ORIGINAL ARTICLE

European Journal of
Haematology   WILEY

Characteristics of peripheral blood differential counts in hospitalized patients with COVID-19

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Abstract

Background: A lot remains unknown about the features and laboratory findings that may predict worse outcomes in patients with coronavirus disease 2019 (COVID-19). The aim of this study was to evaluate the difference in complete blood count parameters and differential counts in patients hospitalized with COVID-19 who survived compared to those who died.

Design: We performed a single-center retrospective study including 242 patients with confirmed COVID-19. We described the characteristics of the complete blood count parameters in these patients. Mann-Whitney *U* test was used to compare hematologic parameters of patients who died and those who survived; multivariate logistic regression was used to look for associations with mortality.

Results: Patients with COVID-19 who died had significantly lower median absolute monocyte count (AMC) (0.4 vs 0.5, *P* = .039) and median platelet count (169 vs 213, *P* = .009) compared to those who survived. Patients who died had a significantly higher neutrophil-to-lymphocyte ratio (6.4 vs 4.5, *P* = .001). The NLR was positively associated with death (OR = 1.038; 95% CI, 1.003-1.074, *P* = .031), while AMC was inversely associated with death (OR = 0.200; 95% CI, 0.052-0.761, *P* = .018).

Conclusion: Among patients with COVID-19, a lower AMC and higher NLR are associated with higher mortality.

KEYWORDS
COVID-19, lymphopenia, monocytes, novel coronavirus

1 | INTRODUCTION

The pandemic of coronavirus disease 2019 (COVID-19), the disease caused by the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus that emerged in Wuhan, China, in December 2019, has led to significant mortality in hospitalized patients in most affected parts of the world. In the United States, New York emerged as the epicenter of the pandemic, with New Jersey following as the state with the greatest number of cases.[1] According to the Centers for Disease Control and Prevention (CDC), as of May 2020, in Pennsylvania, there have been more than 57 000 reported cases with the majority of cases in Philadelphia County, which has also seen more than 850 associated deaths.[2] Among patients who are hospitalized with COVID-19, disease severity also varies, with age and comorbidities associated but not fully accountable for poor outcomes. The pathophysiology of disease as well as the mechanisms

and factors that lead to differential responses in affected patients including mortality are not entirely elucidated. The development of severe disease is currently thought to be associated with an excessive inflammatory response with several studies reporting association of increased serum levels of inflammatory cytokines and chemokines and higher inflammatory markers.[3-6] The involvement of monocytes and macrophages in the unraveling of the inflammatory response has also been highlighted as a key component of the response to SARS-CoV-2, although their role remains elusive but could involve the potential for productive replication, or the potential to serve as reservoirs or become vectors for viral dissemination as is the case for other human coronaviruses.[7,8] Lymphopenia is a feature reported in patients with COVID-19 which is particularly prominent in the presence of severe disease,[9] whereas neutrophilia has been shown to have a positive correlation with the risk of in-hospital death.[10] We therefore sought to explore whether there were substantial differences in the complete blood count parameters and peripheral blood leukocyte differential counts in our sample population with COVID-19 which is predominantly African American.

2 | METHODS

2.1 | Study design, participants, and data collection

This study was a single-center retrospective analysis of all patients >18 years of age who were admitted to Einstein Medical Center Philadelphia from March 1 to April 24, 2020, with a confirmed diagnosis of COVID-19 via reverse transcriptase-polymerase chain reaction (RT-PCR) assays performed on nasopharyngeal swab specimens. Demographic and clinical data, comorbidities, outcomes, and laboratory findings including hematologic profile were obtained. The complete blood count and differential analysis were performed at the laboratory of Einstein Medical Center Philadelphia following standard procedures. Institutional review board (IRB) approval was obtained with IRB number: 2020-436.

2.2 | Statistical analysis

Demographic variables were presented using descriptive statistics and frequencies. Categorical variables were analyzed with chi-square testing. Demographic and clinical variables were tabulated. Kolmogorov-Smirnov test was used to determine normality of distribution of data. Data that were skewed were presented as median with interquartile range (IQR) and non-parametric Mann-Whitney *U* test was used to compare the hematologic variables among those who died compared to those who survived. Multivariable logistic regression was used to evaluate the factors and blood parameters associated with mortality. 95% confidence intervals were used and are presented when appropriate.

