European Review for Medical and Pharmacological Sciences 2023; 27: 2686-2691

Levels of peripheral IL-6 and CD4+ and CD8+ T cells and their prognostic significance in COVID-19

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Abstract. – OBJECTIVE: The aim of this study was to discuss the prognostic significance of peripheral interleukin-6 (IL-6) and CD4+ and CD8+ T cells in COVID-19.

PATIENTS AND METHODS: Eighty-four COVID-19 patients were retrospectively analyzed and classified into three groups, including the moderate group (15 cases), the serious group (45 cases), and the critical group (24 cases). The levels of peripheral IL-6, CD4+, and CD8+ T cells and CD4+/CD8+ were determined for each group. It was assessed whether these indicators were correlated to the prognosis and death risks of COVID-19 patients.

RESULTS: The three groups of COVID-19 patients differed significantly in the levels of peripheral IL-6 and CD4+ and CD8+ cells. The IL-6 levels in the critical, moderate, and serious groups were increased successively, but the changed levels of CD4+ and CD8+ T cells were just opposite to that of IL-6 ($p<0.05$). The peripheral IL-6 level increased dramatically in the death group, while the levels of CD4+ and CD8+ T cells decreased significantly ($p<0.05$). The peripheral IL-6 level was significantly correlated with the level of CD8+ T cells and CD4+/CD8+ ratio in the critical group ($p<0.05$). The logistic regression analysis indicated a dramatic increase in the peripheral IL-6 level in the death group ($p=0.025$).

CONCLUSIONS: The aggressiveness and survival of COVID-19 were highly correlated with the increases in IL-6 and CD4+/CD8+ T cells. The fatalities of COVID-19 individuals remained at increased incidence due to elevated peripheral IL-6 levels.

Key Words:
COVID-19, Interleukin-6, CD4+/CD8+ T cells, Prognosis, Risk.

Introduction

Novel coronavirus-infected pneumonia (NCIP), as known as COVID-19, is the most serious acute infectious respiratory disease in the world in recent years[1]. As of March 10, 2020, the cumulative number of confirmed COVID-19 cases was 80,778 in China, and the cumulative number of COVID-19 deaths was 3,158[2]. SARS-CoV-2 is a linear single-strained RNA virus, an acellular entity that proliferates through host cells. So far, there has been no specific antiviral drug against COVID-19. The pathological injury caused by COVID-19 is closely related to the body's immune system. T cells are important immune cells in the body. The number and proportion of T cells are crucial indicators of the immune level[3,4]. We analyzed the levels of peripheral IL-6 and lymphocyte subsets and their prognostic significance in COVID-19 patients of varied severity. We were also concerned with the value of these indicators in predicting the death risk of critical patients.

Patients and Methods

Data Sources

We randomly collected 84 COVID-19 patients from ordinary wards, Critical Care Unit (CCU), and Intensive Care Unit (ICU) of the Eastern Campus of Hubei Provincial People's Hospital between January 31, 2020, and March 3, 2020. These patients were analyzed retrospectively, including 49 men and 35 women, aged 35-87 (59.23±1.142) years old. Diagnosis and Treatment Plan for COVID-19 (The Seventh Trial Edition)[5] were the diagnostic criteria. Based on the clinical manifestations of COVID-19, there were moderate (15-6 males and 9 females), serious (45-24 males and 21 females), and critical groups (24-19 males and 5 females). Furthermore, they were also divided into death (n=9) and survival groups (n=75) according to the prognosis. Those combined with immune diseases, such as abnormal thyroid function, systemic lupus erythematosus, rheumatoid/rheumatism, HIV, and tumors, were

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Table I. General information of three groups of patients ($\bar{x} \pm s$).

General information	Moderate (n = 15)	Serious (n = 45)	Critical (n = 24)	F/χ^2	*p*
Age (year)	57.00 ± 1.79	61.51 ± 1.59	56.33 ± 2.30	2.408	0.096
Male (%)	6 (40.0)	24 (53.3)	19 (79.2)	3.580	0.032
Female (%)	9 (60.0)	21 (46.7)	5 (20.8)		

excluded. This study was approved by the Research Ethics of Renmin Hospital of Wuhan University (approval No.: WDRY2020-K120).

