Research Article

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Ratios between circulating myeloid cells and lymphocytes are associated with mortality in severe COVID-19 patients

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Abstract: Recent studies indicate that host immune responses are dysregulated with either myeloid cell compartment or lymphocyte composition being disturbed in COVID-19. This study aimed to assess the impact of SARS-CoV-2 viral infection on the composition of circulating immune cells in severe COVID-19 patients. In this retrospective single-center cohort, 71 out of 87 COVID-19 patients admitted to the intensive care unit for oxygen treatment were included in this study. Demographics, clinical features, comorbidities, and laboratory findings were collected on admission. Out of the 71 patients, 5 died from COVID-19. Compared with survived patients, deceased patients showed higher blood cell counts of neutrophils and monocytes but lower cell counts of lymphocytes. Intriguingly, the neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), and basophil-to-lymphocyte ratio (BLR) were markedly higher in deceased patients compared to survived patients. Furthermore, the lymphocyte counts were negatively correlated with D-dimer levels, while the ratios between myeloid cells and lymphocyte (NLR, MLR, and BLR) were positively correlated with D-dimer levels. Our findings revealed that the ratios between myeloid cells and

lymphocytes were highly correlated with coagulation status and patient mortality in severe COVID-19.

Keywords: COVID-19, myeloid cells, lymphocytes, coagulation, D-dimer

1 Introduction

The novel coronavirus disease-2019 (COVID-19) outbreak, mediated by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in Wuhan, China, in December 2019 [1,2]. As with other virulent coronavirus infections such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), SARS-CoV-2 poses a major health threat to human worldwide [3,4]. With the rapid spread worldwide causing high morbidity and mortality, it has been declared a pandemic by the World Health Organization. There have been 24,257,989 confirmed cases and 827,246 deaths reported as of 28 August 2020 [5].

Respiratory compromise characterized by lower respiratory tract symptoms, such as fever, dry cough, and dyspnea, has been reported in patients with COVID-19 [6]. In patients with severe COVID-19, the disease progresses to acute respiratory distress syndrome and life-threatening events including coagulopathy, septic shock, and death [3]. To reduce the morbidity and mortality rates, it is crucial to identify key risk factors associated with poorer prognosis. Basic laboratory tests have been proven crucial in identification of high-risk COVID-19 patients [7]. This allows early intensive care and support to be provided to patients at high risk of mortality.

The immune systems, including the innate and adaptive immune systems, are commonly disturbed in virus infection [8–10]. White blood cells, which are categorized into myeloid cells (neutrophils, monocytes, eosinophils, and basophils) and lymphocytes (T cells, B cells, and natural killer [NK] cells) based on their cell lineage, constitute the first line of defense against invading pathogens

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including viruses [11]. In COVID-19, several recent studies indicate that host immune responses are dysregulated with either myeloid cell compartment [12–14] or lymphocyte composition [15–17] being disturbed. Furthermore, the effectiveness and concerns over immunosuppressive therapies in COVID-19 patients have been discussed [18,19]. However, studies on differential involvement of the myeloid cells and lymphocytes and their correlation with the coagulation status and disease severity in COVID-19 patients are scarce to date.

In this study, we analyzed blood cells, coagulation parameters, and inflammatory markers from routine clinical laboratory tests and discovered the differential impact of SARS-CoV-2 infection on circulating myeloid cells and lymphocytes. Furthermore, we found that the ratios between myeloid cells and lymphocytes were highly correlated with blood coagulation status and disease severity in COVID-19.

Figure 1: Study design.

2 Materials and methods

2.1 Patients and study design

This was a retrospective single-center study with a total of 87 patients tested positive with COVID-19 between 19 January and 23 February 2020 in Wuhan Iron & Steel (Group) Company Second Staff Hospital. Subsequently, only 71 patients with comprehensive medical records were included in this study (Figure 1). The study was conducted in accordance with the principles of Declaration of Helsinki and approved by the Institutional Review Board of Tianjin Chest Hospital (IRB-SOP-016(F)-001-02). The need for informed consent was waived given the observational and retrospective nature of the study.