3 | RESULTS

3.1 | Demographic and clinical characteristics of the patients

A total of 389 patients were evaluated in our hospital and tested positive via RT-PCR for COVID-19. One hundred and twenty-two patients were excluded who were still admitted at the time of analysis, and 25 patients with incomplete clinical data were excluded leaving a final sample of 242 patients (see Figure 1). In the final sample of 242 patients (see Table 1), the mean age ($\pm$SD) was 66 $\pm$ 14.75. 49% were female, and 70% were African American. Chronic medical conditions of these patients included hypertension (74%), diabetes mellitus (49%), and either chronic obstructive pulmonary disease (COPD) or asthma (19%). Out of our final sample, 26 (11%) patients had a diagnosis of a malignancy. The number of in-hospital deaths was 52 (22%).

Looking at the hematologic parameters, patients who died had a significantly lower median absolute monocyte count (AMC) 0.4 × 10^3 (0.2-0.7) vs 0.5 (0.3-0.8), P = .039 and platelet count 169 × 10^3 (136-229) vs 213 (160-265), P = .009 compared to those who survived. On the other hand, patients who died had a significantly higher white blood cell (WBC) count 8.0 × 10^3 (6.1-11.5) vs 6.4 × 10^3 (5.0-9.4), P = .011 and neutrophil-to-lymphocyte ratio (NLR) 6.4 (4.6-9.9) vs 4.5 (3.1-6.8), P = .001 compared to those who survived (see Table 2). There were no significant differences in the hematologic parameters among African Americans vs non–African Americans (Table 3). In multivariable logistic regression, after adjusting for age, demographics, and common comorbidities, NLR was significantly positively associated with inpatient death (OR = 1.038; 95% CI, 1.003-1.074, P = .031), while AMC was significantly inversely associated with inpatient death (OR = 0.200; 95% CI, 0.052 to 0.761, P = .018) (Table 4).

4 | DISCUSSION

Our study showed that patients who died from the disease had lower levels of monocytes and platelets in peripheral blood samples. A higher NLR was associated with increased mortality, while a higher AMC was inversely associated with mortality. Several studies have investigated the characteristics of peripheral blood differential counts of patients with COVID-19 and their association with disease severity. In a study by Yang et al,[11] most patients with COVID-19 presented with mild symptoms and peripheral blood analysis showed that 24.2% had leukopenia, 35.6% had lymphopenia, and 13.4% of patients had lower than normal range platelet counts. Their study also showed that 22.8% of patients with COVID-19 had lower than normal range neutrophil counts. However, Qin et al[9] reported that patients with severe COVID-19 tend to have higher overall leukocyte counts and higher percentage of neutrophils while at the same time having lower lymphocyte counts and lower percentage of monocytes. As a consequence, the

