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Morphological Changes in Blood Cells as Indicators for Disease Progression in COVID-19

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Abstract:

BACKGROUND: COVID-19 as a pandemic has caused an alarming increase in mortality and morbidity. Viral-induced morphologic changes in the peripheral blood cells are well characterized in certain infections and can direct diagnostic workup to ensure timely therapeutic intervention. This study describes the morphological changes of blood cells in various stages of COVID disease.

MATERIALS AND METHODS: A total of thousand COVID-positive patients admitted in the tertiary care center were taken for the study. They were classified as mild, moderate, and severe based on the clinical criteria suggested by World Health Organization. Peripheral smears of the patients were analyzed, and the morphological changes in various blood cells were correlated with the disease stage and coagulation parameters.

RESULTS: The study demonstrated significant morphological changes in the blood cells of COVID patients during the course of disease progression and during the onset of COVID-associated coagulopathy. Leukocytosis, neutrophilia, and toxic changes in neutrophils were seen in the severe stage of the disease and in COVID coagulopathy suggesting these are important indicators of disease severity. Activated lymphocyte was found to be the most common morphological presentation seen in all patients irrespective of the disease stage, whereas plasmacytoid lymphocytes were an important finding in severe-stage disease. Schistocytes an important finding in any other coagulopathy was present only in 1% of cases of COVID coagulopathy.

CONCLUSIONS: The study demonstrated significant morphological changes in the blood cells of COVID-positive patients during the course of disease progression. Comprehensive daily complete blood count and peripheral smear examination should be undertaken in patients hospitalized with COVID-19 to predict potential clinical deterioration and signs of disease progression. These morphological changes in peripheral smear can be used as one of the factors indicating disease progression which can formulate for further evaluation. Since follow-up and post-COVID morphological examination were not done, additional research in this aspect can shed light on the clinical categorization of COVID patients based on the morphological findings.

Keywords:

Activated lymphocytes, blood cell morphology, coagulopathy, COVID-19, neutrophil toxic changes, peripheral smear

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Introduction

A novel highly pathogenic human coronavirus (COVID-19) has been recently recognized in Wuhan, China, as the cause of corona disease outbreak. It has rapidly spread from China to various countries across the world evolving as a pandemic. The pandemic has alarming morbidity and mortality occurring as a result of acute respiratory distress syndrome.[1-3] The virus spreads by droplet infection. The pathogenesis of COVID-19 is complicated, it enters the respiratory tract, causes activation of the innate immune system producing a cytokine storm resulting in a prothrombotic

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state, and finally leading to acute respiratory distress syndrome.[4-7]

Viral-induced morphologic changes in the peripheral blood cells are well characterized in certain infections and can direct diagnostic workup to ensure timely therapeutic intervention. For example, Epstein–Barr virus infection produces atypical lymphocytes, whereas dengue fever caused by *Flaviviridae* produces many activated plasmacytoid and monocytoid lymphocytes. Certain viral infections such as human immunodeficiency (HIV) may not cause any significant morphological changes.

In a study by Singh, the various morphological changes of red blood cells (RBCs), white blood cells (WBCs), and platelets in COVID-19-positive patients have been described and correlated with clinical findings.[8] In a study by Lee and Teo, the various morphological changes of lymphocytes in COVID-19-positive patients have been described.[9]

Peripheral smear is a simple, cost-effective method, and the findings can be used as an early indicator of disease progression that can help in prompt diagnosis and treatment. The study represents a systematic analysis of peripheral smears of COVID-positive patients. The patients were divided into mild, moderate, and severe based on the clinical presentation as suggested by World Health Organization (WHO).[10,11] The peripheral smear findings were then correlated with the clinical stage of the disease and coagulation parameters.

Materials and Methods

This was a prospective study conducted at an academic institute in South India. The study was reviewed and approved by the Institutional Human Ethics Committee, KMCHIHSR, approval number KMCHIHSR/IHEC/2020/Apr/Exp/25.

A total of thousands of reverse transcriptase–polymerase chain reaction COVID-positive patients admitted in the tertiary care center during the study period (October 2020–March 2021) were taken for the study. The patients were classified as mild, moderate, and severe based on the clinical criteria suggested by the WHO, and the findings were documented.

