Clinical Significance of CBC and WBC Morphology in the Diagnosis and Clinical Course of COVID-19 Infection

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ABSTRACT

Objectives: *To investigate the clinical significance of numeric and morphologic peripheral blood (PB) changes in coronavirus disease 2019 (COVID-19)–positive patients in predicting the outcome, as well as to compare these changes between critically ill COVID-19–positive and COVID-19–negative patients.*

Methods: *The study included 90 COVID-19–positive (51 intensive care unit [ICU] and 39 non-ICU) patients and 30 COVID-19–negative ICU patients. We collected CBC parameters (both standard and research) and PB morphologic findings, which were independently scored by two hematopathologists.*

Results: *All patients with COVID-19 demonstrated striking numeric and morphologic WBC changes, which were different between mild and severe disease states. More severe disease was associated with significant neutrophilia and lymphopenia, which was intensified in critically ill patients. Abnormal WBC morphology, most pronounced in monocytes and lymphocytes, was associated with more mild disease; the changes were lost with disease progression. Between COVID-19–positive and COVID-19–negative ICU patients, significant differences in morphology-associated research parameters were indicative of changes due to the severe acute respiratory syndrome coronavirus 2 virus, including higher RNA content in monocytes, lower RNA content in lymphocytes, and smaller hypogranular neutrophils.*

Conclusions: *Hospitalized patients with COVID-19 should undergo a comprehensive daily CBC with manual WBC differential to monitor for numerical and morphologic changes predictive of poor outcome and signs of disease progression.*

Key Points

- More severe disease in coronavirus disease 2019 (COVID-19)–positive patients was associated with more significant neutrophilia and lymphopenia, while more mild disease was associated with more floridly abnormal monocyte and lymphocyte morphology.
- Dynamically, patients who ultimately died became progressively more neutrophilic and lymphopenic from diagnosis until the time of demise.
- Research CBC parameters identified differences in intensive care unit patients with and without COVID-19 infection, suggestive of WBC changes due to SARS-CoV-2.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of an ongoing pandemic of coronavirus disease 2019 (COVID-19), an acute viral illness with a spectrum of disease presentation and severity.[1] During review of peripheral blood of patients with COVID-19 admitted to our hospital, we have noticed significant alterations of WBC morphology. While there are an increasing number of publications and preprints in peer-reviewed and non-peer-reviewed journals regarding COVID-19 pathogenesis, clinical presentation, and treatment, the studies that have addressed the morphologic changes in peripheral blood associated with SARS-CoV-2 are all limited to case and image reports.[2-5] The most recent study by Nazarullah et al[6] that includes a detailed quantitative and qualitative analysis of peripheral blood changes was conducted on 12 COVID-19–positive patients. In addition, very few studies provide correlation between peripheral blood WBC morphologic changes and disease outcomes and address the dynamics of CBC parameters and morphology.[2]

Viral-induced numeric and morphologic changes in the peripheral blood WBC are well characterized in other infections and can direct diagnostic workup to ensure

timely therapeutic intervention. For example, in infectious mononucleosis, caused by the Epstein-Barr virus, there is a significant lymphocytosis with the presence of large atypical lymphocytes, termed Downey cells,[7] while in human immunodeficiency virus infection, the lymphocytes are morphologically unremarkable in the setting of lymphopenia.[8]

This study presents a systematic analysis of peripheral blood CBC, including standard and research parameters, as well as morphologic findings in 90 consecutive patients with COVID-19. Importantly, the study compares the peripheral blood findings between patients with COVID-19 in the intensive care unit (ICU) and non-ICU settings, as well as between COVID-19–positive ICU and COVID-19–negative ICU patients, and demonstrates significant differences between these two groups, suggesting an important role of CBC with manual smear review in patient risk stratification. To our knowledge, this is the first study to monitor dynamic changes in CBC numeric and morphology parameters in COVID-19–positive patients who died of the disease and to investigate morphology-associated research parameters measured by hematology analyzers and compare them between COVID-19–positive and COVID-19–negative patients.

Materials and Methods

The study included 90 consecutive patients with COVID-19 admitted to our hospital between March 14, 2020, and April 14, 2020, as well as 30 ICU patients negative for COVID-19. The study was approved by the Institutional Human Research Committee. Clinical presentation of patients with COVID-19 varied from mild to severe disease: 51 patients were admitted to the ICU, and 39 patients were followed in the non-ICU settings. There were no significant differences in underlying comorbidities between COVID-19–positive ICU and non-ICU patients, with hypertension and cardiovascular disease being the most common (39% vs 36%, respectively), followed by diabetes (21% vs 15%, respectively). Nonhematologic malignancy was present in 15% in both groups, and hematologic malignancy was present in 12% of ICU patients (6% in remission) and 5% of non-ICU patients. Acute kidney injury was present in 6% and 5%, respectively. Approximately 15% of patients in both groups did not have significant underlying comorbidities.

The ICU patients negative for COVID-19 included age- and sex-matched patients who required ICU level of care as a marker for severity of clinical illness similar to COVID-19–positive patients and included patients with severe sepsis and/or acute respiratory distress syndrome to parallel the respiratory distress found in the COVID-19–positive ICU cohort. Overall, 70% of COVID-19–negative ICU patients had bacterial infections, with one patient with concurrent influenza B infection. The underlying comorbidities included cardiovascular disease (80% of patients), ischemic or nonischemic heart failure (65% of patients), diabetes (25% of patients), and atrial fibrillation (20% of patients). To ensure there were no other hospital-based confounding factors that would have skewed the results, we chose patients who were contemporaneously in the ICU with other patients with COVID-19 (but COVID-19 negative themselves).

