Original Article

Peripheral Blood Smear Findings of COVID-19 Patients Provide İnformation about the Severity of the Disease and the Duration of Hospital Stay

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Abstract. *Background:* **Data about the morphological changes in peripheral blood smears during COVID-19 infection and their clinical severity association are limited. We aimed to examine the characteristics of the cells detected in the pathological rate and/or appearance and whether these findings are related to the clinical course by evaluating the peripheral blood smear at the time of diagnosis in COVID-19 patients.**

Methods: **Clinical features, laboratory data, peripheral blood smear of fifty patients diagnosed with COVID-19 by PCR was evaluated at diagnosis. Peripheral smear samples of the patients were compared with the age and sex-matched 30 healthy controls. Pictures were taken from the patient's peripheral blood smear. Patients were divided into two groups. Mild and severe stage patient groups were compared in terms of laboratory data and peripheral smear findings. The relationship between the laboratory values of all patients and the duration of hospitalization was analyzed.**

Results: **The number of segmented neutrophils and eosinophils were low, pseudo-Pelger-Huet, pseudo-Pelger-Huet/mature lymphocyte ratio, atypical lymphocytes, monocytes with vacuoles, bands, and pyknotic neutrophils rates were higher in the peripheral blood smear of the patient group (p <0.05). Increased pseudo-Pelger-Huet anomaly, pseudo-Pelger Huet/mature lymphocyte ratio, a decreased number of mature lymphocytes, and eosinophils in peripheral blood smear were observed in the severe stage patients (p <0.05). A negative correlation was observed between hospitalization duration and mature lymphocyte and monocytes with vacuoles rates (p <0.05).**

Conclusion: **A peripheral blood smear is an inexpensive, easily performed, and rapid test. Increased Pseudo-Pelger-Huet anomaly/mature lymphocyte rate suggests a severe stage disease, while high initial mature lymphocyte and monocytes with vacuoles rates at the time of diagnosis may be an indicator of shortened duration of hospitalization.**

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Introduction. Coronaviruses (CoV) are a large family of viruses that cause diseases in a broad clinical spectrum, from mild common flu infection to Middle East Respiratory Syndrome (MERS-CoV) and Severe Acute Respiratory Syndrome (SARS-CoV-1). The new coronavirus disease was first identified as a result of research in a group of patients who developed respiratory tract symptoms such as fever, cough, shortness of breath in Wuhan Province in China at the end of December.[1,2] On 02/11/2020, the disease caused by a new coronavirus (SARS-CoV-2) was officially named COVID-19 by the World Health Organization (WHO).[3] SARS-CoV-2 was initially detected in people working at the seafood and animal market in this region. It spread from person to person and other cities such as Hubei, and finally, other world countries.[4,5] Ultimately, it was declared a pandemic disease by WHO on March 11, 2020.[3]

Viral infections can change the cell numbers and morphology in the peripheral blood smear. In addition to numerical changes like leukocytosis, leukopenia, neutrophilia, neutropenia, monocytosis, monocytopenia, lymphocytosis, lymphopenia, thrombocytopenia, it can also change the morphology of the cells like atypical lymphocyte, inclusion bodies, vacuolization. Atypical lymphocytes, which are sometimes difficult to distinguish from the blasts, can be seen in many viral infections such as infectious mononucleosis, viral hepatitis, cytomegalovirus infections, human immunodeficiency virus infections (HIV), and COVID-19 infection.[6,7] Some viruses can produce intracytoplasmic or intranuclear inclusions when they grow either in vivo and in vitro (in cell cultures). It is possible to see them easily under a microscope in painted smears. The number of monocytes with vacuoles may increase with infections, malignancies, alcohol use, toxic situations as evidence of active immune response.[8,9,10] Pyknosis and karyorrhexis are known as death phases of cells and appear with rounding and opacity of infected cell nuclei due to the virus infection's cytopathic effect. The cells' band size (club, stab) varies between 10 and 16 microns, and their nuclei are undivided, seen like a horseshoe. Their numbers may increase in infections and bleeding. Two or single lobe neutrophils that have defects of lobulation or maturation are called pseudo-Pelger-Huet anomalies. The hereditary type of pseudo-Pelger-Huet is autosomal dominant, but the acquired type is mostly associated with different pathological states like myelodysplastic syndrome, certain infections, and drug use.[11,12,13]

While it is impossible to obtain the presence, percentage, or counts of some cells with a unique pathological appearance by complete blood count, they can be easily distinguished by peripheral blood smear. Those cells with prognostic significance in several diseases are illustrated as a pseudo-Pelger-Huet anomaly in myelodysplastic syndrome, smudge cells in chronic lymphocytic leukemia, and vacuolar lymphocytes in metabolic diseases.[14,15,16] Cells suggesting viral infection in peripheral blood smears in COVID-19 patients are limited, and a small number of studies showed an association between peripheral blood smear findings and clinical severity of the disease. In our study, we aimed to define cells with the pathological appearance and/or rates in peripheral blood smear at the time of diagnosis and whether these cells are associated with clinical severity in COVID-19 patients.

Materials and Methods.
Patients. The data of laboratory-confirmed COVID-19 patients diagnosed between June 01, 2020, and June 20, 2020, were analyzed retrospectively in the Malatya Training and Research Hospital.

Fifty COVID-19 patients and 30 healthy controls were included in this study. Peripheral blood smear samples of the patient group were compared with the healthy controls initially. All peripheral blood smear samples were obtained with the same techniques. The patient group was divided into two as mild and severe disease groups. Both mild and severe disease groups were compared in terms of age, gender, leukocytes, neutrophils, hemoglobin, hematocrit, lymphocytes, eosinophils, monocytes, platelets in complete blood count, segmental, bands, pseudo-Pelger-Huet anomalies, mature lymphocytes, pseudo-Pelger Huet /mature lymphocytes, atypical lymphocytes, monocytes, monocytes with vacuoles, eosinophils, basophils, pyknotic neutrophils in peripheral blood smear, duration of hospitalization and mortality rates. The relationship between the baseline laboratory characteristics of the patients and the duration of hospitalization was also examined.

