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Clinica Chimica Acta 508 (2020) 122–129

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

The underlying changes and predicting role of peripheral blood inflammatory cells in severe COVID-19 patients: A sentinel?

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ARTICLE INFO

Keywords:
COVID-19
Peripheral blood inflammatory cells (PBICs)
Blood routine test
Lymphocytes subsets
Severe type

ABSTRACT

Background: The underlying changes of peripheral blood inflammatory cells (PBICs) in COVID-19 patients are little known. Moreover, the risk factors for the underlying changes of PBICs and their predicting role in severe COVID-19 patients remain uncertain.

Material and methods: This retrospective study including two cohorts: the main cohort enrolling 45 patients of severe type serving as study group, and the secondary cohort enrolling 12 patients of no-severe type serving as control group. The PBICs analysis was based on blood routine and lymphocyte subsets. The inflammatory cell levels were compared among patients according to clinical classifications, disease-associated phases, as well as one-month outcomes.

Results: Compared with patients of non-severe type, the patients of severe type suffered from significantly decreased counts of lymphocytes, eosinophils, basophils, but increased counts of neutrophils. These PBICs alterations got improved in recovery phase, but persisted or got worse in aggravated phase. Compared with patients in discharged group, the patients in un-discharged/died group suffered from decreased counts of total T lymphocytes, CD4 + T lymphocytes, CD8 + T lymphocytes, as well as NK cells at 2 weeks after treatment. Clinical classification-critically severe was the independently risk factor for lymphopenia (*OR = 7.701, 95%CI:1.265–46.893, P = 0.027*), eosinopenia (*OR = 5.595, 95%CI:1.008–31.054, P = 0.049*), and worse one-month outcome (*OR = 8.984; 95%CI:1.021–79.061, P = 0.048*).

Conclusion: Lymphopenia and eosinopenia may serve as predictors of disease severity and disease progression in COVID-19 patients, and enhancing the cellular immunity may contribute to COVID-19 treatment. Thus, PBICs might become a sentinel of COVID-19, and it deserves attention during COVID-19 treatment.

1. Introduction

Since December 2019, an increasing number of pneumonia cases emerged in Wuhan, and rapidly spread throughout China [1]. The causative virus was officially named as 2019-novel coronavirus (2019-nCoV), and its relevant infected disease was also officially designated

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https://doi.org/10.1016/j.cca.2020.05.027
Received 4 April 2020; Received in revised form 22 April 2020; Accepted 12 May 2020
Available online 14 May 2020

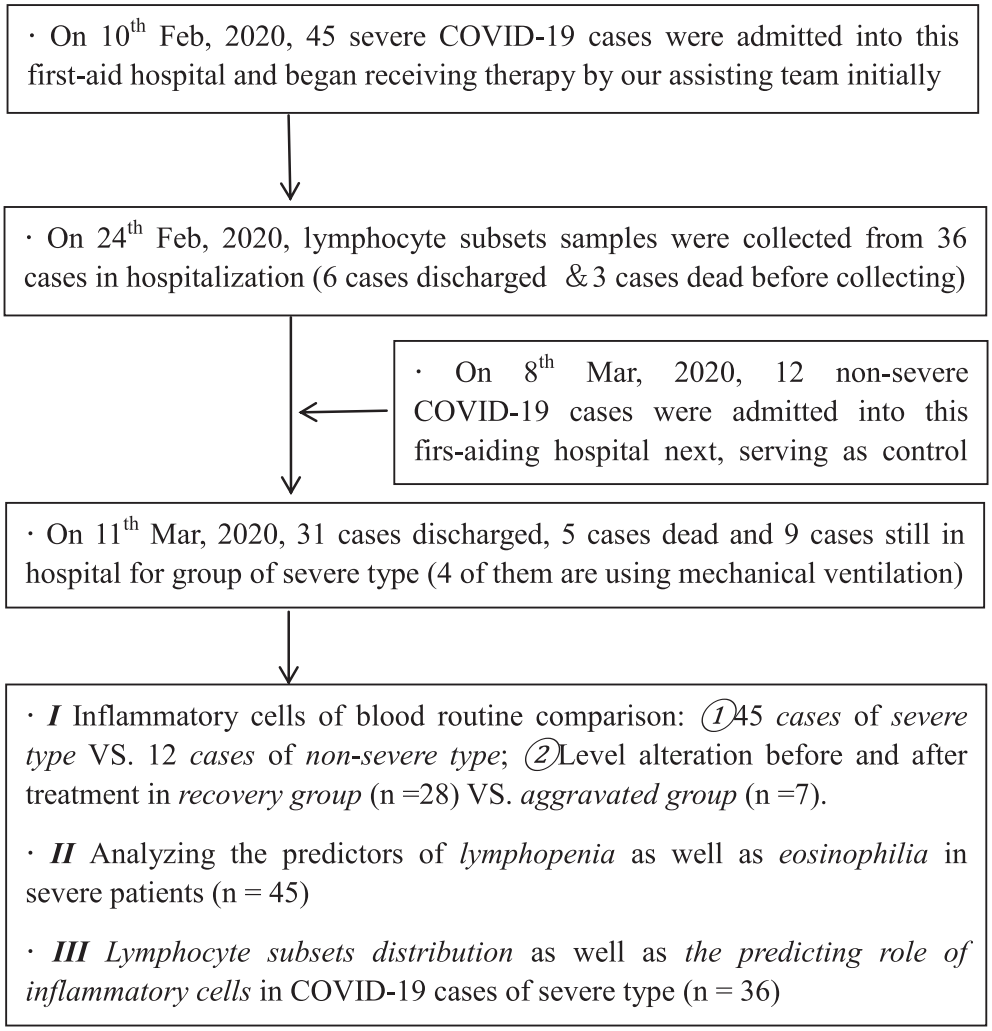

Fig. 1. The flow chart for patients inclusion, sample collection as well as purposes in this study.

coronavirus disease 2019 (COVID-19) by World Health Organization (WHO) [2,3]. As of March 11, 2020, COVID-19 had reached 115 countries, with 119 239 confirmed cases and 4 287 deaths [4]. Currently, the major COVID-19 epidemic hotspots were brought under control in China [5], due to Chinese government's quickly effective response as well as vigorous public health measures. However, the number of COVID-19 cases outside China had increased drastically, with 143 affected countries, states, or territories reporting to WHO by March 16, 2020 [6]. Obviously, the situation of COVID-19 is towards controlling of a pandemic now [6], and study investigating risk factors for severe COVID-19 related outcomes including death is still needed [7].