Routine CBC with WBC differential was performed on or near the date of COVID-19 diagnosis (confirmed by SARS-CoV-2 reverse transcription polymerase chain reaction) and/or admission date (for transferred patients) on Sysmex XN-9000 hematology analyzers as a part of routine clinical care. An automated six-part WBC differential included absolute count of lymphocytes, monocytes, neutrophils, eosinophils, basophils, and immature granulocytes, the latter representing an automated count of promyelocytes, myelocytes, and metamyelocytes in peripheral blood. Research parameters associated with neutrophil, lymphocyte, and monocyte morphology (neutrophil lateral scatter light intensity [NE-SSC], neutrophil fluorescent light intensity [NE-SFL], neutrophil forward scatter light intensity [NE-FSC], lymphocyte lateral scattered light intensity [LY-X], lymphocyte fluorescent light intensity [LY-Y], lymphocyte forward scattered light intensity [LY-Z], monocyte lateral scattered light intensity [MO-X], monocyte fluorescent light intensity [MO-Y], monocyte forward scattered light intensity [MO-Z]), measured on Sysmex-XN hematology analyzers but not reported as a part of CBC, were collected in addition to the routine CBC parameters. WBC morphology was analyzed as changes from normal expected/baseline morphology by two independent board-certified hematopathologists (O.P. and A.S.K.) to review individual abnormal features not encapsulated in the differential count and/or the advanced research parameters. The morphologic changes for neutrophils included toxic granulation, cytoplasmic vacuolization, Howell-Jolly body-like inclusions, and Döhle bodies; for monocytes, the changes included the presence of large coalescing cytoplasmic vacuoles; for lymphocytes, the changes included the presence of cytoplasmic vacuoles, large granular lymphocytes, and atypical lymphocytes, including plasmacytoid forms; and for eosinophils, the changes included the presence of cytoplasmic vacuolization **Image 1**. Independent

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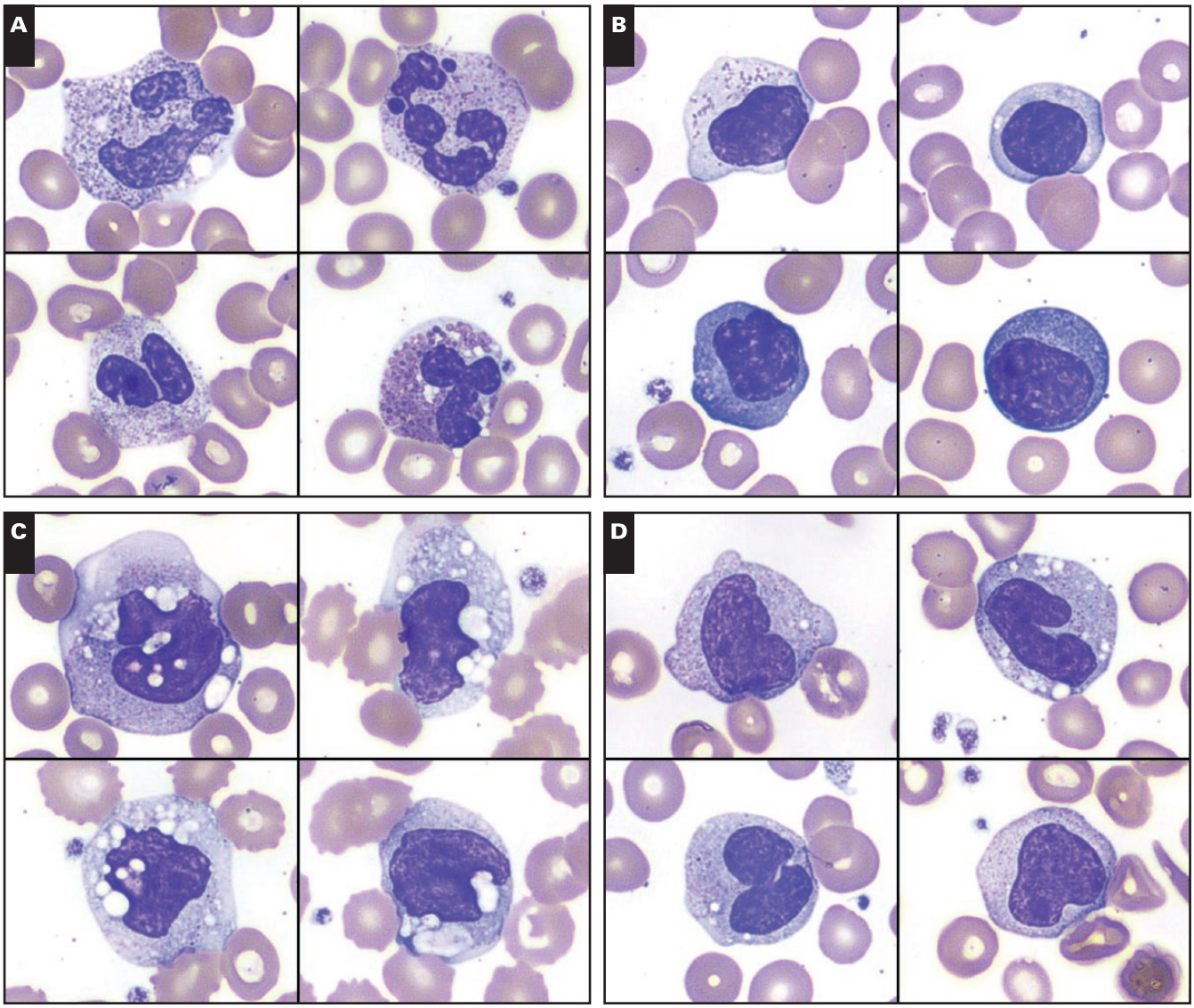

Image 1 A composite image of peripheral blood WBCs showing a spectrum of morphologic changes in coronavirus disease 2019 (COVID-19)–positive patients (**A-C**) and COVID-19–negative patients (**D**) (Wright-Giemsa, ×100). **A**, Segmented neutrophilia with vacuolization and toxic granulation (top left), Howell-Jolly body-like inclusions (top right), pseudo–Pelger-Huet nuclei (bottom left), and eosinophil with cytoplasmic vacuoles (bottom right). **B**, Large granular lymphocyte (top left), lymphocyte with cytoplasmic vacuolization (top right), atypical lymphocyte (bottom left), and plasmacytoid lymphocyte (bottom right). **C**, Atypical monocytes with large coalescing cytoplasmic vacuoles. In contrast, monocytes in COVID-19–negative patients (**D**) show only occasional small cytoplasmic vacuoles.

scoring of WBC morphology (4-point scale: 0, absent; 1, present in up to 10% of cell lineage; 2, present in 11%-25% of cell lineage; or 3, present in >25% of cell lineage) via Cellavision DM9600 was performed by two board-certified hematopathologists (O.P. and A.S.K.), and significant (>1-point) discrepancies were resolved by adjudication. In addition, we collected selected markers to benchmark systemic inflammation: ferritin and C-reactive protein (CRP) levels. These markers were measured on a cobas 8000 analyzer (Roche Diagnostics). For COVID-19–positive patients, CRP and ferritin results were collected at admission or closest to the time of the positive test (generally within 48 hours), in line with the institutional guidelines for patients with COVID-19.