Laboratory Analysis. Real-time reverse transcriptase-polymerase chain reaction (PCR) tests for SARS-CoV-2 RNA were performed using nasopharyngeal swabs. Total nucleic acid extraction of nasopharyngeal swabs of viral isolates was performed using a biospeedy and coyote extraction system (Bioeksen ltd and Coyote Bioscience ltd.). Real-time PCR (RT-PCR) assays for SARS-CoV-2 RNA detection were performed using Biospeedy COVID-19 RT-qPCR Detection Kit (Bioeksen, Istanbul, Turkey).

Disease Severity. Patients were divided into four stages according to their clinical status. Stage 1; asymptomatic

(without any symptoms), stage 2; symptomatic without lung involvement, stage 3; symptomatic with lung involvement, stage 4; acute respiratory distress syndrome, intubated, multiorgan failure.[17,18,19] Stage 1,2 patients were classified as a mild stage, and 3,4 patients were classified as a severe stage.

Collection of Peripheral Smear. For cases meeting the study criteria, the Wright stained smear was subsequently examined by two hematologists (Dr. Aydogdu I, Dr. Berber I). Hematologists evaluated peripheral blood smear preparations blinded in terms of patients, controls, and laboratory results of the subjects. Peripheral blood smears of the patients were prepared with a blood sample taken from an EDTA tube. A peripheral blood smear was performed, using the same technique, in 30 healthy controls. One hundred leukocyte counts were performed on each peripheral blood smear. Pictures were taken from the peripheral blood smears of the patients (**Figure 1-5**). Lymphocytes divided into two groups; mature lymphocytes and atypical or reactive lymphocytes, which have an eccentric nucleus, dark basophilia of the cytoplasm and pale sunflower region adjacent to the nucleus, cells similar to the plasma cell, and single-core lymphocyte-monocyte, dark basophilia or vacuoles in the cytoplasm and sometimes with nucleoli (**Figure 1**). Neutrophils were divided into three subgroups; (I) the nuclei composed of 3-4 segments connected with thin chromatin as segmented neutrophils, (II) single-lobe or bilobed neutrophils as a pseudo-Pelger-Huet anomaly (**Figure 2**), (III) cell size ranging from 10 to 16 microns with horseshoe-shaped undivided nuclei resembling the C, U, S letters as bands (club, stab) (**Figure 3**). The cells with a nucleus having features like loose chromatin network, curved, resembling kidney and located in the middle or edge of the cell, with gray-blue colored cytoplasm, and thin granules