As of now, the number of known risk factors for COVID-19 are relatively limited. Admittedly, older age and diabetes were already reported to be significantly correlated with increased incidence, disease severity, as well as risk of mortality in COVID-19 [8–11]. Besides, increased D-dimer, high N terminal pro B type natriuretic peptide (NT-proBNP), and increased serum amyloid A were also reported to be risk factors for disease severity and prognosis in COVID-19 [12–14]. As components of blood routine test available for almost all hospitalized patients, PBICs usually serve as practical markers in infectious disease. Indeed, leukocytosis, leukopenia, and lymphopenia have been reported to be commonly seen in COVID-19 patients [15]. However, the underlying changes of other peripheral blood inflammatory cells (PBICs) in COVID-19 patients are little known, especially eosinophil level and lymphocyte subsets. Additionally, the risk factors for the underlying changes of these PBICs as well as their roles in COVID-19 patients' outcomes are not well addressed yet.

Herein, the PBICs in COVID-19 patients were compared between group of severe type and group of no-severe type in this research. Meanwhile, the underlying changes as well as the predicting role of PBICs, especially eosinophil level and lymphocyte subsets, in severe COVID-19 patients were also explored.

2. Material and methods

2.1. Participants, treatment and sample collection

Patients included in this study were 2019-nCoV positive based on nucleic acid detection, from the intensive care unit (ICU) of Tongji hospital affiliated to Huazhong University of Science and Technology. The primary cohort included 45 cases of severe type, who were admitted by our assisting team initially on 10th Feb 2020, serving as study group. Subsequently, 12 cases of no-severe type were admitted into this first-aiding hospital by our team on 8th Mar 2020, serving as control group. The major treatments for patients were drugs therapy, including antiviral treatment (*Arbidol tablets* combined with *Lian Hua Qing Wen Jiao Nang),* antibiotic therapy/corticosteroid therapy if necessary, and other supporting measures, such as oxygen or mechanical ventilation or continuous renal replacement therapy or extracorporeal membrane oxygenation.

The aggravated phase in this research was defined in case of using

Table 1
Clinical characteristics of COVID-19 patients included in this study (n = 57).

Parameter	Severe type (n = 45)	No-severe type (n = 12)
Age (years)	67.0 (56.5–72.5)	61.0 (49.0–65.0)
Gender (Male/Female)	24/21	5/7
Interval from illness onset to hospital admission (days)	13.1 ± 4.3	22.2 ± 13.5
Fever (Y/N)	41/4	10/2
Respiratory signs (Y/N)	37/8	9/3
Digestive signs (Y/N)	29/16	3/9
General signs (Y/N)	37/8	3/9
Chronic disease history (Y/N)	32/13	2/10
Tumor history (Y/N)	5/40	0/12
Smoking history (Y/N)	12/33	0/12
Drinking history (Y/N)	12/33	0/12
Temperature after admission (℃)	36.4 (36.3–36.9)	36.3 (36.3–36.4)
Pulse rate after admission (times per min)	82.0 (77.5–94.5)	100.0 (98.0–108.0)
Breath rate after admission (times per min)	20.0 (19.5–24.0)	20.0 (18.0–20.0)
MAP after admission (mmHg)	93.3 (87.7–101.3)	103.0 (100.8–112.7)
BMI (Kg/m^2)	24.0 (21.5–26.0)	26.8 (23.0–29.9)

Abbreviations and illustration: Values are presented as mean ± SD, or median (interquartile range). Y/N, Yes/Not; MAP, mean arterial pressure; BMI, body mass index.

mechanical ventilation. The discharge criteria was defined as following: 'without fever for more than 3 days', 'the signs of respiratory system improved significantly', 'the acute exudative inflammation of lung dissipated or improved significantly', and '2019-nCoV turned into be negative by throat swab at less twice (with the interval ≥ 1 day)'.

For the patients of severe type, lymphocyte subsets samples were simultaneously collected from 36 cases in hospitalization (on 24th Feb 2020), since 6 cases have been discharged and 3 cases were dead before sample collection. The blood routine samples were collected the first day after admission, the day of using mechanical ventilation due to respiratory failure, as well as the day before discharge after recovery. The blood routine test before discharge was available in 28 recovery cases, but unavailable in 3 recovery cases. For aggravated patients, the blood routine test of aggravated phase was examined at the onset of using mechanical ventilation due to respiratory failure, which was available in 7 cases. As components of blood routine test, the leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, and basophils were detected and counted by hemocytometer. Lymphocyte subsets comprising of T cells, CD4 + T cells, CD8 + T cells, B cells and NK cells, were detected and counted by flow cytometer.

By the date of research (11th Mar 2020), all patients of severe type had been observed more than one month. Among them, 31 cases had been discharged, 5 cases were dead, and 9 cases were in hospital because they did not meet recovery criteria. The flow chart in Fig. 1 showed the patients inclusion as well as sample collection in this study. This research was in accordance with the *Declaration of Helsink*i, and this study was also approved by the Ethics Commission of 1st hospital affiliated to Jilin University (No. 2020–171). Written informed consent was waived given the rapid emergence of this infectious disease.