The impact of each parameter on the disease status (ICU vs non-ICU) was estimated using a univariate logistic regression model with $\alpha = 0.05$. From the univariate

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analysis, a pool of significant candidate morphologic predictors adjusted for sex was then selected for the multivariate logistic regression. The candidate multivariate model was chosen, based on parsimony, from the five models with the smallest Akaike information criterion estimated from the backwards selection procedure. All modeling was done using the "glm package" from R version 4.0.0 (Free Software Foundation's GNU project). Overall, we examined 19 laboratory and morphology parameters associated with ICU status. The significance of research CBC parameters between ICU and non-ICU COVID-19–positive patients and COVID-19–positive and COVID-19–negative patients was assessed by Student *t* test using 2018 GraphPad Software.

Results

Comparison of Laboratory Values Between COVID-19–Positive ICU and Non-ICU Patients

COVID-19–positive patients demonstrated striking numerical peripheral blood WBC and inflammatory marker abnormalities (**Table 1** and Supplemental Table 1; all supplemental materials can be found at *American Journal of Clinical Pathology* online). Although overall mean WBC counts were in the normal range, as seen in previous reports,[9,10] patients with COVID-19 had increased ferritin (mean, 1,905 µg/L) and CRP (mean, 124 mg/L) as well as lymphopenia (mean absolute lymphocyte count [ALC], 0.97 × 10^9/L) and monocytopenia (mean absolute monocyte count [AMoC], 0.44 × 10^9/L; Supplemental Table 1). The laboratory values, including both the markers of inflammation and CBC parameters, were significantly different between the ICU and non-ICU COVID-19–positive patients. Patients in the ICU settings had significantly higher ferritin (2,359.22 vs 971.85 µg/L, $P = .013$) and CRP (205.05 mg/L vs 103.03 mg/L, $P < .0001$) levels, a reflection of the hyperinflammatory state that has been described in a subset of COVID-19–positive patients with more severe disease presentation.[1] In addition, patients in the ICU setting had significantly higher WBC counts with associated neutrophilia and left-shifted granulopoiesis (WBC, 9.96 vs 6.00 × 10^9/L, $P = .0009$; absolute neutrophil count, 8.32 vs 4.21 × 10^9/L, $P = .001$; absolute immature granulocyte [IG] count 2.46 vs 0.64 × 10^9/L, $P = .0212$). Importantly, patients in the ICU were more likely to have lymphopenia (ALC, 0.75 vs 1.24 × 10^9/L, $P = .0124$) and increased nucleated RBCs (0.24 vs 0.03, × 10^9/L $P = .0462$), while there was no statistical difference in monocyte count (AMoC, 0.47 vs 0.40 x 10^9/L, $P = .5820$).

Comparison of Cellular Morphology Between COVID-19–Positive ICU and Non-ICU Patients

The most striking findings in patients with COVID-19 were the WBC morphologic changes observed on peripheral blood smears (**Table 2** and Image 1). Abnormal morphologic features were present in 100% of patients with COVID-19 (Supplemental Table 1). Although the

Table 1
Demographic, Laboratory, CBC, and Morphology Parameters in ICU and Non-ICU COVID-19–Positive Patients

	COVID-19 Positive		Univariate Analysis	
Characteristic	**Non-ICU (n = 39)**	**ICU (n = 51)**	**Odds Ratio**	***P* Value[a]**
Demographic and inflammatory parameters, means				
Age, y	58.66	64.12	1.03	.0928
Sex, male/female	0.56	1.55	2.77	**.0207**
Ferritin, µg/L	971.85	2,359.22	1	**.013**
CRP, mg/L	103.03	205.05	1.01	**<.0001**
CBC parameters, means[b]				
WBC, × 10^9/L	6	9.96	1.23	**.0009**
Hb, g/L	117.1	106.5	0.77	**.0199**
Platelets, × 10^9/L	225.82	211.67	0.99	.638
ANC, × 10^9/L	4.21	8.32	1.35	**.0001**
ALC, × 10^9/L	1.24	0.75	0.44	**.0124**
AMoC, × 10^9/L	0.4	0.47	1.23	.582
AIGC, × 10^9/L	0.64	2.46	1.34	**.0212**
nRBC, × 10^9/L	0.03	0.24	29.5	**.0462**

AIGC, absolute immature granulocyte count (promyelocytes, myelocytes, metamyelocytes); ALC, absolute lymphocyte count; AMoC, absolute monocyte count; ANC, absolute neutrophil count; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; Hb, hemoglobin; ICU, intensive care unit; nRBC, nucleated RBCs.
[a]Significant parameters at the .05 level are bolded.
[b]CBC reference ranges: WBC, 4.0-10.0 × 10^9/L; Hb, 135-180 g/L (males) and 110-149 g/L (females); platelets, 150-450 × 10^9/L; ANC, 1.92-7.60 × 10^9/L; ALC, 0.72-4.10 × 10^9/L; AMoC, 0.16-1.10 × 10^9/L; AIGC, 0 × 10^9/L; nRBC, 0 × 10^9/L.