All patients were confirmed of COVID-19 either by SARS-CoV-2 nucleic acid test (63.4%) or by clinical symptoms and computerized tomography (CT) scan imaging (36.6%), according to the Diagnosis and Treatment Protocol for Novel Coronavirus Pneumonia (Trial Version 4.0) released by the National Health Commission of the People's Republic of China [20]. Patient data including demographics, comorbidities, symptoms, and laboratory findings were collected during the hospital admission.

2.2 Laboratory testing

Patient throat swab specimens were collected for the detection of SARS-CoV-2 via the real-time reverse

transcriptase-polymerase chain reaction (RT-PCR) assay. Peripheral blood was collected and analyzed in the clinical laboratory of Wuhan Iron & Steel (Group) Company Second Staff Hospital. The laboratory test results including full blood cell counts, coagulation parameters (prothrombin time [PT], international normalized ratio [INR], activated partial thromboplastin time [APTT], thrombin time [TT], D-dimer, and fibrinogen), and other blood markers (C-reactive protein [CRP] and creatinine kinase [CK]) were assessed for both survived and deceased COVID-19 patients. A full blood count was performed using the XN-10 automated hematology analyzer (Sysmex® Corporation), which generated the white blood cell differential fluorescence (WDF) scattergram [21].

2.3 Statistical analysis

All statistical analyses were performed using SPSS 25.0 (IBM Corp., Armonk, NY, USA). A *p*-value of less than 0.05 is considered statistically significant. Graphs for figures were prepared with GraphPad Prism 8.00 (GraphPad Software, San Diego, CA, USA). Missing data were not imputed. Continuous data were presented as mean ± standard deviation (SD) or median ± interquartile range (IQR), and categorical variables were presented as percentage. The differences were compared by Student's *t*-test, Mann–Whitney *U* test, χ^2 test, or Fisher's exact test, depending on the nature of data. The relationship among biomarkers was assessed using Spearman's correlation analysis.

3 Results

3.1 Patient characteristics

From 19 January to 23 February 2020, a total of 71 patients admitted to Wuhan Iron & Steel (Group) Company Second Staff Hospital with confirmed COVID-19 and comprehensive medical records were included in this study (Figure 1). Five of these 71 patients died from COVID-19 and 66 patients fully recovered and were discharged. As shown in Table 1, the median age of deceased patients was significantly older than those who recovered (74.0 years, IQR: 65.0–83.0 vs 61.0 years, IQR: 50.0–71.0; $p = 0.025$), and there were more males in the deceased patients (100 vs 40.9%; $p = 0.010$). Of all COVID-19 patients, nearly half of them ($n = 34$, 47.9%) had at least one chronic medical condition, with diabetes being the most common comorbidity followed by hypertension, coronary heart disease,

malignancy, cerebrovascular disease, and respiratory disease. Hypertension was more frequently seen among the deceased patients than those who recovered (60.0 vs 13.6%; $p = 0.008$).

3.2 Laboratory parameters of COVID-19 patients

Differences in the initial laboratory parameters, including blood cell counts and biochemical markers, between the deceased patients and those who recovered from COVID-19 are presented in Table 2.

A full blood count and the white blood cell WDF scattergram were generated using the XN-10 automated hematology analyzer (Table 2 and Figure S1). Compared with the survivors, the deceased patients had lower red blood cell counts ($3.46 \times 10^{12}/L$ vs $4.06 \times 10^{12}/L$; $p = 0.010$) and platelet counts ($152.00 \times 10^9/L$ vs $228.00 \times 10^9/L$; $p = 0.004$). Interestingly, deceased patients presented with lower lymphocyte counts ($0.91 \times 10^9/L$ vs $1.57 \times 10^9/L$; $p = 0.014$) but higher neutrophil ($6.83 \times 10^9/L$ vs $3.53 \times 10^9/L$; $p = 0.007$) and monocyte counts ($0.61 \times 10^9/L$ vs $0.41 \times 10^9/L$; $p = 0.008$) (Table 2, Figure 2 and Figure S1). No difference in basophil and eosinophil counts was observed between the two patient groups.