2.2. Clinical classification

All patients were evaluated of clinical classification after admission, according to the diagnostic and therapeutic guideline of COVID-19 (Trial 6th edition) issued by National Health Commission of China [16]. Severe type was defined if any of the following items was met: 'Tachypnea, with breath rate ≥ 30 times per minute', 'Oxygen saturation of fingertip ≤ 93% at resting time', 'Partial arterial oxygen pressure (PaO_2)/oxygen saturation (FiO_2) ≤ 300 mmHg'. Critically severe type was defined if any of the items was presented: 'Respiratory failure needing mechanical ventilation', 'Shock', 'Concomitant dysfunction/failure of other organ, needing monitoring and therapy in intensive care unit'.

2.3. Study purposes

In this research, we firstly compared the inflammatory cells according to blood routine, between groups of severe type and non-severe type. Second, we analyzed the inflammatory cells alterations of blood routine in recovery group, as well as aggravated group. Third, we analyzed the predictors of lymphopenia as well as eosinopenia in patients of severe type. Finally, we investigated the lymphocyte subsets distribution, as well as the role of PBICs in predicting one-month outcome for patients of severe type. Our study purposes could also be seen in Fig. 1.

2.4. Statistical analysis

In this research, SPSS version 23.0 software (SPSS Inc.) was used to perform data analysis. The study subgroups were compared using the chi-square test for categorical variables. If the continuous variables were normally distributed, they would be expressed as mean ± standard deviation (SD), and then compared by *Student's t*-test. Otherwise, the continuous variables would be expressed in median (interquartile range, IOR), and compared by *Mann-Whitney-Wilcoxon W* test. Logistic regression via both univariable and multivariable analysis was used to exploit the risk factors for lymphopenia, eosinopenia, as well as one-month outcome, and odds ratio (OR) with its 95% confidence interval (CI) was utilized as the effect value. In each test, a *P* value with two tails < 0.05 was defined as statistically significant.

3. Results

3.1. The characteristics of included patients

The median age for severe group and non-severe group were 67.0 years (*IOR:* 56.5–72.5) and 61.0 years (*IQR:* 49.0–65.0), and the intervals from illness onset to hospital admission were 13.1 ± 4.3 days and 22.2 ± 13.5 days, respectively. The clinical characteristics for these two groups were not compared, but were shown in Table 1.

3.2. Comparison of blood routine-inflammatory cells in patients of different classifications

When compared with patients of non-severe type, the patients of severe type suffered from significantly decreased counts of lymphocytes (*1.197 ± 0.488 x10^9/L, 1.978 ± 0.507 x10^9/L, P < 0.001*), eosinophils (*M = 0.030, IQR:0.005–0.050 x10^9/L; M = 0.160, IQR:0.123–0.228 x10^9/L, P < 0.001*), basophils (*M = 0.010, IQR:0.010–0.030 x10^9/L; M = 0.030, IQR:0.020–0.038 x10^9/L, P < 0.001*), but increased counts of neutrophils (*M = 4.540, IQR:3.695–6.145 x10^9/L; M = 3.600, IQR:3.078–4.383 x10^9/L, P = 0.023*). On the contrary, no difference was found in the counts of leukocytes (*M = 6.300, IQR:5.420–7.820 x10^9/L; M = 6.370, IQR:5.560–7.450 x10^9/L, P = 0.953*) or monocytes (*M = 0.470, IQR:0.360–0.680 x10^9/L; M = 0.570, IQR:0.490–0.648 x10^9/L, P = 0.200*). Besides, the percentage alteration was in consistent with the absolute counts alteration for each analyzed inflammatory cell (Table 2).

3.3. Predictors of lymphopenia as well as eosinopenia in patients of severe type

Totally, there were 21 patients diagnosed with lymphopenia in group of severe type, with the percentage of 46.67% (21/45). According to the univariable logistic regression, interval from illness onset to hospital admission (*OR = 0.843; 95%CI:0.716–0.992,*

Table 2
Comparison of blood routine-inflammatory cells in COVID-19 patients of different classification (n = 57).

Variable [Reference range]	Severe type (n = 45)	Non-severe type (n = 12)	Z/t value	P value
Le ($x10^9$/L) [3.50–9.50]	6.300 (5.420–7.820)	6.370 (5.560–7.450)	–0.059	0.953
NE ($x10^9$/L) [1.80–6.30]	4.540 (3.695–6.145)	3.600 (3.078–4.383)	–2.271	0.023
Ly ($x10^9$/L) [1.10–3.20]	1.197 ± 0.488	1.978 ± 0.507	–4.890	0.000
Mo ($x10^9$/L) [0.10–0.60]	0.470 (0.36–0.680)	0.570 (0.490–0.648)	–1.283	0.200
Eo ($x10^9$/L) [0.02–0.52]	0.030(0.005–0.050)	0.160 (0.123–0.228)	–4.904	0.000
Ba ($x10^9$/L) [0.00–0.10]	0.010 (0.010–0.030)	0.030 (0.020–0.038)	–3.630	0.000
NE (%) [40.0–75.0]	71.515 ± 11.655	56.798 ± 7.258	4.149	0.000
Ly (%) [20.0–50.0]	19.106 ± 9.107	30.915 ± 5.986	–4.239	0.000
Mo (%) [3.0–10.0]	7.620 (6.020–10.687)	8.983 (7.880–10.385)	–1.233	0.218
Eo (%) [0.4–8.0]	0.437 (0.033–0.799)	2.354 (1.789–3.768)	–4.852	0.000
Ba (%) [0.0–1.0]	0.166 (0.132–0.312)	0.460 (0.294–0.653)	–3.937	0.000

Abbreviations and illustration: Values are presented as mean ± SD, or median (interquartile range); Le, leukocyte; NE, neutrophil; Ly, lymphocyte; Mo, monocyte; Eo, eosinophil; Ba, basophil.