Methodology

After centrifugation, 3 ml of blood was obtained for detection. Peripheral IL-6 level was determined by the ELISA method using the Siemens Advia 2400 biochemistry analyzer (Siemens Healthcare Diagnostics Inc., Deerfield, IL, USA). FACS Calibur Flow Cytometer [Becton Dickinson (BD), NJ, USA] and the relevant antibody reagents by BD were used to determine the contents of peripheral $CD4^+/8^+$ T cells in the EDTA tubes.

Statistical Analysis

Statistical analyses were conducted using SPSS 22.0 (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov normality test was first performed to examine the normally distributed characteristics. The data not normally distributed were expressed as medians, and the normally distributed data were expressed as mean ± standard deviation. Multiple comparisons of quantitative data (normally distributed) were conducted by using one-way ANOVA. The non-normally distributed quantitative data were analyzed by Kruskal-Wallis H-test. Spearman's correlation test was performed to measure the relevance strength. The differences in variables between the survival group and the death group were analyzed by the Mann-Whitney U test. Logistic regression analysis was performed to analyze the relevance between variables and prognosis. $p<0.05$ indicated a statistically significant difference.

Results

General Information

The three groups of COVID-19 patients did not differ significantly in age ($p>0.05$). However, males outnumbered females in the serious group (Table I).

Comparison of the Levels of Peripheral IL-6, and the Content and Ratio of $CD4^+/8^+$ T Cells

A non-parametric test was employed to analyze the indicators among groups (Table II). The levels of peripheral IL-6 and $CD4^+$ and $CD8^+$ T cells were significantly different across the three groups of COVID-19 patients ($p<0.0001$). However, the $CD4^+/CD8^+$ ratio was similar among the three groups ($p=0.590$). A pairwise comparison was conducted between the moderate, serious, and critical patients. The IL-6 levels in the critical, moderate, and serious groups were increased successively, but the changed levels of $CD4^+$ and $CD8^+$ T cells were just opposite to that of IL-6 ($p<0.05$).

Table II. Comparison of the peripheral IL-6 levels, the contents, and the ratio of $CD4^+/8^+$ T cells.

Groups	IL-6 (pg/ml)	$CD4^+$ (cells/μL)	$CD8^+$ (cells/μL)	$CD4^+/CD8^+$
Moderate	5.98 (1.5, 31.36)$^{***\#}$	609.1 (322.0, 1415.0)$^{***\#\#}$	368.1 (125.0, 688.0)$^{***\#\#}$	1.8 (0.6, 3.3)
Serious	22.2 (1.5, 293.4)**	329.9 (68.0, 724.0)*	195.4 (45.0, 671.0)**	2.2 (0.5, 9.9)
Critical	121.3 (3.1, 889.3)	228.6 (56.0, 626.0)	117.1 (16.0, 318.0)	2.4 (1.0, 8.3)
Z-statistic	21.47	22.70	26.402	1.057
p	0.000	0.000	0.000	0.590

Note: (1) $*p < 0.05$ *vs.* critical group; $**p < 0.01$ *vs.* critical group; $***p < 0.001$ *vs.* critical group. (a) IL: Moderate *vs.* critical: Z = -4.468, $p = 0.000$; serious *vs.* critical: Z = -3.298, $p = 0.001$; (b) $CD4^+$: Moderate vs. critical: Z = 4.764, $p = 0.000$; serious *vs.* critical: Z = 2.417, $p = 0.016$; (c) $CD8^+$: Moderate *vs.* critical: Z = 5.131, $p = 0.000$; serious *vs.* critical: Z = 2.807, $p = 0.005$. (2) $^{\#}p < 0.05$ *vs.* critical group; $^{\#\#}p < 0.01$ *vs.* critical group; $^{\#\#\#}p < 0.001$ *vs.* critical group. (a) IL: Moderate vs. critical: Z = -2.137, $p = 0.033$. (b) $CD4^+$: Moderate *vs.* critical: Z = 3.210, $p = 0.001$. (c) $CD8^+$: Moderate *vs.* critical: Z = 3.285, $p = 0.001$.

Table III. Cox PH regression model estimates for the risk of clinical recurrence.