All the patients presented with pro-thrombotic states, including relatively lower PT and INR, and higher D-dimer levels (Table 2). Of all COVID-19 patients with available data of coagulation tests, 22 out of 31 (70.97%) patients presented with D-dimer levels higher than the normal range. However, no significant difference was observed between survived and deceased patients in any of the coagulation parameters measured, including D-dimer levels ($2.01 \mu g/mL$, IQR: 1.17–3.00 for deceased patients vs $0.57 \mu g/mL$, IQR: 0.30–2.10 for survived patients; $p = 0.159$), likely due to a small number of deceased patients in the current study.

The levels of an inflammatory biomarker, CRP, were elevated in most COVID-19 patients whose CRP data were available, with 18 out of 25 (72.00%) patients presented with CRP levels higher than the normal range. Although higher median CRP levels were found in deceased patients (75.25 mg/L , IQR: 48.55–78.63 vs 29.58 mg/L , IQR: 5.00–74.50; $p = 0.203$), the levels did not differ between survived and deceased patients. CK levels, however, were significantly higher in deceased patients (105.00 U/L , IQR: 69.00–165.00) compared with those who survived (37.00 U/L , IQR: 29.00–51.00; $p = 0.019$).

Table 1: Baseline characteristics of the study population

	Alive $n = 66$	Dead $n = 5$	p -value
Demographic			
Age (years)	61.0 (50.0–71.0)	74.0 (65.0–83.0)	0.025
Male (%)	27 (40.9)	5 (100.0)	0.010
Diagnosis			
Nuclear test (%)	42 (63.6)	3 (60.0)	0.871
Clinical test (%)	24 (36.4)	2 (40.0)	0.871
Hospital stay duration (days)	19.5 (16.0–22.0)	6 (3.5–15.0)	0.008
Symptoms			
Fever (%)	40 (60.6)	5 (100.0)	0.078
Cough (%)	35 (53.0)	4 (80.0)	0.243
Comorbidities			
Diabetes mellitus (%)	12 (18.2)	1 (20.0)	0.919
Hypertension (%)	9 (13.6)	3 (60.0)	0.008
Coronary heart disease (%)	8 (12.1)	1 (20.0)	0.610
Cerebrovascular disease (%)	2 (3.0)	1 (20.0)	0.069
Respiratory disease (%)	2 (3.0)	0 (0.0)	0.693
Malignancy (%)	4 (6.1)	0 (0.0)	0.571

Continuous data are presented as mean $\pm$ SD or median $\pm$ IQR, and statistical analysis of continuous data was performed using unpaired Student's t -test or Mann–Whitney U test. Categorical variables are presented as %, with differences between the groups tested with χ^2 . Bold values indicate significant difference with a p -value of less than 0.05.

