International Journal of General Medicine Dovepress

Open Access Full Text Article ORIGINAL RESEARCH

Dynamic Characteristics of Lymphocyte Subsets and Their Predictive Value for Disease Progression and Prognosis in Primary Infection and Unvaccinated COVID-19 Patients

Xinyi Zhang[1,2,*], Zhu Chen[3,*], Jun Zheng[4], Chen Feng[5], Bennan Zhao[1], Lijuan Lan[1], Dafeng Liu[1]

[1]The First Ward of Internal Medicine, Public Health Clinical Centre of Chengdu, Chengdu, People's Republic of China; [2]Department of Endocrinology & Metabolism, Sichuan University West China Hospital, Chengdu, People's Republic of China; [3]Department of Drug Clinical Trial Center, Public Health Clinical Centre of Chengdu, Chengdu, People's Republic of China; [4]Medical Department, Public Health Clinical Centre of Chengdu, Chengdu, People's Republic of China; [5]Legal Services Division, Public Health Clinical Centre of Chengdu, Chengdu, People's Republic of China

*These authors contributed equally to this work

Correspondence: Dafeng Liu, The First Ward of Internal Medicine, Public Health Clinical Centre of Chengdu, No. 377 Jingming Road, Jinjiang District, Chengdu, Sichuan, 610066, People's Republic of China, Email ldf312@126.com

International Journal of General Medicine downloaded from https://www.dovepress.com/
For personal use only.

Aim: Our cohort study aimed to investigate the dynamic changes of lymphocyte subsets and their abilities to predict disease severity and prognosis in primary infection and unvaccinated COVID-19 patients.

Methods: A total of 773 cases, including 718 primary infection and unvaccinated COVID-19 patients and 55 controls. COVID-19 patients were assigned to severe and nonsevere groups according to disease severity, as well as survival and death groups according to prognosis. Serum samples were collected to measure the numbers of total lymphocytes and lymphocyte subsets. The differences among different severity groups were analyzed. Spearman correlation was performed to assess associations between lymphocyte subsets and disease severity and prognosis. Meanwhile, receiver operating characteristic (ROC) curves were also analyzed to find optimal cutoff points.

Results: At admission, the severe group demonstrated significantly lower total lymphocyte counts and percentages, $CD3^+$ and $CD3^+CD4^+$ T cell counts and percentages, $CD3^+CD8^+$ T cell counts, $CD19^+$ B cell counts and $CD56^+$ NK cell counts and percentages than the nonsevere group. Meanwhile, compared with the survival group, the death group also had lower total lymphocyte counts and percentages, $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ T cell counts. Additionally, differences in these parameters were also noticed within four weeks after admission. Furthermore, Spearman analysis reported that disease severity was negatively correlated with lymphocyte counts and percentages, $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ T cell counts, $CD3^+$ and $CD3^+CD4^+$ T cell percentages (r=−0.166, −0.179, −0.173, −0.186, −0.127, −0.117, −0.149, respectively)(all P<0.05). The prognosis of death was also negatively correlated with total lymphocyte counts and percentages, $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ T cell counts (r=−0.125, −0.121, −0.123, −0.123, −0.091, respectively)(all P<0.05).

Conclusion: In primary infection and unvaccinated COVID-19 patients total lymphocytes and T cell, B cell and NK cell subsets at COVID-19 onset play valuable roles in predicting disease severity and prognosis.

Clinical Trial Registry: Chinese Clinical Trial Register ChiCTR2000034563.

Keywords: lymphocyte subsets, coronavirus disease 2019, COVID-19, prediction, severity, prognosis

Introduction

Coronavirus disease 2019 (COVID-19), caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), poses the most urgent threat to global health.[1–5] As of 13 April 2023, the WHO reported that there were over 762 million confirmed cases of COVID-19, including over 6.8 million deaths.[6] For patients hospitalized with COVID-19, one-quarter to one-third of patients will further develop COVID-19-associated acute respiratory distress

Received: 20 July 2024
Accepted: 6 October 2024
Published: 10 October 2024

© 2024 Zhang et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v3.0) License (http://creativecommons.org/licenses/by-nc/3.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

syndrome (CARDS).[7] Additionally, rapid disease progression and comorbidities can result in poor prognosis.[8–14] Therefore, early detection of severe COVID-19 cases and timely treatment are of vital importance.

Pre-existing weakened immune system can result in out of control infections. Defective cellular immunity, an important part of host immune response dysregulation, plays a crucial role in the pathophysiology of COVID-19[9,15–19] and MERS-CoV,[20] ICU-acquired infection[21] and increased mortality of ICU patients.[22–24] SARS-CoV-2 can alter host immune responses, including innate and adaptive immune responses.[25] Overall, decreased lymphocytes and subsets, especially T and B subsets, were found[16,17] and were strongly associated with disease progression and adverse outcomes in COVID-19 patients with diabetes mellitus (DM) or severe disease.[17] One previous study found that a model based on NK cell and $CD4^+$ T cell counts combined with interleukin-8 had good predictive value for COVID-19 patient mortality.[26] Other previous studies found that the $CD8^+$ T-cell level reflected disease severity, and decreased $CD4^+$ T-cell counts were independently associated with increased in-hospital mortality.[27] Meanwhile, lower T lymphocyte subsets were significantly associated with a higher occurrence of composite endpoint events in COVID-19 patients.[28] An increase in $CD4^+$ T cells, $CD8^+$ T cells, and the administration of interferon were independent predictors of clinical response within the first week after admission.[29] Significantly lower lymphocyte subpopulation counts (CD3+ T cells, CD3+CD4+ T cells, CD3+CD8+T cells, CD16+CD56+ natural killer (NK) cells and CD19+ B cells) were found in ICU patients with acquired infection compared those without acquired infection.[21] At ICU admission lymphopenia and T cell depletion were associated with increased mortality of ICU patients.[22–24]

Those above findings supported lymphocytes and subsets as predictive biomarkers and potential therapeutic targets in intensive care medicine. However, most studies on COVID-19 had small sample sizes and did not report dynamic changes in total lymphocytes and their subsets during disease progression. Additionally, associations between different lymphocyte subsets and disease severity and prognosis remain unclear, and optimal values of cutoff points of different lymphocyte subsets are also unknown. Therefore, we conducted a cohort study of 773 COVID-19 cases to investigate the dynamic changes in total lymphocytes and their subsets, as well as their abilities and optimal cutoff points in predicting disease severity and prognosis in primary infection and unvaccinated COVID-19 patients.

Methods

Subjects

This study is a cohort study that recruited a total of 773 patients, including 718 primary infection and unvaccinated COVID-19 patients (COVID-19 group) from the hospital isolation ward[16,18,19] and 55 patients without COVID-19 (control group)[17] from the medical examination clinic who presented to the Public Health Clinical Centre of Chengdu from January 16, 2020, to February 28, 2021 (Figure 1).[16,18,19]

Inclusion and Exclusion Criteria

Both male and female patients who were primarily infected with COVID-19 and unvaccinated were included in the COVID-19 group without any age limitation. Patients with autoimmune diseases or who have used or are using immunosuppressive agents, and patients with previous infection or vaccination were excluded from this study.

Criteria for Disease Diagnosis, Clinical Typing and Cure Definition

The criteria of disease diagnosis, clinical typing and cure definition in COVID-19 patients were based on the seventh Trial Version of the Novel Coronavirus Pneumonia Diagnosis and Treatment Guidance.[8]

The diagnostic criteria were cases with one of the following etiological pieces of evidence: positive results of SARS-CoV-2 detected by real-time fluorescence reverse transcription polymerase chain reaction (RT–PCR) and positive results from viral gene sequencing.

The clinical types were classified as asymptomatic infection, mild, common, severe and critical illness. Specific classification criteria were as follows: (1) asymptomatic infection means that there are no clinical symptoms and no pneumonia manifestations on imaging; (2) mild illness means that the clinical symptoms are mild, and there are no pneumonia manifestations on imaging; (3) common illness refers to patients with clinical symptoms including fever,

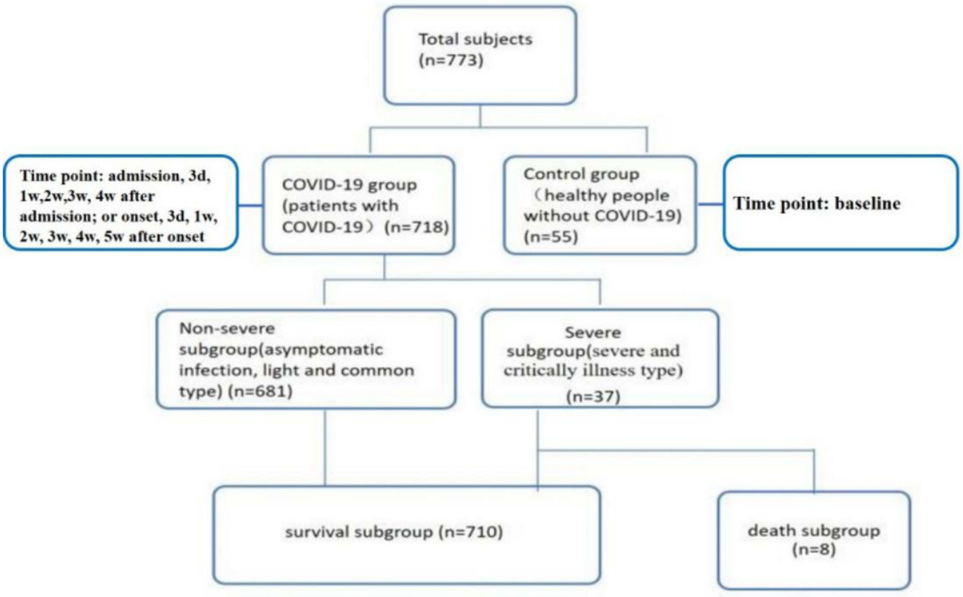

Figure 1 Patient data. Nonsevere refers to the clinical type of COVID-19 that is asymptomatic, light, and common. Severe refers to the clinical type of COVID-19 that is associated with severe and critical illness.

symptoms of respiratory tract, and pneumonia can be seen on imaging; (4) severe illness refers to patients with any of the following items: ① respiratory distress, respiratory rate (RR) ≥30 times/min; ② oxygen saturation ≤93% in the resting state; ③ arterial blood oxygen partial pressure (PaO2)/oxygen concentration (FiO2)≤300 mmHg (1 mmHg = 0.133 kPa) (in areas with high altitude (over 1000 meters above sea level), PaO2/FiO2 should be corrected according to the following formula: PaO2/FiO2 * [atmospheric pressure (mmHg)/760]); ④ pulmonary imaging shows lesions with significant progress over 50% within 24–48 hours; (5) criteria for critical illness contains one of the following conditions: ① occurrence of respiratory failure which needs mechanical ventilation; ② occurrence of shock; ③ concurrence of other organ failure which requires intensive care unit.

Patients who meet all of the following criteria can be regarded as cured and can be discharged from the hospital: (1) body temperature returns to normal for more than 3 days; (2) respiratory symptoms improve significantly; (3) lung imaging shows a significant improvement in acute exudative lesions; and (4) two consecutive sputum, nasopharyngeal swab or other respiratory specimen tests are negative for nucleic acid (sampling intervals are at least 24 hours).

Grouping Standards

Among all 773 cases, there were 718 patients in the COVID-19 group[18,19] and the remaining 55 in the control group[17] (Figure 1). According to disease severity, these patients were classified into the asymptomatic infection group (237 cases), mild illness group (73 cases), common illness group (371 cases), severe illness group (18 cases) and critical illness group (19 cases). Furthermore, 681 patients with asymptomatic infection or mild or common illness were assigned to the nonsevere group, while the remaining 37 severe and critical illness patients were assigned to the severe group (Figure 1).

Additionally, 710 surviving patients were included in the survival subgroup, while 8 dead patients were included in the death subgroup (Table 1 and Figure 1). For the survival subgroup, specifically, there were 237 cases with asymptomatic infection, 73 cases with mild illness, 371 cases with common illness, 18 cases with severe illness and 11 cases with critical illness (Figure 1). For the death subgroup, all 8 patients who died were initially diagnosed with critical illness.

