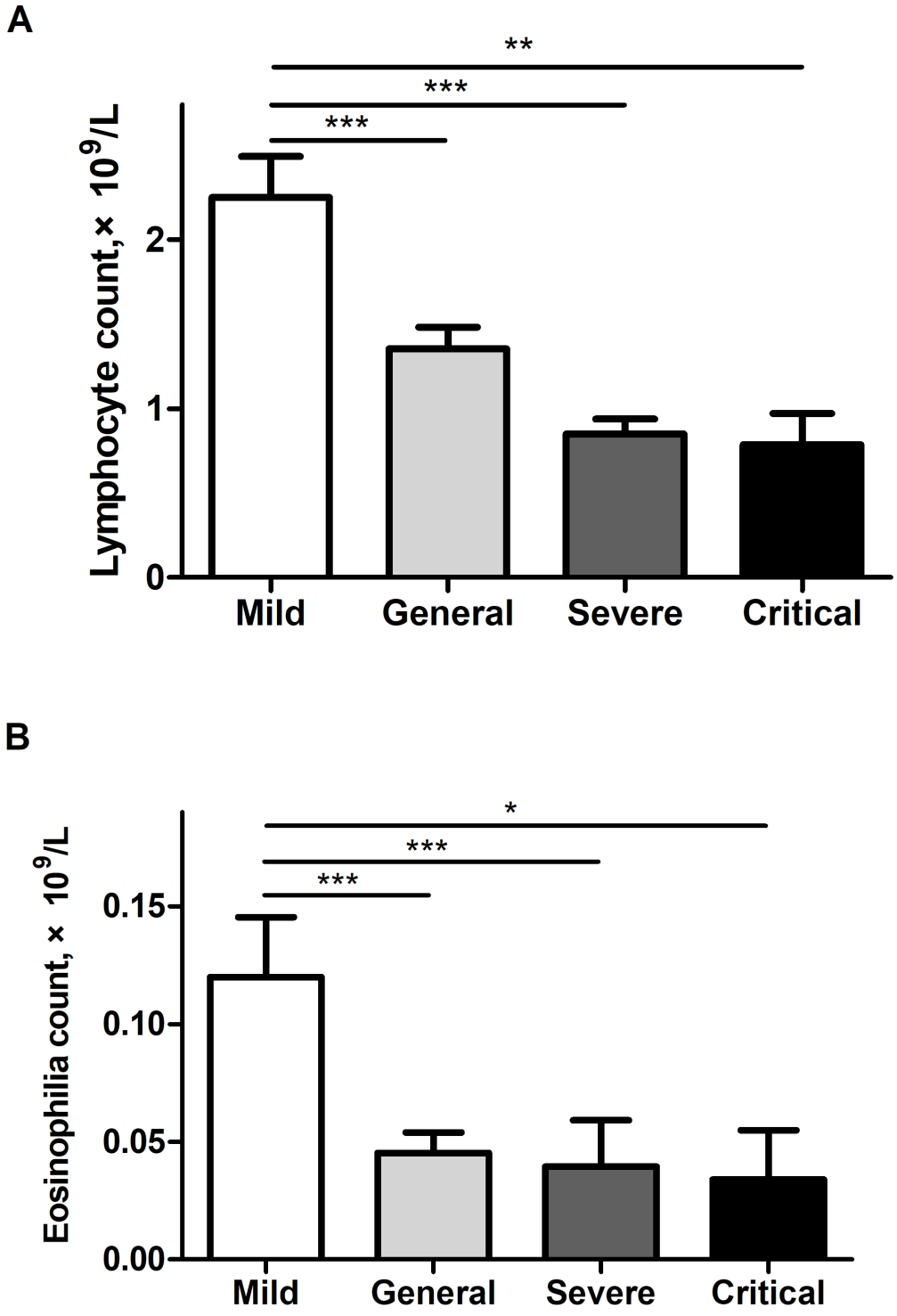


Figure 1

Lymphocyte count and eosinophil count in different groups of illness severity of COVID-19. (A) lymphocyte count in severe and critical cases were significantly lower than that in mild cases. (B) eosinophil count in severe and critical cases were significantly lower than that in mild cases. ***, P<0.001; **, P<0.01; *, P<0.05.

