Decreased Counts of T Lymphocyte Subsets Predict Prognosis in SARS-CoV-2-Infected Pneumonia in Wuhan, China: a Retrospective Study

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Abstract

Background The outbreak and the rampant spread of SARS-CoV-2-infected pneumonia (COVID-19) first identified in Wuhan, China, has infected thousands of patients and killed more than two thousand. We aimed to find indicators that could predict the progression of SARS-CoV-2 pneumonia.

Methods Medical history, clinical features, laboratory and radiological results were retrospectively reviewed from 112 patients with clinically diagnosed SARS-CoV-2 pneumonia in Renmin Hospital of Wuhan University from Jan 1 to Jan 31, 2020. Clinical outcomes were followed up to Feb 9, 2020.

Results Based on their outcomes, we divided these patients into groups of remission, deterioration and death respectively, and analyzed the counts of lymphocyte and its subsets. A decreased combination of CD3+, CD4+ and CD8+ T lymphocyte counts was observed as the SARS-CoV-2 pneumonia progressed. Among them, the CD4+ T lymphocyte counts were reduced at the early stage, while CD8+ counts were decreased at advanced stage or end stage.

Conclusions We identified in our study of 112 hospitalized patients that CD3+, CD4+ and CD8+ T lymphocyte counts were useful markers to predict the clinical progression of SARS-CoV-2 pneumonia at different stages. Considering the large number of patients with severe pneumonia, and the urgency of this ongoing global public health emergency, the counts of lymphocyte and its subsets from laboratory examinations could be easy and useful indicators for physicians to determine a timely and proper therapeutic strategy for patients and an early warning sign for predicting or reducing mortality in SARS-CoV-2-infected pneumonia.

Background

Since December 2019, an outbreak of novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diseases (COVID-19) has occurred in Wuhan, Hubei Province, and spread rapidly to other parts of China and abroad within a short period of time, which challenged the public health [1–4]. Studies have shown that at an early stage of the outbreak, the majority of the infected were epidemiologically linked to Huanan Seafood Wholesale Market where wild animals might be illegally sold, but the current mode of transmission is mainly from person to person [5–8]. As of February 17, 2020, there were a total of 71902 cases confirmed and 1775 deaths in 30 countries/regions, most of which occurred in the center of the epidemic area, Wuhan, Hubei Province, China [9]. Depending on the severity of the clinical symptoms and medical examination results, clinical syndromes associated with SARS-CoV-2 infection encompass a broad spectrum of conditions, from uncomplicated illness, through mild pneumonia, to severe pneumonia and ultimately acute respiratory distress syndrome (ARDS), septic shock and other serious complications [10, 11]. Although China has more than 80 clinical trials on potential treatments for SARS-CoV-2 infection [12], there are currently no specific drugs and vaccines. The prognosis of SARS-CoV-2-infected pneumonia depends largely on the immune capacity of the body, in addition to supportive therapies.

Based on phylogeny, taxonomy and established practice, the 2019 novel coronavirus has been officially named SARS-CoV-2 [13], despite there is considerable genetics distance between SARS-CoV-2 and SARS-CoV, and even greater distance from Middle East respiratory syndrome coronavirus (MERS-CoV) [14]. Although the sequences in S-protein between SARS-CoV and SARS-CoV-2 were diverse, the RBD domains of them had a similar ability of binding to human ACE2 [14]. Thus, the mechanisms of SARS-CoV-2 that humans infected from animals may be identical to the mechanism of human-to-human transmission, which is via the S protein-ACE2 binding pathway. A better understanding of these mechanisms should shed light on novel therapeutic targets for developing drugs and vaccines against SARS-CoV-2.

Generally, viral infections, immunodeficiency diseases, and other infectious diseases result in innate and adaptive immune responses in vivo. Previous studies have suggested that during the course of SARS-CoV or MERS-CoV infection, the CD4 + T lymphocyte played vital roles in immune response [15–17]. At present, information regarding the association between immune capacity and clinical outcome of pneumonia infected with SARS-CoV-2 is scarce. In our previous study, we found that an abnormal accumulation of CD4 + T lymphocyte and CD163 + M2 macrophage in the lung tissue and suggest a role of immune dysfunction in pathogenesis by case report (under review). In this study, we found CD4 + T lymphocyte and CD8 + T lymphocyte could not only predict the prognosis of SARS-CoV-2-infected pneumonia, but also monitor dynamically the trend of the disease, which was consistent with our previous pathological findings.