Table 2: Laboratory parameters of COVID-19 patients

	Alive <i>n</i> = 66	Dead <i>n</i> = 5	<i>p</i> -value
Full blood count indices			
RBC count ($\times 10^{12}/L$)	4.06 (3.80–4.29)	3.46 (3.15–3.90)	0.010
Hemoglobin (g/L)	125.00 (118.00–133.00)	116.00 (106.00–135.00)	0.237
Hematocrit (%)	36.90 (34.80–39.45)	35.50 (31.35–38.90)	0.261
MCV (fL)	91.70 (89.10–93.90)	92.10 (89.95–101.55)	0.370
MCH (pg)	30.90 (30.00–32.10)	32.10 (29.95–34.75)	0.251
MCHC (g/L)	337.50 (330.50–344.00)	330.00 (319.50–341.00)	0.146
RDW-CV (%)	12.00 (11.60–12.60)	12.50 (11.75–12.95)	0.389
RDW (fL)	46.00 (44.20–48.30)	49.30 (44.05–52.45)	0.153
Platelet ($\times 10^9/L$)	228.00 (184.00–264.00)	152.00 (88.00–178.00)	0.004
PDW (fL)	16.10 (15.80–16.40)	15.90 (15.35–17.00)	0.282
MPV (fL)	8.90 (8.30–9.40)	9.00 (8.75–9.55)	0.567
PCT (%)	0.19 (0.17–0.22)	0.21 (0.09–0.23)	0.836
WBC ($\times 10^9/L$)	5.85 (4.55–6.61)	8.43 (5.38–10.49)	0.056
Neutrophil count ($\times 10^9/L$)	3.53 (2.54–4.27)	6.83 (4.16–8.32)	0.007
Lymphocyte count ($\times 10^9/L$)	1.57 (1.20–1.88)	0.91 (0.61–1.31)	0.014
Monocyte count ($\times 10^9/L$)	0.41 (0.33–0.49)	0.61 (0.46–0.80)	0.008
Basophil count ($\times 10^9/L$)	0.03 (0.02–0.03)	0.06 (0.02–0.18)	0.110
Eosinophil count ($\times 10^9/L$)	0.15 (0.11–0.22)	0.11 (0.08–0.17)	0.256
Coagulation panel			
PT (s)	11.00 (10.60–11.30)	11.40 (10.75–14.70)	0.173
INR	0.85 (0.80–0.85)	0.89 (0.81–1.17)	0.268
APTT (s)	32.60 (29.30–38.45)	29.40 (27.30–32.80)	0.190
TT (s)	14.20 (13.70–14.65)	14.80 (13.70–15.40)	0.290
Fibrinogen (g/L)	2.49 (2.07–2.97)	2.43 (1.97–2.93)	0.679
D-dimer ($\mu g/mL$)	0.57 (0.30–2.10)	2.01 (1.17–3.00)	0.159
Others			
C-reactive protein (mg/L)	29.58 (5.00–74.50)	75.25 (48.55–78.63)	0.203
Creatine kinase (U/L)	37.00 (29.00–51.00)	105.00 (69.00–165.00)	0.019

Data are presented as median $\pm$ IQR and statistical analysis was performed using the Mann–Whitney *U* test. Abbreviations: RBC, red blood cell; MCV, mean cell volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red cell distribution width – coefficient of variation; RDW, red cell distribution width; PDW, platelet distribution width; MPV, mean platelet volume; PCT, plateletcrit; WBC, white blood cell; PT, prothrombin time; INR, international normalized ratio; APTT, activated partial thromboplastin time; TT, thrombin time. Bold values indicate significant difference with a *p*-value of less than 0.05.

3.3 Differential influence of SARS-CoV-2 on circulating myeloid cells and lymphocytes

Given the different profiles of circulating myeloid cells and lymphocytes from peripheral blood of survived and deceased patients (Table 2 and Figure 2), we further analyzed ratios of these two cell populations, including the neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), basophil-to-lymphocyte ratio (BLR), and eosinophil-to-lymphocyte ratio (ELR). Strikingly, as shown in Figure 3, deceased patients had significantly higher ratios of myeloid cells over lymphocytes: NLR (6.19 vs 2.27; $p < 0.001$), MLR (0.67 vs 0.26; $p < 0.001$), and BLR (0.07 vs 0.02; $p = 0.004$). Of note, although the

ELR appeared higher in deceased patients (0.15 vs 0.09; $p = 0.418$), the difference was not statistically significant (Figure 3d), likely due to the small sample size available for this study.

3.4 Correlation of D-dimer levels with circulating immune cell counts

Since both coagulation parameters and the immune systems are disturbed in COVID-19, we analyzed the correlation between D-dimer levels and different immune cells (and their ratios). As shown in the correlation matrix (Figure 4), D-dimer levels were negatively correlated

Figure 2: Myeloid cells (a, neutrophils; b, monocytes) and lymphocytes (c) in deceased patients and those recovered from COVID-19. Data are presented as median $\pm$ IQR, and statistical analysis was performed using the Mann–Whitney U test. The region highlighted in pink is the normal ranges of the respective cell counts.