were evaluated monocytes. Monocytes containing vacuoles in their cytoplasm were recorded as monocytes with vacuoles (**Figure 4**). Pyknosis is the shrinkage of the cell nucleus. Karyolysis is the melting of nucleus chromatin with enzymes (nucleases) released from the dead cell's lysosomes. Karyorrhexis is the rupture of the nuclear membrane, chromatin division into small basophilic granules, and spreading into the cytoplasm (**Figure 5**). Cells with a bilobed nucleus and large pink granules in their cytoplasm were recorded as eosinophils.
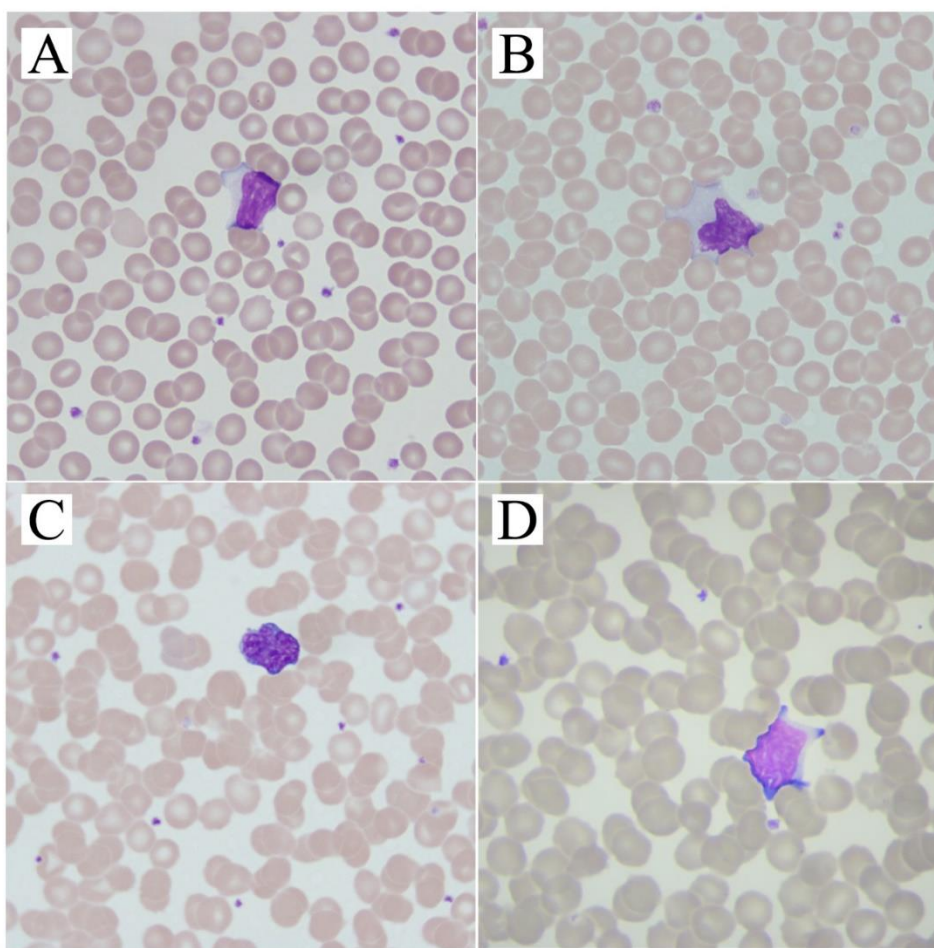
Figure 1. Atypical lymphocytes in patients' peripheral blood smear **A, B, C**. There were lymphocytes with a broad cytoplasm, loose chromatin network and lobuled appearance. Lymphocytes were larger than they should be. The cytoplasm of the lymphocytes was scattered looking adherent to the erythrocytes. Their appearance was particularly similar to stimulated lymphocytes seen in viral infections, and especially "Downey" cells. **D.** Blast-like lymphocytes.
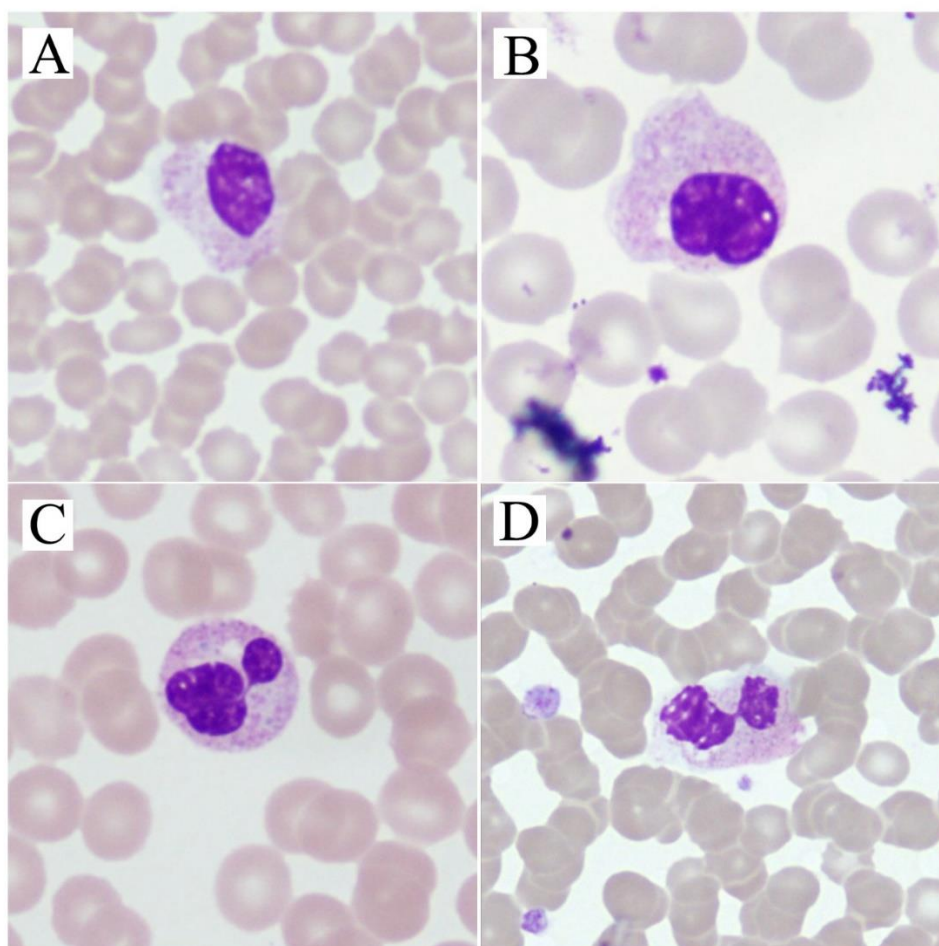
Figure 2. Different degrees of abnormal maturation (Pseudo-Pelger Huet type). There may be single or double lobed neutrophils. **A.** Neutrophil granulocytes with unsegmented nucleus with coarsely clumped chromatin. **B.** Toxic granulations are suggestive of infection in the cytoplasm **C.** The bilobed neutrophil with vacuoles, which we are accustomed to seeing mostly in patients with myelodysplastic syndrome. **D.** Bilobed neutrophil with dysplasia.
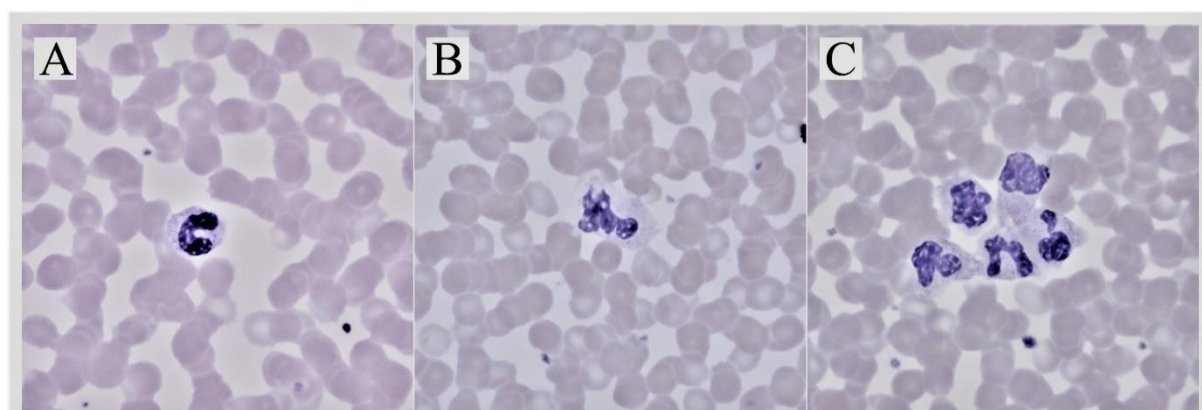

Figure 3. A. Band cell that we are accustomed to seeing in bacterial infections whose nucleus is "C" shaped. They are also known as club or stab cells. **B.** A dysplastic neutrophil that we see mostly in patients with myelodysplastic syndrome. Hypogranulation is at the forefront of the neutrophil's cytoplasm. **C.** Cell group consisting of bands and dysplastic neutrophils.

Statistical Analysis. Data analysis was performed using IBM SPSS v26 software. Descriptive statistics were used to summarize data. Variables were assessed for normal distribution with the Kolmogorov Smirnov test. Categorical data were presented as number-percentages, and numerical data were presented as median, minimum, and maximum. Differences between categorical variables were analyzed with the Chi-Square test, and numeric variables were compared with the Mann-Whitney U test. A two-sided p-value ≤ 0.05 was considered statistically significant.