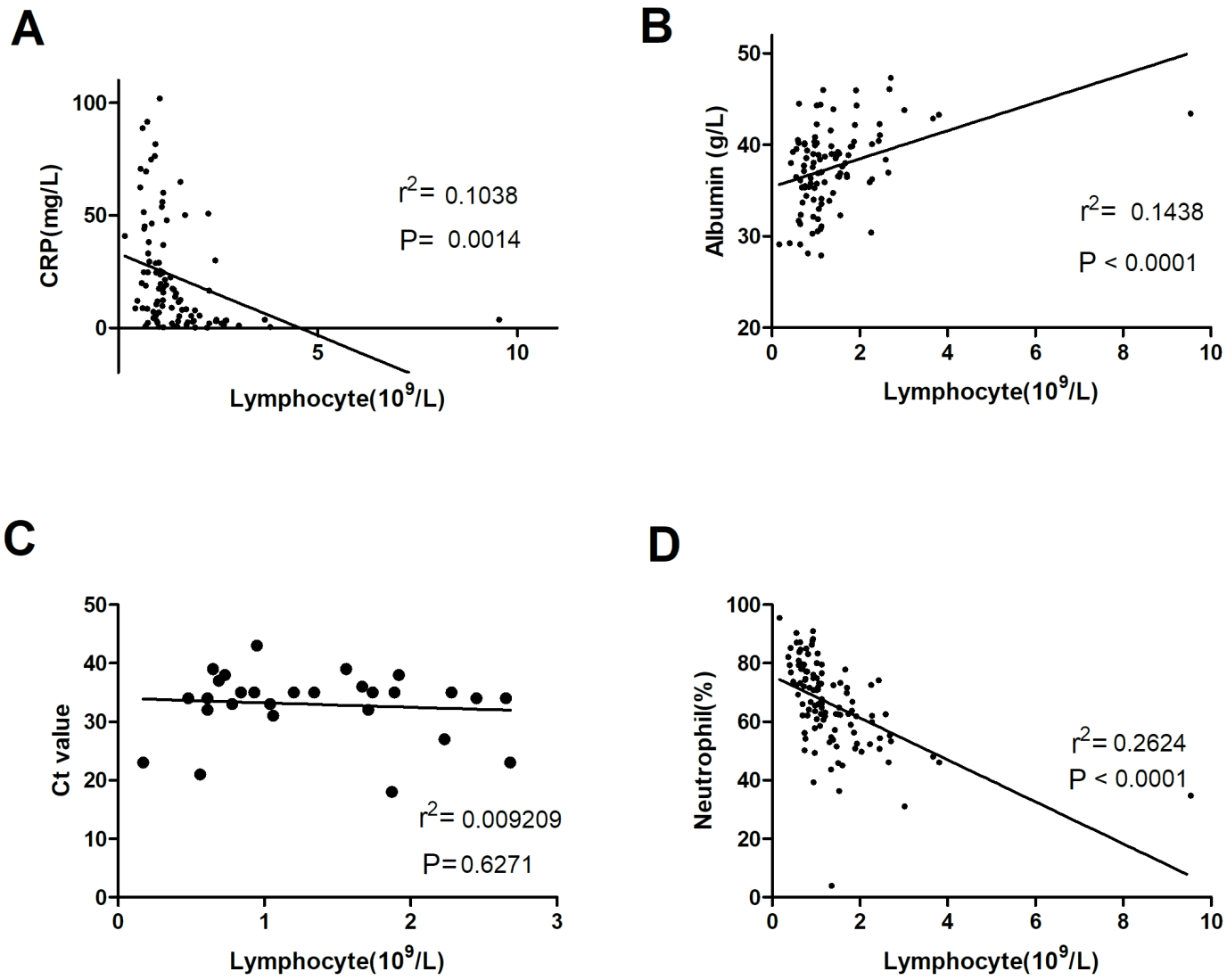


Figure 2

The correlation of lymphocyte count and laboratory test. Lymphopenia was highly correlated with CRP level (A), serum albumin level (B) and neutrophil count (D). No significant correlation was found between lymphocyte count and Ct value (C). Spearman rank correlation analysis (r) and P values are provided in each graph.

