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The clinical course and its correlated immune status in COVID-19 pneumonia

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ABSTRACT

Objectives: To explore the clinical course and its dynamic features of immune status in COVID-19 patients and find predictors correlated with severity and prognosis of COVID-19.

Methods: The electronic medical records of 204 patients with COVID-19 pneumonia confirmed by nucleic acid testing were retrospectively collected and analyzed.

Results: All patients were divided into severe (69) and non-severe group (135). Lymphocyte subsets count, including CD3⁺ T cell, CD4⁺ T cell, CD8⁺ T cell, B cell (CD19⁺) and NK cell (CD16⁺ 56⁺), were significantly lower in severe group (P < 0.001). The dynamic levels of T lymphocyte in severe group were significantly lower from disease onset, but in the improved subgroup the value of T lymphocyte began to increase after about 15-day treatment and finally returned to the normal level. The cut-off value of the counts of CD3⁺ (576), CD4⁺ (391) and CD8⁺ (214) T cell were calculated and indicated significantly high sensitivity and specificity for severity of COVID-19.

Conclusion: Our results shown that the decrease of CD3⁺, CD4⁺ and CD8⁺ T lymphocyte correlated with the course of patients with COVID-19 pneumonia, especially in severe cases. The level of T lymphocyte could be used as an indicator for prediction of severity and prognosis of patients with COVID-19 pneumonia. The application of glucocorticoid should be cautious in severe cases.

1. Introduction

In December 2019, the acute viral respiratory diseases named as Coronavirus Disease 2019 (COVID-19) was reported in Wuhan, China. COVID-19 is caused by a novel coronavirus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). The World Health Organization (WHO) has listed the novel coronavirus pneumonia epidemic as a public health emergency of international concern. As of April 1, 2020, over 700,000 SARS-CoV-2 infection had been confirmed all over the world.

The clinical presentation of COVID-19 varies from mild to severe, but most elder patients or patients with known comorbidities, such as hypertension, diabetes, cardiomyopathy, chronic renal failure, chronic obstructive pulmonary disease (COPD) and decreased immunity appear to be at a higher risk of developing severe disease, resulting in a high case-fatality rate¹,². A retrospective study showed the mortality was 61.5% in severe patients with COVID-19³. Early identification and intervention for the underlying severe patients was crucial for reduction of mortality.

Due to the lack of specific antiviral drugs and vaccine, own immune status become one of the most crucial factors affecting disease progression and prognosis. The clinical laboratory data of patients revealed that most COVID-19 cases displayed significantly low circulating lymphocyte counts, especially severe patients admitted to ICU¹,²,⁴.

We aimed to characterize the immune status between the severe and non-severe patients with COVID-19 pneumonia, review the correlation of the clinical course and dynamic changes of T lymphocyte subsets in those patients, and attempt to find an indicator for early identification of the underlying severe patients with COVID-19 pneumonia.
2. Methods

2.1. Study design and Participants

This is a retrospective study. All patients confirmed by fluorescence reverse-transcriptase-polymerase chain reaction (RT-PCR) assay for SARS-CoV-2 at Renmin Hospital of Wuhan University between January 10, 2020 and February 13, 2020 were enrolled. All patients were divided into severe and non-severe groups according to 'Pneumonia diagnosis and treatment program for novel coronavirus infection (trial version 5)' issued by National Health Commission of the People’s Republic of China (http://www.nhc.gov.cn/). The classification criteria were summarized as Table S1. The end of follow-up was February 13, 2020.

2.2. Data Collection

The electronic medical records, including the epidemiological, clinical, laboratory, and radiological findings of patients were collected and analyzed. The laboratory results were obtained within the early stage of the disease. The cases with incomplete data were excluded.

2.3. Statistical Analysis

Statistical analysis was performed using SPSS 23.0 software and GraphPad prism 8.0 software. Percentages and frequency rates were used to describe categorical variables, mean, median, interquartile range (IQR) and standard deviation (SD) were used to describe continuous variables. T test or Mann-Whitney test was used to compare mean for continuous variables. χ2 test or Fisher exact test was used to compare reference between non-severe and severe group (Table 2), including median white blood cell count (P = 0.021), neutrophil count (P < 0.001), lymphocyte count (P < 0.001), platelet count (P < 0.001), D-dimer (P = 0.018), aspartate aminotransferase (P = 0.002), urea (P < 0.001), lactate dehydrogenase (P < 0.001) and C-reactive protein (P < 0.001).