Table 1 Baseline Information of Controls and COVID-19 Patients (n=773)

Variables	Control group (*n*=55)		COVID-19 group (*n*=718)	
	χ±SD or Case (%)	Range	χ±SD or case (%)	Range
Age (years)	55.54±7.79	36–68	38.48±14.15	0.17~87
Male (case, %)	25(45.45)		529(73.68)	
Duration (day)			1.74±1.20	1~30
LY (cells/μL)	1477.18±319.02	1138~2913	2061.83±916.87	256~7641
LY% (%)	17.10±4.71	6.17~29.40	23.49±7.86	2.14~55.52
$CD3^+$ (cells/μL)	1066.77±210.48	824~1968	1489.14±668.19	168~4757
$CD3^+CD4^+$ (cells/μL)	668.70±113.46	482~1075	840.32±410.07	88~2430
$CD3^+CD8^+$ (cells/μL)	334.21±85.56	246~783	549.56±293.42	40~2684
$CD3^+$% (%)	72.30±2.35	63.30~79.30	72.37±6.96	39.98~89.26
$CD3^+CD4^+$% (%)	45.44±2.57	31.83~50.37	40.64±8.00	18.02~70.92
$CD3^+CD8^+$% (%)	22.61±1.79	20.04~26.87	27.00±7.58	10.28~48.84
Ratio of $CD4^+$ to $CD8^+$	2.03±0.23	1.18~2.51	1.70±0.75	0.42~5.36
$CD19^+$ (cells/μL)	207.06±34.74	131~273	226.01±171.07	23~1331
$CD56^+$ (cells/μL)	249.14±151.98	155~778	183.96±160.96	13~1109
$CD19^+$% (%)	14.41±2.69	7.20~20.07	12.66±4.85	1.85~32.95
$CD56^+$% (%)	17.34±3.59	11.86~24.49	11.07±6.21	0.85~35.06
Virus negative conversion time (day)			15.48±11.18	2~53
In-hospital time (day)			18.28±11.16	2~56
Severe (case, %)			37(5.15)	
Prognosis				
Survival (case, %)			710(98.89)	
Death (case, %)			8(1.11)	

Abbreviation: LY, lymphocytes.

Definition of Disease Severity and Prognosis

The prognosis was classified as survival or death within four weeks after admission or within five weeks after onset. The disease severity included nonsevere (COVID-19 patients with asymptomatic infection, mild or common illness clinic type) and severe (COVID-19 patients with severe or critical illness clinic type).

Detection Methods and Analysis Methods of Lymphocytes and Subsets

Peripheral blood (2 mL) samples with EDTA anticoagulation were collected from COVID-19 patients and controls and were tested by Beckman Coulter flow cytometry (FCM) within 6 hours after collection. Two million cells were resuspended in PBS and blocking buffer (2% bovine serum albumin, 2% rabbit serum, 2% human serum, 2% mouse serum, 2% rat serum) to block unspecific binding of antibodies. Briefly, $CD19^+$ B cell, $CD3^+$ T cell, $CD4^+$ T cell, $CD8^+$ T cell and $CD56^+$ NK cell counts (cells/μL) were measured by multiple-color FCM with human monoclonal anti-CD19-phycoerythrin (PE), anti-CD3-fluorescein isothiocyanate (FITC), anti-CD4-PE, anti-CD8-allophycocyanin (APC) and anti-CD56-PE antibodies (BD Multitest) according to the manufacturer's instructions. After 30 min of light-protected incubation, the cells were washed, centrifuged, and subjected to flow-cytometric analysis using a FACS Canto-II (BD, Heidelberg, Germany). Cell populations were defined after exclusion of doublets by antibody positivity in the following manner: T cells as CD3+CD56- (subsequent analysis for CD4 and CD8), natural killer cells as CD3-CD19-CD56+ (subsequent analysis of CD16 and CD56), and B cells as CD19+. A total of 1200 $CD3^+$ cells were recorded per tube, and FCM data were analyzed using the Beckman application software CyExpert for DxFLEX.[16,18,19,23,30]

The samples from patients with COVID-19 were collected at different time points, including the admission day, the third day, the first week, the second week, the third week and the fourth week after admission or the day of onset, the third day, the first week, the second week, the third week, the fourth week and the fifth week after onset.

Data Collection

All data of 773 cases, including clinical and demographic information, lymphocytes and subsets at all follow-up time points, were collected to establish databases. Researchers strictly controlled the accuracy, completeness and authenticity of all data.

Statistical Analysis

SPSS 26.0 (SPSS, Chicago, IL, USA) and GraphPad Prism 8 (GraphPad, CA, USA) were used for statistical analyses. Measurement data with normal distributions are presented as the mean and standard deviation (SD), while those with nonnormal distributions are presented as the median and standard error. Categorical data are expressed as percentages or proportions.

Data with normal distributions and homogeneity of variance between multiple groups were compared using one-way or two-way ANOVA, and further comparison between two groups was performed by using the least significant difference (LSD) *t*-test. Data with normal distributions and homogeneity of variance between two groups were compared using the independent samples *t*-test. Enumeration data are presented as percentages or proportions, and data between two or multiple groups were compared using a chi-square test. Two-factor correlation analysis was performed with Spearman correlation analysis. Receiver operating characteristic (ROC) analysis was used to assess the capacity of different lymphocytes and their subsets to distinguish cases of the nonsevere group from the severe group, as well as those who survived from dead COVID-19 patients. A $P<0.05$ was considered statistically significant.

Patient and Public Involvement

Patients and the public were not involved in the design or conduct of this study. All patients recruited in this study received verbal and written information about this research. The burden of the intervention was carefully assessed by the investigators. Participants were assessed for eligibility, and data collection was performed. Dissemination of the general results (without personally identifying data) will occur on demand.

Our study was approved by the Ethics Committee of the Public Health Clinical Centre of Chengdu (ethics approval number: PJ-K2020-26-01). Written informed consent was waived by the Ethics Commission of the designated hospital because this study is related to emerging infectious diseases.

Results

Baseline Information

A total of 718 COVID-19 patients (medium age, 38.48 years; 73.68% male) and 55 control patients (medium age, 55.54 years; 45.45% male) were included. Other baseline information, including duration, lymphocyte subsets at admission, virus-negative conversion time, in-hospital time, proportion of severe cases and proportion of deaths, is shown in Table 1 and Figure 1. Among 718 COVID-19 patients, the severity rate was 5.15% (37/718), and the overall mortality rate was 1.11% (8/718) (Table 1).

The time of onset and admission in the asymptomatic infection group was the same day. The average time from onset to admission in the mild illness group and the common illness group was approximately 3–4 days, and the average time from onset to admission in the severe illness group and the critical illness group was approximately 6–7 days.

COVID-19 patients in the nonsevere subgroup and in the survival group were significantly younger than healthy controls in the control group (Table 2). While COVID-19 patients in the severe subgroup were similar old as healthy controls in the control group, but those in the nonsurvival group were older than healthy controls in the control group (Table 2).

For COVID-19 patients, those in the survival group were significantly younger than those in the death group ($P<0.0001$) (Table 2). Meanwhile, those in the severe subgroup were also older than those in the nonsevere subgroup (Table 2). There was also a difference in sex between the severe COVID-19 group, the survival COVID-19 group, the death COVID-19 group and the control group (all $P<0.0001$) (Table 2).

Table 2 Comparison of Baseline Conditions Between the Three Groups (n=773)[15]

Variable	Controlgroup (*n*=55)	COVID group (*n*=718)				
		Total (*n*=718)	Nonsevere Subgroup (*n*=681)	Severe Subgroup(*n*=37)	Survival Subgroup (*n*=710)	Deadly Subgroup (*n*=8)
Age (year)	55.54±7.79	38.48±14.15	37.44±13.14	57.70±18.08	38.11±13.68	71.38±17.45
Male (case, %)	25 (45.45)	529 (73.68)	505 (74.16)	24(64.87)	527(74.23)	2(25.00)

Notes: The chi-square test was used for the comparison of sex between the control group and COVID-19 group, COVID-19 nonsevere subgroup, COVID-19 severe subgroup, COVID-19 survival subgroup, and COVID-19 death subgroup, all P<0.0001. Unpaired *t*-tests were used for comparisons of age between the control group and COVID-19 group, COVID-19 nonsevere subgroup, COVID-19 severe subgroup, COVID-19 survival subgroup, and COVID-19 death subgroup, P<0.0001, <0.0001, 0.4341, <0.0001, <0.0001, respectively. Unpaired *t*-tests were used for comparisons of age between the COVID-19 nonsevere subgroup and the COVID-19 severe subgroup and between the COVID-19 survival subgroup and the COVID-19 death subgroup, all P<0.0001.

The Characteristics of Lymphocyte Subsets at Admission in Patients with COVID-19

At admission, different groups demonstrated different lymphocyte subsets. In terms of disease severity, compared with patients in the control group, the nonsevere group had higher lymphocyte counts and percentages (Figure 2A and B), $CD3^+$ T cell counts (Figure 2C) (all $P<0.05$), similar $CD3^+$ T cell percentages and $CD19^+$ B cell counts (Figure 2D and E) (all $P>0.05$), while it showed lower $CD19^+$ B cell percentages (Figure 2F), $CD56^+$ NK cell counts and percentages (Figure 2G and H), also had higher $CD3^+CD4^+$ T cell counts (Figure 3A), $CD3^+CD8^+$ T cell counts and percentages (Figure 3C and D) (all $P<0.05$), and lower $CD3^+CD4^+$ T cell percentages (Figure 3B) and the ratio of $CD4^+$ T cells to $CD8^+$ T cells (Figure 3E) (all $P<0.05$). For the severe group, despite the age similarity, the lymphocyte counts and percentages, $CD3^+$ T cell counts and percentages, $CD19^+$ B cell counts and percentages, $CD56^+$ NK cell counts, $CD3^+CD4^+$ T cell counts and percentages, $CD3^+CD8^+$ T cell counts and the ratio of $CD3^+CD4^+$ T cells to $CD3^+CD8^+$ T cells were lower than those in the control group (Figure 2A–G and Figure 3A–C, E) (all $P<0.05$), while the $CD3^+CD8^+$ T cell percentages (Figure 3D) was higher than that in the control subgroup ($P<0.05$). Moreover, compared with the nonsevere group, lymphocyte counts and percentages, $CD3^+$ T cell counts and percentages, $CD19^+$ B cell counts, $CD56^+$ NK cell counts, $CD3^+CD4^+$ T cell counts and percentages, and $CD3^+CD8^+$ T cell counts (Figure 2A–E, H and Figure 3A–C) in the severe group were significantly lower (all $P<0.05$), while the $CD56^+$ NK cell percentages (Figure 2G) was higher than that in the nonsevere subgroup ($P<0.05$).

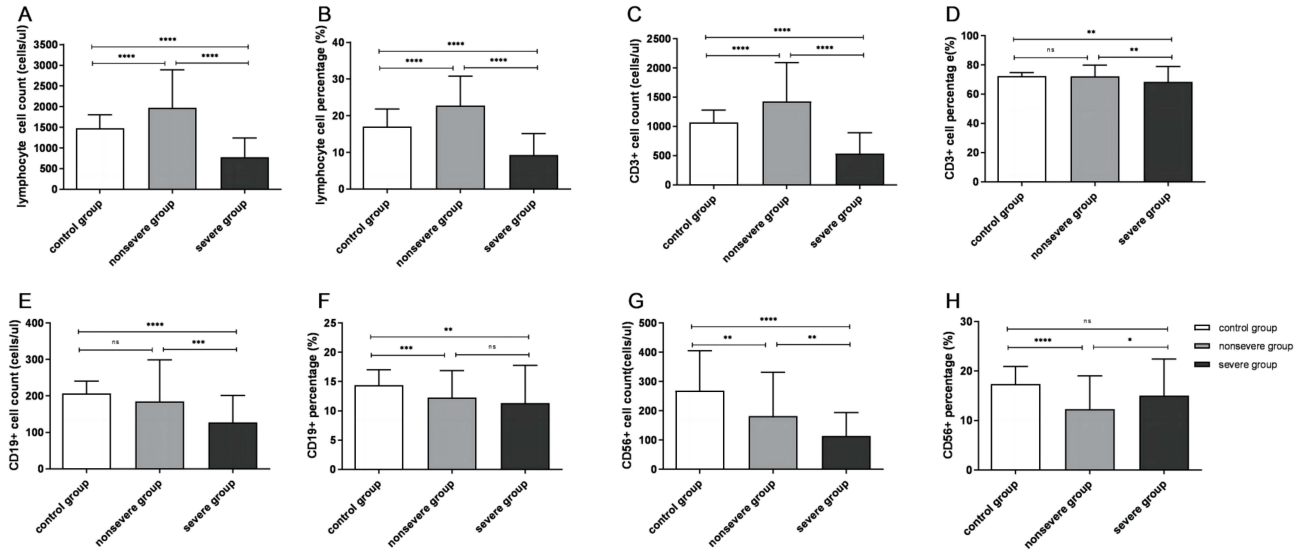

Figure 2 Comparison of baseline levels of lymphocyte and T, B and NK cell counts and percentages among the control group, the nonsevere COVID-19 group and the severe COVID-19 group (n=773; control, severe and nonsevere groups, n=55, 37 and 681, respectively). Abbreviations: COVID-19, coronavirus disease 2019. (**A**) Lymphocyte count. (**B**) Lymphocyte percentage. (**C**) T lymphocyte count. (**D**) T lymphocyte percentage. (**E**) B lymphocyte count. (**F**) B lymphocyte percentage. (**G**) NK lymphocyte count. (**H**) NK lymphocyte percentage. The measurement data are expressed as χ±SD. Unpaired one-way ANOVA was used for intergroup comparisons (**A–C, G** and **H**), P all<0.0001; (**E**), P<0.001; (**D** and **F**), P all<0.01). Unpaired *t*-tests were used for comparisons with the control group or with the nonsevere COVID-19 group, ^{NS}P>0.05, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