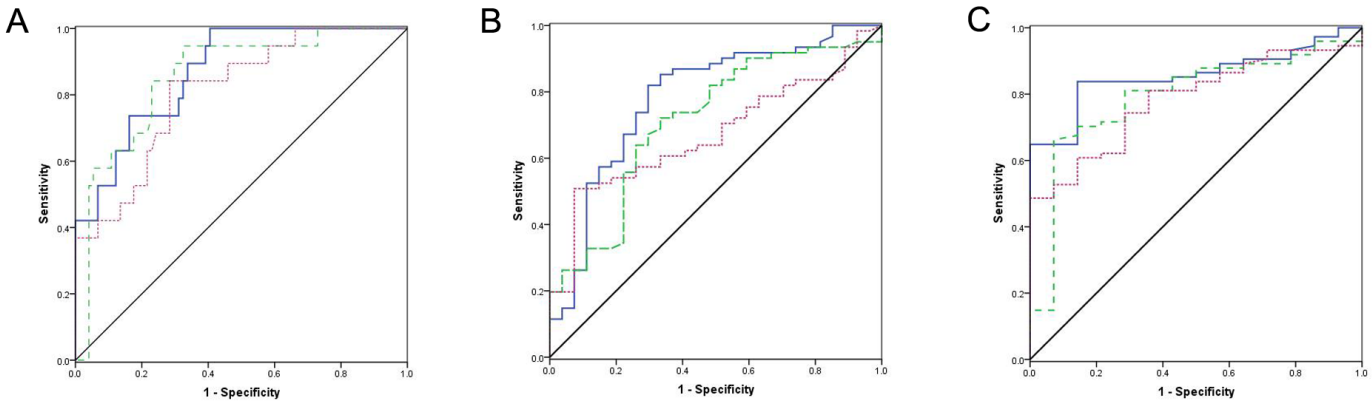


Figure 3

ROC plots express the prognostic value of illness severity of lymphopenia compared with CRP and LDH level. ROC curve to predict patients with (A) pneumonia of severe grade or critical grade, (B) bilateral lung involvement in lung CT scan, (C) abnormal lung image on discharge. The diagonal line indicates an AUC of 0.5 (no discrimination between two states). LYM, lymphopenia; CRP, C-reactive protein; LDH, lactate dehydrogenase; AUC, area under the curve; ROC, receiver-operator curve.