Table 3
Analyzing predictors of lymphopenia in COVID-19 patients of severe type (n = 45).

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	P value	OR	95% CI	P value
Age (≥67/ < 67)	2.800	0.829–9.458	0.097			
Gender (Male/Female)	0.450	0.136–1.488	0.191			
Interval from illness onset to hospital admission (days)	0.843	0.716–0.992	0.040	0.843	0.706–1.007	0.060
Chronic disease history (Y/N)	4.286	0.988–18.586	0.052			
Tumor history (Y/N)	1.833	0.276–12.191	0.531			
Smoking history (Y/N)	3.600	0.823–15.742	0.089			
Drinking history (Y/N)	6.786	1.280–35.966	0.024	4.694	0.717–30.721	0.107
Clinical type (Critically/No-critically)	8.250	1.529–44.528	0.014	7.701	1.265–46.893	0.027
BMI (Kg/m^2)	0.893	0.762–1.047	0.164			
Le ($x\ 10^9$/L)	1.021	0.831–1.254	0.846			

Abbreviations: Y/N, Yes/Not; Le, leukocyte; BMI, body mass index.

P = 0.040), drinking history (*OR = 6.786; 95%CI:1.280–35.996, P = 0.024*), and clinical classification-critically severe (*OR = 8.250; 95%CI:1.529–44.528, P = 0.014*) were significantly associated with lymphopenia. However, when these associated factors were analyzed according to multivariable logistic regression, only clinical classification-critically severe was found to be associated with lymphopenia (*OR = 7.701; 95%CI:1.265–46.893, P = 0.027*) (Table 3).

Meanwhile, 16 patients were diagnosed with eosinopenia in the group of severe type, with the percentage of 35.56% (16/45). Univariable logistic regression results demonstrated that gender (*OR = 0.239; 95%CI:0.065–0.882, P = 0.032*), interval from illness onset to hospital admission (*OR = 0.830; 95%CI:0.698–0.988, P = 0.036*), drinking history (*OR = 9.167; 95%CI:1.058–79.386, P = 0.044*), and clinical type-critically severe (*OR = 4.861; 95%CI:1.145–20.632, P = 0.032*) were found to be associated with eosinopenia. Similarly, only clinical classification-critically severe was found to be associated with eosinopenia (*OR = 5.595; 95%CI:1.008–31.054, P = 0.049*) when analyzed via multivariable logistic regression (Table 4).

3.4. Alteration of blood routine-inflammatory cells in patients of severe type

The blood routine samples immediately after admission and before discharge were available in 28 cases of the 31 recovery COVID-19 patients. When compared with patients in recovery phase, patients in acute phase suffered from significantly decreased counts of lymphocytes (*1.383 ± 0.442 $x10^9$/L, 1.655 ± 0.571 $x10^9$/L, P = 0.001*), eosinophils (*0.031 ± 0.024 $x10^9$/L; 0.133 ± 0.082 $x10^9$/L, P < 0.001*), basophils (*M = 0.010, IQR:0.010–0.020 $x10^9$/L; M = 0.030, IQR:0.020–0.040 $x10^9$/L, P < 0.001*), but increased counts of leukocytes (*M = 6.235, IQR:5.368–7.370 $x10^9$/L; M = 5.690, IQR:4.795–6.523 $x10^9$/L, P = 0.015*), neutrophils (*M = 4.365, IQR:3.400–5.005 $x10^9$/L; M = 3.130, IQR:2.603–3.910 $x10^9$/L, P = 0.003*) as well as monocytes (*M = 0.550, IQR:0.443–0.780 $x10^9$/L; M = 0.505, IQR:0.420–0.643 $x10^9$/L, P = 0.018*). The percentage alteration was in consistent with the absolute counts alteration for each analyzed inflammatory cell, except monocyte for which no significant alteration was identified after recovery.

On the contrary, 7 patients developed respiratory failure and used mechanical ventilation during the treatment, which were defined as cases with aggravation. The inflammatory cells comparison between acute phase and aggravated phase could be seen in Table 5. No significant alteration between these two phases was found, but the situation of lymphopenia became worse and the situation of eosinopenia persisted in aggravated phase (Table 5).

3.5. Lymphocyte subsets distribution in patients of severe type

The lymphocyte subsets distribution was available in 36 patients of severe type. According to one-month outcome, these patients were divided into discharged group (n = 25) and un-discharged/died group (n = 11). Compared with patients in discharged group, the cases in un-discharged/died group suffered from decreased counts of total T lymphocytes (*1089.680 ± 290.154/μl, 698.455 ± 393.675/μl, P = 0.002*), CD4 + T lymphocytes (Th) (*686.96 ± 225.383/μl, 427.091 ± 251.712/μl, P = 0.004*), CD8 + T lymphocytes (Ts) (*359.840 ± 11.279/μl, 247.818 ± 153.638/μl, P = 0.019*), as well as NK cells (*M = 222.000, IQR: 159.000–332.000/μl; M = 117.000, IQR:74.000–246.000/μl; P = 0.01*). On the contrary, there was no difference between these two groups, regarding Th/Ts ratio, or total B lymphocytes counts with its percentage. However, the percentages of total T cells, CD4 + T cells, CD8 + T lymphocytes as well as NK cells were equivalent between these two groups (Table 6).

Table 4
Analyzing predictors of eosinophilia in COVID-19 patients of severe type (n = 45).