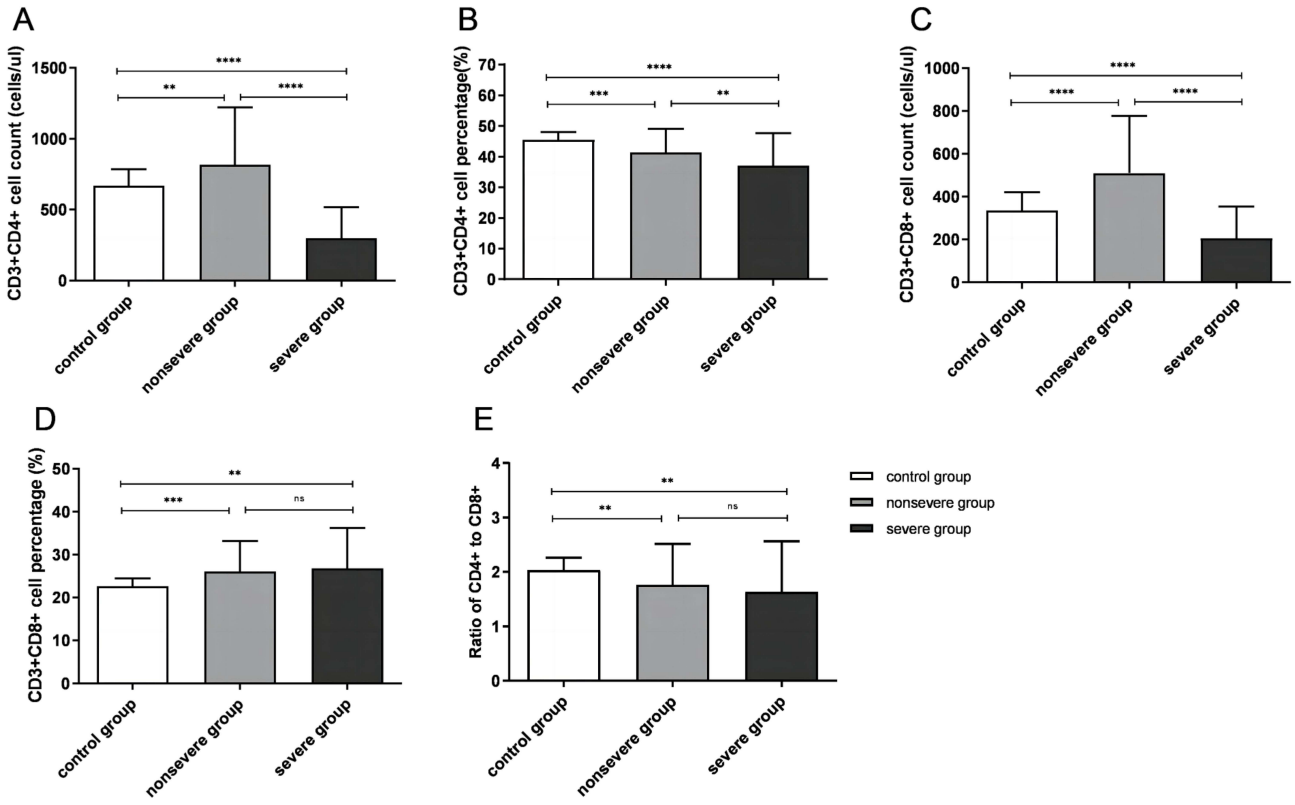

Figure 3 Comparison of baseline T lymphocyte subset counts and percentages among the control group, the severe COVID-19 group and the nonsevere COVID-19 group (n=773; control, severe and nonsevere groups, n=55, 37 and 681, respectively). (**A**) CD3+CD4+ count. (**B**) CD3+CD4+ percentage. (**C**) CD3+CD8+ count. (**D**) CD3+CD8+ percentage. (**E**) Ratio of CD4+/CD8+ cells. The measurement data are expressed as $\chi \pm SD$. Unpaired one-way ANOVA was used for intergroup comparisons (**A**–**C**), P all<0.0001; (**D**), P<0.01; (**E**), P<0.05). Comparisons with the control group or with the nonsevere COVID-19 group using unpaired *t*-tests, ^{NS}P>0.05, **P<0.01,***P<0.001, ****P<0.0001.

Regarding disease prognosis, compared with the control group, the survival group also demonstrated higher lymphocyte counts and percentages (Figure 4A and B), $CD3^+$ T cell counts (Figure 4C) (all $P<0.05$), similar $CD3^+$ T cell percentages and $CD19^+$ B cell counts (Figure 4D and E), while it harbored lower $CD19^+$ B cell percentages (Figure 4F), $CD56^+$ NK cell counts and percentages (Figure 4G and H), and also had higher CD3+CD4+ T cell counts (Figure 5A), and CD3+CD8+ T cell counts and percentages (Figure 5C and D) (all P<0.05), lower $CD3^+CD4^+$ T cell percentages (Figure 5B) and the ratio of $CD4^+$ T cells to $CD8^+$ T cells (Figure 5E) (all $P<0.05$). For the death group, the lymphocyte counts and percentages (Figure 4A and B), $CD3^+$ T cell counts (Figure 4C), $CD19^+$ B cell counts and percentages (Figure 4E and F), $CD56^+$ NK cell counts (Figure 4G), $CD3^+CD4^+$ T cell counts (Figure 5A) and $CD3^+CD8^+$ T cell counts (Figure 5C) were lower than those in the control group (all $P<0.05$), while the $CD3^+CD8^+$ T cell percentages (Figure 5D) was higher than that in the control subgroup ($P<0.05$). Furthermore, in the death group, lymphocyte counts and percentages, $CD3^+$ T cell counts, $CD3^+CD4^+$ T cell counts and $CD3^+CD8^+$ T cell counts (Figures 4A–C and 5A, C) were significantly lower than the corresponding values in the survival group (all $P<0.05$).

Dynamic Characteristics of Lymphocyte Subsets Within Four Weeks After Admission in Patients with COVID-19

From admission to the fourth week after admission, significant differences between the severe group and the nonsevere group were noticed in lymphocyte counts, lymphocyte percentages, $CD19^+$ B cell counts, $CD56^+$ NK cell percentages, $CD3^+$ T cell counts, $CD3^+CD4^+$ T cell counts and $CD3^+CD8^+$ T cell counts. In the nonsevere group, lymphocyte counts (Figure 6A) and lymphocyte percentages (Figure 6B) decreased gradually from admission to the third week and rose slightly in the fourth week. In the severe COVID-19 group, lymphocyte counts (Figure 6A) and lymphocyte percentages (Figure 6B) were always lower than

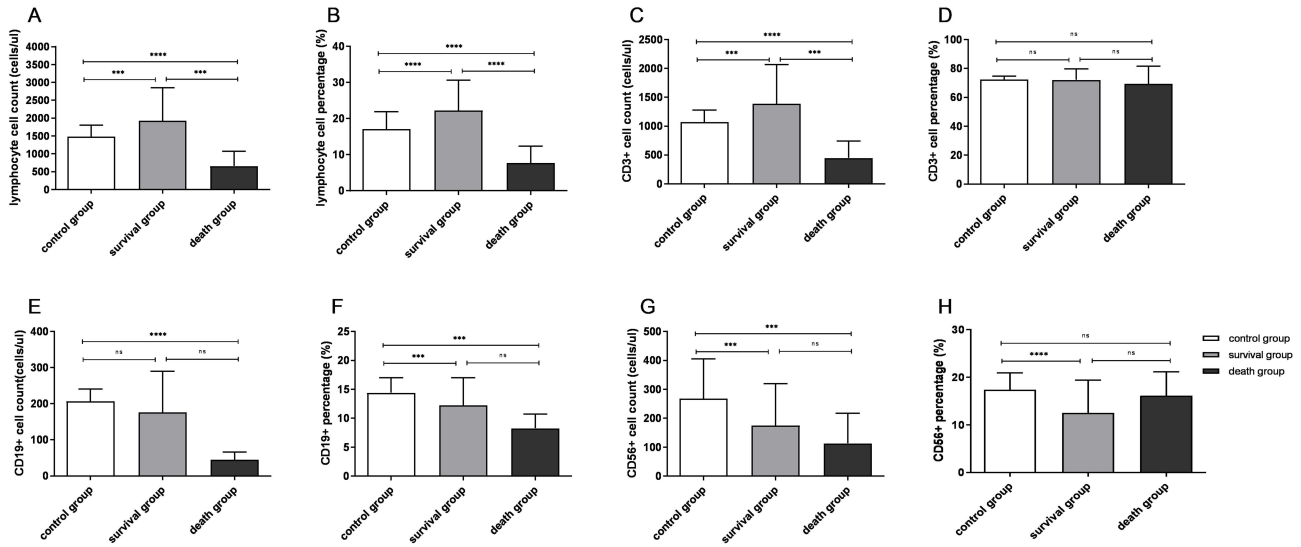

Figure 4 Comparison of baseline levels of lymphocyte and T, B and NK cell counts and percentages among the control group, the death COVID-19 group and the survival COVID-19 group (n=773; control, death and survival groups, n=55, 8 and 710, respectively). (**A**) Lymphocyte count. (**B**) Lymphocyte percentage. (**C**) T lymphocyte count. (**D**) T lymphocyte percentage. (**E**) B lymphocyte count. (**F**) B lymphocyte percentage. (**G**) NK lymphocyte count. (**H**) NK lymphocyte percentage. The measurement data are expressed as χ±SD. Intergroup comparisons were performed using unpaired one-way ANOVA (**A**–**C, G** and **H**), all P all<0.0001; (**D**), P>0.05, (**E** and **F**), P<0.001). Comparisons with the control group or with the surviving COVID-19 group were performed using unpaired *t*-tests, ^{NS}P>0.05, ***P<0.001, ****P<0.0001.

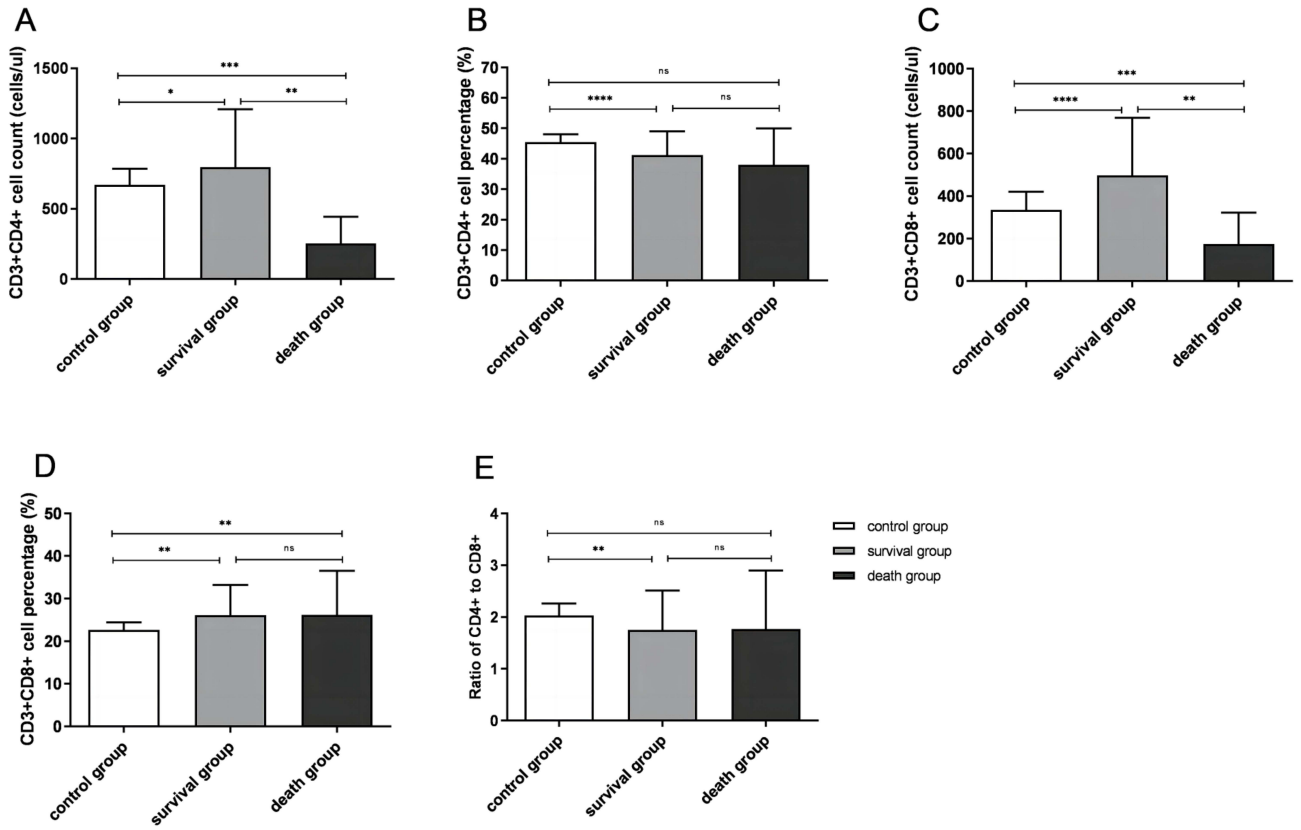

Figure 5 Comparison of baseline levels of T lymphocyte subset counts and percentages among the control group, the survival COVID-19 group and the death COVID-19 group (n=773; control, survival and death groups, n=55, 710 and 8, respectively). (**A**) CD3+CD4+ count. (**B**) CD3+CD4+ percentage. (**C**) CD3+CD8+ count. (**D**) CD3+CD8+ percentage. (**E**) Ratio of CD4+/CD8+ cells. The measurement data are expressed as χ±SD. Intergroup comparisons were performed using unpaired one-way ANOVA (**A** and **C**), P all<0.0001; (**B**) P<0.001; (**D**) P<0.01; (**E**), P<0.05). Comparisons with the control group or with the surviving COVID-19 group using unpaired *t*-tests, ^{NS}P>0.05, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