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Table 2
WBC Morphology Grades in Non-ICU and ICU COVID-19–Positive Patients

	Non-ICU (n = 39)		ICU (n = 51)		Univariate Analysis	
Characteristic	Grade 0	Grade ≥1	Grade 0	Grade ≥1	Odds Ratio	*P* Value[a]
MO vacuolization, %	5	34	21	30	0.21	**.0051**
LY vacuolization, %	12	27	22	29	0.59	.2322
Atypical LY, %	13	26	36	15	0.21	**.0006**
Large granular LY, %	4	35	9	42	0.53	.328
Myeloid left-shift, %	26	13	20	31	3.1	**.0109**
NE, toxic granulation, %	2	37	1	50	2.7	.424
NE, vacuolization, %	7	32	8	43	1.18	.775

ICU, intensive care unit; LY, lymphocytes; MO, monocytes; NE, neutrophils.
[a]Significant parameters at the .05 level are bolded.

constellation of morphologic findings was distinct, some of them resembled changes associated with other viral or bacterial infections. The most uniform morphologic finding was cytoplasmic vacuolization that was present in multiple cell types with varying frequency. Monocytes demonstrated the most impressive vacuolization, with numerous large coalescing vacuoles seen in 71% of patients. Smaller cytoplasmic vacuoles were also present in neutrophils (83%), lymphocytes (53%), and eosinophils (13%). Only two patients with COVID-19 did not demonstrate any cytoplasmic vacuoles. Neutrophil toxic granulation (97%), large granular lymphocytes (LGLs, 86%), and atypical lymphocytes (54%) were also frequent in patients with COVID-19. Importantly, there were different patterns of abnormal morphologic changes in lymphocytes and monocytes between ICU and non-ICU patients with COVID-19. Monocytes with large coalescent cytoplasmic vacuolization or atypical lymphocytes (grade >0) were more prevalent in non-ICU patients (odds ratios [ORs], 0.21, $P = .0051$ and 0.21, $P = .0006$, respectively), while myeloid left shift was associated with ICU status (OR, 3.10, $P = .0109$). The latter confirmed the CBC finding of increased immature granulocytes in the ICU COVID-19–positive patients (see above). When considering only grade 3 morphologic changes (present in >25% of cell lineage), there was a trend for an inverse correlation of LGLs and ICU status (OR, 0.43, $P = .0528$; data not shown).

From the pool of candidate predictors, we estimated a multivariate logistic regression model for ICU outcome in COVID-19 infection, regressing upon sex, monocyte vacuolization status, presence of atypical lymphocytes, and left-shifted myeloid cells (Table 2). While holding other predictors constant, male sex increased the odds for being in the ICU 3.9-fold, and the presence of any left-shifted myeloid cells increased the odds 3.7-fold. Conversely, the presence of monocyte vacuolization or atypical lymphocytes decreased the odds of being in the ICU for COVID-19–positive patients by 0.21-fold and 0.23-fold, respectively.

Comparison of Laboratory Values and Cellular Morphology Between COVID-19–Positive Deceased and Alive Patients

There was a high mortality rate among the COVID-19–positive ICU patients of 39.2%: 20 patients died of the disease, while 31 patients completely recovered. To better understand the disease course and identify potential predictors of inferior outcome, we evaluated laboratory and WBC morphologic parameters at the time of diagnosis between these two groups of patients Table 3 and Table 4. The deceased group constituted an older patient population (mean age, 71.35 vs 59.42 years, $P = .0065$), but there was no significant difference in sex distribution, with males comprising most patients in both groups (male to female ratio of 1.50 and 1.58, $P = .9266$). Both groups had markedly elevated ferritin and CRP levels. No differences were observed in the CBC values and overall WBC morphology at the time of diagnosis. As noted above, the COVID-19–positive ICU patients had significant lymphopenia compared with the non-ICU COVID-19–positive patients. However, there was a trend to even more significant lymphopenia at presentation in the deceased group compared with ICU survivors (0.54 vs 0.90 × 10^9/L , $P = .0495$). The morphologic WBC changes, such as monocyte, lymphocyte, and neutrophil vacuolization; the presence of atypical lymphocytes or LGLs; and toxic granulation in neutrophils were similar between these two groups. The only significant difference was the much higher number of left-shifted/immature granulocytes (promyelocytes, myelocytes, and metamyelocytes) on peripheral blood of the deceased patients (60% vs 6%, $P = .00003$) noted on Cellavision morphologic evaluation (Table 4). However, there was no difference in immature granulocytes, as evaluated by the automated IG parameter (absolute IG count of 0.20 vs 0.29 × 10^9/L, $P = .4776$). This discrepancy between the manual and automated evaluation possibly reflects the selection bias of Cellavision in capturing leukocytes for manual differential analysis and its lower sensitivity due

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Table 3
Comparison of CBC, Laboratory, and Morphology Parameters Between COVID-19 Deceased and Alive Patients

Characteristic	ICU Patients		Univariate Analysis
	COVID-19 Deceased (n = 20)	COVID-19 Alive (n = 31)	*P* Value[a]
Demographic and inflammatory parameters, means			
Age, y	71.35	59.42	**.0065**
Sex, male/female	1.50	1.58	.9266
Ferritin, µg/L	2,544.95	2,351.06	.0807
CRP, mg/L	193.75	233.84	.184
CBC parameters, means			
WBC, × 10^9/L	9.47	10.28	.6103
Hb, g/L	101.0	110.0	.1438
Platelets, × 10^9/L	186.05	228.19	.2178
ANC, × 10^9/L	8.23	8.39	.9144
ALC, × 10^9/L	0.54	0.90	**.0495**
AMoC, × 10^9/L	0.42	0.51	.6991
AIGC, × 10^9/L	0.20	0.29	.4776
nRBC, x 10^9/L	0.67	0.59	.8299

AIGC, absolute immature granulocyte count (promyelocytes, myelocytes, metamyelocytes); ALC, absolute lymphocyte count; AMoC, absolute monocyte count; ANC, absolute neutrophil count; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; Hb, hemoglobin; ICU, intensive care unit; nRBC, nucleated RBCs.
[a]Significant parameters at the .05 level are bolded.