Roc analysis was performed to find a cut-off point for differential morphological aspects between mild and severe disease stages.
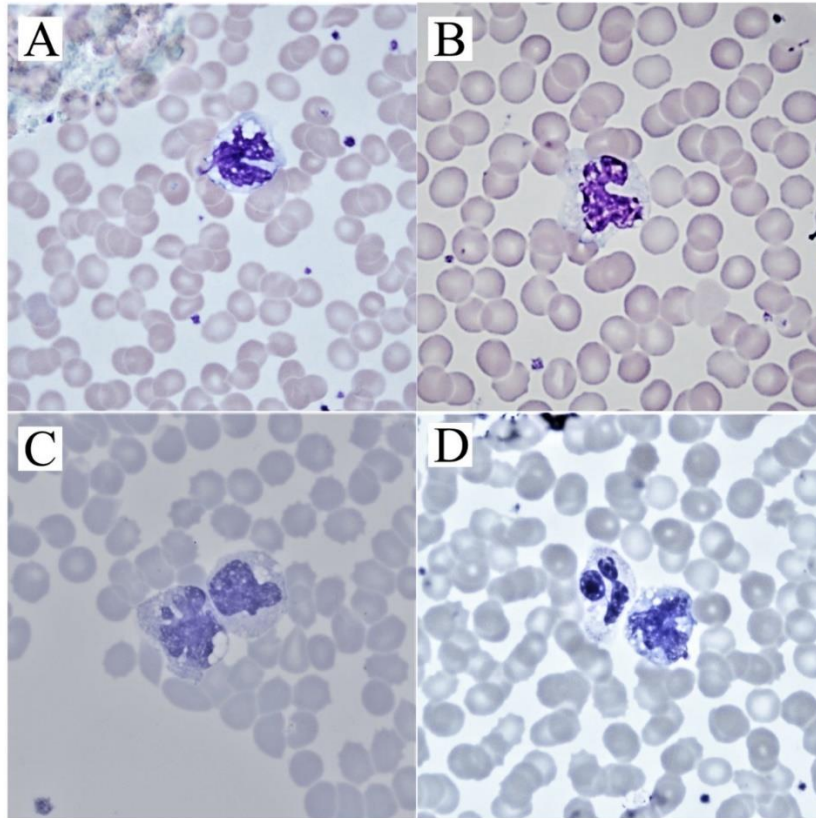
Figure 4. A, B. The monocytes with vacuoles that generally increased during infections. Vacuoles are often considered to be evidence of the fight against infectious agents. **C.** A picture to show the severity of vacuolization in patients. **D.** Beside a monocyte with vacuole, a neutrophil with dysplasia and vacuole.
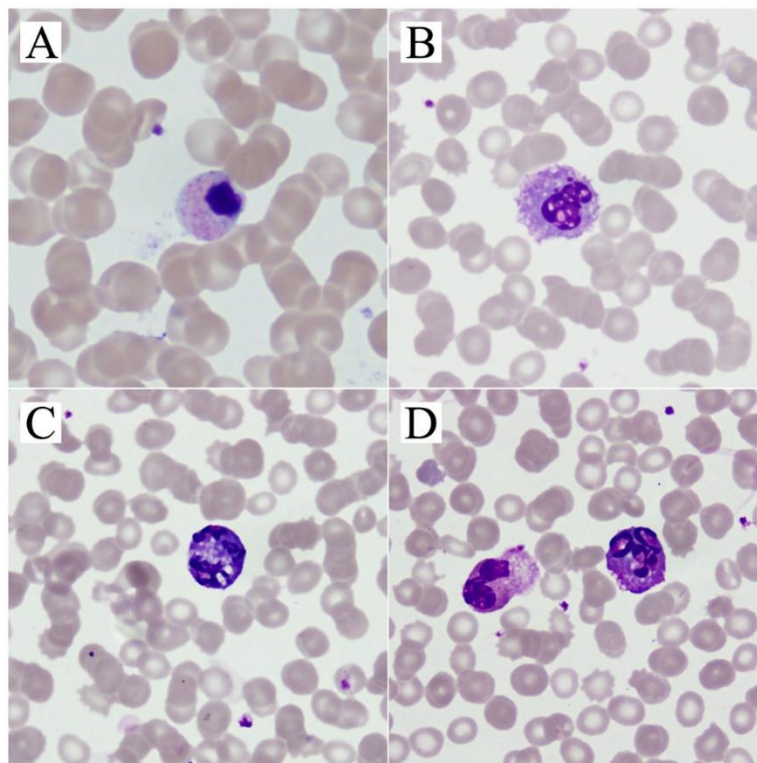
Figure 5. Pyknosis, karyolysis and karyorrhexis are the death steps of the cell nucleus as a result of the cytopathic effect of the virus. **A.** Pyknosis is the shrinkage of the cell nucleus. Neutrophils with concentrated and basophilic nuclei showing pyknosis. Toxic granulations are suggestive of infection. **B.** Karyolysis is the melting of nucleus chromatin with enzymes (nucleases) released from the lysosomes of the dead cell. The nuclear membrane is preserved. **C, D.** Karyorrhexis is the rupture of the nuclear membrane, division of chromatin into small basophilic granules and spreading into the cytoplasm. Neutrophils that have undergone karyorrhexis are seen. Moreover, a dysplastic neutrophil with increased basophilic staining in one part of the cytoplasm and vacuolization in the other part.

Table 1. Clinical features, laboratory values, and peripheral blood smear findings of all the patients and healthy controls.