those in the nonsevere COVID-19 group but showed a small increase from admission to the fourth week. $CD19^+$ B cell counts (Figure 6C) remained stable at a low level in the severe COVID-19 group but showed some fluctuations around a higher level in the nonsevere COVID-19 group. In the nonsevere group, $CD19^+$ B cell percentages (Figure 6D) and $CD56^+$ NK cell counts (Figure 6E) remained stable from admission to the fourth week, but in the severe COVID-19 group $CD19^+$ B cell percentages (Figure 6D) remained stable only from admission to the first week, then decreased gradually from the first week to the fourth week, and $CD56^+$ NK cell counts (Figure 6E) increased gradually from admission to the fourth week. In contrast, $CD56^+$ NK cell percentages (Figure 6F) stayed at a stable low level in the nonsevere COVID-19 group, while they were significantly higher in the severe COVID-19 group. In the nonsevere group, $CD3^+$ T cell counts and percentages (Figure 7A and B), $CD3^+CD4^+$ T cell counts (Figure 7C) and $CD3^+CD8^+$ T cell counts (Figure 7E) decreased gradually from admission to the third week and rose slightly in the fourth week In the severe COVID-19 group, lymphocyte counts (Figure 6A), lymphocyte percentages (Figure 6B), $CD3^+$ T cell counts (Figure 7A), $CD3^+CD4^+$ T cell counts (Figure 7C) and $CD3^+CD8^+$ T cell counts (Figure 7E) were always lower than those in the nonsevere COVID group but showed a small increase from admission to the fourth week. $CD19^+$ B cell counts (Figure 6C) remained stable at a low level in the severe COVID-19 group but showed some fluctuations around a higher level in the nonsevere COVID-19 group. In contrast, $CD56^+$ NK cell percentages (Figure 6F) stayed at a stable low level in the nonsevere COVID-19 group, while they were significantly higher in the severe COVID-19 group. Whether in the nonsevere COVID-19 group or in the severe COVID-19 group, $CD3^+CD4^+$ T cell percentages (Figure 7D) and $CD3^+CD8^+$ T cell percentages (Figure 7F) remained stable from admission to the fourth week, and the ratio of $CD3^+CD4^+$ T cells to $CD3^+CD8^+$ T cells (Figure 7G) also remained stable from admission to the third week then increased in the nonsevere COVID-19 group but decreased in the severe COVID-19 group in the fourth week.

For the death group and survival group, significant differences were shown in lymphocyte counts, lymphocyte percentages, $CD3^+$ T cell counts, $CD3^+CD4^+$ T cell counts and $CD3^+CD8^+$ T cell counts. In the survival group, the lymphocyte counts (Figure 8A) and lymphocyte percentages (Figure 8B) declined in the first three weeks after admission and increased slightly at the fourth week, $CD19^+$ B cell counts and percentages (Figure 8C and D), $CD56^+$ NK cell counts and percentages (Figure 8E and F),

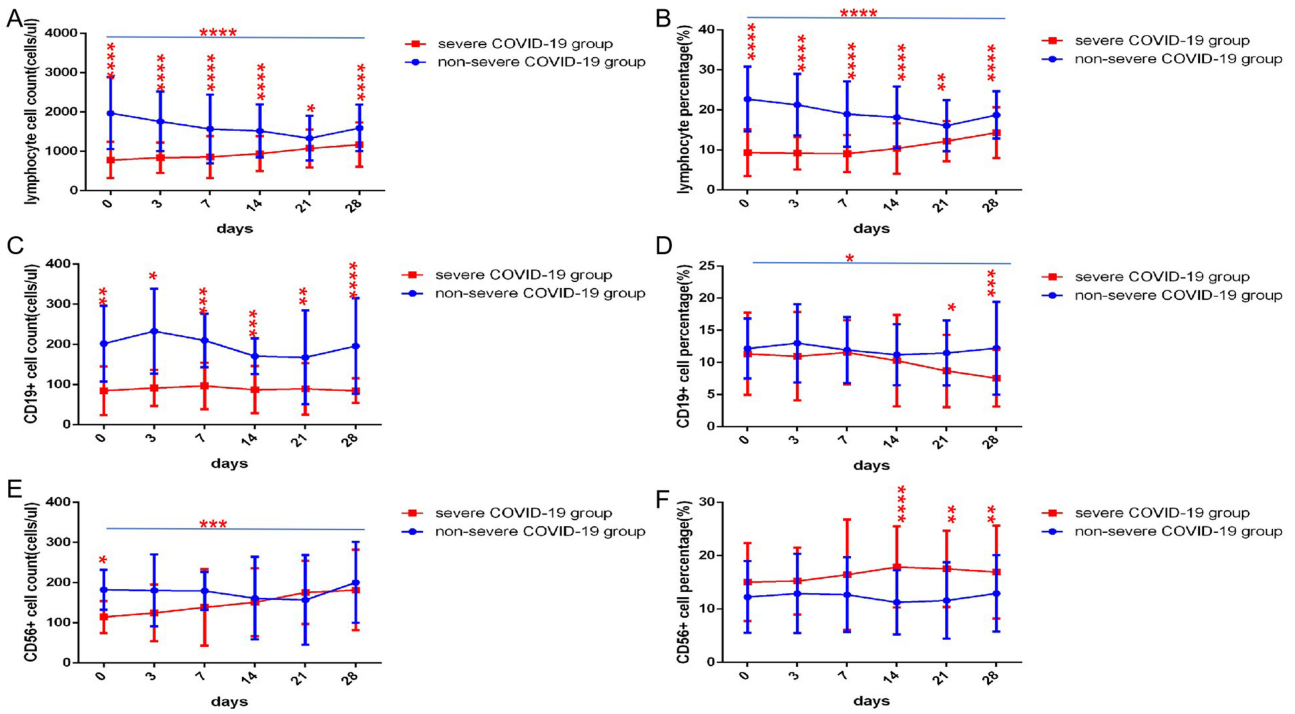

Figure 6 Comparison of the dynamic characteristics of lymphocyte and B and NK cell counts and percentages between the nonsevere COVID-19 group and the severe COVID-19 group (n=718; nonsevere and severe groups, n=681 and 37, respectively). (**A**) Lymphocyte count. (**B**) Lymphocyte percentage. (**C**) CD19+ count. (**D**) CD19+ percentage. (**E**) CD56+ count. (**F**) CD56+ percentage. The measurement data are expressed as $\chi \pm SD$. Two-way ANOVA was used for comparisons between two subgroups from admission to the fourth week after admission (interaction, (**A** and **B**), all P<0.0001, (**D**) P<0.05, (**E**) P<0.001, (**C** and **F**) all P>0.05; row factor, (**A** and **F**) all P>0.05, (**B** and **C**) all P<0.05, (**D**) P<0.01, (**E**) P<0.001; column factor, (**A**–**F**) all P<0.0001). Unpaired *t*-tests were used for comparisons between the nonsevere group and the severe group at the same time point, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

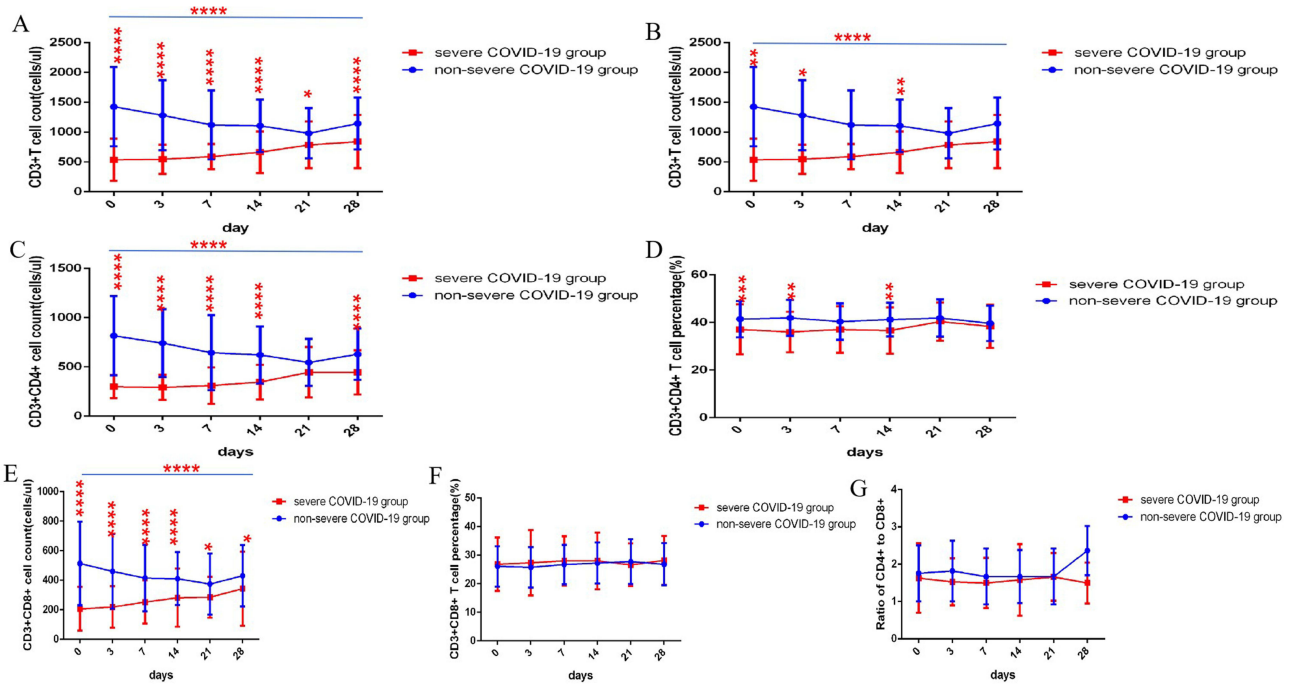

Figure 7 Comparison of dynamic characteristics of T lymphocyte subset counts and percentages among the severe COVID-19 group and the nonsevere COVID-19 group (n=718; severe and nonsevere groups, n=37 and 681, respectively). (**A**) CD3+ count. (**B**) CD3+ percentage. (**C**) CD3+CD4+ count. (**D**) CD3+CD4+ percentage. E. CD3 +CD8+ count. (**F**) CD3+CD8+ percentage. (**G**) Ratio of CD4+/CD8+ cells. The measurement data are expressed as χ±SD. Two-way ANOVA was used for comparisons between two subgroups from admission to the fourth week after admission (interaction, (**A–C, E** and **G**), all P<0.0001, (**D** and **F**), all P>0.05; row factor, (**B**) P<0.0001, (**G**) P<0.01, (**A**) and (**C–F**), all P>0.05; column factor, (**A–E** and **G**), all P<0.0001, (**F**), P>0.05). Unpaired *t*-tests were used for comparisons between the nonsevere group and the severe group at the same time point, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

$CD3^+CD4^+$ T cell percentages (Figure 9D) and $CD3^+CD8^+$ T cell percentages (Figure 9F) and the ratio of $CD3^+CD4^+$ T cells to $CD3^+CD8^+$ T cells (Figure 9G) remained stable from admission to the fourth week, $CD3^+$ T cell counts (Figure 9A), $CD3^+CD4^+$ T cell counts (Figure 9C) and $CD3^+CD8^+$ T cell counts (Figure 9E) declined in the first three weeks after admission and increased slightly at the fourth week, and $CD3^+$ T cell percentages (Figure 9B) remained stable from admission to the first week then increased from the first week to the fourth week. For the death group, those parameters remained similar trends and were always remarkably lower than those in the survival group (Figure 8A–F and Figure 9A–G).