The coagulation parameters such as prothrombin time (PT), activated partial thromboplastin time (APTT), and D-dimer of the patients were analyzed in blood collected in blue top vacutainers (3.2% sodium citrate). The preanalytical procedures were strictly adhered to and all the samples were run within 2 h of collection in an instrumentation and laboratory (IL-ACL) semi-automated coagulation analyzer. The values were documented simultaneously.

The ethylenediaminetetraacetic acid sample of the COVID patients was used to analyze the blood counts. All the samples were run in the Sysmex XP-100 three-part automated analyzer. The same samples were used to make peripheral smears according to the standard operating protocol. The stained smears were analyzed simultaneously by two trained pathologists, and the findings were documented in a formulated template. The template included morphological features of RBCs, various WBCs, and platelets.

The various morphological parameters included in the template are described below. Morphological changes in RBCs included the presence of schistocytes, whereas in neutrophils, it was toxic changes and shift to the left. Toxic change is defined as the presence of coarse purple cytoplasmic granules and cytoplasmic vacuoles. Morphological changes in lymphocytes were divided into two groups, activated monocytoid lymphocytes and plasmacytoid lymphocytes. Monocytoid lymphocytes have abundant vacuolated cytoplasm with a large sometimes lobated nucleus and cytoplasmic vacuoles resembling a monocyte. Plasmacytoid lymphocytes are cells with dark basophilic cytoplasm, round peripherally placed nucleus, condensed chromatin, and sometimes with a perinuclear hoff resembling a plasma cell. Morphological changes in monocytes such as cytoplasmic vacuolations were documented. The presence of large/giant platelets was looked for and documented. Giant platelets are those platelets which are almost the size of RBC, whereas large platelets are somewhat smaller in size when compared to RBCs. Since these peripheral smear changes can be found in a variety of infections, liver diseases etc., such parameters were considered and excluded when the smears were analyzed.

The peripheral smear findings documented were then correlated with the coagulation parameters and the disease severity. The data were analyzed using SPSS, (IBM, Chicago US). Descriptive statistics were used to summarize data. Categorical data were presented as number percentages, and numerical data were presented as median, minimum, and maximum. Roc analysis was performed to find a cutoff point for differential morphological patterns between mild, moderate, and severe disease stages.

Definitions

The WHO criteria for clinical classification of COVID-19-positive patients

Mild

Symptomatic patients meeting the case definition for COVID-19 without evidence of viral pneumonia or hypoxia.

Moderate

Clinical signs of pneumonia (fever, cough, dyspnea, and tachypnea) but no signs of severe pneumonia, including SpO2 ≥90% on room air.

Severe

With clinical signs of pneumonia (fever, cough, dyspnea, and fast breathing) plus one of the following: respiratory rate >30 breaths/min; severe respiratory distress; or SpO2 <90% on room air.

Results

Thousand patients of COVID-19 admitted during the study period of 6 months were taken for the study. The patients were classified as mild (Stage 1), moderate (Stage 2), and severe (Stage 3) based on the clinical criteria suggested by WHO. In the 1000 patients, 79% belonged to mild category, 16% belonged to moderate, and 5% belonged to severe disease. The majority of the patients (79%) had mild forms of the disease (Stage 1) but were admitted for monitoring.

Comparison of peripheral smear findings with clinical stage

The clinical stage of the patient was correlated with the total WBC count and differential count. WBC count of more than 11,000/μL was taken as leukocytosis, a differential neutrophil count of more than 75% was taken as neutrophilia, and a platelet count of <1,00,000/μL was taken as thrombocytopenia.

The variation of total WBC count, differential neutrophil count, differential lymphocyte count, and differential monocyte count between Stages 1 and 2 and Stages 1 and 3 were significant ($P < 0.005$).

The mean blood count values in different clinical stages of the disease are described in Table 1. An increase in mean WBC count, differential neutrophil count, and a decrease in differential lymphocyte count was seen in Stage 3 disease.