Characteristics and laboratory values			All patients median (min-max)	Controls median (min-max)	P
Total number of patients			50	30	
Median Age			44 (18-88)	45(18-75)	>0.05
Gender (Number/percent)	Female (%)		25 (50)	15 (50)	
	Male (%)		25 (50)	15 (50)	
Stage (Number. %)	Early	Stage I	1 (2)		
		Stage II	23 (46)		
	Severe	Stage III	24 (48)		
		Stage IV	2 (4)		
Complete blood count (Median)	Leukocyte		5.19 (2.83-20.36)	5.21(2.4-11.3)	>0.05
	Neutrophil		3.27(0.86-18.81)	3.22(1.1-10.3)	>0.05
	Hemoglobin		13.95 (8.5-17.9)	14,03 (10,2-17.1)	>0.05
	Hematocrit		40.75 (25.6-52.7)	42.09(30,6-51.3)	>0.05
	Lymphocyte		**1.28 (0.39-2.53)**	**2.1(2-2.5)**	**0.008**
	Monocyte		0.47 (0-1.16)	0.41 (0-0.9)	>0.05
	Eosinophil		0.02 (0-0.6)	0.02 (0-0.42)	>0.05
	Platelet		210 (116-389)	216(110-344)	>0.05
Peripheral blood smear (%)	Neutrophils	Segmental	**19.5 (0-57)**	**54 (36-60)**	**<0,001**
		Band	7.5 (0-44)	2,5 (0-8)	**0,019**
		Pseudo-Pelger Huet	**10 (0-46)**	**0 (0-2)**	**<0,001**
	Lymphocyte	Mature lymphocyte	28 (0-65)	30,5 (24-49)	0,126
		Atypical lymphocyte	**8 (0-26)**	**0 (0-3)**	**<0,001**
	Monocyte	Mature Monocyte	5.5 (0-20)	5 (0-10)	0,486
		Monocyte with vacuoles	2 (0-22)	0 (0-0)	**0,002**
	Basophils		0 (0-4)	0 (0-2)	0,592
	Eosinophils		**0 (0-7)**	**4 (0-5)**	**0,001**
	Pyknotic neutrophils		**0 (0-67)**	**0 (0-0)**	**0,014**
	Pseudo-Pelger Huet/ Mature lymphocyte ratio		**0.44 (0.00-7.00)**	**0,12(0-18)**	**<0.001**
Length of hospital stay (day)			5 (1-17)		
Mortality (Number/Percent)			2 (4)		

Spearman correlation coefficient correlates with hospital stay length and baseline laboratory values and morphological findings in the peripheral blood smears.

The study was approved by the research ethics committee of Inonu University, Faculty of Medicine (date/reference number: 30-06-2020/892). All analyses were performed in accordance with the principles of the Declaration of Helsinki.

Results. Patients and healthy controls were similar in terms of age and sex. In the peripheral blood smear, the number of segmented neutrophils, eosinophils were low; the band, pseudo-Pelger-Huet, atypical lymphocytes, monocytes with vacuoles, pseudo-Pelger-Huet/mature lymphocyte ratio, and pyknotic neutrophils were higher in the patient group (**Table 1**) (p<0,05).

Twenty-four patients in the mild-stage and 26 patients in the severe stage were compared in terms of clinical features and laboratory values (**Table 2**). No statistically significant difference was found between mild and severe stage in terms of complete blood count. In peripheral blood smears of severe-stage patients, it was observed that the number of mature lymphocytes and eosinophils decreased, and pseudo-Pelger-Huet anomaly, pseudo-Pelger Huet anomaly/mature lymphocytes ratio increased when compared with the mild stage group (p< 0.05). The number of band cells, mature monocytes, and monocytes with vacuoles were increased in the severe stage, although they did not reach the statistical significance (p>0.05). The length of hospital stay was significantly higher in severe-stage patients (p<0.05). A cut-off point for differential morphological aspects between the mild and severe stages of the disease was tried to be determined by Roc analysis. Unfortunately, a specific value could not be found because the area under the curve (AUC) was not significant. While none patients died in the mild stage group, two patients died in the severe stage (p> 0.05).

Laboratory values of all patients were compared with the duration of hospitalization. A significant positive correlation between a longer hospitalization duration and an increased number of neutrophils in the complete blood count was observed. A negative correlation between the duration of hospitalization and lymphocyte count in hemogram, mature lymphocyte, and monocytes with vacuoles rates in peripheral blood smear was observed (p<0.05) (**Table 3**).

Table 2. Comparison of laboratory values, peripheral blood smear findings, and clinical features of Mild and severe stage patients.

Characteristics and laboratory values			Mild Stage Median (min-max)	Severe Stage Median (min-max)	p
Total number of patients			24	26	
Median Age (year)			**27.5 (18-52)**	**57.5 (27-88)**	**<0.001**
Gender	Female (number/percent)		10 (41.7)	15 (57.7)	0.396
	Male (number/percent)		14 (58.3)	11 (42.3)	
Stage (Number. (%))	Early	Stage I	1 (4.2)		
		Stage II	23 (95.8)		
	Severe	Stage III		24 (92.3)	
		Stage IV		2 (7.7)	
Complete blood count	Leukocyte		5.54 (3.25-9.09)	5.13 (2.83-20.36)	0.398
	Neutrophil		3.33(1.63-6.84)	3.26(0.86-18.81)	0.614
	Hemoglobin		14.3 (10.5-17.2)	13.65 (8.5-17.9)	0.244
	Hematocrit		41.25 (35.2-52.2)	40.25 (25.6-52.7)	0.541
	Lymphocyte		1.21 (0.61-2.53)	1.39 (0.39-2.32)	0.778
	Monocyte		0.49 (0.00-1.12)	0.43 (0.32-1.16)	0.122
	Eosinophil		0.05 (0.00-0.60)	0.01 (0.00-0.21)	0.058
	Platelet		221 (152-389)	196 (116-309)	0.062
Peripheral blood smear (%)	Neutrophil	Segmental	23.5 (0-55)	17 (0-57)	0.062
		Band	5 (0-40)	9 (0-44)	0.094
		Pseudo-Pelger Huet	**6 (0-46)**	**18.5 (0-36)**	**0.007**
	Lymphocyte	Mature lymphocyte	**31 (0-65)**	**17 (4-44)**	**0.010**
		Reactive lymphocyte	9 (1-21)	8 (0-26)	0.366
	Monocyte	Mature Monocyte	5 (0-20)	6.5 (1-18)	0.271
		Monocyte with vacuoles	0 (0-22)	4 (0-16)	0.159
	Basophils		0 (0-2)	0 (0-4)	0.234
	Eosinophils		**1 (0-7)**	**0 (0-4)**	**0.008**
	Pyknosis		1 (0-18)	0 (0-67)	0.162
	Pseudo-Pelger Huet/ Mature lymphocyte		**0.1538 (0.00- 1.44)**	**0.7305 (0.00-7.00)**	**0.002**
Lenght of hospital stay (day)			**5 (1-17)**	**10 (1-16)**	**0.037**
Mortality (Number. %)			0 (0)	2 (7.7)	0.491

Discussion. This study shows that an increased pseudo-Pelger Huet, pseudo-Pelger Huet/ mature lymphocyte ratio, and decreased mature lymphocyte and eosinophil rates in peripheral blood smear were found in COVID patients with severe diseases stage. Moreover, at the onset of the disease, patients with an increased number of mature lymphocytes and monocytes with vacuoles in the peripheral blood smear had a short hospitalization duration.