Lymphocyte subsets count, including CD3+ T cell, CD4+ T cell, CD8+ T cell and B cell (CD19+) and NK cell (CD16/56+), were significantly lower in severe group than those in non-severe group (all P < 0.001). For the humoral immune function, the significantly higher level of IgG (P = 0.024) and Complement C3 (P = 0.045) and lower IgM (P = 0.009) were detected in patients of severe group. The level of IL-4 (P = 0.005) and TNF-α (P = 0.004) were significantly higher in severe group.

Patients also were divided into age < 60 and age ≥ 60 group. There were significant differences in count of lymphocyte subsets between two groups (Fig. S1a). Compared to patients without comorbidities, the count of T lymphocyte and value of humoral immune, such as IgM, IgA and C4, were significantly lower in patients with comorbidities (Fig. S1g, j).

3.3. Dynamic profile of lymphocyte subsets

The results shown that the level of all lymphocyte subsets was normal during hospitalization in non-severe group (Fig. 1). The level of T lymphocyte was significant lower in patients of COVID-19 pneumonia in severe group (Fig. 1a–c). To further investigate the relationship between level of lymphocyte subsets with clinical course of COVID-19, according to the results of treatment, 15 patients in severe group were divided into improved subgroup (7 patients) and dead subgroup (8 patients).

The dynamic results indicated that the level of T lymphocyte in dead subgroup continued to decrease until death. However, the count of T
lymphocyte began to increase after 15-day treatment, finally returned to the normal level after 25-day treatment in patients of improved subgroup (Fig. 2a). The time of recovery of lymphocyte count was approximately consistent with the time point of improvement of clinical course. The levels of B cell and NK cell were close to normal range and approximately consistent with the time point of improvement of clinical symptoms. The ventilator was required in 10 patients (4.90%). As of February 13, 134 (65.69%) patients were discharged, 44 (21.57%) patients were hospitalized and 26 (12.75%) patients were dead.

### 3.5. The ROC curve (AUC) in severe patients

Our results have indicated that the level of lymphocyte subsets was strongly correlated with the course of COVID-19 patients. ROC curve and area under ROC curve (AUC) were used to assess the diagnostic value of them for identification of severe COVID-19. Our results shown that the count of CD3^+^, CD4^+^ and CD8^+^ T cell had significantly high sensitivity and specificity (Fig. 1f), and the AUC were 0.983 (95%CI, 0.966-0.995), 0.972 (95%CI, 0.954-0.990) and 0.953 (95%CI, 0.896-0.969), respectively in severe patients with COVID-19 pneumonia. Simultaneously, the cut-off value was calculated according to ROC curve, including CD3^+^ T cell (576), CD4^+^ T cell (391), CD8^+^ T cell (214), B cell (111) and NK cell (128). The sensitivity and specificity of humoral immune parameters were not excellent and the AUC of them were at the range from 0.5 to 0.612 and lower than AUC of T lymphocytes (Fig. 1g).

### 3.4. Treatment and prognosis

All patients were isolated for standardized treatment followed 'Pneumonia diagnosis and treatment program for novel coronavirus infection' updated in real time issued by National Health Commission of the People’s Republic of China. Treatment mainly included antiviral therapy (98.53%, oseltamivir, Arbidol, Ganciclovir and Lopinavir and Ritonavir Tablets), antibiotic therapy (97.02%, moxifloxacin, levofloxacin, cefasporins), albumin infusion (25.98%). Glucocorticoid (25.00%) was used at appropriate time. The ventilator was required in 27 patients (13.24%). Invasive mechanical ventilation was required in 10 patients (4.90%). As of February 13, 134 (65.69%) patients were discharged, 44 (21.57%) patients were hospitalized and 26 (12.75%) patients were dead.
Fig. 1. Dynamic changes of lymphocyte subsets count between non-severe and severe group. The count of CD3+ T cell (A), CD4+ T cell (B), CD8+ T cell (C), B cell (D) and NK cell (E) were illustrated in chronological order. The solid lines (black) show the lower normal limit of each parameter. The dotted line (red) show the cut-off value calculated by ROC analysis. The sensitivity and specificity of lymphocyte subsets count (F) and level of humoral immune function (G) for classification of COVID-19. Error bars, mean ± sem.
Fig. 2. Dynamic changes of lymphocyte subsets count between improved and dead group. The severe patients were divided into improved and dead groups according to the outcomes as of February 13. The count of CD3\(^+\) T cell (A), CD4\(^+\) T cell (B), CD8\(^+\) T cell (C), B cell (D) and NK cell (E) were illustrated in chronological order. The solid lines (black) show the lower normal limit of each parameter. The dotted line (red) show the cut-off value calculated by ROC analysis. Scatter plot described the correlation between TNF with CD3\(^+\) T cell (F), CD4\(^+\) T cell (G) and CD8\(^+\) T cell (H), respectively.
with dynamic data. The 7 patients in severe group improved and transformed into non-severe type after 20-day treatment, and the cut-off value of T lymphocytes also excellently predicted the dynamic trends of diseases.

