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RESEARCH ARTICLE

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Expression of eosinophil in peripheral blood of patients with COVID-19 and its clinical significance

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Abstract

Aims: To investigate the eosinophil cell (EC) expression in peripheral blood of patients infected with severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) and its clinical significance of diagnosis and prognosis.

Methods: 95 patients, whose nucleic acid test of SARS-CoV-2 was positive to make a definite diagnosis of COVID-19, were selected as the study group. They were admitted at the Chengdu Public Health Clinical Medical Center from January 21 to March 2, 2020. Another 95 healthy subjects and 95 non-infectious fever patients during the same period were selected as the control group. The BC-6900 blood cell analyzer was used to continuously observe and detect ECs in 95 patients with COVID-19 and the control group. The differences in expression levels of ECs in peripheral blood were analyzed.

Results: ECs were significantly decreased in 95 (75.8%) COVID-19 patients ($P < .01$). The absolute EC count IQR was 0.01×10^9/L (0×10^9/L – 0.04×10^9/L), and the EC percentage IQR was 0.3% (0.1% – 0.8%). As the patients' condition improved, the ECs returned to normal, but for those without improvement, ECs continued to decline.

Conclusions: ECs decreased remarkably in patients with COVID-19, and gradually returned to normal after the improvement of the patients' condition, while EC continued to decrease in patients without improvement. It is suggested that ECs have certain clinical significance in the diagnosis and prognosis of COVID-19, and may be a useful index in the early warning of acute infectious diseases.

KEYWORDS

COVID-19, eosinophil cell, SARS-CoV-2

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1 | INTRODUCTION

Since December 2019, multiple cases of patients infected by SARS-CoV-2 have been discovered in Wuhan, China. It has transmitted in an extremely short period to more than 100 countries, resulted in the infection of more than 100,000 people by March 07, 2020. SARS-CoV-2 belongs to *Betacoronavirus Lineage β, Sarbecovirus*. Current research indicated that SARS-CoV-2 showed a resemblance of more than 85% to batSL-CoVZC45.[1] Transmission occurs primarily via respiratory droplets from coughs or sneezes and close contact. The main clinical manifestations of COVID-19 patients are fever, dry cough, and fatigue. Currently, the gold standard for the diagnosis of COVID-19 patients is the detection of SARS-CoV-2 nucleic acid positive by real-time reverse transcriptional polymerase chain reaction (RT-PCR) reagent.[1] Current research indicated the decreased white blood cell and lymphocyte count, increased C-reactive protein (CRP) and blood sedimentation, and abnormal procalcitonin in most patients. In severe cases, D-dimer increased and blood lymphocytes decreased progressively. When analyzing the complete blood counts of patients with COVID-19, we found abnormal changes in eosinophil cell (EC) values in many patients. However, there are few related studies on EC detection in COVID-19 patients. This study explores the expression level and clinical significance of EC in COVID-19 patients, in order to provide laboratory basis for the early diagnosis and treatment of COVID-19.

2 | METHODS

2.1 | Study population

95 patients, diagnosed with COVID-19, were selected as the research objects. They are all admitted at the Chengdu Public Health Clinical Medical Center from January 21 to March 2, 2020. The diagnostic criteria and clinical classification were determined by the new Coronavirus Pneumonia Diagnosis and Treatment Program (version 3-6) promulgated by the National Health Commission of China. The diagnostic criteria are that the detection of SARS-CoV-2 nucleic acid is positive via real-time reverse transcriptional polymerase chain reaction (RT-PCR) reagent, and it is consistent with relevant epidemiological, clinical, laboratory, and imaging characteristics. According to the diagnosis and treatment program, clinical classification is divided into mild, ordinary, and severe, and critical severe COVID-19 patients meet one of the following conditions: ①shortness of breath, RR > 30 counts per minute; ② resting state, oxygen saturation <93%; ③partial pressure of artery (PaO2)/fraction of inspiration (FiO2) <300 mmHg; and ④pulmonary imaging showed that the lesions progress more than 50% within 24-48 hours. Critical COVID-19 patients meet one of the following conditions: ①Respiratory failure occured and requires mechanical ventilation. ②Shock occurred; ICU monitoring and treatment were required for combined organ failure. Another 95 healthy subjects and 95 non-infectious fever patients during the same period were non-randomly selected as the control groups. There was no significant difference in comparison of baseline characteristics data such as age and gender between the three groups.