Dynamic Characteristics of Lymphocyte Subsets within Five Weeks After Onset in COVID-19 Patients with Different Disease Severity

The lymphocyte subsets among different groups with different disease severity also showed similar dynamic changes from onset to five weeks after onset. Lymphocyte counts (Figure 10A), lymphocyte percentages (Figure 10B), $CD3^+$ T cell counts (Figure 10C) and $CD19^+$ B cell counts (Figure 10E) were all significantly higher in the asymptomatic group than those in the critically ill group and severe group, while those in the common group and mild group stayed in the middle. In addition, all parameters above showed a slight decrease in the asymptomatic group but demonstrated a small increase in the critically ill group and severe group. In all groups $CD3^+$ T cell percentages (Figure 10D), $CD19^+$ B cell percentages (Figure 10F), $CD56^+$ NK cell counts and percentages (Figure 10G and H) remained relatively stable or small fluctuations. In contrast, $CD56^+$ NK cell percentages (Figure 10H) remained stable at a low level in the asymptomatic group but showed a higher level in the critically ill group and severe group. Moreover, $CD3^+CD4^+$ T cell counts (Figure 11A) and $CD3^+CD8^+$ T cell counts (Figure 11C) were all significantly higher in the asymptomatic group than those in the critically ill group and severe group, while those in the common group and mild group stayed in the middle. In all groups $CD3^+CD4^+$ T cell percentages (Figure 11B), $CD3^+CD8^+$ T cell percentages (Figure 11D) and the ratio of $CD3^+CD4^+$ T cell to $CD3^+CD8^+$ T cell (Figure 11E) remained relatively stable or small fluctuations. In addition, all parameters above showed a slight decrease in the asymptomatic group but demonstrated a small increase in the critically ill group and severe group.

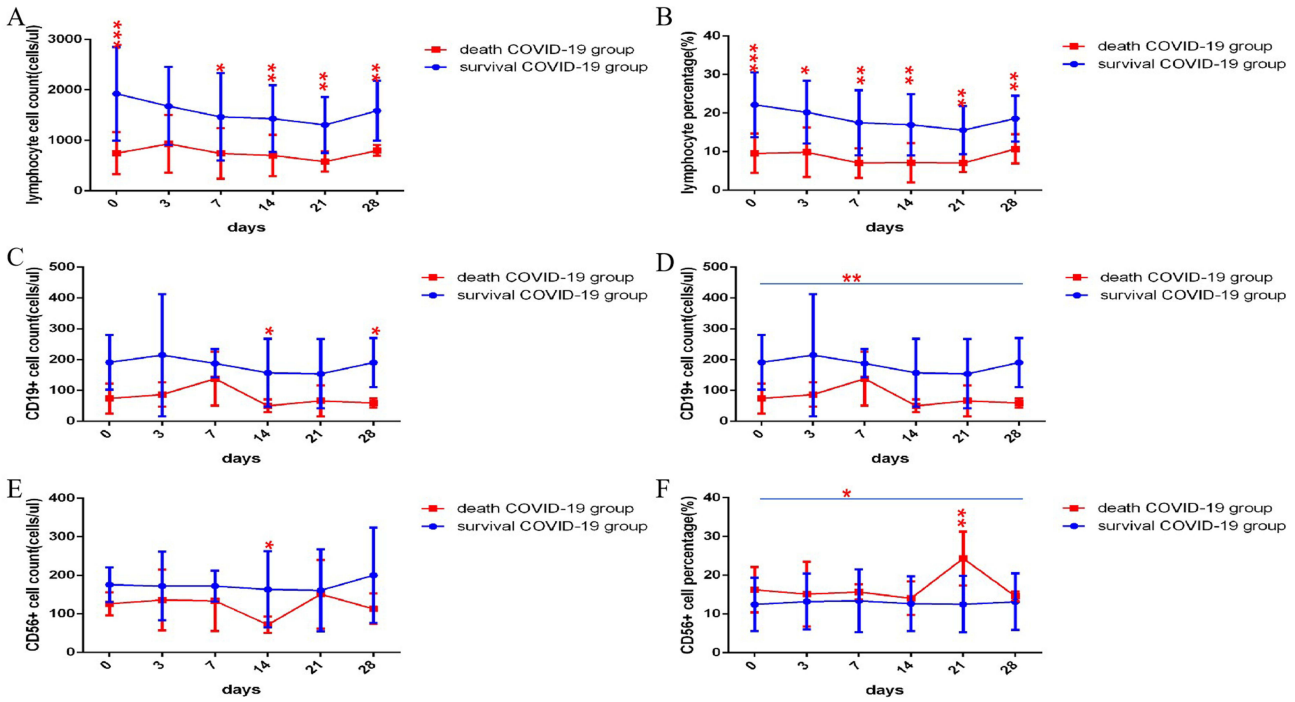

Figure 8 Comparison of dynamic characteristics of lymphocytes and B and NK cells and percentages among the surviving COVID-19 group and the nonsurviving COVID-19 group (n=718; the surviving COVID-19 group and the nonsurviving COVID-19 group, n=710 and 8, respectively). (**A**). Lymphocyte count. (**B**). Lymphocyte percentage. (**C**) CD19+ count. (**D**) CD19+ percentage. (**E**). CD56+ count. (**F**) CD56+ percentage. The measurement data are expressed as χ±SD. Two-way ANOVA was used for comparisons between two subgroups from admission to the fourth week after admission (interaction, (**A**–**C** and **E**), all P>0.05, (**D**) P<0.01, (**F**) P<0.05; row factor, (**A**–**C, E** and **F**), all P>0.05, (**D**) P<0.05; column factor, (**A**–**C** and **E**), all P<0.0001, (**D**), P<0.05, (**F**), P<0.001). Unpaired *t*-tests were used for comparisons between the surviving COVID-19 group and the nonsurviving COVID-19 group at the same time point, *P<0.05, **P<0.01, ***P<0.001.

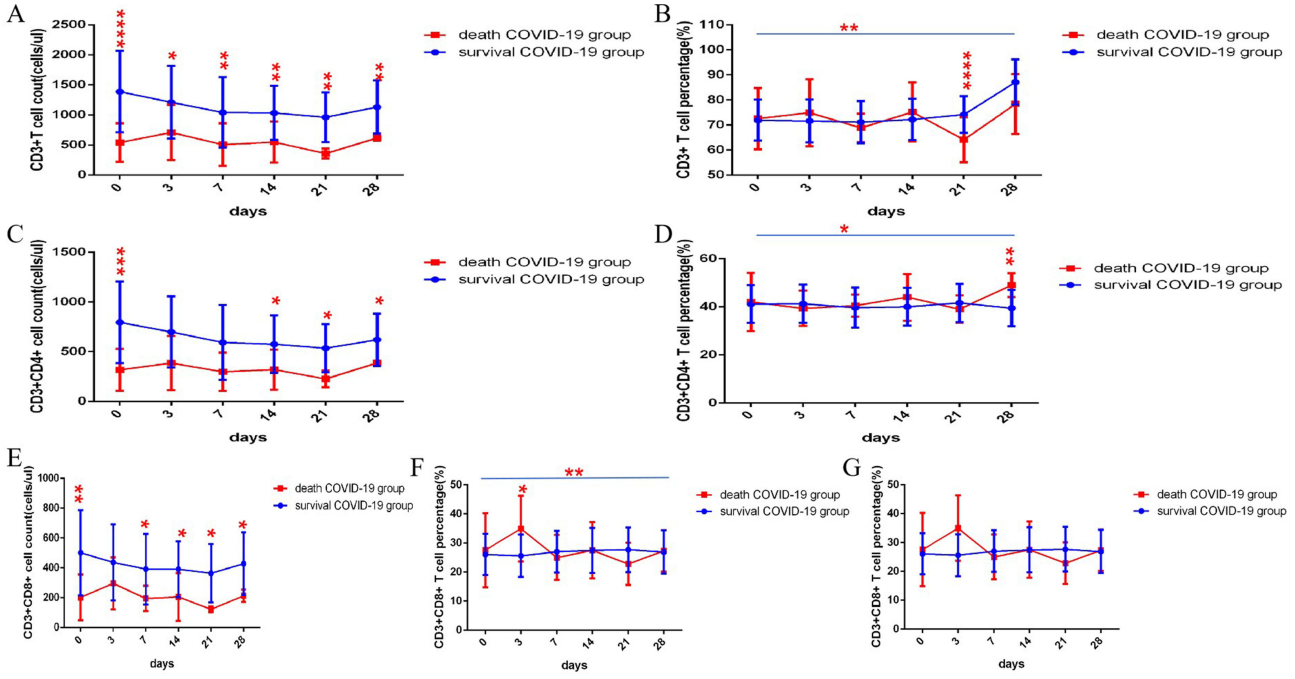

Figure 9 Comparison of dynamic characteristics of T lymphocyte levels and percentages among the nonsurviving COVID-19 group and the surviving COVID-19 group (n=718; death and survival group, n=8 and 710, respectively). (**A**). CD3+ count. (**B**). CD3+ percentage. C. CD3+CD4+ count. (**D**). CD3+CD4+ percentage. (**E**) CD3+CD8 + count. F. CD3+CD8+ percentage. (**G**). Ratio of CD4+/CD8+ cells. The measurement data are expressed as χ±SD. Two-way ANOVA was used for comparisons between two subgroups from admission to the fourth week after admission (interaction, (**A**–**E** and **G**, all P>0.05, F, P<0.01; row factor, **B**, P<0.0001, **G**, P<0.05, **A**–**F**, all P>0.05; column factor, **A**–**C**, all P<0.0001, **D**–**G**, all P>0.05). Unpaired *t*-tests were used for comparisons between the surviving COVID-19 group and the nonsurviving COVID-19 group at the same time point, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

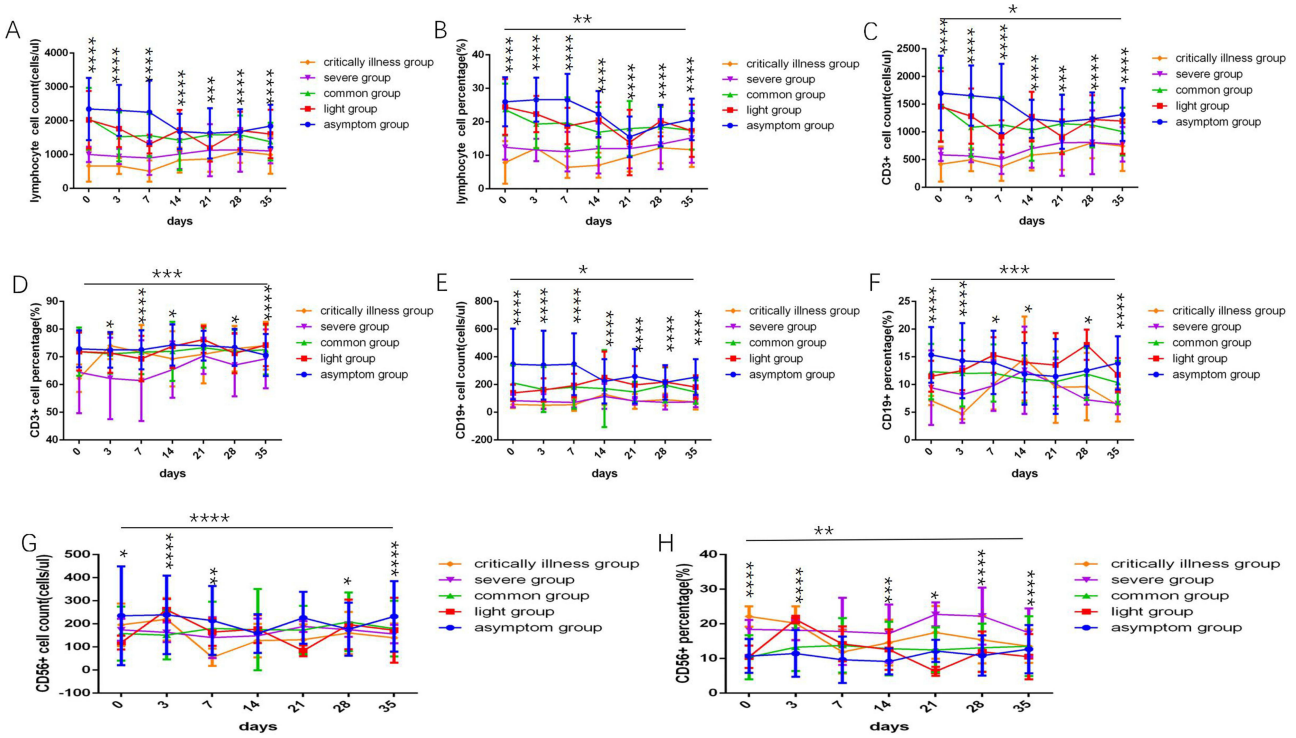

Figure 10 Comparison of the dynamic characteristics of lymphocytes and T, B and NK cell counts and percentages among the asymptomatic infection, light, common, severe, and critical illness COVID-19 groups (n=718; the asymptomatic infection, light, common, severe, and critical illness groups, n=237, 73, 371, 18 and 19, respectively). (**A**). Lymphocyte count. (**B**). Lymphocyte percentage. (**C**) T lymphocyte count. (**D**) T lymphocyte percentage. (**E**) B lymphocyte count. (**F**) B lymphocyte percentage. (**G**) NK lymphocyte count. (**H**) NK lymphocyte percentage. The measurement data are expressed as χ±SD. Two-way ANOVA was used for comparisons among subgroups from onset to the fifth week after onset (interaction, (**A, C**–**E** and **G**), P all>0.05, (**B** and **F**), all P<0.01, (**H**), (**G**), P all>0.05, (**B** and **F**), P all<0.01, (**D** and **H**), P all <0.05; column factor, (**A**–**F** and **H**), P all<0.0001, (**G**), P<0.05). One-way ANOVA was used for comparisons among groups at the same time point, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