In general, viral infections are known to be manifested by atypical lymphocytes, inclusion bodies, monocytes with vacuoles, and pyknosis in leukocytes of the peripheral blood smear. Information about the morphological changes of COVID-19 infection in peripheral blood smears and the significance of these changes on the disease's clinical course is limited.

Dan Zhang et al. reported COVID-19 induces readily detectable morphological and inflammation-related phenotypic changes in peripheral blood monocytes, whose entity correlates with patient outcome. They detected an increased number of larger, atypical, vacuolated monocytes, not generally seen in healthy individuals' peripheral blood smear. We confirmed the presence of the same vacuolated monocytes in our previous study.[20] Delphine Gérard et al. reported that peripheral blood film examination revealed a highly pleomorphic atypical lymphocyte population, and many cells were large (15–30 µm). The cytoplasm was sometimes granulated. Cytoplasmic basophilia was sometimes generalized and sometimes confined to the cytoplasmic margins. Some cells had one or more nucleoli and could have been mixed with blast cells.[6] Alia Nazarullah et al. reported peripheral blood examination findings in SARS-CoV-2 infection. They showed that the pseudo-Pelger-Huet anomaly was recorded in all cases of COVID-19, affecting more than 5% of granulocytes in most cases.[21] Yunus Murat Akcabelen et al. reported dysplastic changes in peripheral blood cells of COVID-19 patients. We also observed atypical lymphocytes, pseudo-Pelger-Huet anomaly, and dysplastic changes in peripheral blood cells in a previous study.[22]

Maryame et al. found neutrophil granulocyte with dysmorphic morphology marked by hypogranular

Table 3. Correlation analysis between the length of hospital stay, and baseline laboratory characteristics, morphological findings of the patients.

Baseline laboratory characteristics and morphological findings			Correlation Coefficient (Spearman's rho)	p-value
Complete blood count		Leukocyte	0,69	0.632
		Neutrophil	**0.352**	**0.012**
		Hemoglobin	-0.185	0.197
		Hematocrit	-0.160	0.268
		Lymphocyte	**-0.308**	**0.03**
		Monocyte	-0.185	0.197
		Eosinophil	-0.072	0.617
		Platelet	-0.94	0.525
Peripheral blood smear (%)	Neutrophils	Segmental	-0.13	0.928
		Band	0.34	0.815
		Pseudo-Pelger Huet	-0.96	0.506
	Lymphocyte	**Mature lymphocyte**	**-0,315**	**0.026**
		Reactive lymphocyte	-0.197	0.171
		Mature Monocyte	-0.181	0.208
	Monocyte	**Monocyte with vacuoles**	**-0.376**	**0.007**
		Basophils	-0.002	0.990
		Eosinophils	-0.140	0.331
		Pyknosis	0.107	0.460
		Pseudo-Pelger Huet/ Mature lymphocyte	-0.95	0.515

cytoplasm and hyposegmented nucleus, atypical eosinophils containing multiple vacuoles, rare, activated lymphocytes, and large monocytes in some peripheral blood films in COVID-19 patients.[23] They did not show any association of these changes with the clinical course of the disease. In this study, we observed a large number of dysplasia, hypolobular, hypergranular neutrophils, and a small number of hypogranular neutrophils. It was observed that atypical lymphocytes increased compared to the control group but did not significantly affect hospitalization duration. Cantu MD et al. reported blue-green neutrophil and monocyte cytoplasmic inclusions in peripheral blood smear of critically ill SARS-CoV-2 positive patients; these inclusions were still present 20 days after the diagnosis of COVID-19. They showed that blue-green inclusions might correlate with short-term mortality.[24] Similar inclusions were found by us in one patient's lymphocytes with a severe stage (Figure 1H), showing that they are not specific for myeloid lineage. Alterations of granulocytes can be different in COVID-19; Christian Salib et al. showed hypersegmented granulocytes infected patients;[25] in our study, we found that pseudo-Pelger-Huet anomalies were significantly present, especially in severe patients. Similarly, Gina Zini et al. reported morphological anomalies of circulating blood cells in COVID-19 patients. These anomalies were unsegmented or bilobular nuclei like pseudo-Pelger-Huet, hypergranular with basophilic cytoplasm, hypogranular and agranular areas, multiple vacuoles in neutrophil granulocyte,

immature circulating cells with blasts-like reticular chromatin and rare thin azurophilic granules, likely pre-apoptotic, circulating apoptotic neutrophil, apoptotic cells with blue cytoplasm, of possible lymphocyte origin, large polyploid reactive lymphocyte with hyperbasophilic cytoplasm, giant vacuolated platelets.[26] Anupam Mitra et al. reported a leukoerythroblastic reaction in a 46-year-old previously healthy female with COVID-19 infection. In peripheral blood smear, they found nucleated erythroid, rare blast, with prominent nucleoli and immature chromatin pattern, a left-shifted myeloid series with immature promyelocytes and metamyelocytes, and occasional monocytes.[27] Chuan Qin et al. reported 452 patients with COVID-19 infection, 286 of whom were diagnosed as a severe infection. Severe cases tend to have lower lymphocyte counts, higher leukocyte counts, neutrophil-lymphocyte ratio (NLR), and lower percentages of monocytes, eosinophils and basophils.[28] Man Kong et al. reported a higher level of neutrophil-to-lymphocyte is associated with severe COVID-19.[29] Ai-Ping Yang et al. reported that elevated neutrophil-to-lymphocyte ratio could be considered independent biomarkers for indicating poor clinical outcomes.[30]

The cells we examined in peripheral blood smear were morphologically similar to the others published in the literature. We did not see a leukoerythroblastic reaction in any of our patients. Especially in severe patients, we found a higher number of band cells and a lower number of segmented neutrophils, but these

results did not reach statistically significant levels, and that could be due to increased cytokine levels in the severe stage. We observed an increased pseudo-Pelger Huet anomaly, pseudo-Pelger Huet anomaly/mature lymphocyte ratio, decreased mature lymphocytes, and eosinophils rates in the severe stage patients. Impaired immunity due to decreased lymphocyte count and an increase in dysfunctional neutrophil count due to pseudo-Pelger Huet anomaly may be the answer to why these patients are admitted to the hospital at an severe stage.