	Moderate group		Serious group		Critical group	
	r	*p*	*r*	*p*	*r*	*p*
$CD4^+$	-0.247	0.374	-0.069	0.652	-0.045	0.835
$CD8^+$	-0.047	0.869	-0.494	0.001	0.011	0.958
$CD4^+/CD8^+$	-0.240	0.389	0.331	0.026	0.041	0.848

Correlation Between the Peripheral IL-6 Levels, the Contents, and the Ratio of $CD4^+/8^+$ T Cells in COVID-19 Patients

As shown in Table III, Spearman's correlation indicated that the contents of peripheral IL-6, $CD8^+$ T cells, and $CD4^+/CD8^+$ ratio were correlated in the serious group ($p<0.05$). However, no such correlations were observed in other groups.

Differences in Peripheral IL-6 Levels, and the Contents and Ratio of $CD4^+/8^+$ T Cells Between Survival and Death Groups

The Mann-Whitney U test was performed to compare the differences between the survival and the death groups in the peripheral IL-6 levels, and the contents and ratio of $CD4^+/8^+$ T cells (Table IV). It was found that the survival and the death groups did not differ significantly in the levels of peripheral IL-6 and $CD4^+$and $CD8^+$ T cells. The peripheral IL-6 level increased dramatically in the death group, while the levels of peripheral $CD4^+$ and $CD8^+$ T cells decreased dramatically ($p<0.05$). However, the $CD4^+/CD8^+$ ratio was similar in the two groups.

Predictive Value of Peripheral IL-6 Levels, and the Contents and Ratio of $CD4^+/8^+$ T Cells

The death risk of COVID-19 patients was predicted using binary unconditional logistic regression. A COVID-19 patient was considered to have an underlying condition if they also had chronic bronchitis (or chronic obstructive pulmonary disease), coronary heart disease, hypertension or diabetes. Concurrent bacterial infection was defined as a pro-calcitonin level >0.1 ng/ml upon admission. Having an underlying condition=1, not having an underlying condition=0; having concurrent bacterial infection=1, not having concurrent bacterial infection=0; aged above 59=1, aged 59 and below=0; smoker=1, non-smoker=0. The continuous variables measured were directly introduced into the equation. As shown in Table V, the fatalities of COVID-19 individuals remained at increased incidence due to elevated peripheral IL-6 levels ($p=0.025$).

Discussion

A primary feature of COVID-19 is lung inflammation or even systematic inflammation

Table IV. Differences in the peripheral IL-6 levels, and the contents and ratio of $CD4^+/8^+$ T cells in survival and death groups.

Groups	IL-6 (pg/ml)	$CD4^+$ (cells/μL)	$CD8^+$ (cells/μL)	$CD4^+/CD8^+$
Survival group (n = 68)	19.46 (1.50, 293.38)	368.67 (68.00, 1415.00)	214.41 (43.00, 688.00)	2.15 (0.51, 9.87)
Death group (n = 9)	282.11 (23.20, 889.27)	202.22 (56.00, 404.00)	116.11 (16.00, 318.00)	2.22 (1.04, 5.07)
Z	4.449	-2.329	-2.235	0.419
p	0.000	0.020	0.025	0.675

Table V. Predictive value of the contents of peripheral IL-6 and $CD4^+/8^+$ T cells.

Factors	B	Wald	OR	95% CI	*p*
IL-6	0.024	0.011	1.025	1.003-1.047	0.025
$CD4^+$	-0.012	0.008	0.988	0.974-1.003	0.124
$CD8^+$	0.003	0.007	1.003	0.989-1.017	0.649
Infection	-1.396	1.395	0.248	0.016-3.809	0.317

caused by SARS-CoV-2. COVID-19 is highly contagious. Males are considered more susceptible to COVID-19 than females, possibly due to the benefits of the female X chromosome and the protective effect of female sex hormones[6]. However, the severity of COVID-19 varies little in males and females. In the present study, the males with critical COVID-19 outnumbered the females. This might be attributed to the biases in few cases. The immune system is the body's important defense against invading pathogenic microorganisms, including viruses. The immune system is momentous in clearing viruses. The number and dynamic balance of T cell subsets exert a decisive impact on the human immune status[7].

T cells are mainly derived from the pluripotent stem cells in the bone marrow and are divided into different subsets based on the surface CD antigens. $CD4^+$ T cells play a central role in immune protection. They are effector cells inhibiting viral replication by direct killing or secreting inflammatory cytokines, such as interferons and tumor necrosis factors[8]. $CD8^+$ T cells are cytotoxic T cells, which directly and continuously kill the target cells by specifically recognizing antigens to trigger the specific immune response. Generally speaking, a dynamic balance is maintained between $CD4^+$ and $CD8^+$ T cells, which regulates cellular immunity in humans[9,10]. The human immunodeficiency virus (HIV) genome consists of two identical positive-stranded RNA molecules and mainly attacks human helper T cells. HIV-infected patients usually have decreased contents and ratio of $CD4^+/8^+$ T cells[11]. Plasma viral load varies in asymptomatic HIV infection, symptomatic HIV infection, and AIDS. The decreased $CD4^+/8^+$ T cells reflect the degree of the injured immune system in HIV-infected patients[12].