Chengdu Public Health Clinical Medical Center is one of the designated hospitals for inpatient treatment of COVID-19 patients. Demographic data, clinical characteristics (including medical history, exposure history, comorbidities, surgery history, signs and symptoms), chest computed tomography (CT) or X-ray results, and laboratory test results of each patient were obtained from the electronic medical record system. Analysis was performed by three independent researchers. Our research was approved by the Research Ethics Committee, West China Hospital, Sichuan University.

2.2 | Materials

The BC-6900 blood cell analyzer was applied to continuously observe and detect ECs in 95 patients with COVID-19 and the control groups via three-dimensional cell analysis technology. By analyzing the forward scattered light signal, side scattered light signal, and fluorescent signal of the cell particles, it achieves accurate detection of various cells and abnormal cells. Reference interval of EC count was 0.05×10^9/L − 0.5×10^9/L; reference interval of EC percentage was 0.5%-5%.

2.3 | Data analysis

Continuous variable descriptions use median and interquartile range (IQR) values (when n ≤ 3, the median, maximum, and minimum values are used for description). In order to compare the continuous variables of different patient groups, Mann-Whitney test was applied. The data were collected and organized with Microsoft Excel 2016, and the data analysis is completed by IBM SPSS statistics 21 software. Significance was defined at the 5% level ($P < .05$).

3 | RESULTS

3.1 | Level of EC in COVID-19 group, non-infectious fever group, and normal control group

In this study, 75.8% of patients with COVID-19 had lower absolute EC values than normal. The absolute value and relative value of the first EC measurement in COVID-19 patients were great lower than those in the normal control group, and there was significant difference between the two groups ($P < .01$). The absolute and relative values of EC in the non-infectious fever group were also lower than those in the normal control group ($P < .01$). Compared with non-infectious fever control group, the EC Value of COVID-19 patients was lower. According to the results, the relative value and the absolute value were both remarkably different ($P < .05$). (Table 1).

TABLE 1 Level of EC in COVID-19 group, non-infectious fever group, and normal control group

	Number of cases	Cases of absolute value lower than normal value	Eosinophils ($\times 10^9$/L)	Eosinophil percentage (%)
COVID-19 group	95	72 (75.8%)	0.01 (0-0.04)	0.3 (0.1-0.8)
Non-infectious fever group	95	48 (50.5%)	0.04 (0.01-0.12)	0.5 (0.1-1.6)
Normal control group	95	1 (1.0%)	0.13 (0.09-0.20)	2.2 (1.6-3.3)

3.2 | EC level of COVID-19 patients before and after treatment

According to the dynamic observation of ECs in 95 patients with COVID-19 (3 dead cases included), 92 patients' EC count recovered with the improvement of their condition ($P < .01$). The absolute value of EC in 3 cases of death continued to be low, sometimes the test value was zero, which was not improved compared with that at the time of admission ($P < .01$).(Tables 2 and 3).

3.3 | The lymphocyte number, lymphocyte percentage, procalcitonin, CRP, CD4/CD8 ratio of patients with COVID-19

According to the laboratory examination results of patients diagnosed with COVID-19 (Table 4), the number of lymphocytes in most patients decreased, the percentage of lymphocytes decreased, the concentration of CRP increased, while the patients with abnormal serum procalcitonin concentration and CD4/CD8 ratio accounted for less in all patients. According to the classification criteria, we included mild and ordinary patients in non-severe group, while severe and critical patients in severe group. There were significant differences in lymphocyte number, lymphocyte percentage, CRP, EC number, and EC percentage between the non-severe (mild and ordinary type) and severe (severe and critical type) groups. The decrease of lymphocytes and ECs was more obvious in severe groups. The concentration of CRP was higher.