Methods

Study Design and Participants

This study (WDRY2020-K044) was approved by the Ethics Committee of Renmin Hospital of Wuhan University and written informed consent was waived by the same committee. For this retrospective, single-center study, 112 patients confirmed with SARS-CoV-2 infection were enrolled from Jan 1, 2020 to Jan 31, 2020. According to the study design, the length of the patient's stay in hospital was artificially divided into three periods, early stage, advanced stage and late stage. Early stage refers to the period between the time of admission to the hospital and the occurrence of respiratory symptoms without diagnosis. Advanced stage refers to the period from the occurrence of respiratory symptoms to the diagnosis of nosocomial infections, or the state of the patient with community-acquired infections when admitted to the hospital. Late stage refers to the state of the confirmed patient after 7.9 days (mean) of standardized treatment. Clinical outcomes were followed up to Feb 9, 2020. Based on Diagnosis and Treatment of Pneumonitis Caused by Novel Coronavirus (trial version 5) [11], standardized treatment includes anti-infective therapy based on antiviral (aerosolized inhaled interferon, intravenous or oral antiviral drugs), and other symptomatic supportive therapies such as oxygen inhalation; on this basis, severe patients were treated with low-dose hormones and gamma globulin, as well as other advanced life support treatments. After the treatment, patients were divided into the following 3 groups based on short-term clinical outcomes: remission group, deterioration group, and death group. The remission refers to that for mild pneumonia, the criteria satisfies any of the following conditions (1. Patient's fever /

respiratory symptoms improve; 2. CT or chest radiograph shows pulmonary inflammation absorption; 3. The SARS-CoV-2 test results change from positive to negative); for severe pneumonia, the criteria satisfies any of the following conditions (1. Patient's respiratory distress symptoms improve; 2. Oxygen saturation rises above 94%). The criteria of deterioration satisfy any of the following conditions (1. Patient's fever / respiratory symptoms worsen; 2. CT or chest radiograph shows pulmonary inflammation is further aggravated; 3. Oxygen saturation continued to decrease).

Procedures

Initially, we collected data from 178 patients infected with SARS-CoV-2 admitted to Renmin Hospital of Wuhan University, and 112 patients were eventually enrolled after excluding hematological diseases and incomplete data. The information on epidemiology, clinical feature, laboratory tests, radiographic results and treatment and outcomes were obtained with standardised data collection forms from electronic medical records. Three researchers recorded the data independently and performed cross-checking. Data analysis was performed by other researchers.

Nasal and pharyngeal swabs specimens were obtained and performed for the detection of SARS-CoV-2 utilizing real-time RT-PCR assays. The primers and probe target to envelope gene of SARS-CoV-2 were employed and the sequences were as follows: forward primer 5'-CCCTGTGGGTTTTACACTTAA-3'; reverse primer 5'-ACGATTGTGCATCAGCTGA-3'; and the probe 5'-FAM-CCGTCTGCGGTATGTGGAAGGTTATGG-BHQ1-3'. According to the protocol, real-time RT-PCR assay was employed by the following conditions: 50 °C for 15 min, 95 °C for 3 min, followed by 45 cycles of 95 °C for 15 s and 60 °C for 30 s.

Statistical Analysis

Categorical variables were described as count and percentages and compared by the use of χ^2 or Fisher's exact test. Continuous variables were expressed as mean $\pm$ S.E. and one-way analysis of variance (ANOVA) was applied for multiple comparisons with LSD post hoc analysis for data meeting homogeneity of variance or with Tamhane's T2 analysis for data of heteroscedasticity. $P < 0.05$ was considered to be significant. Statistical analyses were performed with SPSS for Windows software (version 19.0, IBM Corp.).