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	P value	OR	95% CI	P value
Age (≥67/< 67)	1.200	0.352–4.094	0.771			
Gender (Male/Female)	0.239	0.065–0.882	0.032	0.437	0.075–2.544	0.357
Interval from illness onset to hospital admission (days)	0.830	0.698–0.988	0.036	0.850	0.700–1.031	0.099
Chronic disease history (Y/N)	2.281	0.524–9.924	0.272			
Tumor history (Y/N)	3.115	0.462–20.988	0.243			
Smoking history (Y/N)	1.950	0.443–8.579	0.377			
Drinking history (Y/N)	9.167	1.058–79.386	0.044	3.900	0.290–52.518	0.305
Clinical type (Critically/No-critically)	4.861	1.145–20.632	0.032	5.595	1.008–31.054	0.049
BMI (Kg/m^2)	0.993	0.891–1.107	0.896			
Le (x 10^9/L)	1.026	0.830–1.268	0.814			

Abbreviations: Y/N, Yes/Not; Le, leukocyte; BMI, body mass index.

3.6. Risk factors for one-month outcomes in patients of severe type

The overall one-month survival rate for all included patients of severe type was 88.89% (40/45), with the mortality rate of 11.11% (5/45). In order to explore the role of PBICs in patients' outcome as much as possible, we utilized the inflammatory cells of blood routine (the first day after admission), lymphocyte subsets (2 weeks after admission), as well as other clinical characteristics. Univariable logistic regression results showed that the risk factors for one-month outcome were clinical classification-critically severe (*OR = 13.800; 95%CI:2.127–89.524, P = 0.006*), total lymphocyte counts (*OR = 9.562; 95%CI:1.666–54.890, P = 0.011*), and total NK cell counts (*OR = 9.187; 95%CI:1.803–46.828, P = 0.008*). But when analyzed via multivariable logistic regression analysis, only clinical classification-critically severe was associated with worse one-month outcome (*OR = 8.984; 95%CI:1.021–79.061, P = 0.048*) (Table 7).

4. Discussion

The pathological findings of COVID-19 patient demonstrated that there were diffuse alveolar damage as well as interstitial mononuclear inflammatory infiltrates, dominated by lymphocytes in bilateral lungs [17]. Meanwhile, cytometric analysis of this patient showed that CD4 + and CD8 + T cells were substantially reduced but with hyperactivated status in the peripheral blood [17]. Accordingly, the researchers proposed that the over-activation and high cytotoxicity of lymphocytes partly contributed to the severe immune injury in this patient [17]. Besides, lung pathology of severe acute respiratory syndrome CoV (SARS-CoV) and Middle East respiratory syndrome CoV (MERS-CoV) patients also showed that there were extensive cellular infiltrates in the interstitium and alveoli, with the neutrophils and macrophages being the predominant cell type [18]. Based on these background along with the decrease of multiple PBICs in COVID-19 patients, we hypothesized that neutrophils, eosinophils and lymphocytes migrate from peripheral blood into the lung tissue, resulting in neutropenia, lymphopenia, and eosinopenia in peripheral blood, as well as acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) in COVID-19 patients.

Notably, several points have been raised in our study. First, COVID-19 patients of severe type suffer from decreased counts of lymphocytes, eosinophil, and basophils, but increased counts of neutrophils, when compared with COVID-19 patients of non-severe type. Second, clinical classification-critically severe is the independent risk factor for lymphopenia and eosinopenia. Third, the situation of inflammatory cells decrease gets improved in recovery phase of COVID-19 patients, but this situation persists like this or gets worse in aggravated phase of COVID-19 patients. As for the fourth point, when compared with cases in discharged group, the cases in un-discharged/died group suffered from poor cellular immunity, characterized by reduced level of total T lymphocytes as well as NK cells. Last but not the least, clinical classification-critically severe is the independently risk factor of one-month outcome in patients of severe type. Nevertheless, some of our results are consistent with the scientific results raised by other researchers, which will be explained step by step as followings.

First of all, several studies have raised the issue of leukopenia, lymphopenia, eosinopenia, monopenia, and/or leukocytosis in COVID-19 patients. One study based on 452 cases (286 severe cases and 266 non-severe cases) demonstrated that severe cases had higher counts of leukocytes (*5.6x10^9/L vs 4.9x10^9/L; P < 0.001*) and neutrophils (*4.3x10^9/L vs 3.2x10^9/L; P < 0.001*), but lower percentages of monocyte (*6.6% vs 8.4%; P < 0.001*), eosinophil (*0.0% vs 0.2%; P < 0.001*), and basophil (*0.1% vs 0.2%; P = 0.015*) [19]. Another

Table 5
The alteration of blood routine-inflammatory cells in COVID-19 patients (severe type) of different developing trends (n = 35).

Variable [Reference range]	Recovery group (n = 28)				Aggravated group (n = 7)			
	Acute phase	Recovery phase	Z/t value	P value	Acute phase	Aggravated phase	Z/t value	P value
Le (x10^9/L) [3.50–9.50]	6.235 (5.368–7.370)	5.690 (4.795–6.523)	–2.437	0.015	8.200 ± 4.335	13.433 ± 5.847	–1.639	0.152
NE (x10^9/L) [1.80–6.30]	4.365 (3.400–5.005)	3.130 (2.603–3.910)	–3.006	0.003	7.073 ± 4.012	12.423 ± 5.890	–1.693	0.141
Ly (x10^9/L) [1.10–3.20]	1.383 ± 0.442	1.655 ± 0.571	–3.664	0.001	0.680 (0.400–0.780)	0.440 (0.400–0.860)	–0.676	0.499
Mo (x10^9/L) [0.10–0.60]	0.550 (0.443–0.780)	0.505 (0.420–0.643)	–2.369	0.018	0.320 (0.310–0.680)	0.420 (0.290–0.550)	–0.169	0.866
Eo (x10^9/L) [0.02–0.52]	0.031 ± 0.024	0.133 ± 0.082	–7.122	0.000	0.000 (0.000–0.010)	0.000 (0.000–0.010)	–0.378	0.705
Ba (x10^9/L) [0.00–0.10]	0.010 (0.010–0.020)	0.030 (0.020–0.040)	–4.107	0.000	0.010 (0.000–0.020)	0.010 (0.010–0.030)	–0.647	0.518
NE (%) [40.0–75.0]	66.714 ± 9.743	57.962 ± 8.034	3.340	0.002	84.145 ± 6.614	91.221 ± 4.358	–1.908	0.105
Ly (%) [20.0–50.0]	22.570 (15.035–27.671)	28.556 (23.568–35.864)	–3.484	0.000	8.609 ± 2.633	4.983 ± 3.036	1.823	0.118
Mo (%) [3.0–10.0]	9.479 (7.516–11.549)	9.969 (7.710–11.587)	–0.182	0.855	5.650 (5.087–7.226)	2.701 (2.178–4.471)	–1.859	0.063
Eo (%) [0.4–8.0]	0.483 (0.101–0.759)	1.937 (1.370–3.514)	–4.623	0.000	0.000 (0.000–0.106)	0.000 (0.000–0.091)	–0.135	0.893
Ba (%) [0.0–1.0]	0.185 (0.136–0.344)	0.509 (0.346–0.804)	–4.463	0.000	0.134 (0.000–0.159)	0.090 (0.064–0.120)	–0.169	0.866