The morphological abnormalities in all cell lines may be a prognosticator for increased mortality of this disease. There was a negative correlation between monocytes with vacuoles, lymphocyte counts, and duration of hospitalization. These observations can be explained by the degree of suppressing the SARS-CoV-2 virus through inflammatory mechanisms on hematopoiesis and the immune system that reduce these cells' number.

Our study has some strengths. To our knowledge, this is the first article showing an association between the stages, the clinical course of the disease, and cell morphology in peripheral blood smears of COVID-19 patients. Our study has several limitations: the low number of the study population with a low mortality rate did not permit establishing a relationship between peripheral blood smear findings and disease prognosis. Moreover, we could not examine pathological cells' disappearance because the peripheral blood smear samples could not be taken during the follow-up period. Increased pseudo-Pelger Huet anomaly and pseudo-Pelger Huet/mature lymphocyte ratio in peripheral blood smear may suggest the advanced stage disease. Increased monocytes with vacuoles and mature lymphocytes may indicate shorter duration of hospitalization. As a result, peripheral blood smear is an inexpensive, easily performed, and rapid test. Peripheral blood smear assessment at the time of diagnosis in COVID-19 patients can provide information about the stage and severity of the disease and the length of hospital stay. Studies with larger number of patients are needed in order to increase the reliability of this information and associate the findings of peripheral smear with the prognosis of the disease.

References:

1. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, Ren R, Leung KSM, Lau EHY, Wong JY, Xing X, Xiang N, Wu Y, Li C, Chen Q, Li D, Liu T, Zhao J, Liu M, Tu W, Chen C, Jin L, Yang R, Wang Q, Zhou S, Wang R, Liu H, Luo Y, Liu Y, Shao G, Li H, Tao Z, Yang Y, Deng Z, Liu B, Ma Z, Zhang Y, Shi G, Lam TTY, Wu JT, Gao GF, Cowling BJ, Yang B, Leung GM, Feng Z. Early Transmission Dynamics in Wuhan, China, of Novel Coronavirus-Infected Pneumonia. N Engl J Med. 2020 March 26;382(13):1199-1207.
https://doi.org/10.1056/NEJMoa2001316
PMid:31995857 PMCid:PMC7121484
2. Pal M, Berhanu G, Desalegn C, Kandi V. Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2): An Update. Cureus. 2020;12(3):e7423. Published 2020 March 26.
https://doi.org/10.7759/cureus.7423
3. World Health Organization Press Conference. The World Health Organization (WHO) Has Officially Named the Disease Caused by the Novel Coronavirus as COVID-19. Available online: (accessed on May 18 2020).
4. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, Tsoi HW, Lo SK, Chan KH, Poon VK, Chan WM, Ip JD, Cai JP, Cheng VC, Chen H, Hui CK, Yuen KY. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet. 2020 Feb 15;395(10223):514-523.
https://doi.org/10.1016/S0140-6736(20)30154-9
5. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020 February 20;382(8):727-733.
https://doi.org/10.1056/NEJMoa2001017
PMid:31978945 PMCid:PMC7092803
6. Gérard D, Henry S, Thomas B. SARS-CoV-2: a new aetiology for atypical lymphocytes. Br J Haematol. 2020 Jun;189(5):845.
https://doi.org/10.1111/bjh.16730
PMid:32311762 PMCid:PMC7264674
7. El Jamal SM, Salib C, Stock A, Uriarte-Haparnas NI, Glicksberg BS, Teruya-Feldstein J, Dembitzer FR, Nadkarni GN, Firpo-Betancourt A. Atypical lymphocyte morphology in SARS-CoV-2 infection. Pathol Res Pract. 2020 Sep;216(9):153063. doi: 10.1016/j.prp.2020.153063. Epub 2020 June 10.
https://doi.org/10.1016/j.prp.2020.153063
PMid:32825937 PMCid:PMC7284261
8. Ponder E., Ponder R.V. Cytology of polymorphonuclear leucocyte in toxic conditions. J Lab Clin Med. 1942; 28:316-322.
9. GORDIN R. Toxic granulation in leukocytes; development and relation to cloudy swelling. Acta Med Scand Suppl. 1952;270:1-50.
10. Jordans G.H.W. The familial occurrence of fat containing vacuoles in the leucocytes diagnosed in 2 brothers suffering from dystrophia musculorum progressiva. Acta Med Scand. 1953;145:419-423.
https://doi.org/10.1111/j.0954-6820.1953.tb07038.x
PMid:13079655
11. Harald T, Heinz D, Torsten H. Color Atlas of Hematology. 2004.
12. Löffler H, Rastetter J, Haferlach T. Atlas of Clinical Hematology. Sixth Revised Edition. 2005
13. Aydogdu I, Kuku I, Kaya E, Gödekmerdan A. Kan Hastalıkları Atlası. 2003
14. Tassin F, Dewé W, Schaaf N, Herens C, Ravoet C, Albert A, Beguin Y, Paulus JM. A four-parameter index of marrow dysplasia has predictive value for survival in myelodysplastic syndromes. Leuk Lymphoma. 2000 Feb;36(5-6):485-96.
https://doi.org/10.3109/10428190009148396
PMid:10784393
15. Gogia A, Raina V, Gupta R, Gajendra S, Kumar L, Sharma A, Kumar R, Vishnubhatla S. Prognostic and predictive significance of smudge cell percentage on routine blood smear in chronic lymphocytic leukemia. Clin Lymphoma Myeloma Leuk. 2014 Dec;14(6):514-7.
https://doi.org/10.1016/j.clml.2014.02.007
PMid:24656596
16. Anderson G, Smith VV, Malone M, Sebire NJ. Blood film examination for vacuolated lymphocytes in the diagnosis of metabolic disorders; retrospective experience of more than 2,500 cases from a single centre. J Clin Pathol. 2005 Dec;58(12):1305-10.
https://doi.org/10.1136/jcp.2005.027045
PMid:16311352 PMCid:PMC1770783
17. Lauer SA, Grantz KH, Bi Q, Jones FK, Zheng Q, Meredith HR, Azman AS, Reich NG, Lessler J. The Incubation Period of Coronavirus Disease 2019 (COVID-19) From Publicly Reported Confirmed Cases: Estimation and Application. Ann Intern Med. 2020 May 5;172(9):577-582.
https://doi.org/10.7326/M20-0504
PMid:32150748 PMCid:PMC7081172
18. Ferguson N.M, Laydon D., Nedjati-Gilani G., Imai N., Ainslie K., Baguelin M., Bhatia S., Boonyasiri A., Cucunubá Z., Cuomo-Dannenburg G., Dighe A., Dorigatti I., Fu H., Gaythorpe K., Green W., Hamlet A.,