Results

112 patients infected with SARS-CoV-2 were included in this study. 52 patients were men, with a mean age of 53 years. 50 (44.6%) patients had underlying disease history, including cardiovascular and cerebrovascular diseases, chronic kidney disease, chronic liver disease, respiratory system disease, and malignant tumor respectively. 77 (68.8%) patients had fever, and 57 (50.9%) patients had dry cough on admission. Other main symptoms included myalgia and diarrhea. Among the 112 patients who were enrolled analyzed, 74 patients had significant improvements on CT examination and clinical symptoms after treatment, while 26 patients continued to deteriorate, and 12 patients died after treatment. The 12 dead patients were senior, with an average age of 66.67 (34–87) years and 3 males among them.

Regarding the causes of death, 6 patients died from circulatory failure and cardiac arrest; 6 patients died from respiratory failure. The patients who died often had underlying diseases (mainly include acute cerebrovascular disease, hypertension and diabetes), especially, two cases died from nosocomial infections after lung surgery and pancreatic surgery. Besides, majority of the patients who died at the end stage had fever on admission (Table 1).

Table 1
Baseline characteristics of patients infected with SARS-CoV-2 based on the outcome

	No. (%)				P value*
	Total (n = 112)	Remission (n = 74)	Deterioration (n = 26)	Death (n = 12)	
Age, mean (SD), y	53.00 (17.87)	48.22 (16.70)	60.11 (17.68)	66.67 (14.35)	< 0.001
Sex					
Male	52 (46.4)	32 (43.2)	17 (65.4)	3 (25.0)	0.045
Female	60 (53.6)	42 (56.8)	9 (34.6)	9 (75.0)	
Comorbidities					
Diabetes	16 (14.3)	7 (9.5)	4 (15.4)	5 (41.7)	0.017
Hypertension	27 (24.1)	10 (13.5)	8 (30.8)	9 (75.0)	< 0.001
Cardiovascular disease	6 (5.4)	2 (2.7)	2 (7.7)	2 (16.7)	0.071
COPD	7 (6.3)	3 (4.1)	3 (11.5)	1 (8.3)	0.316
Chronic kidney disease	6 (5.4)	2 (2.7)	4 (15.4)	0 (0.0)	0.047
Chronic liver disease	2 (1.8)	0 (0.0)	2 (7.7)	0 (0.0)	0.113
Tumor	14 (12.5)	4 (5.4)	8 (30.8)	2 (16.7)	0.003
Cerebrovascular disease	7 (6.3)	0 (0.0)	4 (15.4)	3 (25.0)	< 0.001
Early signs and symptoms					
Fever	77 (68.8)	45 (60.8)	20 (76.9)	12 (100.0)	0.009
Dry cough	57 (50.9)	39 (52.7)	9 (34.6)	9 (75.0)	0.059
Myalgia	19 (17.0)	13 (17.6)	3 (11.5)	3 (25.0)	0.503
Diarrhea	7 (6.3)	6 (8.1)	0 (0.0)	1 (8.3)	0.377
Abbreviations: SD, standard deviation; COPD, chronic obstructive pulmonary disease; the data were analysis through the outcome of patients. *P value indicate differences among three groups. P ≤ 0.05 was considered statistically significant. Clinical characteristics were compared by the use of χ^2 or Fisher's exact test for categorical variables.					

According to the patients' disease progressions from Jan 1, 2020 to Feb 9, 2020, we divided them into three groups including remission, deterioration and death. We monitored the count of peripheral lymphocyte and its lymphocyte subsets, including CD3+, CD4+, CD8+, CD56 + T lymphocytes, and CD19

+ B lymphocytes. In the remission group, the patients' lymphocyte counts in their blood samples were kept at a relative high level ($> 1.1 \times 10^9 / L$) compared with the other two groups. In the deterioration group, the counts of peripheral lymphocytes in patients was at normal level initially ($1.34 \pm 0.13 \times 10^9 / L$) and continued to decline at advanced stage (From $0.88 \pm 0.08 \times 10^9 / L$ to $0.80 \pm 0.09 \times 10^9 / L$). In the death group, the total lymphocyte count ($0.95 \pm 0.12 \times 10^9 / L$) was below the lower limit of the normal range at the onset timepoint of admission to hospital. With the diagnosis and progression of SARS-CoV-2-infected pneumonia, the lymphocyte count continued to have a sharp drop, with the lowest count value at $0.49 \pm 0.11 \times 10^9 / L$ (Fig. 1).