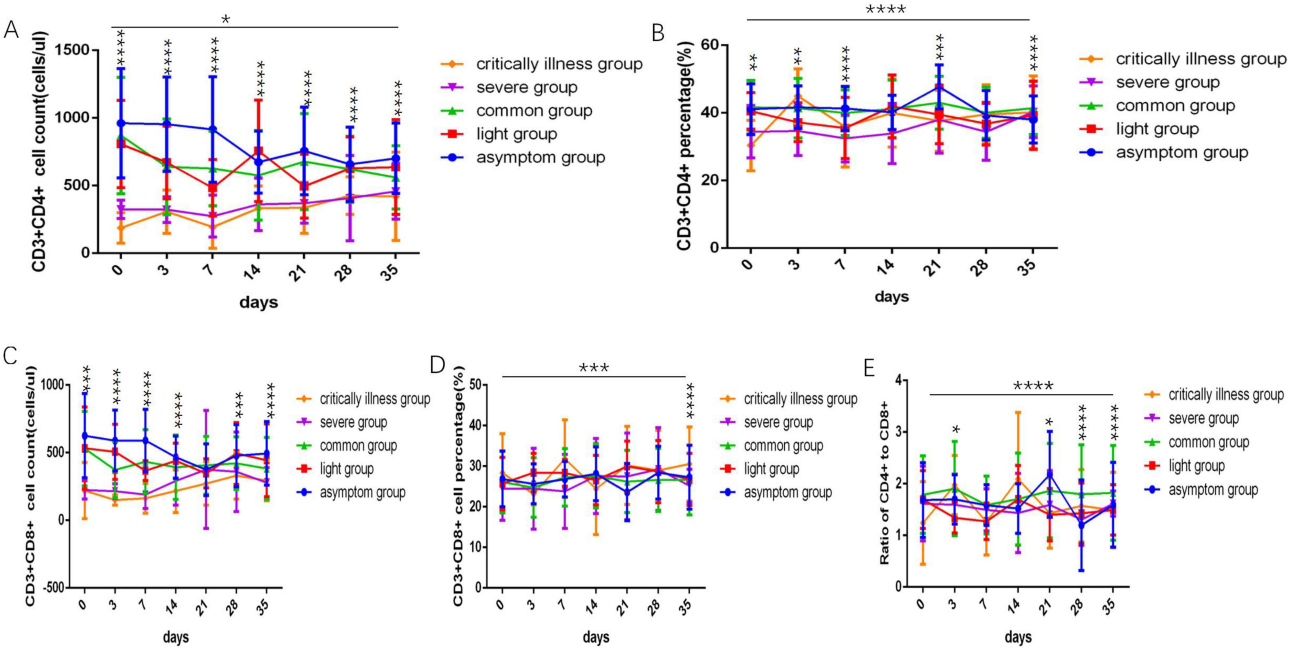

Figure 11 Comparison of dynamic characteristics of T lymphocyte subset counts and percentages among the asymptomatic infection, light, common, severe, and critical illness COVID-19 groups (n=718; the asymptomatic infection, light, common, severe, and critical illness groups, n=237, 73, 371, 18 and 19, respectively). (**A**). CD3+CD4+ count. (**B**). CD3+CD4+ percentage. (**C**) CD3+CD8+ count. (**D**). CD3+CD8+ percentage. (**E**). Ratio of CD4+/CD8+ cells. The measurement data are expressed as χ±SD. Two-way ANOVA was used for comparisons among groups from onset to the fifth week after onset (interaction, (**B**), P<0.05, (**A** and **C**–**E**), P>0.05; row factor, (**B**) P<0.05, (**A** and **C**–**E**), P>0.05; column factor, (**A**–**C**), P all<0.0001, (**D** and **E**), P all>0.05). One-way ANOVA was used for comparisons among subgroups at the same time point, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.

The Relationship of Disease Progression and Prognosis with Lymphocyte Subsets in Patients with COVID-19

Spearman correlation analysis (Table 3) reported that when considering patients' lymphocyte subsets at admission, disease severity was negatively correlated with lymphocyte counts (r=−0.166, P<0.0001), lymphocyte percentages (r=−0.179, P<0.0001), $CD3^+$ T cell counts (r=−0.173, P<0.0001), $CD3^+CD4^+$ T cell counts (r=−0.186, P<0.0001), $CD3^+CD8^+$ T cell counts (r=−0.127, P=0.004), $CD3^+$ T cell percentages (r=−0.117, P=0.008), and $CD3^+CD4^+$ T cell percentages (r=−0.149, P=0.001). Meanwhile, when considering patients' lymphocyte subsets at admission, the prognosis of death was negatively correlated with lymphocyte counts (r=−0.125, P=0.005), lymphocyte percentages (r=−0.121, P=0.006), $CD3^+$ T cell counts (r=−0.123, P=0.005), $CD3^+CD4^+$ T cell counts (r=−0.123, P=0.005), and $CD3^+CD8^+$ T cell counts (r=−0.091, P=0.04). In contrast, the prognosis of death was positively related to $CD56^+$ NK cell counts (r=0.204, P=0.033).

Receiver operating characteristic curve (ROC) analysis also found that lymphocyte subsets at onset had a good ability to distinguish severe cases and nonsevere cases, as well as surviving cases and dead cases. The cutoff points of different lymphocyte subsets, including lymphocyte counts, lymphocyte percentages, $CD3^+$ T cell counts and percentages, $CD3^+CD4^+$ T cell counts, $CD3^+CD8^+$ T cell counts and percentages, $CD19^+$ B cell counts and percentages and $CD56^+$ NK cell counts, are shown in Tables 4 and 5.

Table 3 Spearman Correlation Analysis of Disease Severity, Virus Negative Conversion Time, Prognosis and Lymphocytes and Subsets (n=718)

Variable	Disease severity (1=Nonsevere, 2=Severe)		Virus Negative Conversion Time (Day)		Prognosis (1=Survival, 2=Death)	
	r	p	r	p	r	p
LY (cells/ul)	−0.166	<0.0001	−0.266	<0.0001	−0.125	0.005
LY% (%)	−0.179	<0.0001	−0.311	<0.0001	−0.121	0.006
$CD3^+$ (cells/ul)	−0.173	<0.0001	−0.279	<0.0001	−0.123	0.005
$CD3^+CD4^+$ (cells/ul)	−0.186	<0.0001	−0.286	<0.0001	−0.123	0.005
$CD3^+CD8^+$ (cells/ul)	−0.127	0.004	−0.205	<0.0001	−0.091	0.040
$CD3^+$% (%)	−0.117	0.008				
$CD3^+CD4^+$%	−0.149	0.001	−0.102	0.022		
$CD3^+CD8^+$%			0.120	0.007		
The ratio of $CD4^+$ to $CD8^+$			−0.118	0.008		
$CD19^+$ (cells/ul)			−0.189	0.048		
$CD56^+$ (cells/ul)					0.204	0.033

Abbreviation: LY, lymphocytes.

Table 4 The Performance of Various Parameters at COVID-19 Onset for Distinguishing Between Severe Cases and Nonsevere Cases (n =718)

Variables	Cutoff point	AUC (95% CI)	Sensitivity	Specificity	False Positive	False Negative
Lymphocyte (cells/ul)	448	1.000(1.000~1.000)	100.00%	100.00%	0.00%	0.00%
Lymphocyte (%)	5.575	1.000(1.000~1.000)	100.00%	100.00%	0.00%	0.00%
$CD3^+$ (cells/ul)	340	1.000(1.000~1.000)	100.00%	100.00%	0.00%	0.00%
$CD3^+CD4^+$(cells/ul)	161	1.000(1.000~1.000)	100.00%	100.00%	0.00%	0.00%
$CD3^+CD8^+$(cells/ul)	131	1.000(1.000~1.000)	100.00%	100.00%	0.00%	0.00%
$CD3^+$%(%)	65.865	0.824(0.695~0.952)	82.40%	100.00%	17.60%	0.00%
$CD3^+CD8^+$%(%)	18.02	0.912(0.816~1.000)	91.20%	100.00%	7.80%	0.00%
$CD19^+$ (cells/ul)	30	1.000(1.000~1.000)	100.00%	100.00%	0.00%	0.00%
$CD56^+$ (cells/ul)	62	0.853(0.734~0.972)	85.30%	100.00%	14.70%	0.00%
$CD19^+$%(%)	7.98	0.971(0.914~1.000)	97.10%	100.00%	2.90%	0.00%

Abbreviations: AUC, area under the curve; CI, confidence interval.

Table 5 The Performance of Various Parameters at COVID-19 Onset for Distinguishing Between Surviving Cases and Dead Cases (n =718)

Variables	Cutoff Point	AUC (95% CI)	Sensitivity	Specificity	False Positive	False Negative
Lymphocyte (cells/ul)	448	1.000(1.000~1.000)	100.00%	100.00%	0.00%	0.00%
Lymphocyte (%)	5.575	1.000(1.000~1.000)	100.00%	100.00%	0.00%	0.00%
$CD3^+$ (cells/ul)	340	1.000(1.000~1.000)	100.00%	100.00%	0.00%	0.00%
$CD3^+CD4^+$(cells/ul)	161	1.000(1.000~1.000)	100.00%	100.00%	0.00%	0.00%
$CD3^+CD8^+$(cells/ul)	131	1.000(1.000~1.000)	100.00%	100.00%	0.00%	0.00%
$CD3^+$%(%)	65.865	0.824(0.695~0.952)	82.40%	100.00%	17.60%	0.00%
$CD3^+CD8^+$%(%)	18.02	0.912(0.816~1.000)	91.20%	100.00%	7.80%	0.00%
$CD19^+$ (cells/ul)	30	1.000(1.000~1.000)	100.00%	100.00%	0.00%	0.00%
$CD56^+$ (cells/ul)	62	0.853(0.734~0.972)	97.10%	100.00%	2.90%	0.00%
$CD19^+$%(%)	7.98	0.971(0.914~1.000)	85.30%	100.00%	14.70%	0.00%

Abbreviations: AUC, area under the curve; CI, confidence interval.

Discussion

COVID-19, caused by a virus called SARS-CoV-2, can cause a systemic inflammatory response and significant tissue damage, leading to rapid disease progression and even death. In this condition, white blood cell populations, including monocytes, lymphocytes and neutrophils, play a critical role.