Abbreviations and illustration: Values are presented as mean ± SD, or median (interquartile range); Le, leukocyte; NE, neutrophil; Ly, lymphocyte; Mo, monocyte; Eo, eosinophil; Ba, basophil.

Table 6
The distribution of lymphocyte subsets in COVID-19 patients (severe type) by different one-month outcomes (n = 36).

Variable [Reference range]	Discharged group (n = 25)	Un-discharged/Died group (n = 11)	Z/t value	P value
Total T lymphocyte (CD3 + CD19-) [50%-84%]	71.194 ± 6.521	66.509 ± 13.746	−1.400	0.171
Total T lymphocyte (CD3 + CD19-) [955–2860/μl]	1089.680 ± 290.154	698.455 ± 393.675	−3.337	0.002
Total B lymphocyte (CD3-CD19 +) [5%-18%]	10.837 ± 5.432	14.392 ± 5.937	1.759	0.088
Total B lymphocyte (CD3-CD19 +) [90–560/μl]	139.000 (109.000–185.000)	105.000 (73.000–215.000)	−0.721	0.471
Helper/induced T lymphocyte(CD3 + CD4 +) [27%-51%]	44.279 ± 6.872	40.184 ± 10.969	−1.365	0.181
Helper/induced T lymphocyte (CD3 + CD4 +) [550–1440/μl]	686.960 ± 225.383	427.091 ± 251.712	−3.077	0.004
Inhibitory/cytotoxic lymphocyte(CD3 + CD8 +)[15%-44%]	21.920 (19.250–26.680)	24.540 (13.860–26.650)	−0.155	0.877
Inhibitory/cytotoxic lymphocyte(CD3 + CD8 +) [320–1250/μl]	359.840 ± 111.279	247.818 ± 153.638	−2.472	0.019
NK cell (CD3-/CD16 + CD56 +) [7%–40%]	15.410 (13.260–18.940)	15.760 (8.620–25.130)	−0.395	0.693
NK cell (CD3-/CD16 + CD56 +) [150–1100/μl]	222.000 (159.000–332.000)	117.000 (74.000–246.000)	−2.593	0.010
T + B + NK [95.00%–100%]	99.070 (98.580–99.415)	99.000 (97.760–99.590)	−0.515	0.606
T + B + NK [Not available/μl]	1518.720 ± 407.291	984.455 ± 481.608	−3.430	0.002
Th/Ts [0.71–2.78]	1.993 ± 0.606	1.957 ± 0.905	−0.139	0.890

Abbreviations and illustration: Values are presented as mean ± SD, or median (interquartile range). NK, natural killer cell; Th, helper T cells; Ts, suppressor T cells.

study based on 1 099 cases from 552 hospitals showed that lymphopenia and leukopenia were present in 83.2% and 33.7% of the patients on admission [20]. The other study enrolling 138 patients revealed that eosinopenia was seen in 60.7% of the severe patients (34/56), according to blood cell test results on the first day of admission. Meanwhile, this study also revealed that absolute numbers of circulating eosinophils and lymphocytes correlated positively with each other, especially for the second test during hospitalization [21]. Moreover, another study showed that the incidence rate of eosinopenia in COVID-19 was 70.0% (7/10), but the incidence rate in patients with other viral pneumonia is only 16.7% (5/30) [22], suggesting that COVID-19 patients are susceptible to eosinopenia.

The alterations of these above blood inflammatory cells have also been shown to be associated with COVID-19 patients' outcome. One study based on 33 cases reported that the non-survivors suffered from severe lymphopenia, neutrophilia, as well as leukocytosis when compared with survivors [23]. In parallel with the above results, another study enrolling 191 cases showed that lymphopenia was more commonly seen in non-survivors (76%) when compared with survivors (26%) [9]. Additionally, the lymphopenia (median counts 0.42–0.67 $x10^9$/L) persisted in non-survivors as long as 25 days from illness onset, but the median lymphocyte counts increased from 1.08 $x10^9$/L to 1.43 $x10^9$/L in survivors [9]. Moreover, as continuous variable, lymphocyte was also reported to be associated with in-hospital death (*OR = 0.02, 95%CI: 0.01–0.08, P < 0.0001*) by univariable logistic regression, but this association was not found by multivariate regression anlaysis in this above study [9]. Coincidently, this above logistic regression results were in line with those in our study, in which the situation of eosinopenia get improved during recovery phase and eosinopenia did not correlate with worse one-month outcome via multivariable logistic regression analysis. Interestingly, one more study based on ten COVID-19 patients was also identified, showing that counts of lymphocyte increased persistently after sustained lopinavir-combined regimen, and eosinophil counts were low on initial hospitalization, but all returned to normal before discharge [24]. These above results suggesting that lymphopenia and eosinopenia are associated with disease severity, and the alterations of lymphocytes and eosinophils significantly correlated with disease progression in COVID-19 patients.