Table 4
Comparison of WBC Morphology Grades Between Deceased and Alive COVID-19 Intensive Care Unit Patients

Characteristic	Deceased (n = 20)		Alive (n = 31)		Univariate Analysis *P* Value[a]
	Grade 0	Grade ≥1	Grade 0	Grade ≥1	
MO vacuolization, %	7	13	14	17	.4716
LY vacuolization, %	11	9	11	20	.1695
Atypical LY, %	14	6	21	10	.8653
Large granular LY, %	2	18	6	25	.3698
Myeloid left-shift, %	12	8	2	29	**.00003**
NE, toxic granulation, %	0	20	1	30	.7499
NE, vacuolization, %	1	19	7	24	.9189

ICU, intensive care unit; LY, lymphocytes; MO, monocytes; NE, neutrophils.
[a]Significant parameters at the .05 level are bolded.

to a much smaller number of cells for analysis (115 leukocytes for Cellavision vs ~30,000 leukocytes for CBC).

Serial CBC data were available for 18 (90%) deceased patients, allowing us to assess the CBC numerical and morphology parameter dynamics from the ICU admission to the date of death. Despite the lack of differences in CBC at the time of the ICU admission between the patients who subsequently died or recovered, the former patients demonstrated a significant increase in absolute neutrophil count (ANC) over the course of the disease in 14 (77.8%) patients with the overall percent increase from already elevated ANC values that ranged from 4.8% to 208.3%, with the majority demonstrating greater than a 30% ANC increase over the baseline values (10/15, 66.6%). Only four (22.2%) patients demonstrated a decrease in ANC, with only two of them with percent change over 20% Figure 1A. WBC showed similar dynamics (data not shown). Another fascinating finding was a continuing decline in ALC from the ICU admission to demise. Nine (50%) of 18 patients demonstrated a decrease in ALC by 28.3% to 100%, while among the nine patients who showed an ALC increase, only four demonstrated normalized ALC of greater than 1 × 10^9/L Figure 1B. Interestingly, although AMoC fluctuated, it remained within the normal reference range during the course of the disease (data not shown). In terms of WBC morphology, it appeared that striking vacuolization with coalescing vacuoles in monocytes was less prominent in peripheral blood smears closer to demise (data not shown).

Comparison of Laboratory Values Between COVID-19–Positive and COVID-19–Negative ICU Patients

To rule out the possibility that the CBC and morphologic changes seen in the ICU COVID-19–positive patients could be attributed to the ICU environment rather than SARS-CoV-2 infection, we compared the findings between COVID-19–positive ICU patients (51 patients)

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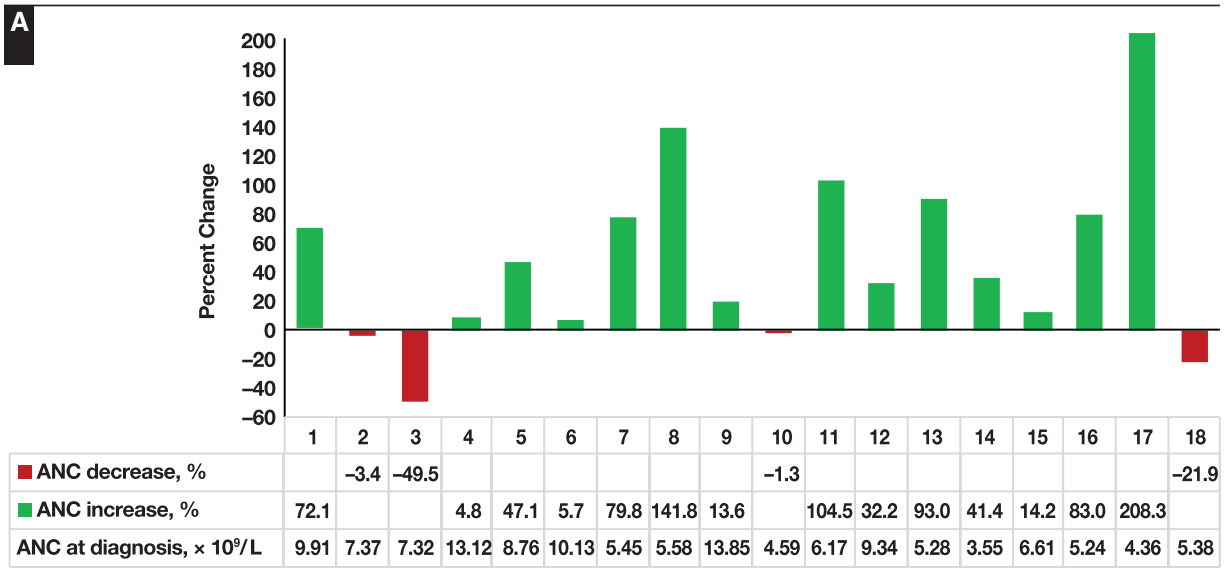
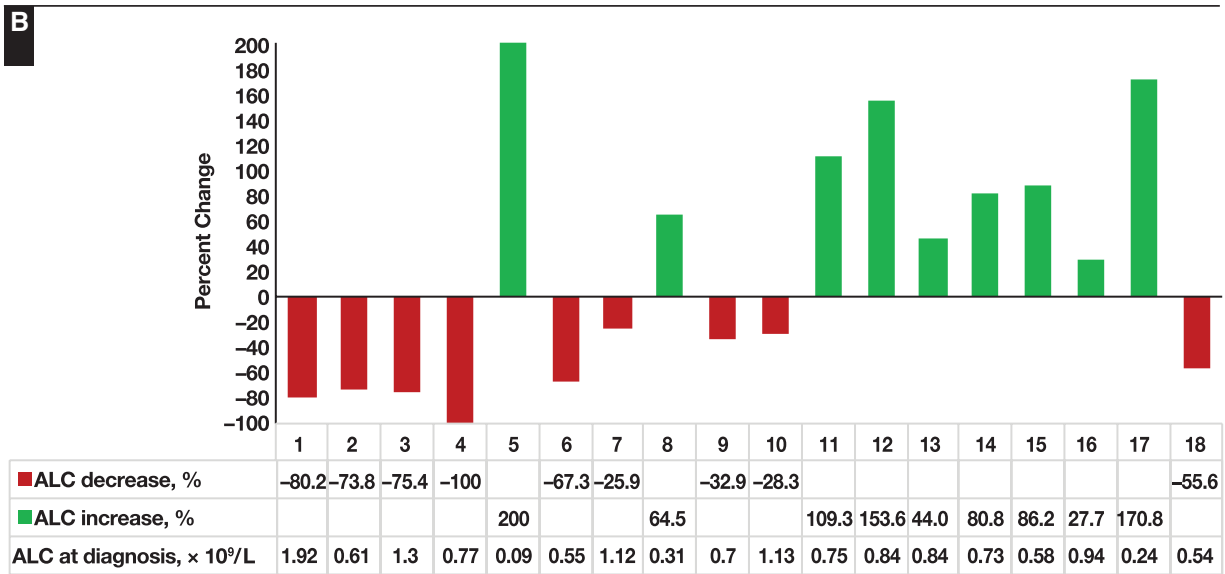