Our study found that nonsevere and severe COVID-19 patients, survivors and nonsurvivors of COVID-19 infection, demonstrated different lymphocyte subsets, whether at admission, four weeks after admission, or five weeks after disease onset. At admission, compared with nonsevere COVID-19 patients, severe COVID-19 patients demonstrated lower lymphocyte counts and percentages, $CD3^+$ T cell counts and percentages, $CD19^+$ B cell counts and percentages, $CD56^+$ NK cell counts, $CD3^+CD4^+$ T cell counts and percentages and $CD3^+CD8^+$ T cell counts. Meanwhile, at admission, nonsurvivors also had lower lymphocyte counts and percentages, $CD3^+$ T cell counts, $CD3^+CD4^+$ T cell counts, and $CD3^+CD8^+$ T cell counts. For the follow-up period after admission or onset, the nonsevere group showed higher lymphocyte counts and percentages, $CD19^+$ B cell counts, $CD56^+$ NK cell percentages, $CD3^+$ T cell counts, $CD3^+CD4^+$ T cell counts and $CD3^+CD8^+$ T cell counts than the severe group. These phenomena were also found between survivors and nonsurvivors. Therefore, it seemed that the lymphocyte subsets mentioned above were correlated with disease severity and prognosis. Our Spearman correlation analysis proved that when considering patients' lymphocyte subsets at admission, disease severity was negatively correlated with lymphocyte counts and percentages, $CD3^+$ T cell counts and percentages, $CD3^+CD4^+$ T cell counts and percentages, and $CD3^+CD8^+$ T cell percentages. Meanwhile, when considering patients' lymphocyte subsets at admission, the prognosis of death was negatively correlated with lymphocyte counts and percentages, $CD3^+$ T cell counts, $CD3^+CD4^+$ T cell counts, and $CD3^+CD8^+$ T cell counts. Furthermore, our ROC analysis also found that lymphocyte subsets at COVID-19 onset had a good capacity to distinguish severe and nonsevere cases, as well as survivors and nonsurvivors. These findings showed that in primary infection and unvaccinated COVID-19 patients total lymphocytes and T cell, B cell and NK cell subsets at COVID-19 onset play valuable roles in predicting disease severity and prognosis.

Our results were consistent with previous studies. Several observational studies have suggested the relationship between immune parameters and clinical severity and disease prognosis. Lymphopenia seemed to be the leading characteristic of COVID-19-infected patients, as a great number of clinical studies have reported decreased lymphocyte numbers in a large proportion of COVID-19 patients.[9,31–42] In addition, some studies further suggested that absolute numbers of lymphocytes were correlated with disease severity: significantly lower levels of lymphocyte numbers were noticed in severe and critically ill COVID-19 cases than in mild cases.[31,43,44] Increases in CD4+ and particularly CD8+ T lymphocyte counts were also noticed in severe COVID-19 patients after treatment.[45] Some studies have also suggested the role of a higher neutrophil-to-lymphocyte ratio (NLR) in predicting the severity of COVID-19.[46,47]

For subpopulations of lymphocytes, remarkable differences in $CD4^+$ and $CD8^+$ T cells were noticed between mild and severe/critical COVID-19 patients.[42] Furthermore, some studies also suggested cut-off points for different lymphocyte subsets

to predict further disease deterioration and in-hospital death. Diao et al[48] reported that for nonintensive care unit patients, those with total T cell counts lower than 800/μL, CD4+ T cell counts lower than 400/μL, or CD8+ T cell counts lower than 300/μL were prone to further deterioration. Moreover, Xu et al[49] also found that lower levels of T lymphocyte counts and their subsets (total CD3+ T cells<200/μL, $CD4^+$ T cells<100/μL, and $CD8^+$ T cells<100/μL) were remarkably associated with an increased risk of in-hospital death owing to COVID-19. Our ROC analysis also demonstrated that lymphocyte counts and their subsets at disease onset had a good capacity to predict future disease severity and prognosis. The cutoff points were as follows: lymphocyte counts 448/μL, lymphocyte percentages 5.575%, $CD3^+$ T cell counts 340/μL, $CD3^+$ T cell percentages 65.865%, $CD3^+CD4^+$ T cell counts 161/μL, $CD3^+CD8^+$ T cell counts 131/μL, $CD3^+CD8^+$ T cell percentages 65.865%, $CD19^+$ B cell counts 30/μL, $CD19^+$ B cell percentages 7.98%, and $CD56^+$ NK cell counts 62/μL.

In contrast, with decreased levels of most lymphocyte subsets, including $CD3^+$ T cells, $CD4^+$ T cells, $CD8^+$ T cells and $CD19^+$ B cells, $CD56^+$ NK cells were the only subset that had an elevated level in more severe COVID-19 cases. However, our findings were different from previous results that reported a decreased level of NK cell counts in patients with more severe disease.[50–52] This inconsistency might be attributed to our small sample size of severe cases. Kram et al[52] also found that NK cells were functionally impaired and expressed higher levels of the apoptosis markers active caspase-3 and CD95.

Those above findings supported lymphocytes and subsets as predictive biomarkers and potential therapeutic targets in primary infection and unvaccinated COVID-19 patients. The mechanism behind lower lymphocyte counts and subsets in more severe COVID-19 cases might be the result of inflammatory responses and cytokine storms induced by virus infection. Ni et al[45] found that the levels of proinflammatory factors increased significantly in severe COVID-19 cases, and most of these factors, including IL-2R, IL-6, TNF-α, and CRP, decreased significantly after treatment. These proinflammatory factors can lead to lymphocyte activation initially; however, continuous active responses can subsequently contribute to lymphocyte exhaustion. A previous study also reported increased numbers of activated $CD8^+$ T cells and NK cell populations in COVID-19 patients, and with increasing severity, failure of clonal expansion in $CD8^+$ T effector and central memory cells and depletion of COVID-19 clonotypes were observed.[53] Furthermore, associations with severity for exhaustion markers and specific activated NK and CD69+ MAIT cell populations were also noticed.[53] Meanwhile, CD4+ and CD8+ cell exhaustion, with increased concentration of proinflammatory cytokines and chemokines and decreased T regulatory cells, could lead to an excessive inflammatory response that is out of control, which causes a vicious circle.[54]

It has been widely recognized that immune function declines with age.[55,56] Our study found that healthy controls and COVID-19 patients, whether nonsevere COVID-19 patients or sever COVID-19 patients, whether survivors of COVID-19 infection or nonsurvivors of COVID-19 infection, all demonstrated different lymphocyte subsets at admission. Compared with healthy controls, nonsevere COVID-19 patients and survivors of COVID-19 infection all demonstrated higher lymphocyte counts and percentages, CD3+ T cell counts, CD3+CD4+ T cell counts, CD3+CD8+ T cell counts and percentages, lower CD19+ B cell percentages, CD56+ NK cell counts and percentages, CD3+CD4+ T cell percentages and the ratio of CD3+CD4+ T cells to CD3+CD8+ T cells, and nonsevere COVID-19 patients also demonstrated lower CD19+ B cell counts. Compared with healthy controls, severe COVID-19 patients and nonsurvivors of COVID-19 infection all demonstrated lower lymphocyte counts and percentages, CD3+ T cell counts, CD19+ B cell counts and percentages, CD56+ NK cell counts, CD3+CD4+ T cell counts and CD3+CD8+ T cell counts, higher CD3+CD8+ T cell percentages. Severe COVID-19 patients also demonstrated lower CD3+ T cell percentages, CD3+CD4+ T cell percentages and the ratio of CD3+CD4+ T cells to CD3+CD8+ T cells. These changes may be age-related, but not exclusively age-related, as nonsevere COVID-19 patients and survivors of COVID-19 infection were significantly younger than healthy controls. These changes showed that these nonsevere COVID-19 patients and survivors of COVID-19 infection did not respond to the infection with a robust immune response. Because of these nonsevere COVID-19 patients and survivors of COVID-19 infection were primary infection and unvaccinated COVID-19 patients, so these changes was not due to already pre-infection. Although these nonsevere COVID-19 patients and survivors of COVID-19 infection had higher lymphocyte and T cell subset, but B cell and NK cell subset, CD4+ T cell percentages and the ratio of CD4+ T cells to CD8+ T cells were decreased. Moreover, although severe COVID-19 patients were similar old as healthy controls, and nonsurvivors of COVID-19 infection were significantly older than healthy controls, severe COVID-19 patients and nonsurvivors of COVID-19 infection all had overall decreased lymphocyte and T cell, B cell and NK cell subsets, these changes showed that severe COVID-19 patients and nonsurvivors of COVID-19 infection responded to the

infection with a robust immune response. Previous studies showed that in old age the innate immune system is characterized by chronic low-grade inflammation and a simultaneous reduction of an adequate response to pathogens, the adaptive immune system is characterized by a reduced hematopoiesis of new naïve lymphocytes and a reduction of diversity describe.[56] Immunosenescence, as age-related impairment of immune function, play an important role in age-related morbidity and mortality, and infectious disease.One aspect of immunosenescence is age-related natural killer (NK) cell dysfunction, demonstrated decreased target cell cytotoxicity and reduced cytokine secretion, increased NK cell numbers, which play central actors in the immunosurveillance of senescent cells. NK cell dysfunction is implicated in the increasing burden of infection, malignancy, inflammatory disorders, and senescent cells with age.[55]

This study findings showed that in primary infection and unvaccinated COVID-19 patients total lymphocytes and T cell, B cell and NK cell subsets at COVID-19 onset play valuable roles in predicting disease severity and prognosis. But this is not COVID-19 specific, not even infection specific, and a general and well-known observation for patients ending up in ICU. A study found that Mpox patients showed as elevated CD3+CD8+T counts and inverted ratio of CD3+CD4+T cell to CD3 +CD8+T cell, significantly decreased CD3+CD4+T counts in mpox patients co-infected with HIV compared to the pre-infection level.[57] Other studies found that ICU patients with acquired infection had significantly lower lymphocyte sub-population counts (CD3+ T cells, CD3+CD4+ T cells, CD3+CD8+T cells, CD16+CD56+ natural killer (NK) cells and CD19+ B cells) compared those without acquired infection, CD3+T cells and CD3+CD4+ T cells were independent significant risk factors for ICU-acquired infections,[21] and lymphopenia and T cell depletion at ICU admission were associated with increased mortality of ICU patients.[22–24] Those above findings supported lymphocytes and subsets as predictive biomarkers and potential therapeutic targets in intensive care medicine, yet the underlying mechanisms remain unknown.

It is clear that SARS-CoV-2 infection results in a good and robust immune response. However, not in all patients. The reason for this unknown, and likely not SARS-CoV-2 specific, but may be due to low cell counts at the start or other reasons these subjects are more vulnerable. However, the initial blood assessment can provide a good indication of the level of treatment required and predict disease progression.

There were several limitations in our study. First, it was a single-center, retrospective study. Second, the numbers of control cases and severe cases, especially dead cases, were small. Therefore, some bias may be caused by the imbalanced patient numbers between the nonsevere and severe groups and the survival and death groups.

Conclusions

In primary infection and unvaccinated COVID-19 patients total lymphocytes and T cell, B cell and NK cell subsets at COVID-19 onset play valuable roles in predicting disease severity and prognosis. These findings provide a reference for clinicians for the early identification of patients with immunodeficiency and timely immunomodulatory treatment to slow disease progression and improve prognosis.

Data Sharing Statement

All data, models, or code generated or used during the study are available from the corresponding author by request: Dafeng Liu, E-mail: liudf312@126.com.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Public and Health Clinic Centre of Chengdu (ethics approval number: PJ-K2020-26-01), and the Ethics Committee waived written informed consent because of emerging infectious diseases.

Consent for Publication

All of the participants understand that the information will be published without their child or ward's/their relative's (circle as appropriate) name attached but that full anonymity cannot be guaranteed. All of the participants understand that the text and any pictures or videos published in the article will be freely available on the internet and may be seen by the general public. The pictures, videos and text may also appear on other websites or in print and may be translated into other languages or used for commercial purposes. All of the participants were offered the opportunity to read the manuscript.

Acknowledgments

We thank Dr. Xiu Li and Yaling Liu (the Public and Health Clinic Center of Chengdu, rehabilitation division).

This manuscript has been presented as a preprint in the research section according to the following link: https://www.researchsquare.com/article/rs-1121580/private/timeline, https://www.researchsquare.com/article/rs-1048607/private/timeline.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This research was supported by the Chengdu Science and Technology Bureau (2021-YF05-00536-SN).

Disclosure

The authors declare that they have no competing interests in this work.