Next, we investigated the specific lymphocyte populations in patients' blood samples. Of the 112 patients, 78 had blood tests for lymphocyte subsets information at the advanced stage. We evaluated the levels of CD3 + cells as the marker for T lymphocytes and CD19 + cells as the marker for B lymphocytes. Notably, the CD3 + T lymphocyte count was significantly decreased in death group ($313.29 \pm 41.71 / \mu l$) compared with groups of remission ($718.44 \pm 48.76 / \mu l$, $p < 0.001$) and deterioration ($528.76 \pm 60.11 / \mu l$, $p = 0.022$), which was also reduced in deterioration group compared with remission group although no significance ($p = 0.056$) was observed in these latter two groups (Fig. 2A). Contrarily, the counts of CD19 + B lymphocyte in these groups were in normal range, and there was no significant differences among them ($p = 0.71$; Fig. 2B).

We then further analyzed the counts of various populations of T lymphocytes. Compared with remission group ($384.61 \pm 26.80 / \mu l$), the CD4 + T lymphocyte count was significantly decreased in both groups of deterioration ($277.47 \pm 36.148 / \mu l$, $p = 0.038$) and death ($220.57 \pm 44.868 / \mu l$, $p = 0.028$), with no significant differences between the latter two groups ($p = 0.49$; Fig. 3A). Meanwhile, the CD8 + T lymphocyte count was significantly decreased in death group ($86 \pm 18.35 / \mu l$) compared with the other two groups (remission, $288.81 \pm 24.95 / \mu l$, $p < 0.001$; deterioration, $227.76 \pm 35.55 / \mu l$, $p = 0.006$), no significant difference was observed between groups of remission and deterioration ($p = 0.43$; Fig. 3B). These data suggested that the count of CD4 + T lymphocyte was decreased earlier than that of CD8 + T lymphocyte, indicating that CD4 + T lymphocyte count could be used as an earlier indicator of disease progression and the CD8 + T lymphocyte count as a marker for advanced stage or end stage of SARS-CoV-2-infected pneumonia. Additionally, the absolute counts of CD16 + and CD56 + B lymphocyte did not alter among all groups ($p = 0.42$), and they were all in normal range (Fig. 3C).

Besides, we monitored the dynamic changes of the peripheral blood CD4 + and CD8 + T lymphocyte counts in 11 patients with different prognosis over a hospitalized period of nearly one month. We found that the levels of both CD4 + and CD8 + T lymphocyte counts in remission group were above normal, while the majority of these values of patients in groups of deterioration and death were below normal. It is worth noting that, the counts of both the CD4 + and CD8 + T lymphocyte were being increased in remission group when tested twice during the one-month period, which were instead being reduced in both groups of deterioration and death in the same period (Fig. 4A and 4B).

Discussion

We here present a retrospective study of 112 hospitalized patients with SARS-CoV-2 infection in Wuhan. Levels of lymphocytes and lymphocyte subsets are of great importance to keep the immune system functional. Usually viral infection, immunodeficiency diseases, and other infectious diseases lead to abnormal changes in the levels of lymphocyte subsets [18]. We confirmed in our cases that the clinical symptoms and the outcome of these patients with SARS-CoV-2 infection are closely related to the functions of immune system, as previously reported [19]. Notably, based on our findings in these 112 patients, total lymphocyte counts, absolute CD3+, CD4 + and CD8 + T lymphocyte counts were dramatically decreased as the pneumonia continues to progress. Among them, CD4 + and CD8 + T lymphocytes can be evaluated as a standard laboratory marker for disease progression and a dominant prognostic and predictive factor for SARS-CoV-2 infection.