4 | DISCUSSION

This study included 95 community-infected COVID-19 patients. Analysis of EC of the 95 patients with COVID-19 showed that 75.8% of them had significantly lower absolute and relative EC values than normal at the time of first admission. It was suggested that the decreased EC might be an indicator for SARS-CoV-2 infection in suspected patients. At present, there are many studies on inflammatory indexes such as lymphocytes in COVID-19, and few reports on ECs only point out the phenomenon of eosinopenia in patients.[2] The main difficulty in the diagnosis of fever of unknown origin is the lack of effective and rapid diagnostic methods. The unknown epidemiological history and non-specific laboratory results often make the clinical diagnosis of the disease in trouble.[3] However, the current study on many inflammatory indicators has not found specific indicators that are helpful to distinguish from common infection. In this study, compared with the non-infectious fever patients, the absolute value of EC in patients with COVID-19 was lower, and the difference was significant ($P < .01$), which may be helpful for the diagnosis and differential diagnosis of COVID-19. According to the current report, the patients with COVID-19 have the phenomenon of decrease in the total number of leukocytes and lymphocyte count in the early stage of the disease,[2] while for the atypical patients without lymphocyte reduction and imaging changes, eosinopenia may become an important diagnostic clue.

ECs are a variety of white blood cells and one of the immune system components, which can produce a variety of pro-inflammatory mediators and immunoregulatory molecules. During the Th2 immune response, ECs from the bone marrow and blood are recruited to the site of inflammation to play a role.[4] Clinically, eosinopenia is common in typhoid fever, paratyphoid fever, severe tissue damage after surgery, and after application of adrenocortical hormone or adrenal hormone. We found the phenomenon of eosinopenia in COVID-19 patients, which may be of reference value for improving the differential diagnosis of eosinopenia-related diseases.

Dynamic observation of ECs in COVID-19 patients showed that ECs gradually returned to normal as their condition improved, while ECs continued to decrease in severe or fatal cases. In 2 of the 3 fatal cases, the absolute count of the last EC measurement before the death of patients decreased to 0. The decrease of ECs might

TABLE 2 EC absolute value of COVID-19 patients before and after treatment

		Eosinophils ($\times 10^9$/L)		
Number of cases		Test value at admission	Test value at discharge/improved condition	*P* value
Improved group	92	0.01 (0-0.05)	0.1 (0.07-0.2)	<.01
Death group	3	0.01 (0-0.04)	0 (0-0.03)	>.1

Note: P values denoted the comparison between test value at admission and test value at discharge/improved condition.

TABLE 3 EC percentage of COVID-19 patients before and after treatment

Number of cases		Eosinophil percentage (%)		*P* value
		Test value at admission	Test value at discharge/ improved condition	
Improved group	92	0.3 (0.1-0.8)	1.8 (1.1-3.2)	<.01
Death group	3	0.2 (0-0.8)	0.1 (0-0.2)	>.1

Note: P values denoted the comparison between test value at admission and test value at discharge/improved condition.

TABLE 4 The lymphocyte number, lymphocyte percentage, procalcitonin, C-reactive protein, and CD4/CD8 ratio of patients with COVID-19