References

1. Wu G, Gao GF, Tan W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. 2020;382(8):727–733. doi:10.1056/NEJMoa2001017
2. Lu H, Stratton CW, Tang YW. Outbreak of pneumonia of unknown etiology in Wuhan China: the Mystery and the Miracle. *J Med Virol*. 2020;92(4):401–402. doi:10.1002/jmv.25678
3. Ji W, Wang W, Zhao X, et al. Cross-species transmission of the newly identified coronavirus 2019-nCoV. *J Med Virol*. 2020;92(4):433–440. doi:10.1002/jmv.25682
4. Gates B. Responding to COVID-19- A once-in-a-century pandemic? *N Engl J Med*. 2020;382(18):1677–1679. doi:10.1056/NEJMp2003762
5. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. *Nature*. 2020;579:265–269. doi:10.1038/s41586-020-2008-3
6. World Health Organization. *COVID-19 weekly Epidemiological Update*. Geneva: World Health Organization; 2023.
7. Attaway AH, Scheraga RG, Bhimraj A, Biehl M, Hatipoğlu U. Severe covid-19 pneumonia: pathogenesis and clinical management. *BMJ*. 2021;372:n436. doi:10.1136/bmj.n436
8. National Health Commission of the People's Republic of China. The seventh trial version of the novel coronavirus pneumonia diagnosis and treatment guidance. Available from: http://www.nhc.gov.cn/xcs/zhengcwj/202003/46c9294a7dfe4cef80dc7f5912eb1989.shtml. Accessed October 08, 2024.
9. Huang C, Wang Y, Li X, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;395(10223):497–506. doi:10.1016/S0140-6736(20)30183-5
10. Chen N, Zhou M, Dong X, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. *Lancet*. 2020;395(10223):507–513. doi:10.1016/S0140-6736(20)30211-7
11. Wang D, Hu B, Hu C, et al. Clinical characteristics of 138hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. *JAMA*. 2020;323(11):1061–1069. doi:10.1001/jama.2020.1585
12. Guan WJ, Ni ZY, Hu Y, et al. Clinical Characteristics of 2019 Novel Coronavirus Infection in China. *N Engl J Med*. 2020;382(18):1708–1720. doi:10.1056/NEJMoa2002032
13. Wilson N, Kvalsvig A, Barnard LT, et al. Case-fatality risk estimates for COVID-19 calculated by using a lag time for fatality. *Emerg Infect Dis*. 2020;26(6):1339–1441. doi:10.3201/eid2606.200320
14. de Almeida-Pititto B, Dualib PM, Zajdenverg L, et al.; Brazilian Diabetes Society Study Group (SBD). Severity and mortality of COVID 19 in patients with diabetes, hypertension and cardiovascular disease: a meta-analysis. *DiabetolMetabSyndr*. 2020;12:75.
15. Qin C, Zhou L, Hu Z, et al. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. *Clin Infect Dis*. 2020;71(15):762–768. doi:10.1093/cid/ciaa248
16. Liu D, Lan L, Luo D, et al. Lymphocyte subsets with the lowest decline at baseline and the slow lowest rise during recovery inCOVID-19 critical illness patients with diabetes mellitus. *Diabet Res Clin Pract*. 2020;167:108341. doi:10.1016/j.diabres.2020.108341
17. Liu D, Wang Y, Zhao B, et al. Overall reduced lymphocyte especially T and B subsets closely related to the poor prognosis and the disease severity in severe patients with COVID-19 and diabetes mellitus. *Diabetol Metab Syndr*. 2021;13(1):5. doi:10.1186/s13098-020-00622-3
18. Liu D, Zheng Y, Kang J, et al. Not only high number and specific comorbidities but additionally, age are closely related to progression and poor prognosis in patients with COVID-19. *Front.Med*;2022. 736109. doi:10.3389/fmed.2021.736109
19. Liu D, Yuan X, Gao F, et al. High Number and Specific Comorbidities Could Impact the Immune Response in COVID-19 Patients. *Front Immunol*. 2022;13:899930. doi:10.3389/fimmu.2022.899930
20. Mahallawi WH, Khabour OF, Zhang Q, et al. MERS-CoV infection in humans is associated with a pro-inflammatory Th1 and Th17cytokine profile. *Cytokine*. 2018;104:8–13. doi:10.1016/j.cyto.2018.01.025

21. Zhao J, Dai RS, Chen YZ, Zhuang YG. Prognostic significance of lymphocyte subpopulations for ICU-acquired infections in patients with sepsis: a retrospective study. *J Hosp Infect*. 2023;140:40–45. doi:10.1016/j.jhin.2023.05.022
22. Sheikh Motahar Vahedi H, Bagheri A, Jahanshir A, Seyedhosseini J, Vahidi E. Association of lymphopenia with short term outcomes of sepsis patients; a brief report. *Arch Acad Emerg Med*. 2019;7(1):e14.
23. Hohlstein P, Gussen H, Bartneck M, et al. Prognostic relevance of altered lymphocyte subpopulations in critical illness and sepsis. *J Clin Med*. 2019;8(3):353. doi:10.3390/jcm8030353
24. Luperto M, Zafrani L. T cell dysregulation in inflammatory diseases in ICU. *Intensive Care Med Exp*. 2022;10(1):43. doi:10.1186/s40635-022-00471-6
25. Lin L, Luo S, Qin R, et al. Long-term infection of SARS-CoV-2changed the body's immune status. *Clin Immunol*. 2020;218:108524. doi:10.1016/j.clim.2020.108524
26. Luo Y, Mao L, Yuan X, et al. Prediction model based on the combination of cytokines and lymphocyte subsets for prognosis ofSARS-CoV-2 infection. *J Clin Immunol*. 2020;40(7):960–969. doi:10.1007/s10875-020-00821-7
27. Wen X, Jiang D, Gao L, et al. Clinical characteristics and predictive value of lower CD4 + T-cell level in patients with moderate and severeCOVID-19: a multicenter retrospective study. *BMC Infect Dis*. 2021;21:57. doi:10.1186/s12879-020-05741-w
28. Zhang W, Li L, Liu J, et al. The characteristics and predictive role of lymphocyte subsets in COVID-19 patients. *Int J Infect Dis*. 2020;99:92–99. doi:10.1016/j.ijid.2020.06.079
29. Rezaei M, Marjani M, Mahmoudi S, Mortaz E, Mansouri D. Dynamic changes of lymphocyte subsets in the course of COVID-19. *Int Arch Allergy Immunol*. 2021;182(3):254–262. doi:10.1159/000514202
30. Wong CK, Lam CW, Wu AK, et al. Plasma inflammatory cytokines and chemokines in severe acute respiratory syndrome. *Clin Exp Immunol*. 2004;136(1):95–103. doi:10.1111/j.1365-2249.2004.02415.x
31. Chen G, Wu D, Gue W, et al. Clinical and immunological features in severe and moderate coronavirus disease. *J Clin Investig*. 2019;130:2620–2629. doi:10.1172/JCI137244
32. Ding Q, Lu P, Fan Y, Xia Y, Liu M. The clinical characteristics of pneumonia patients coinfected with 2019 novel coronavirus and influenza virus in Wuhan, China. *J Med Virol*. 2020;92:1549–1555. doi:10.1002/jmv.25781
33. Guan W, Ni Z, Hu Y, et al. Clinical characteristics of coronavirus disease 2019 in China. *N Engl J Med*. 2020;382:1708–1720. doi:10.1056/NEJMoa2002032
34. Han R, Huang L, Jiang H, Dong J, Peng H, Zhang D. Early clinical and CT manifestations of coronavirus disease 2019 (COVID-19) pneumonia. *AJR Am J Roentgenol*. 2020;17:1–6. doi:10.2214/AJR.20.22961
35. Lin L, Lu L, Cao W, Li T. Hypothesis for potential pathogenesis of SARS-CoV-2 infection – a review of immune changes in patients with viral pneumonia. *Emerg Microb Infect*. 2020;9:727–732. doi:10.1080/22221751.2020.1746199
36. Liu K, Chen Y, Lin R, Han K. Clinical feature of COVID-19 in elderly patients: a comparison with young and middle-aged patients. *J Infect*. 2020;80:e14–e18. doi:10.1016/j.jinf.2020.03.005
37. Lupia T, Scabini S, Pinna SM, Di Perri G, De Rosa FG, Corcione S. 2019-novel coronavirus outbreak: a new challenge. *J Glob Antimicr Res*. 2020;21:22–27. doi:10.1016/j.gar.2020.02.021
38. Mo P, Xing Y, Xiao Y, et al. Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China. *Clin Infect Dis*. 2020. doi:10.1093/cid/ciaa270
39. Sun S, Cai X, Wang H, et al. Abnormalities of peripheral blood system in patients with COVID-19 in Wenzhou, China. *Clin. Chim. Acta*. 2020;507:174–180. doi:10.1016/j.cca.2020.04.024
40. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China. summary of a report of 72 314 cases from the Chinese center for disease control and prevention. *JAMA*. 2020;323:1239. doi:10.1001/jama.2020.2648
41. Yang W, Cao Q, Qin L, et al. Clinical characteristics and imaging manifestations of the 2019 novel coronavirus disease (COVID-19): a multi-center study in Wenzhou city, Zhejiang, China. *J Infect*. 2020;86:388–393. doi:10.1016/j.jinf.2020.02.016
42. Zhang MQ, Wang XH, Chen YL, et al. Clinical features of 2019 novel coronavirus pneumonia in the early stage from a fever clinic in Beijing. *Zhonghua Jie He He Hu Xi Za Zhi*. 2020;43:215–218. doi:10.3760/cma.j.issn.1001-0939.2020.0013
43. Peng YD, Meng K, Guan HQ, et al. Clinical characteristics and outcomes of 112 cardiovascular disease patients infected by 2019-nCoV. *Zhonghua Xin Xue Guan Bing Za Zhi*. 2020;48:E004. doi:10.3760/cma.j.cn112148-20200220-00105
44. Wan S, Yiang Y, Fang W, et al. Clinical features and treatment of COVID-19 patients in Northeast Chongqing. *J Med Virol*. 2020;92:797–806. doi:10.1002/jmv.25783
45. Ni M, Tian FB, Xiang DD, Yu B. Characteristics of inflammatory factors and lymphocyte subsets in patients with severe COVID-19. *J Med Virol*. 2020;92(11):2600–2606. doi:10.1002/jmv.26070
46. Jimeno S, Ventura PS, Castellano JM, et al. Prognostic implications of neutrophil-lymphocyte ratio in COVID-19. *Eur J Clin Invest*. 2021;51(1):e13404. doi:10.1111/eci.13404
47. Buonacera A, Stancanelli B, Colaci M, Malatino L. Neutrophil to lymphocyte ratio: an emerging marker of the relationships between the immune system and diseases. *Int J Mol Sci*. 2022;23(7):3636. doi:10.3390/ijms23073636
48. Diao B, Wang C, Tan Y, et al. Reduction and functional exhaustion of T cells in patients with coronavirus diseases 2019 (COVID-19). *Front Immunol*. 2020;11:827. doi:10.3389/fifimmu.2020.00827
49. Xu B, Fan CY, Wang AI, et al. Suppressed T cell-mediated immunity in patients with COVID-19: a clinical retrospective study in Wuhan, China. *J Infect*. 2020;81:e51–e60. doi:10.1016/j.jinf.2020.04.012
50. Li M, Guo W, Dong Y, et al. Elevated exhaustion levels of NK and CD8(+) T Cells as indicators for progression and prognosis of COVID-19 disease. *Front Immunol*. 2020;11:580237. doi:10.3389/fimmu.2020.580237
51. Qin R, He L, Yang Z, et al. Identification of parameters representative of immune dysfunction in patients with severe and fatal COVID-19 infection: a systematic review and meta-analysis. *Clin Rev Allergy Immunol*. 2023;64(1):33–65. doi:10.1007/s12016-021-08908-8
52. Krämer B, Knoll R, Bonaguro L, et al. Early IFN-α signatures and persistent dysfunction are distinguishing features of NK cells in severe COVID-19. *Immunity*. 2021;54(11):2650–69.e14. doi:10.1016/j.immuni.2021.09.002
53. Ahern DJ, Ai Z, Ainsworth M. A blood atlas of COVID-19 defines hallmarks of disease severity and specificity. *Cell*. 2022;185(5):916–38.e58. doi:10.1016/j.cell.2022.01.012

54. Jesenak M, Brndiarova M, Urbancikova I, et al. Immune parameters and COVID-19 infection - associations with clinical severity and disease prognosis. *Front Cell Infect Microbiol*. 2020;10:364. doi:10.3389/fcimb.2020.00364
55. Brauning A, Rae M, Zhu G, et al. Aging of the immune system: focus on natural killer cells phenotype and functions. *Cells*. 2022;11(6):1017. doi:10.3390/cells11061017
56. Großkopf A, Simm A. Alterung des Immunsystems [Aging of the immune system]. *Z Gerontol Geriatr*. 2022;55(7):553–557. doi:10.1007/s00391-022-02107-6
57. Zhao B, Liu Q, Du Q, et al. Characteristics and differences in mpox patients with and without hiv infection: a retrospective cross-sectional study in Chengdu, China. *Int J Gen Med*. 2024;17:1381–1393. doi:10.2147/IJGM.S456198

International Journal of General Medicine Dovepress

Publish your work in this journal

The International Journal of General Medicine is an international, peer-reviewed open-access journal that focuses on general and internal medicine, pathogenesis, epidemiology, diagnosis, monitoring and treatment protocols. The journal is characterized by the rapid reporting of reviews, original research and clinical studies across all disease areas. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/international-journal-of-general-medicine-journal