FIGURE 1 Study population [Colour figure can be viewed at wileyonlinelibrary.com]
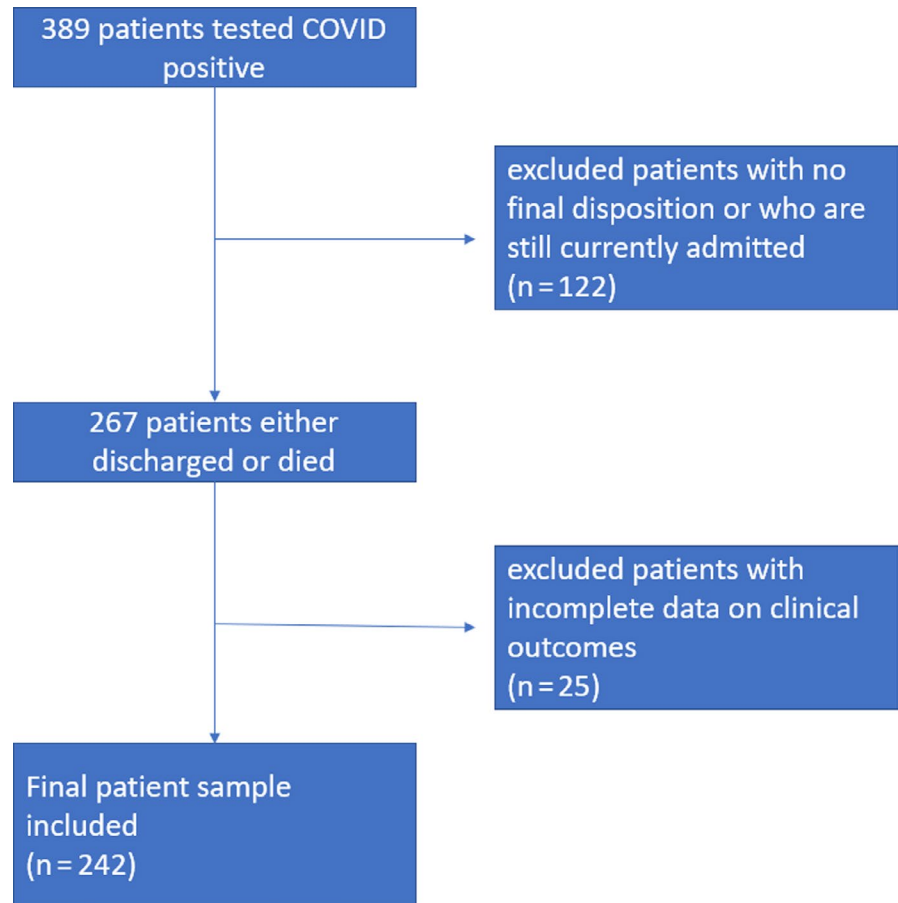
NLR tended to be higher in those with severe infection. Another study from Wuhan, China by Mo et al[12] showed that patients with refractory COVID-19 had no significant difference in the overall leukocyte count compared to general patients but had significantly higher counts of neutrophils and lower counts of platelets. Monocyte counts were not reported. Liu et al[10] also reported that a higher NLR was associated with a higher risk of inpatient mortality. It seems plausible to argue that leukocyte differential counts vary depending on disease severity with mild disease presenting with leukopenia and neutropenia, while severe disease presents with neutrophilia and lymphopenia consistent with our study findings where NLR was significantly associated with mortality. The lymphopenia observed in these patients may reflect massive recruitment of T cells and monocytes to inflamed tissues, the use of steroids, or may be the result of high levels of apoptosis.[7] A case report from a postmortem analysis from a patient who died from COVID-19 showed substantial interstitial mononuclear inflammatory infiltrates, dominated by lymphocytes in the lungs and heart[13] supporting the concept of lymphocyte depletion from peripheral blood.

We also found that non-survivors had lower AMC and that it was inversely associated with increased mortality. In contrast, Qin et al[9] reported lower percentage of monocytes in patients with severe disease but that AMC was not different between patients with severe disease and general patients. The difference could

potentially be attributed to differences in ethnic characteristics of patients included, as 70% of the patients in our study were African American. To our knowledge, this is the first report of the inverse association of AMC with inpatient mortality in patients with COVID-19. Our finding of lower monocyte counts in non-survivors may represent the result of more extensive tissue inflammation and recruitment of those cells at those sites of inflammation, and possibly direct productive viral infection of these cells as SARS-CoV-2 nucleoprotein has been detected in secondary lymphoid tissue macrophages.[14] Mononuclear phagocytes accounted for as much as 80% of cells in bronchoalveolar lavage fluid (BALF) from patients with severe COVID-19, with an abundance of inflammatory monocyte-derived macrophages.[15] In cases of mild COVID-19, the BALF had a minimal amount of inflammatory monocyte infiltration but rather a predominance of tissue-resident memory T cells.[16] Merad et al[7] report that these memory T cells may have cross-reactivity against SARS-CoV-2 and enable rapid control of the virus thus limiting progression of inflammation and accumulation of macrophages. Thus, it seems like activation of the adaptive immune response is essential in successful control of COVID-19 which can be the result of prior cross-reactivity against SARS-CoV-2 as mentioned above, while excess activation of just the innate immune response (neutrophils, macrophages, monocytes)[15] can lead to an excessive inflammatory response potentially associated with poor outcomes.