The pneumonia associated with the fast-growing outbreak of 2019 novel coronavirus, therefore it is an immediate urgency to have medicines before a vaccine can be produced. However, no specific treatment is currently available for SARS-CoV-2 pneumonia to date. Several antiviral agents are being identified to further examine their potential use and development as therapeutic drugs based on sequencing data of SARS-CoV-2, coupled with molecular modelling according to the genomes of related virus proteins [14], such as antiviral agent remdesivir, virus inhibitor chloroquine analogs and the anti-HIV lopinavir plus ritonavir combination [20, 21]. Some Chinese medicine prescriptions have also been used to reduce viral entry into cells and minimize inflammatory storms in patients, yet the therapeutic effects of these medications are very limited. Currently one of the main treatments for SARS-CoV-2 pneumonia patients is immune support therapy, which might also cause excessive immune responses and consequently trigger inflammatory storms in patients [22]. Therefore, a further understanding on the pathogenesis of SARS-CoV-2 pneumonia and dynamic monitoring of clinical symptoms and pathological indicators in patients are urgent needs for more effective drug development and personalized treatment plans.

In general, SARS-CoV-2 pneumonia is an acute respiratory disease with a fatality rate of ~ 2% mainly due to dyspnea. A just published work have first characterized the pathological features of a patient who died from SARS-CoV-2 by obtaining biopsy samples at autopsy [23]. They observed the substantially reduced counts of peripheral CD4 + and CD8 + T lymphocytes with hyper-activated status, here we further confirmed in our 112 hospitalized cases that, total lymphocyte counts, absolute CD3+, CD4 + and CD8 + T lymphocyte count were gradually decreased as the pneumonia continues to progress by evaluating SARS-CoV-2 pneumonia patients at different stages including remission, deterioration and death. Meanwhile, we monitored and dynamically assessed the peripheral lymphocytes in 11 patients among 112. The counts of CD4 + and CD8 + T lymphocyte decreased during deterioration while increased in the process of remission. Particularly, total lymphocyte counts were maintained at a certain level among patients in remission, by contrast, the lymphocyte counts decreased dramatically when pneumonia advanced, especially at late stage. We further notice that the decrease of CD3 + T lymphocyte count was gradual in groups in line with the progression of pneumonia. In addition, CD4 + T lymphocyte count was significantly reduced in both deterioration and death groups comparing with remission patients, while the

significant drop in CD8 + T lymphocyte count was observed in cases of the death compared to the other two groups. These data suggest that CD3 + T lymphocyte count is potentially used as a surrogate marker of SARS-CoV-2 pneumonia progression, CD4 + T lymphocyte count can be used as an early indicator of disease progression while CD8 + T lymphocyte count is an endpoint marker for critically advanced stage of SARS-CoV-2 pneumonia.

Moreover, the report has also revealed the pathological characteristics, pulmonary oedema with hyaline membrane formation, interstitial mononuclear inflammatory infiltrates dominated by lymphocytes, desquamation of pneumocytes and multinucleated syncytial cells with atypical enlarged pneumocytes [23], which resembled those seen in SARS [24] and MERS [25]. Besides the pathological findings, the clinical and CT imaging features of SARS-CoV2 patients are similar to those observed in SARS and MERS [26], patients usually presented with fever, dry cough, ground-glass opacity in CT images, dyspnea and dense consolidation in lung can also be observed in patients with severe pneumonia, as suggested by our data as well as many other reports [27]. These evidence indicate that the pathological mechanisms could be similar among SARS-CoV2, SARS-CoV and MERS-CoV infections.

The pathophysiology of SARS is related to the dysfunction of immune system, including hyperinduction of chemokines and cytokines, abnormal cellular immune response and insufficient interferon reactions [28]. In the case study of 38 SARS patients, the researchers found that the absolute counts of lymphocyte subsets related to immune functions, such as CD4 + and CD8 + T cells, were significantly decreased in SARS patients, which can be monitored as a prognosis predictor for SARS [17]. In our present study, the decreased counts of T lymphocyte subsets including CD3, CD4 + and CD8 + T cells were also gradually reduced in patients of SARS-CoV-2 pneumonia as disease progressed, suggesting their potential role in predicting prognosis in SARS-CoV-2-infected pneumonia. Uniquely, we did not observe significant decreases in B lymphocytes and NK T cells in SARS-CoV-2 infected patients, indicating a relatively weak inflammation caused by SARS-CoV-2 compared with SARS-CoV and MERS-CoV. This could also explain the lower lethality in SARS-CoV2 than SARS-CoV and MERS-CoV, which needs to be confirmed by further investigations.