Laboratory parameters	All patients (n = 95)	Disease severity		*P* value
		Non-severe patients (n = 65)	Severe patients (n = 30)	
Lymphocytes ($\times 10^9$/L; normal range 1.1-3.2)	1.05 (0.64-1.50)	1.24 (0.79-1.68)	0.77 (0.48-1.24)	<.01
Lymphocyte percentage (%; normal range 20-50)	20.9 (14.8-26.6)	23.6 (17.6-28.9)	15.5 (7.93-19.1)	<.01
Procalcitonin (PCT) (ng/mL; normal range 0-0.1)	0.02 (0.01-0.03)	0.02 (0.02-0.03)	0.03 (0.02-0.05)	<.05
C-reactive protein (CRP) (mg/L; normal range 0-3)	14.0 (5.62-32.1)	8.90 (4.85-16.7)	33.8 (19.3-55.0)	<.01
CD4/CD8 ratio(normal range 1.4-2.0)	1.54 (1.08-2.27)	1.59 (1.28-2.27)	1.46 (0.74-2.33)	>.05
Eosinophils ($\times 10^9$/L; normal range 0.05-0.5)	0.01 (0.0-0.04)	0.02 (0.01-0.06)	0.01 (0.0-0.01)	<.01
Eosinophil percentage (%; normal range 0.5%-5%)	0.3 (0.1-0.8)	0.4 (0.1-1.0)	0.1 (0.0-0.33)	<.01

Note: P values denoted the comparison between non-severe and severe subgroups.

be caused by the secretion of adrenocorticosteroids to enhance the anti-infective ability.[4] This also suggested that the detection of ECs could have certain clinical significance in the development of COVID-19 and prognosis. After all, it is still difficult to judge whether the patient's condition is really improved clinically. The detection of virus in throat swab, anal swab, and alveolar lavage fluid is widely used in hospitals. However, the test results will be affected by many factors in the collection process and test process, and false negative rate is not low.

PCT, as an index of inflammation, plays an important role in the diagnosis and treatment of infectious diseases. However, the inflammatory index pct of all patients was within the normal range, and there was no remarkable difference between mild and severe patients ($P > .05$). In contrast, there were obvious differences in lymphocyte number, lymphocyte percentage, CRP, EC number, and EC percentage between the non-severe (mild and ordinary type) and severe (severe and critical type) groups, which indicated that the degree of EC reduction was related to the severity of the disease, and can be used as one of the indicators of COVID-19 clinical typing. This has a certain clinical significance for the timely adjustment of the treatment plan of patients in the course of the disease.

Once the positive feedback loop in the patient's immune effect is out of control, a large number of immune cells are activated to secrete a large number of cytokines, which will cause cytokine storm, that is, inflammation storm. When acute respiratory distress syndrome is caused by inflammatory storm, it is often time to use extracorporeal membrane oxygenation (ECMO).[5] However, the inflammatory indexes and imaging manifestations that are used to judge the coming of inflammatory storm sometimes cannot change in time, leading to the delay of ECMO use. The change of ECs provides the possibility for us to predict the progression of the disease and adjust the treatment plan ahead of schedule.

Current research indicated that SARS-CoV-2 showed a resemblance of more than 85% to batSL-CoVZC45. The phenomenon of eosinopenia in COVID-19 patients has also drawn our attention to the change of EC count in Middle East respiratory syndrome coronavirus (MERS-CoV)– and severe acute respiratory syndrome coronavirus (SARS-CoV)–related diseases. Studies found that patients with severe acute respiratory syndrome (SARS) also had remarkable EC decline. The percentage and count of blood ECs in patients with SARS in the acute phase had a great difference compared with the suspected SARS ($P < .01$), which has certain reference value for the diagnosis of SARS.[6-8] Another retrospective study based on the hospital found that the EC count of the patients with positive MERS-CoV was lower than that of the patients with negative MERS-CoV (0.27 ± 0.43 vs 2.13 ± 2.01, $P < .001$). This phenomenon may be related to acute viral infection and stress.[9] The phenomenon of

eosinopenia in these three infectious diseases further enhanced the role of ECs detection in the early warning of acute infectious diseases.