4. Discussion

SARS-CoV-2 has high infectivity and pathogenicity. The basic reproductive number R0 of SARS-CoV-2 was estimated to be 3.77, much higher than SARS-CoV(R0 = 2.7) and MERS-CoV (R0 < 1)\(^\text{5-7}\). The affinity between SARS-CoV-2 and ACE2 receptor is 10-20 times higher than that of SARS-CoV\(^\text6\). Without a therapeutic vaccine or specificity between SARS-CoV-2 and ACE2 receptor is 10-20 times higher than in regulating and maintaining the stability of the internal immune environment. Compared to young patients with COVID-19 pneumonia, the lymphocytes in dead subgroup of severe patients continued to fall until death. The time of recovery of T lymphocyte count was approximately consistent with the clinical course. In contrast, the level of T lymphocytes with severity of COVID-19 pneumonia. From the course of COVID-19 further confirmed the correlation between the level of T lymphocytes and other immunologically active cells such as macrophages and B lymphocytes\(^\text2\).

The complement system was a critical part of host defense to many bacterial, viral, and fungal infections. But the complement system was also strictly regulated through a number of inhibiting proteins in the serum, because of its potential to damage host tissues\(^\text2\). Our results showed that complement C3 were significantly higher in severe patients with COVID-19 pneumonia than that in non-severe patients. It had been found that complement activation regulates a systemic proinflammatory response to SARS-CoV infection and the inhibition of complement C3 can reduced systemic inflammation\(^\text2\). The anaphylatoxins C3a, C4a, and C5a, which were produced during activation of complement signaling, had potent proinflammatory properties and can aggravate the viral infection and acute lung injury\(^\text2\). The complement system may play an essential role in COVID-19 pneumonia. The inhibition of complement C3 in patients may reduce the proinflammation response and acute lung injury induced by SARS-CoV-2 infection, which may provide a new therapy for COVID-19.

The results of ROC curve further confirmed that the levels of CD3\(^+\), CD4\(^+\) and CD8\(^+\) T cell performed excellent sensitivity and specificity for severity of COVID-19. The cut-off value of T lymphocytes also excellently predicted the dynamic trends of diseases, facilitate to detect and intervene the underlying severe patients earlier, which was crucial for reduction of mortality.

The results of our study also provided some new perspectives for treatment of severe COVID-19. Glucocorticoids which can suppress cell-mediated immunity, promote the decrease of T lymphocyte and delay the virus clearance\(^\text2\), may aggravate the impaired function of T lymphocytes in patients with SARS-CoV-2. Therefore, the use of glucocorticoid should be cautious in severe patients with COVID-19 pneumonia, and the timing of glucocorticoid application deserves further exploration. Improvement of T lymphocyte function maybe a promising therapy for severe patients with COVID-19 pneumonia.

5. Conclusions

In conclusion, we characterized and analyzed the clinical course and its correlated immune function of patients with COVID-19 pneumonia. The decrease of CD3\(^+\), CD4\(^+\) and CD8\(^+\) T lymphocyte correlated with the course of patients with COVID-19 pneumonia, especially in severe cases. The level of T lymphocyte could be used as an indicator for prediction of severity and prognosis of patients with COVID-19 pneumonia. The use of glucocorticoid should be cautious in severe patients, the timing of glucocorticoid application deserves further exploration. Improvement of T lymphocytes function maybe a potential therapeutic strategy for severe patients with COVID-19 pneumonia.

Author Contributions

Conceptualization, Ruyuan He, Zilong Lu and Qing Geng; Data curation, Qing Geng; Formal analysis, Ruyuan He and Zilong Lu; Funding acquisition, Qing Geng; Methodology, Ruyuan He, Zilong Lu, Tao Fan, Rui Xiong and Xiaokang Shen; Project administration, Ruyuan He and Qing Geng; Supervision, Ruyuan He and Qing Geng; Validation, Zilong Lu, Lin Zhang, Wenyang Jiang and Qing Geng; Visualization, Ruyuan He; Writing – original draft, Ruyuan He; Writing – review &
Declaration of Competing Interest

number: WDRY2020-K009). Ethics Committee of Renmin Hospital of Wuhan University (approved Ethics approval and consent to participate

Acknowledgment

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version, at doi: https://doi.org/10.1016/j.jcv.2020.104361.

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