	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
ANC decrease, %		–3.4	–49.5							–1.3								–21.9
ANC increase, %	72.1			4.8	47.1	5.7	79.8	141.8	13.6		104.5	32.2	93.0	41.4	14.2	83.0	208.3	
ANC at diagnosis, × 10^9/L	9.91	7.37	7.32	13.12	8.76	10.13	5.45	5.58	13.85	4.59	6.17	9.34	5.28	3.55	6.61	5.24	4.36	5.38



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
ALC decrease, %	–80.2	–73.8	–75.4	–100		–67.3	–25.9		–32.9	–28.3								–55.6
ALC increase, %					200			64.5			109.3	153.6	44.0	80.8	86.2	27.7	170.8	
ALC at diagnosis, × 10^9/L	1.92	0.61	1.3	0.77	0.09	0.55	1.12	0.31	0.7	1.13	0.75	0.84	0.84	0.73	0.58	0.94	0.24	0.54

Figure 1 **A**, Percent absolute neutrophil count (ANC) change between diagnosis and demise in coronavirus disease 2019 (COVID-19)–positive intensive care unit (ICU) patients. **B**, Percent absolute lymphocyte count (ALC) change between diagnosis and demise in COVID-19–positive ICU patients.

with COVID-19–negative ICU patients (30 patients). The data were collected at the time of diagnosis for COVID-19–positive patients and at the time of ICU admission for COVID-19–negative patients.

The demographic, laboratory, CBC, and morphology parameters are presented in **Table 5** and **Table 6** and Supplementary Table 1. There was no significant difference in age and sex between these two groups. Ferritin and CRP levels were significantly higher in COVID-19–positive ICU patients than in COVID-19–negative ICU patients (2,359.22 vs 549.70, P = .0040; and 205.05 vs 100.40, P = .0002, respectively), supporting the finding of a robust inflammatory response in COVID-19 infection. While WBCs were elevated in both groups, COVID-19–positive patients had significantly lower WBC values than COVID-19–negative patients (9.96 vs 13.39 × 10^9/L , P = .0130). Hemoglobin values were significantly higher in COVID-19–positive ICU patients than in COVID-19–negative ICU patients (106.5 vs

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Table 5
Demographic, Laboratory, CBC, and Morphology Parameters in COVID-19–Positive and COVID-19–Negative ICU Patients

	ICU Patients		Univariate Analysis	
Characteristic	**COVID-19 Negative (n = 39)**	**COVID-19 Positive (n = 51)**	**Odds Ratio**	***P* Value[a]**
Demographic and inflammatory parameters, means				
Age, y	60.39	64.12	1.02	.2910
Sex, male/female	1.50	1.55	1.03	.9440
Ferritin, µg/L	549.7	2,359.22	1.00	**.0040**
CRP, mg/L	100.4	205.05	1.01	**.0002**
CBC parameters, means				
WBC, × 10^9/L	13.39	9.96	0.90	**.0130**
Hb, g/L	90.0	106.5	1.36	**.0075**
Platelets, × 10^9/L	184.37	211.67	1.00	.3020
ANC, × 10^9/L	10.76	8.32	0.92	.0527
ALC, × 10^9/L	0.83	0.75	0.85	.6356
AMoC, × 10^9/L	0.86	0.47	0.53	.0629
AIGC, × 10^9/L	1.05	2.46	1.22	.0842
nRBC, x 10^9/L	2.53	0.24	0.52	**.0279**

AIGC, absolute immature granulocyte count (promyelocytes, myelocytes, metamyelocytes); ALC, absolute lymphocyte count; AMoC, absolute monocyte count; ANC, absolute neutrophil count; COVID-19, coronavirus disease 2019; CRP, C-reactive protein; Hb, hemoglobin; ICU, intensive care unit; nRBC, nucleated RBCs.
[a]Significant parameters at the .05 level are bolded.

Table 6
WBC Morphology Grades in COVID-19–Positive and –Negative Intensive Care Unit Patients

	Negative (n = 39)		Positive (n = 51)		Univariate Analysis	
Characteristic	**Grade 0**	**Grade ≥1**	**Grade 0**	**Grade ≥1**	**Odds Ratio**	***P* Value[a]**
MO vacuolization, %	5	25	21	30	0.29	**.027**
LY vacuolization, %	10	20	22	29	0.66	.3846
Atypical LY, %	24	6	36	15	1.67	.3530
Large granular LY, %	2	28	9	42	0.33	.1797
Myeloid left-shift, %	15	15	20	31	1.55	.3450
NE, toxic granulation, %	0	30	1	50	0.00	.9920
NE, vacuolization, %	4	26	8	43	0.83	.7740

LY, lymphocytes; MO, monocytes; NE, neutrophils.
[a]Significant parameters at the .05 level are bolded.

90.0 g/L, $P = .0075$), suggesting more underlying comorbidities and prolonged disease state in the latter group. Univariate analysis of the WBC morphologic features demonstrated that most of the parameters were similar in the ICU patients with and without COVID-19. However, the ICU COVID-19–positive patients continued to show significantly less monocytes with abnormal vacuolization compared with the ICU COVID-19–negative patients by both univariate analysis (OR, 0.29, $P = .0270$) and multivariate analysis (Table 7; OR of 0.10 for grade 1 vacuolization, $P = .0035$, and OR of 0.17 for grade 3 vacuolization, $P = .0146$), a finding similar to the non-ICU comparison. In the multivariate analysis, the presence of atypical lymphocytes, especially plasmacytoid forms, was more predictive of the COVID-19 infection (OR, 5.52, $P = .0392$).