Previous studies have shown that the mechanism of eosinophilia was mainly induced by mast cells through the production of chemokines, which were induced by pro-inflammatory mediators (such as IL-1 and TNF-α) and eosinophil-directed cytokines (such as IL-4 and IL-5).[4] However, there are few studies about the specific mechanism of eosinopenia. According to the current research, we can put forward three hypotheses about the mechanism of eosinopenia. Firstly, eosinopenia in COVID-19 patients might be related to stress response to acute infectious diseases. When an infection occurs, the secretion of adrenal corticosteroids increases, preventing the release of ECs from the bone marrow, and promoting the infiltration of ECs into the tissues, resulting in eosinopenia in the peripheral blood.[4] On the other hand, according to the latest diagnostic and therapeutic guidelines, patients with COVID-19 may be accompanied by a decreasing number of bone marrow cells. Therefore, although the damage of COVID-19 to the hematopoietic system and the immune system was not clear, it cannot be ruled out as another possible cause of eosinopenia in patients. The proliferation and differentiation of ECs are based on totipotent hematopoietic stem cells, and the storage pool of ECs in bone marrow provides the kinetic basis for its rapid mobilization. The ultra-micro-pathological results of patients with SARS virus infection showed severe atrophy of the spleen white pulp and decreased myeloid hyperplasia.[10] COVID-19, a coronavirus, may also damage the bone marrow, resulting in a decrease in EC production. Furthermore, research on the mechanism of severe pneumonia caused by SARS-CoV has shown that it is related to the host's excessive immune response to its nucleocapsid protein. Significant up-regulation of Th1 (IFNç, IL-2) and Th2 (IL-4, IL-5) cytokines and down-regulation of anti-inflammatory cytokines (IL-10, TGF-þ) make neutrophils, ECs, and lymphocyte infiltrate into the lung in large quantities, and ECs in peripheral blood decrease.[11] Studies on the infection mechanism of MERS-CoV have also shown that the characteristic inflammatory cells of infected lung tissues are ECs and neutrophils, respectively.[12] Therefore, the decrease of ECs in peripheral blood of patients with COVID-19 may also be related to this mechanism.

The study on the mechanism of eosinophilic granulocytopenia has also led us to further explore the immune damage of coronavirus. The evaluation of immunopathological mechanism in vaccine development is a basic and key step, which includes the reaction of immunoglobulin and cytokines to vaccine, and the test of antigen antibody complex in the tissues where the reaction occurs. In a study of SARS vaccine, after quantitative RT-PCR analysis of virus replication, it was found that virus replication is likely to be required for immunopathology induced by vaccination.[10] This suggests that the research on immune damage of SARS-CoV-2 may be of some value for vaccine research and development.

In summary, continuous observation and detection of ECs in 95 patients with COVID-19 in this study showed that ECs decreased significantly in patients with COVID-19. When the condition improved, the ECs returned to normal, while the ECs of the patients who did not improve continued to decline. It is suggested that ECs have clinical significance in the diagnosis and prognosis of COVID-19, and may be a useful index in the early warning of acute infectious diseases. At present, the epidemic situation is grim. In Europe, many countries are facing the shortage of virus nucleic acid detection kits. More comprehensive diagnosis through clinical indicators is beneficial to relieve the pressure on medical resources. In addition, the decrease of ECs in three kinds of diseases caused by coronavirus, COVID-19, Middle East respiratory syndrome, and severe acute respiratory syndrome, suggests that coronavirus may damage the immune function of human body and cause immune damage syndrome by attacking the cells in bone marrow. Further study of this mechanism requires a large number of samples and subsequent retrospective analysis. It can be confirmed that this will play a beneficial role in the treatment, prevention, and vaccine development of coronavirus infection-related diseases.

CONFLICTS OF INTEREST

To the best of our knowledge, the named authors have no conflict of interest, financial or otherwise.

AUTHORS' CONTRIBUTION

Tong Mu, Zumu Yi, and Minjin Wang conceived the study design, wrote the original draft, and collected the data. Junren Wang conceptualized the study and provided the resources. Chongwei Zhang analyzed and interpreted the data. Hong Chen collected the data. Mingxuan Bai analyzed and interpreted the data. Lingyu Jiang revised the study. Yuwei Zhang conceptualized the study and wrote, reviewed, and edited the article.

EMPLOYMENT OR LEADERSHIP

None declared.

HONORARIUM

None declared.

DATA AVAILABILITY STATEMENT

The datasets of the present study are available from the corresponding author on reasonable request.

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