Second, the lymphocyte subsets distribution as well as their role in COVID-19 patients are investigated by only few studies till now. One study based on 249 cases of mild type showed that 45.5% of COVID-19 patients suffered from decreased CD4 + T cells counts, and 92.8% of the patients had normal CD4 + T/CD8 + T ratio [25]. Meanwhile, this study also revealed that CD4 + T cell count (per 100 cells/μl increase) was independently associated with ICU admission (*OR = 0.55,95%:0.33–0.92, P = 0.02*) according to multivariate logistic regression [25]. In another study based on 44 patients, lymphocyte subsets analyzed results showed that the total number of B cells, T cells and natural killer (NK) cells decreased in the whole group, and this decrease became more evident in the cases of severe type when compared with non-severe type (*743.6/uL vs 1020.1/uL; P = 0.032*) [19]. In more detail, T cells along with NK cells were below normal levels, but B cells were within normal range, with the situation becoming more worse in patients of severe type [19], which coincided with the results from the other study enrolling 37 COVID-19 cases according to blood sample before initial treatment [26]. Additionally, there was an

Table 7
Univariate and multivariate logistic analyses of risk factors for one-month outcomes in COVID-19 patients of severe type (n = 36).

Variable	Univariate analysis			Multivariate analysis		
	OR	95% CI	P value	OR	95% CI	P value
Age (years)	1.078	0.997–1.165	0.059			
Gender (Male/Female)	0.556	0.133–2.325	0.421			
Interval from illness onset to hospital admission (days)	0.844	0.695–1.026	0.089			
Chronic disease history (Y/N)	0.571	0.098–3.333	0.534			
BMI (Kg/m^2)	0.943	0.843–1.056	0.310			
Clinical type (Critically/No-critically)	13.800	2.127–89.524	0.006	8.984	1.021–79.061	0.048
Ly (< 1.10×10^9/L **Vs.** ≥ 1.10×10^9/L)	9.562	1.666–54.890	0.011	4.791	0.628–36.559	0.131
T cells (< 0.95×10^9 **Vs.** ≥ 0.95×10^9)	3.719	0.839–16.474	0.084			
B cells (< 0.05×10^9 **Vs.** ≥ 0.05×10^9)	0.997	0.987–1.006	0.483			
NK cells (< 0.15×10^9 **Vs.** ≥ 0.15×10^9)	9.187	1.803–46.828	0.008	3.579	0.485–26.412	0.211
Eo (< 0.02×10^9/L Vs. ≥ 0.02×10^9/L)	3.086	0.707–13.465	0.134			
Hemoglobin (< 116 g/L **Vs.** ≥ 116 g/L)	1.630	0.232–11.455	0.624			
Albumin (< 35 g/L **Vs.** ≥ 35 g/L)	3.111	0.712–13.601	0.132			

Abbreviations: Ly, lymphocyte; Eo, eosinophil; NK, natural killer cell.

increase of CD8 + T cells and B cells in patients with clinical response, but there was no significant change of any lymphocyte subset detected in patients without clinical response [26]. These results are consistent with our results, regarding the total counts alteration of T cells, B cells, and NK cells in patients with different classifications. Differently, we retrospectively investigated the distribution of lymphocyte subsets (2 weeks after treatment rather than pretreatment) in patients with one-month outcome in COVID-19 patients and also analyzed its predicting role in one-month outcome (*Recovery* versus *Un-discharged/died*), which may be useful in judging patients' prognosis.

Recently, study reported that SARS-CoV-2 possessed a unique immune pathology compared with other coronaviruses [27]. The frequency of multi-functional CD4 + T cells (defined as positive at least two of these three molecules: interferon-γ, tumor necrosis factor-α, and interleukin-2) was significantly lower in COVID-19 patients of severe type than the healthy control and mild group. Meanwhile, the frequency of non-exhausted CD8 + T cells (PD-1 negative, CTLA-4 negative, and TIGIT negative) significantly decreased when compared with the other two groups. However, the functional blockade of PD-1, CTLA-4, and TIGIT will enhance CD8 + T cells effector function, resulted in viral clearance [28]. Thus, it is assumed by this study that the functional damage of CD4 + T cells makes COVID-19 patients susceptible to severe disease, and the excessive exhaustion of CD8 + T cells impairs cellular immune response to 2019-nCoV in severe patients [27]. In consistent with this above standpoint, transplantation of angiotensin I converting enzyme 2 receptor (ACE2) negative mesenchymal stem cells (free from 2019-nCoV infection), has been reported to be safe and effective for treatments in COVID-19 patients [16].

Thirdly, is there an inter-relationship between the alterations of PBICs and ALI/ARDS in COVID-19 patients? As a common cause of respiratory failure, ARDS is present in approximately 10% of all ICU patients worldwide [29]. ARDS is currently regarded as a response to various injuries all evolving through a number of different phases: alveolar and capillary damage to lung resolution with or without a fibroproliferative phase [30]. In fact, there is emerging evidence showed that immune molecular regulation involved in the pathogenesis of ARDS, including neutrophil netosis, the pro-inflammatory response of Th17 subsets, and the anti-inflammatory and regenerative role of T regulatory cells subsets [31]. Besides, inflammatory responses are reported to have key effects on every phase of ARDS, and their cascades damage vascular endothelial barrier and increase vascular permeability [32]. Moreover, according to the surgical pathology of 5 patients diagnosed with swine-origin influenza type A (H1N1) and acute respiratory failure, macrophages, CD4 + T helper cells, CD8 + T cytotoxic cells, CD20 + B cells, CD1a + dendritic cells, S100 + dendritic cells, and NK cells were aggregated around vessels and bronchioles in all patients' specimens [33]. In line with this, another study based on 44 patients with alveolar damage showed that there was higher level of neutrophils and macrophages in small airways and parenchyma, but the H1N1 group suffered from higher percentage of CD4 + and CD8 + T lymphocytes, CD83 + dendritic cells, and NK cells in the lung parenchyma [34].