Table 7
Multivariate Analysis in COVID-19–Positive and COVID-19–Negative ICU Patients

Coefficient	**Odds Ratio**	***P* Value**
Intercept	7.57	.0018[a]
MO vacuolization grade 1	0.10	.0035[a]
MO vacuolization grade 2	0.44	.3666
MO vacuolization grade 3	0.17	.0146[b]
LY vacuolization grade 1	1.41	.6544
LY vacuolization grade 2	0.16	.0125[b]
LY vacuolization grade 3	0.37	.2964
Plasmacytoid LY grade 1, 2, or 3	5.52	.0392[b]

LY, lymphocytes; MO, monocytes.
[a]Significant parameters below the .005 level.
[b]Significant parameters below the .05 level.

Exploratory Analysis of Morphology-Associated Research Parameters in COVID-19–Positive and COVID-19–Negative Patients

Striking WBC morphology findings and differences between COVID-19–positive and COVID-19–negative

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patients, as well as COVID-19–positive ICU and non-ICU patients, prompted us to explore WBC research morphology-associated parameters that are measured (but not reported) on Sysmex XN-9000 hematology analyzers with all routine CBC on the WBC differential channel. These parameters are reported as a mean number of coordinates of ~30,000 cells distributed across x, y, and z axes and, to the best of our understanding, are based on the cell complexity (NE-SSC, LY-X, MO-X), size (NE-FSC, LY-Y, MO-Y), and level of maturity (NE-SFL, LY-Z, MO-Z). Table 8 summarizes the data for research morphologic parameters in neutrophils, lymphocytes, and monocytes between three groups of patients: COVID-19–positive ICU and non-ICU and COVID-19–negative ICU patients. Interestingly, despite striking differences in neutrophil, monocyte, and lymphocyte morphology between COVID-19–positive ICU and non-ICU patients observed on peripheral blood smears, all research morphology parameters showed similar values between these two groups, indicating the importance of morphologic review by an experienced professional. On the other hand, all neutrophil morphology parameters (NE-SSC, NE-FSC, and NE-SFL), as well as LY-Y and MO-Y, were significantly different between COVID-19–positive ICU and COVID-19–negative ICU patients, identifying a potential role of these research parameters beyond the manual morphologic review. Due to the lack of experience with these morphology research parameters, it is somewhat difficult to know the significance of these observed differences. However, our data suggest that neutrophils in COVID-19–positive ICU patients are less granular and smaller than neutrophils in COVID-19–negative ICU patients (NE-SSC, 150.99 vs 154.41, $P = .0142$; NE-FSC, 83.79 vs 86.82, $P = .0051$). Lymphocytes appear to contain lower RNA content, as suggested by the LY-Y parameter in COVID-19–positive ICU patients (LY-Y, 71.11 vs 74.22, $P = .0367$), while monocytes demonstrated significantly higher fluorescence light intensity consistent with higher RNA content (124.09 vs 115.15, $P = .0227$), suggesting that the monocytes might be more transcriptionally active. These exploratory data need to be confirmed by more extensive multi-institutional analyses, but they clearly indicate the added value of the research parameters to the conventional CBC parameters and morphologic analysis of peripheral blood smear.

Discussion

Our study demonstrates significant numerical and atypical WBC morphologic changes associated with SARS-CoV-2 infection and shows that the severity of changes is distinct not only between mild and severe disease but also between critically ill patients with and without COVID-19 infection. Our data comparing patients with COVID-19 requiring ICU stays support other published studies that found that COVID-19 clinical course is associated with markedly different peripheral blood WBC findings in response to viral infection, with the more severe disease presenting with more pronounced leukocytosis, neutrophilia, and lymphopenia.[11-14] However, to date, only a handful of publications with small cohort sizes have addressed the dynamic CBC changes during the course of COVID-19 in recovered and deceased patients.[15,16] Such studies are of crucial importance as monitoring CBC parameters changes could be a fast and

Table 8
Comparison of Morphology-Associated Research WBC Parameters in COVID-19–Positive and COVID-19–Negative Patients

	COVID-19 Positive[a]		COVID-19 Negative	Univariate Analysis[b]
Characteristic	Non-ICU (n = 39)	ICU (n = 51)	ICU (n = 30)	*P* Value, ICU COV+ vs ICU COV–
NE-SSC	152.18	150.99	154.41	**.0142**
NE-SFL	51.49	52.83	49.97	.0565
NE-FSC	83.73	83.79	86.82	**.0051**
LY-X	81.19	83.04	82.82	NS
LY-Y	72.35	71.11	74.22	**.0367**
LY-Z	58.4	57.92	58.37	NS
MO-X	124.41	125.88	124.63	NS
MO-Y	120.28	124.09	115.15	**.0227**
MO-Z	65.89	66.31	66.6	NS

COV–, COVID-19 negative; COV+, COVID-19 positive; COVID-19, coronavirus disease 2019; ICU, intensive care unit; LY-X, lymphocyte lateral scattered light intensity; LY-Y, lymphocyte fluorescent light intensity; LY-Z, lymphocyte forward scattered light intensity; MO-X, monocyte lateral scattered light intensity; MO-Y, monocyte fluorescent light intensity; MO-Z, monocyte forward scattered light intensity NE-FSC, neutrophil forward scatter light intensity; NE-SFL, neutrophil fluorescent light intensity; NE-SSC, neutrophil lateral scatter light intensity; NS, not significant.
[a]There were no statistically significant differences in any morphology-associated research parameters in COVID-19–positive patients in the ICU and non-ICU settings.
[b]Significant parameters at the .05 level are bolded.