This study is limited by the small sample size and retrospective method. Several considerations should be taken into account when interpreting the findings. First, only 112 patients with confirmed SARS-CoV-2 pneumonia were included; hematological diseases and incomplete data cases were ruled out in the analyses. And patients in other cities in China, and even in other countries would be better to be included to get a more comprehensive understanding of SARS-CoV-2 pneumonia progression. Second, the present data are based on the clinical observations of nearly one-month period of these patients. Longer monitoring will give us a more comprehensive and accurate analysis on the pathological parameters and immune functions of the patients for further understanding on the pathological mechanisms of SARS-CoV-2 and therapeutic developments. However, the data in this study permit helpful assessment on clinical characteristics and early prediction for disease progression and mortality of SARS-CoV-2 pneumonia before the obvious symptoms appear in patients in Wuhan, China.

Conclusion

In conclusion, we found CD3+, CD4 + and CD8 + T lymphocyte counts are useful markers to predict the clinical progression of SARS-CoV-2 pneumonia at various stages. Considering the urgency of this ongoing global public health emergency, we believe that our findings reported here can be key indicators for physicians to formulate a timely and proper therapeutic strategy for patients and an early warning model for predicting or reducing mortality in SARS-CoV-2 pneumonia.

Declarations

Author Contributions: XL, Y-a J, ZC and X-a L conceived the study. WJ and ZZ designed the study. XL, Y-a J and X-a L supervised the overall study. WJ, DF and WbJ collected Clinical data. SX and ZZ did the statistics analysis. WJ and ZZ made the tables and figures. DF and YZ searched the literature. WJ, HX and ZZ drafted the manuscript. XL, Y-a J, ZC and X-a L critically revised the final manuscript.

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Conflicts of interests

All authors declare no competing interests.

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Figures

Figure 1

Statistical analyses of the peripheral blood lymphocyte count in the different disease stages of the remission group, deterioration group, and death group. Mean $\pm$ standard error. *P < 0.05, remission group vs deterioration group or death group in the advanced stage. **P < 0.05, remission group vs deterioration group or death group in the late stage. one-way ANOVA.

Figure 2

(A) Statistical analyses of the peripheral blood CD3+ T lymphocyte count in the remission group (n=54), deterioration group (n=17), and death group (n=7). (B) There was no significant difference in the peripheral blood CD19+ B lymphocyte count among the three prognosis groups. The red dotted line represents the lowest value in the normal range of the cell count. Mean $\pm$ standard error. *P < 0.05, remission group or deterioration group vs death group. one-way ANOVA.

Figure 3

(A) Statistical analyses of the peripheral blood CD4+ T lymphocyte count in the remission group (n=54), deterioration group (n=17), and death group (n=7). *P < 0.05, remission group vs deterioration group or death group. (B) Statistical analyses of the peripheral blood CD8+ T lymphocyte count among the three

different prognosis groups. *P < 0.05, remission group or deterioration group vs death group. (C) There was no significant difference in the peripheral blood CD16+/CD56+ NK T lymphocyte count among the three prognosis groups. The red dotted line represents the lowest value in the normal range of the cell count. Mean $\pm$ standard error. one-way ANOVA.

Figure 4

Dynamic changes of the peripheral blood CD4+ (A) and CD8+ (B) T lymphocyte count in 11 patients with different prognosis. Remission group, n=6. Deterioration group, n=4. Death group, n=1. The 1st test of the peripheral blood CD4+ or CD8+ T lymphocyte count was at the time of confirmed diagnosis. The 2nd test of the peripheral blood CD4+ or CD8+ T lymphocyte count was the last test before the end of this study. The red dotted line represents the lowest value in the normal range of the cell count.