Hinsley W., Okell L.C., Elsland S.V., Thompson H., Verity R., Volz E., Wang H., Wang Y., Walker P.G.T., Walters C., Winskill P., Whittaker C., Donnelly C.A., Riley S., Ghani A.C. Impact of non-pharmaceutical interventions (NPIs) to reduce COVID-19 mortality and healthcare demand. Imperial College COVID-19 Response Team. 2020;10:77482. URL:
https://www.imperial.ac.uk/media/imperial-college/medicine/sph/ide/gida-fellowships/Imperial-College-COVID19-NPI-modelling-16-03-2020.pdfm

19. Liu Y., Yan L.M., Wan L., Xiang T.X., Le A., Liu J.M., Peiris M., Poon L.L.M., Zhang W. Viral dynamics in mild and severe cases of COVID-19. The Lancet Infectious Diseases. 2020.
https://doi.org/10.1016/S1473-3099(20)30232-2

20. Zhang D., Guo R., Lei L., Liu H., Wang Y., Wang Y., Dai T., Zhang T., Lai Y., Wang J. Liu Z., He A., O'Dwyer M., Hu J. COVID‑19 infection induces readily detectable morphologic and inflammation-related phenotypic changes in peripheral blood monocytes. Journal of Leukocyte Biology. 2020.
https://doi.org/10.1002/JLB.4HI0720-470R
PMid:33040384 PMCid:PMC7675546

21. Nazarullah A., Liang C., Villarreal A., Higgins R.A., Mais D.D. Peripheral Blood Examination Findings in SARS-CoV-2 Infection. American journal of clinical pathology. 2020; 154(3), 319-329.
https://doi.org/10.1093/ajcp/aqaa108
PMid:32756872 PMCid:PMC7454310

22. Akcabelen Y.M., Gurlek G.D., Yaralı N. Dysplastic Changes of Peripheral Blood Cells in COVID-19 Infection. Turk J Haematol. 2020 August 19.
https://doi.org/10.4274/tjh.galenos.2020.2020.0342
PMid:32812414

23. Ahnach M, Ousti F, Nejjari S, Houssaini MS, Dini N. Peripheral Blood Smear Findings in COVID-19. Turk J Haematol. 2020 June 26.
https://doi.org/10.4274/tjh.galenos.2020.2020.0723
PMCid:PMC7702645

24. Cantu MD, Towne WS, Emmons FN, Mostyka M, Borczuk A, Salvatore SP, Yang HS, Zhao Z, Vasovic LV, Racine-Brzostek SE. Clinical significance of blue-green neutrophil and monocyte cytoplasmic inclusions in SARS-CoV-2 positive critically ill patients. Br J Haematol. 2020 Jul;190(2):e89-e92.
https://doi.org/10.1111/bjh.16882
PMid:32453859 PMCid:PMC7283650

25. Salib C, Teruya-Feldstein J. Hypersegmented granulocytes and COVID-19 infection. Blood. 2020;135(24):2196.
https://doi.org/10.1182/blood.2020006483
PMid:32526026 PMCid:PMC7290095

26. Zini G, Bellesi S, Ramundo F, d'Onofrio G. Morphological anomalies of circulating blood cells in COVID-19. Am J Hematol. 2020 Jul;95(7):870-872.
https://doi.org/10.1002/ajh.25824
PMid:32279346 PMCid:PMC7262044

27. Mitra A, Dwyre DM, Schivo M, Thompson GR 3rd, Cohen SH, Ku N, Graff JP. Leukoerythroblastic reaction in a patient with COVID-19 infection. Am J Hematol. 2020 Aug;95(8):999-1000.
https://doi.org/10.1002/ajh.25793
PMid:32212392 PMCid:PMC7228283

28. Qin C., Zhou L., Hu Z., Zhang S., Yang S., Tao Y., Xie C., Ma K., Shang K., Wang W., Tian D.S. Dysregulation of immune response in patients with COVID-19 in Wuhan, China. Clinical Infectious Diseases. 2020.
https://doi.org/10.1093/cid/ciaa248
PMid:32161940 PMCid:PMC7108125

29. Kong M, Zhang H, Cao X, Mao X, Lu Z. Higher level of neutrophil-to-lymphocyte is associated with severe COVID-19. Epidemiol Infect. 2020 July 9;148:e139.
https://doi.org/10.1017/S0950268820001557
PMid:32641174 PMCid:PMC7360950

30. Yang A.P., Liu J., Tao W., Li H.M. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. International immunopharmacology, 2020. 106504.
https://doi.org/10.1016/j.intimp.2020.106504
PMid:32304994 PMCid:PMC7152924