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simple method to predict disease outcome in hospitalized patients, ensuring earlier therapeutic interventions at the first signs of decline. Similar to the study by Pan et al,[15] we show that patients who ultimately died of disease continued to develop neutrophilia with percent changes from the baseline exceeding 30% in most cases. Granulocytes are stimulated by granulocyte colony-stimulating factor, which has been shown to be increased in critically ill COVID-19–positive patients. Most patients with fatal disease also demonstrate progressive lymphopenia, with only four (22%) patients demonstrating ALC normalization at demise. It has been suggested that viral-induced apoptosis may explain the lymphopenia.[17] Our preliminary data of bone marrow assessment from deceased COVID-19–positive patients show hypercellularity with marked myeloid hyperplasia, which could be a result of a hyperinflammatory state due to increased cytokines. In addition, the bone marrows of patients with COVID-19 display prominent hemophagocytosis (unpublished data), and it is interesting to contrast the inflammatory milieu seen in COVID-19 to that in hemophagocytic lymphohistiocytosis (HLH).[18,19] Marked elevation in cytokines in COVID-19 is associated with increased inflammatory markers, especially ferritin, a diagnostic criterion for HLH; however, the lack of severe cytopenias in most cases of COVID-19 despite other markers of macrophage activation suggests different underlying pathophysiologies at play.

WBC morphologic changes are also different between disease stages in COVID-19–positive patients. The initial stages and/or more mild disease are associated with exuberant coalescent monocyte vacuolization and expansion of atypical lymphocytes (Image 1), while disease progression and/or more severe disease are associated with loss of these changes in the setting of increasing neutrophilia and left-shifted myeloid maturation. Interestingly, these striking changes in monocyte morphology with extensive unusual cytoplasmic vacuolization that have also been described in a few prior studies and case reports were not contingent upon AMoC, which remained low to low/normal although it fluctuated.[2,5,20] Monocytopenia has been described in COVID-19–positive patients, with some studies showing even lower monocytes counts in the ICU patients, which we did not observe.[10,11] It has been suggested that peripheral blood monocytes may be reduced due to recruitment to sites of inflammation (ie, lungs) by CXCL10 and CCL2, which are significantly elevated in critically ill COVID-19–positive patients.[21] Regardless, the findings of different monocyte morphology may signify the presence of different monocyte subsets with different functional characteristics in mild and severe disease states. This notion is supported by the recent findings of the presence of a significantly higher percentage of CD14+ CD16+ inflammatory monocytes in peripheral blood of COVID-19–positive patients compared with normal controls; these nonclassical monocytes were characterized by higher forward scatter on flow cytometry and expression of CD68, CD80, CD163, and CD206, and they were enriched for in a more severe disease.[2] These inflammatory monocytes/macrophages secrete granulocyte macrophage colony-stimulating factor, inducing granulocytes and macrophage production, as well as interleukin 6, which is significantly elevated in the ICU patients and has been hypothesized to be a driving factor in monocyte differentiation to macrophages as opposed to dendritic cells inducing subsequent lung damage along with sustaining a hyperinflammatory state.[22,23] In addition, some studies have shown that monocytes develop alkaline vacuoles and that the pH of vacuoles may differ between monocyte subsets, explaining different monocyte morphology in different disease states.[24]

Interestingly, despite a clearly significant role that monocytes/macrophages play in sustaining a hyperinflammatory response in SARS-CoV-2 infection, it appears that not all patients with COVID-19 display monocyte morphologic changes in peripheral blood that we describe in our study. Nazarullah et al[6] have reported quantitative and qualitative peripheral blood changes in 12 COVID-19–positive patients and found that in their cohort, the most significant morphologic changes were related to nuclear morphology in neutrophils showing acquired Pelger-Huët anomaly and monolobate neutrophils. While we did find similar nuclear changes, they were present in a small number of neutrophils (predominantly <5% of neutrophils) and were not specific to COVID-19–positive patients; similar findings were present in COVID-19–negative ICU patients. However, we noticed another nuclear change in neutrophils in the form of Howell-Jolly body-like inclusions that was seen in 10% of patients with COVID-19. Similar to our findings, Nazarullah et al[6] have reported the presence of neutrophil toxic granulation and myeloid left shift, as well as the increased frequency of plasmacytoid lymphocytes in COVID-19–positive patients.

We recognize that analysis of morphologic changes in WBC is operator dependent and as a result could be subjective. In an attempt to standardize morphologic review of peripheral blood smears in patients infected with COVID-19, we have performed an exploratory analysis of morphology-associated research parameters, which are measured but not reported with every CBC with automated differential at our institution. Our analysis revealed significant differences in the research parameters related to morphology of neutrophils, monocytes, and lymphocytes between COVID-19–positive and COVID-19–negative ICU patients. Our experience with these

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parameters is scant, limiting the interpretation of these findings. However, the presence of different parameter values between COVID-19–positive and COVID-19–negative patients and the absence of such difference between COVID-19–positive ICU and non-ICU patients suggests that some of these changes could be attributed to the SARS-CoV-2 virus. For example, one could wonder if the differences in monocyte RNA content, as suggested by the significantly higher MO-Y value in COVID-19–positive ICU patients compared with COVID-19–negative ICU patients (124.09 vs 115.15, $P = .0227$), could be due to possible direct infection of monocytes by COVID-19, similar to the SARS-CoV virus.[25] It would be of great interest to investigate the research morphology-associated parameters in other viral or parasitic infections known to induce peripheral blood morphologic changes. There have been a few studies exploring research hematology analyzer parameters predominantly in the setting of infection. The study by Henriot et al[26] showed that the lymphocyte research parameters AS-Lymph % and Re-Lymph %, measured on the Sysmex XN-10, may be useful in distinguishing between viral and bacterial infections in febrile children. Some research hematology parameters have been investigated as possible predictors of sepsis. For example, the analysis of neutrophil and monocyte volume parameters measured on the UniCel DxH 800 analyzer concluded that the inclusion of monocyte distribution width along with WBC count improves detection of sepsis at the time of the ICU admission.[27,28]

In conclusion, the newly diagnosed hospitalized patients with COVID-19 should undergo a comprehensive manual WBC differential analysis to look for morphologic predictors of poor outcome, as well as daily CBC with manual WBC differential to monitor for numerical and morphologic alterations suggesting potential clinical deterioration due to disease progression.

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