TABLE 1 Demographics and clinical characteristics of patients

	N = 242
Age (mean ± SD)	66.03 ± 14.75
Female gender, n (%)	34 (50)
Ethnicity, n (%)	
African American	171 (70)
Caucasian	17 (7)
Hispanic	26 (11)
Other	28 (12)
Comorbidities [n (%)]	
BMI (mean ± SD)	29.39 ± 9.22
COPD	30 (12)
Asthma	18 (7)
Heart failure	35 (15)
Atrial fibrillation	24 (10)
Liver cirrhosis	8 (3)
Diabetes	118 (49)
Chronic kidney disease	42 (17)
End-stage renal disease on dialysis	19 (8)
Coronary artery disease	45 (19)
Hypertension	180 (74)
HIV	7 (3)
Malignancy	26 (11)
Clinical and laboratory parameters (mean ± SD)	
FiO$_2$% requirement on admission	39 ± 28
Serum ferritin on admission	1888 ± 2836
D-dimer on admission	3855 ± 6143
CRP on admission	145 ± 116
LDH on admission	469 ± 323
COVID-19 treatment [n (%)]	
Hydroxychloroquine	145 (60)
Steroids	55 (23)
Tocilizumab	21 (9)
Clinical outcomes	
Inpatient death	52 (21)
Need for CRRT/HD	24 (10)
Need for vasopressors	49 (20)
Need for intubation	54 (22)
Hematologic parameters [medial (IQR)]	
WBC count	6.6 (5.2-9.8)
Platelet	239 (207-264)
Absolute neutrophil count (ANC)	4.9 (3.4-7.7)
Absolute lymphocyte count (ALC)	1.0 (0.7-1.4)
Absolute monocyte count (AMC)	0.5 (0.3-0.8)
Absolute eosinophil count (AEC)	0 (0-0.04)
Absolute bands	0 (0-0.29)
Absolute basophil	0 (0-0.02)

(Continues)

TABLE 1 (Continued)

	N = 242
Neutrophil-to-lymphocyte ratio (NLR)	4.9 (3.2-8.2)
Lymphocyte-to-monocyte ratio (LMR)	2.1 (1.2-3.5)
Platelet-to-lymphocyte ratio (PLR)	200 (132-297)

Note: Data reported as mean ± SD, n (%), or median (IQR).
Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; CRRT/HD, continuous renal replacement therapy/hemodialysis; FiO$_2$, fraction of inspired oxygen; IQR, interquartile range; LDH, lactate dehydrogenase; SD, standard deviation; WBC, white blood cell.

TABLE 2 Comparison of hematologic parameters among patients who survived vs those who died from COVID-19

Parameters	Death	Survived	*P*-value
WBC count	8.0 (6.1-11.5)	6.4 (5.0-9.4)	.011
Platelet	169 (136-229)	213 (160-265)	.009
ANC	6.1 (4.5-9.1)	4.6 (3.2-7.3)	.006
ALC	0.9 (0.6-0.9)	1.0 (0.7-1.4)	.098
AMC	0.4 (0.2-0.7)	0.5 (0.3-0.8)	.039
AEC	0 (0-0.2)	0.01 (0-0.1)	.250
Absolute basophil	0.01 (0-0.02)	0.01 (0-0.02)	.293
Absolute bands	0 (0-1.0)	0 (0-0.2)	.486
NLR	6.4 (4.6-9.9)	4.5 (3.1-6.8)	.001
LMR	2.2 (1.1-3.9)	2 (1.2-3.2)	.582
PLR	204 (114-288)	200 (133-301)	.752

Note: Data are presented as median (IQR). Numbers reported as ×10^3/μL.
Abbreviations: AEC, absolute eosinophil count; ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; WBC, white blood cell.

Yang et al[17] showed that thrombocytopenia is associated with increased risk of in-hospital mortality, while Tang et al[18] showed that platelet count was negatively correlated with mortality. This is consistent with our study findings as well. Thrombocytopenia in COVID-19 can be linked to possible increased consumption of platelets as a large percentage of patients with severe disease develop a coagulopathy that meets criteria for disseminated intravascular hemorrhage.[19]

In summary, our study found similar results with increased WBC counts, neutrophils, neutrophil-to-lymphocyte ratio, and decreased platelet counts in patients with severe COVID-19. These findings seem to be consistent even in the setting of a predominantly African American sample population where variations in hematologic parameters such as benign ethnic neutropenia can occur.[20] However, our study found that the AMC is inversely associated with mortality in patients with COVID-19 providing insight into the role of the innate immune system to COVID-19 disease pathophysiology.