with lymphocyte counts ($r = -0.600$; $p < 0.001$), whereas there was no correlation between D-dimer levels and other immune cells including neutrophils, monocytes, basophils, and eosinophils. The correlations between D-dimer levels and the inflammatory marker CRP or the tissue damage marker CK were also analyzed but did not reach statistical significance. Intriguingly, D-dimer levels in COVID-19 patients were positively correlated with NLR ($r = +0.608$; $p = 0.001$), MLR ($r = +0.500$; $p = 0.007$), ELR ($r = +0.424$; $p = 0.025$), and BLR ($r = +0.525$; $p = 0.004$). These findings indicate that the imbalance between myeloid

cells and lymphocyte counts is highly correlated with the pro-thrombotic state in COVID-19.

4 Discussion

In this single-center retrospective study, we analyzed the clinical features, blood cells, coagulation parameters, and inflammatory markers of severe COVID-19 patients and uncovered that circulating myeloid cells and

Figure 3: Ratios of myeloid cells and lymphocytes, including (a) neutrophil:lymphocyte, (b) monocyte:lymphocyte, (c) basophil:lymphocyte, and (d) eosinophil:lymphocyte, in deceased patients and those recovered from COVID-19. Data are presented as median $\pm$ IQR, and statistical analysis was performed using the Mann–Whitney U test.

lymphocytes were differentially affected. In particular, the ratios of myeloid cells and lymphocytes were overly associated with disease severity and highly correlated with the pro-thrombotic state in COVID-19.

The deceased patients in this study were older, predominantly male, and the majority had hypertension. These clinical features of severe COVID-19 patients are in agreement with previous reports [3,4,6]. Ageing commonly contributes to more severe COVID-19 and higher risk of death, which is likely associated with uncontrolled innate immune responses that cause cytokine storm and altered T cell responses [22]. The severity of COVID-19 was also reported to be inversely correlated with age in hospitalized children [23]. Recent studies also suggest that the phenotype and function of circulating monocytes, a type of innate immune cells, are changed in both aging and COVID-19 [24,25]. On top of the compromised immune system in aging population, comorbidities such as hypertension may explain the higher risk of death in elderly COVID-19 patients.

Activation of the immune systems has been well documented in viral infections including the SARS, MERS, and COVID-19 [26–28]. Neutrophilia and lymphopenia are associated with more severe disease symptoms and death in COVID-19 [29,30]. In agreement, we found higher counts of neutrophil and monocyte in deceased patients than in survived patients. In addition, we found lower lymphocyte counts in deceased patients. The differential changes in myeloid cells and lymphocytes result in markedly higher NLR, MLR, and BLR in deceased COVID-19 patients (Figure 3). The higher ratios of myeloid cells and lymphocytes in the more critically ill patients (deceased) may reflect the pathogenesis of COVID-19. Activation of neutrophils releases reactive oxygen species and cytokines as well as neutrophil extracellular traps, therefore, constitutes the first line of defense in response to viral infection [31,32]. Given the expression of virus-recognizing immune receptor, Toll-like receptor 7 [33], in monocytes, higher levels of monocytes in the blood may lead to over-reaction to SARS-CoV-2 infection. As Toll-like receptor 7 has been recently reported to deteriorate cardiovascular disease [34,35], higher levels of activated monocytes are expected to increase the risk of mortality in COVID-19 patients with pre-existing cardiovascular disease [4,6,36]. Indeed, using high-dimensional flow cytometry analysis and single-cell RNA sequencing, Silvin et al. recently uncovered a disturbed balance between non-classical CD14^{Low}CD16^{High} monocytes and HLA-DR^{Low} classical monocytes (Human Leukocyte Antigen-DR isotype) in the peripheral blood

of severe COVID-19 patients [12]. Whether this change is associated with activation of monocytic Toll-like receptor 7 by SARS-CoV-2 is unknown. Nevertheless, uncontrolled activation of innate immune cells including neutrophils and monocytes may contribute to the detrimental cytokine storm in COVID-19 patients [6,8,37].