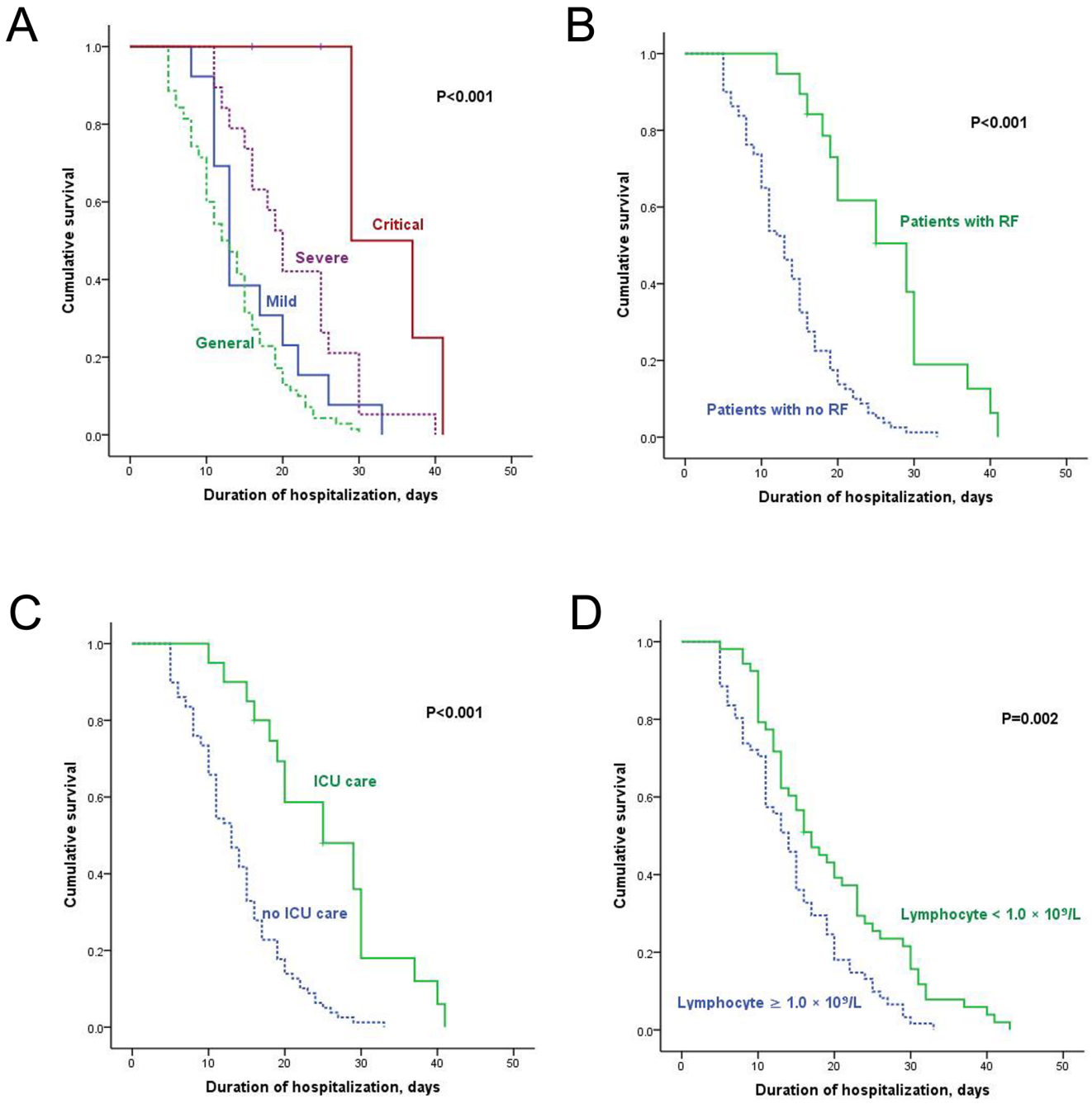
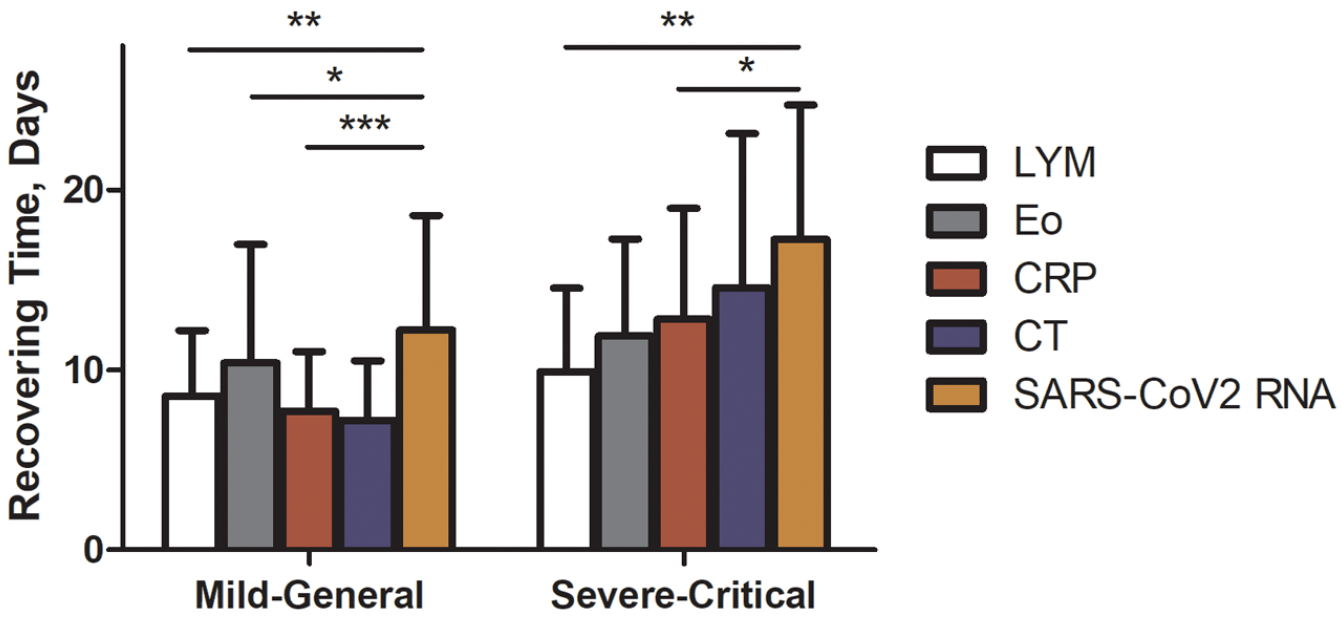


Figure 4

Kaplan-Meier curves for duration of hospitalization in patients within different categories. (A) Kaplan-Meier curves for duration of hospitalization according to severity grades of COVID-19; (B) Kaplan-Meier curves for duration of hospitalization according to the presentation of respiratory failure; (C) Kaplan-Meier curves for duration of hospitalization according to the requirement of ICU care; (D) Kaplan-Meier curves for duration of hospitalization according to lymphocyte count. RF, respiratory failure; ICU, intensive care unit.



	Mild and general cases (N=87)		Severe and critical cases (N=28)	
	Median Recovery time	Range	Median Recovery time	Range
LYM	9.0	3.0-14.0	9.0	3.0-23.0
Eo	9.0	2.0-26.0	12.0	3.0-23.0
CRP	7.5	1.0-14.0	12.0	4.0-23.0
CT	7.0	2.0-16.0	12.5	4.0-32.0
SARS-CoV2	11.0	3.0-32.0	15.0	8.0-31.0

Figure 5

The recovery time (from admission to the date of normalization) of lymphopenia, eosinophilia, CRP level, chest radiograph and clearance time of SARS-CoV2 RNA (from admission to the date of second negative detection result of SARS-CoV2 RNA). LYM, lymphopenia; Eo, eosinophilia; CRP, C-reactive protein. ***, P<0.001; **, P<0.01; *, P<0.05.