The T cell subsets in 84 COVID-19 patients were explored in our study. Various contents of peripheral $CD4^+/8^+$ T cells existed across different COVID-19 patients. $CD4^+/8^+$ T cells in the critical, moderate, and serious groups were increased successively. However, the related mechanism by which COVID-19 damages the immune system remains unclear. There may be some similarities between COVID-19 and HIV infection. The immune cells may be over-activated by viral antigens on the infected cells, followed by delivering lymphocytes, monocytes, macrophages, and inflammatory cytokines in large amounts. These cytokines recruit $CD4^+/8^+$ T cells to the infected positions, causing an infiltration[7]. These T cells kill the neighboring cells by cell-cell contact, inhibiting the immune response. This is possibly due to the consumption of these T cells or the inhibited generation and differentiation of the T cells. But unlike the HIV infection, the $D4^+/CD8^+$ ratio was similar across the groups in our study. This was probably because the $CD4^+/8^+$ T cells were consumed simultaneously. Compared with the survival group, the levels of $CD4^+/8^+$ T cells decreased considerably in the death group ($p<0.001$). A larger death risk was predicted for those with a marked decrease in these indicators, and immune enhancement therapy is recommended. Since COVID-19 attacks the immune system, early immunotherapy is necessary once there is a decrease in peripheral $CD4^+/8^+$ T cells to prevent the progression of moderate, serious or critical COVID-19. Our findings showed a considerable increase in the peripheral IL-6 level in the serious and critical groups compared to the moderate group. IL-6, produced by mononuclear macrophages, has an immunoregulatory effect on infections. IL-6 induces secretion and differentiation of B cells as well as participates in the inflammatory response. IL-6 can be produced massively by activated T cells[13]. We observed a significant increase in the IL-6 level in the death group ($p<0.05$). IL-6 is an important cytokine triggering the inflammatory storm in COVID-19. This cytokine may further cause a systemic inflammatory response, multiple organ failure, or even death[14]. For example, Pesaresi et al[15] reported that SARS-CoV-2 could also be detected in human heart and kidney tissues by transmission electron microscopy and scanning electron microscopy. Therefore, we should fully consider the immune problems of heart and kidney tissues caused by SARS-CoV-2 in clinical practice. In addition, the logistic regression analysis showed that IL-6 was a risk factor for COVID-19 deaths[16]. Clinically, the IL-6 level may be used as an early predictor of death or even an indicator of the severity of infection. A continuous increase in the peripheral IL-6 level deserves due attention and early interventional therapies, including anti-inflammatory, antiviral, and immune enhancement therapies, to reduce mortality. Spearman's correlation showed that the levels of peripheral IL-6, $CD8^+$ T cells, and $CD4^+/CD8^+$ ratio were correlated in the serious group ($p<0.05$). However, no such correlations were observed in other groups, probably due to the small sample size.

Limitations

However, there are also some limitations in this study. First of all, this is a single-center retrospective study. Secondly, the sample size is small. Besides, only critically ill patients are included in this study. In future research, we should conduct multi-center research with a larger and richer sample size to verify our results.

Conclusions

COVID-19 patients are usually combined with an impaired immune system. Lower levels of $CD4^+/8^+$ T cells plus a high IL-6 level predicted a more severe disease. An elevated IL-6 level was a risk factor for COVID-19 death. However, there may be some biases with few cases. Besides, there was no dynamic monitoring of the involved indicators. The levels of peripheral IL-6, $CD4^+$, and $CD8^+$ T cells determined upon admission can be used for preliminary prognostic evaluation. Dramatic changes in the above indicators may imply disease deterioration. Early treatments are necessary to increase the cure rate and decrease mortality in such patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval

This study was approved by the Research Ethics of Renmin Hospital of Wuhan University (approval No.: WDRY2020-K120).

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Informed Consent

Not applicable due to the retrospective nature of the study.

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