The majority of lymphocytes are T cells and B cells which constitute the major cellular components of adaptive immune system [38]. Given the emerging roles of T cells in COVID-19 and the critical role of B cells in producing antibodies [38], the lower levels of lymphocytes in deceased COVID-19 patients in the current study reflect a weaker adaptive immune response in fighting against SARS-CoV-2 infection. The significantly lower lymphocyte levels in deceased patients may be attributable to stress-induced apoptosis and exhaustion of antiviral lymphocytes [39]. In addition, the levels of circulating NK cells, a type of anti-viral innate immune cells which constitute a minor population of lymphocytes, also decrease in severe COVID-19 patients [15]. As a result, higher ratios of myeloid cells and lymphocytes (NLR, MLR, and BLR) indicate a more severe imbalance of innate and adaptive immune responses to SARS-CoV-2 infection and consequently an unfavorable outcome in such patients contracted with COVID-19. In agreement with our findings, the NLR was reported to be positively associated with disease severity in COVID-19 [40–43]. However, to our knowledge, no prior study has systemically assessed the ratios of different myeloid cell populations and lymphocytes in severe COVID-19.

Figure 4: Correlation matrix showing the strength of correlation between D-dimer levels and inflammatory cells and ratios. Values in cells are Spearman correlation coefficient. All correlations were statistically significant ($p < 0.05$).

Inflammatory responses have been associated with thrombotic and bleeding manifestations in sepsis [44,45]. In the context of COVID-19, early studies have shown the association between elevated D-dimer levels and mortality [4]. D-dimer is a fibrin degradation product, and it serves as a marker of fibrinolytic activity [46]. There is evidence that D-dimer correlates with proinflammatory cytokine levels in critically ill patients [47]. In consistence, majority of the patients in this study presented with D-dimer levels higher than the normal range. We did not find significant differences in D-dimer and CRP levels between deceased patients and recovered patients, as it is likely because all the patients included in this study were severe cases. However, by correlation matrix analysis, we found a significant correlation between D-dimer levels and the ratios of myeloid cells and lymphocytes (NLR, MLR, BLR, and ELR) as well as lymphocyte counts (Figure 4). This raises an open question: are the elevated D-dimers because of coagulopathy arising from viral infection, or are the imbalanced innate and adaptive immune responses attributable to activation of coagulation in viral infection? Further studies are warranted.

Besides coagulation parameters and inflammatory markers, we also observed a higher CK level in deceased patients compared with survived patients. Elevated CK levels were previously reported in the SARS and MERS outbreak [48]. Although rhabdomyolysis has been reported as potential late complication associated with COVID-19 [49], we did not have data regarding the symptoms of rhabdomyolysis in patients with elevated CK levels. The increase in CK levels may be attributed to hypovolemia that causes renal impairment in COVID-19 patients [50].

This study has several limitations. First, it is a single-center retrospective study with a small number of patients. Second, laboratory parameters were not complete for each individual patient and the number of deceased patients in this study is rather small, therefore, may result in underpowered statistical analysis. Furthermore, as only clinical laboratory data are available for this study, we are not able to analyze subpopulations of lymphocytes such as T-cells. Multi-center studies with a larger cohort of patients are therefore required to confirm our findings. Moreover, multi-dimensional analysis of subpopulations of immune cells will help to delineate the impact of SARS-CoV-2 infection on innate and adaptive immunity in COVID-19. Nevertheless, given the fact that the overwhelming cases of COVID-19 lead to shortage of medical resources worldwide, our findings may facilitate the development of diagnostic protocols for patient stratification based on the routine laboratory tests available in most clinical laboratories.

5 Conclusion

In conclusion, innate and adaptive immune systems were affected differentially among severe COVID-19 patients. Our findings suggest that the ratios of myeloid cells (except eosinophils) and lymphocytes are highly associated with pro-thrombotic state and disease severity in COVID-19 and may serve as potential biomarkers for risk stratification of COVID-19 patients, allowing early intensive care and support for those of higher risk of death.

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Data availability statements: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Appendix

Figure S1: Representative white blood cell differential fluorescence (WDF) scattergrams of (a) survived and (b) deceased patients from severe COVID-19.