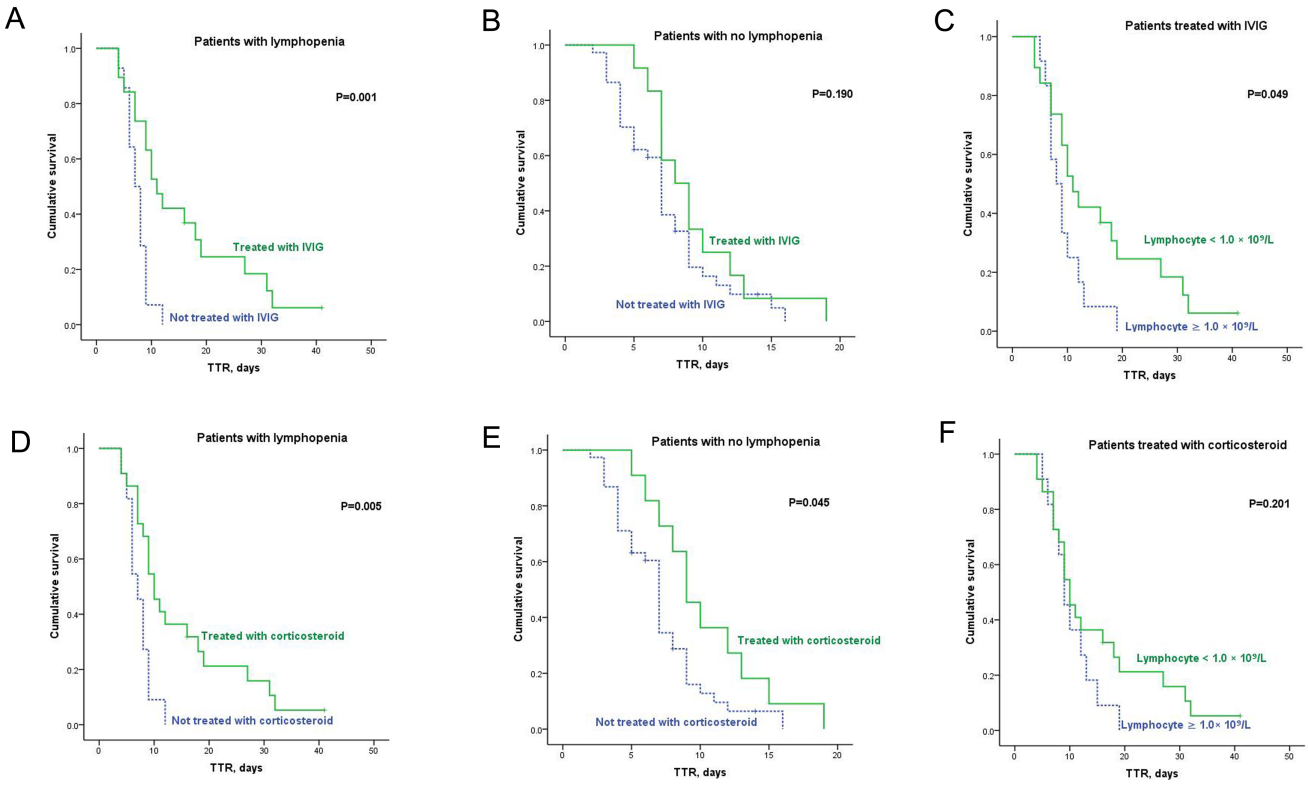


Figure 6

Kaplan-Meier curves for TTR in patients received different treatment. (A) Kaplan-Meier curves for TTR according to treatment of IVIG in patients with lymphopenia; (B) Kaplan-Meier curves for TTR according to treatment of IVIG in patients with no lymphopenia; (C) Kaplan-Meier curves for TTR according to lymphocyte count in patients treated with IVIG; (D) Kaplan-Meier curves for TTR according to treatment of corticosteroid in patients with lymphopenia; (E) Kaplan-Meier curves for TTR according to treatment of corticosteroid in patients with no lymphopenia; (F) Kaplan-Meier curves for TTR according to lymphocyte count in patients treated with corticosteroid. IVIG, intravenous human γ-immunoglobulin; TTR, time to recover.

Supplementary Files

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- FigureS1.tif