TABLE 3 Comparison of hematologic parameters among African American patients vs non–African Americans

Parameters	African Americans	Non–African Americans	*P*-value
WBC count	6.5 (5.1-9.8)	6.9 (5.2-9.8)	.661
Platelet	213 (153-276)	199 (152-255)	.496
ANC	4.9 (3.3-7.6)	5.3 (3.7-7.7)	.488
ALC	1.1 (0.7-1.5)	0.9 (0.6-1.3)	.124
AMC	0.5 (0.3-0.8)	0.5 (0.3-0.7)	.775
AEC	0 (0-0.04)	0 (0-0.04)	.335
Absolute basophil	0 (0-0.02)	0 (0-0.02)	.059
Absolute bands	0 (0-0.2)	0 (0-0.7)	.204
NLR	4.7 (3.1-7.5)	5.4 (3.5-8.9)	.099
LMR	2.1 (1.3-3.5)	1.9 (1.0-3.0)	.130
PLR	194 (130-260)	217 (132-318)	.151

Note: Data are reported as median (IQR). Numbers reported as $\times 10^3/\mu L$. Abbreviations: AEC, absolute eosinophil count; ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; LMR, lymphocyte-to-monocyte ratio; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; WBC, white blood cell.

TABLE 4 Multivariate logistic regression showing factors associated with mortality including blood parameters

Characteristics	Odds ratio (95% CI)	*P*-value
Age	1.054 (1.015-1.094)	.006
BMI	1.015 (0.960-1.074)	.598
Male	*Referrant*	
Female	0.867 (0.366-2.053)	.746
African American	*Referrant*	
Caucasian	4.016 (0.632-25.53)	.141
Hispanic	0.652 (0.129-3.289)	.605
Others	2.325 (0.764-7.079)	.138
COPD and asthma	0.457 (0.120-1.747)	.253
DM	1.258 (0.513-3.086)	.616
HTN	1.466 (0.473-4.542)	.507
HF	0.607 (0.155-2.377)	.473
CKD	0.793 (0.256-2.450)	.686
NLR	1.038 (1.003-1.074)	.031
AMC	0.200 (0.052-0.761)	.018
Platelets	0.997 (0.992-1.003)	.352

Abbreviations: AMC, absolute monocyte count; BMI, body mass index; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; HF, heart failure; HTN, hypertension; NLR, neutrophil-to-lymphocyte ratio.

4.1 | Limitations

Limitations to our study would include the relatively small number of patients included and the fact that this was a single-center retrospective study. Several patients in our study received treatments either with hydroxychloroquine, corticosteroids, tocilizumab, or a combination of these therapies, which may affect the peripheral blood differential counts. We were unable to assess the significance of other hematologic parameters such as hemoglobin, hematocrit, and other red blood cell parameters. We were also unable to include peripheral smear characteristics as our data were derived retrospectively from review of electronic medical records and only the actual numerical blood counts were available. In our study sample, Caucasians were underrepresented which may limit generalizability of our study findings.

5 | CONCLUSION

Among patients with COVID-19, a lower AMC is associated with higher mortality. A higher NLR is also associated with increased mortality.

CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

AUTHOR CONTRIBUTIONS

I. Pakos drafted the manuscript; K.B. Lo analyzed the data; G. Salacup, J. Pelayo, R. Bhargav, E. Peterson, F. Gul, R. DeJoy III, and J. Albano collected the data; and G. Patarroyo Aponte, J. Rangaswami, and Z. Azmaiparashvili supervised the project. All authors were involved in the conceptualization of the study.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This was a retrospective study, and formal consent is not required.

INFORMED CONSENT

The data used in this study were already existing and de-identified. Informed consent was waived. IRB approval was obtained with IRB number: 2020-436.

NOVELTY STATEMENTS

Our work explores characteristics of peripheral blood differential counts that were associated with worse outcomes in patients affected by COVID-19. Our findings indicate that a lower absolute monocyte count is associated with increased mortality in patients hospitalized with the disease. This finding can help identify patients at increased risk for mortality due to COVID-19.

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