Neutrophils are immune cells that are well known to be present in various lung diseases, including viral respiratory disease [35]. As a hallmark of the pathophysiology, it is widely accepted that neutrophils can exit the circulation into the airways, either through the post-capillary venule in the systemic circulation or through the capillary in the pulmonary circulation [36,37]. When recruited and activated in pulmonary tissue in large number, polymorphonuclear neutrophils (PMNs) can cause ALI by increasing permeability of the pulmonary vasculature and extensive damage of interstitial lung tissues [38]. In detail, the neutrophil-derived microparticles activated several target cells which caused lung injury, including endothelial cells, neutrophils, macrophages, and platelets [39].

Lymphocytes exist in relatively small numbers in the lung. In the process of ALI, lymphocytes have been reported to migrate to the lung, where they maintain, enhance, and regulate immune response [40]. According to pig model induced by swine influence H1N1 virus, higher frequencies of cytotoxic T lymphocytes, immunosuppressive T cells, activated T cells, dendritic cells, and CD4 + and CD8 + T cells were found in the infected lungs [41]. Besides, one study showed that T follicular regulatory cells (Tfr) strongly enriched and infiltrated the human airway during the onset of ARDS, and Tfr also regulated the development of B regulatory cells [42]. It is well known that NK cells are effector lymphocytes of the innate immune system. As regulatory cells engaged in reciprocal interactions with T cells, macrophages, and dendritic cells, NK cells can be redundant during immune challenge, and they can exacerbate immune responses [43]. It is revealed that NK cells are sparse in lung tissue of fatal cases, and granzyme B-expressing NK cells are accumulated in the respiratory tracts of cases diagnosed respiratory syncytial virus (RSV) bronchitis [44]. Meanwhile, RSV infection can cause recruitment and activation of lung NK cells at early stage of infection, then activated NK cells became functional NK cells because they produce large amount of gamma interferon (IFN-γ), resulting in acute lung immune injury [45]. Moreover, NK cells could also promote neutrophils recruitment by accelerating the production of pulmonary chemokine CXC ligand (CXCL) 1 and CXXL2 during the lung injury [46].

Eosinophils are derived from CD34 + stem cells in the bone marrow [47], and they are terminally differentiated and will not proliferate once leaving the bone marrow [48]. Eosinophils presented in small numbers in the peripheral blood, and can also be found in lung tissue, adhering to the endothelium as well as in sputum [49]. When recruited or activated, eosinophils would cause airway inflammation or damage by activating various mediators, including major basic protein (MBP), eosinophilic catinonic protein (ECP), eosinophil peroxidase (EPO) as well as cytokines [49]. Then, persistent eosinophilic inflammation can lead to decline and exacerbations in the lung function [47]. Consistently, research in vivo revealed that viruses induced EPO release when coincubated in the presence of antigen-presenting cells and T cells, and this virus-mediated release was associated with proliferation of CD3 + CD4 + T cells and release of cytokines [50].

These above clinical results based on patients, molecular mechanism in vivo, along with the pathological results of COVID-19 patient [17], coincided with our conjecture once again– '*Neutrophils, eosinophils and lymphocytes migrate from peripheral blood into the the lung tissue, resulting in neutropenia, lymphopenia and eosinopenia in peripheral blood, as well as accelerating AKI/ARDS*'. Additionally, some extra strengths were raised in our own study, when compared with previous results. First, we discovered that clinical classification-critically severe was the risk factor for eosinopenia, lymphopenia as well as one-month outcomes in COVID-19 patients. Second, we identified the alteration regularity of PBICs in COVID-19 patients during treatment, not only in recovery patients but also in aggravated patients. Third, severe COVID-19 patients suffered from weak cellular immunity, including reduced counts of T cells and NK cells. Importantly, it may be that the functional damage of CD4 + T cells that makes COVID-19 patients susceptible to severe disease, and the excessive exhaustion of CD8 + T cells that impairs cellular immune response to 2019-nCoV in severe patients according to the literature review [27]. Finally, we put forward that PBICs might serve as indicators of disease severity and signals of disease progression in COVID-19.

However, there are some limitations in our study. The primary concern with our study is that the study sample size was relatively small in this study. The second issue is that the lymphocyte subsets was collected 2 weeks after treatment rather than the first day or within 7 days after admission. Third, the risk factors for neutropenia, neutrocytosis and monopenia were not thoroughly investigated in our study, since the neutrophils can be affected by too many other factors including other infection, and the lower limit for monocyte counts is zero according to normal range. Further more, the PBICs were not analyzed in the sputum of patients in our study, and the pathological

findings of COVID-19 was based on only one case's autopsy. Moreover, the definations of acute phase, recovery phase and aggravated phase were raised by ourselves rather than explicit guideline. Finally, the alterations of PBICs except for leukocytes can also be caused by other confounding factors, such as severe infection, which were not described at length. Hence, clinical study with large scale and well-designed quality is still needed before using PBICs as diagnostics and prognostication in COVID-19 patients.

5. Conclusion

In conclusion, lymphopenia and eosinopenia may serve as predictors of disease severity and disease progression in COVID-19 patients, and enhancing the cellular immunity may contribute to COVID-19 treatment. Also, we inferred that neutrophils, eosinophils and lymphocytes migrate from peripheral blood into the the lung tissue, resulting in neutropenia, lymphopenia and eosinopenia in peripheral blood, as well as accelerating AKI/ARDS in COVID-19 patients. Thus, PBICs might become a sentinel of COVID-19, and it deserves attention during COVID-19 treatment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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