In total, 12% of cases (out of 1000 cases) showed leukocytosis. Eight percentage of cases in Stage 1 (out of 789 cases), 27% of cases in Stage 2 (out of 163 cases), and 32% of cases in Stage 3 (out of 48 cases) showed leukocytosis. Thirty-five percentage of cases (out of 1000 cases) showed neutrophilia. Twenty-five percentage of cases in Stage 1 (out of 789 cases), 72% of cases in Stage 2 (out of 163 cases), and 89% of cases (out of 48 cases) in Stage 3 showed neutrophilia. Hence, an increase in the neutrophil count was found to be a common presentation in Stage 3 disease. About 14.7% of cases (out of 1000 cases) showed thrombocytopenia. There was no significant difference in platelet count between groups.

Table 1: Mean blood cell counts in different stages of coronavirus

	Stage 1	Stage 2	Stage 3
Hemoglobin (g/dL)	13.2	12.2	11.8
Total WBC count (10^3 cells/mL)*	6905	9953	10,036
Differential neutrophil count (%)	67	79	84
Differential lymphocyte count (%)	26	15	11
Differential monocyte count (%)	4	3	2
Platelet count (10^3 cells/mL)*	238,400	238,500	223,700

WBC=White blood cell, *Normal WBC count- ($4*10^3$-$11*10^3$/mL)

The morphological changes of the blood cells and their presentations in various disease stages are discussed in Table 2. The presence of neutrophilia with toxic changes was more prevalent in Stage 3 disease demonstrating that an increase in neutrophil count and the occurrence of toxic changes in neutrophils is an important finding in disease progression [Figure 1]. Activated lymphocytes and monocytoid lymphocytes were common in all stages signifying it as a common morphological parameter seen in all COVID patients irrespective of the stage of the disease [Figure 2a]. Whereas the occurrence of plasmacytoid lymphocytes was more common in Stage 3 disease [Figure 2b].

Monocyte cytoplasmic vacuolation [Figure 3] and giant platelets [Figure 4] were other morphological findings observed in the peripheral smear but there was no significant correlation between these changes and the disease stage

A graph representing variation in leukocyte count and morphology in different clinical stages of COVID-19 is described in Figures 5a and b.

Comparison of peripheral smear changes with coagulation parameters (prothrombin time, activated partial thromboplastin time, and D-dimer)

The coagulation parameters of patients taken were all measured before the start of an anticoagulant to exclude

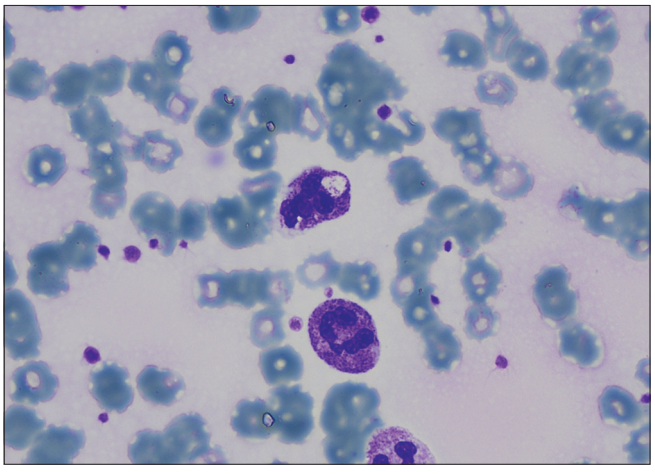

Figure 1: Neutrophil toxic change - Neutrophils exihibiting toxic granules (coarse purplish granules) and vacuoles in the cytoplasm – ×100 Leishman

Table 2: Morphological changes of blood cells in different clinical stages of coronavirus disease

Morphological presentation	Mild stage 1 (out of 789 cases) (%)	Moderate stage 2 (out of 163 cases) (%)	Severe stage 3 (out of 48 cases) (%)
Neutrophil toxic changes	47	73	80
Neutrophil shift to left	1.5	2.5	2.5
Activated lymphocytes	60	69	68
Monocytoid lymphocytes	54	61.4	57
Plasmacytoid lymphocytes	10.6	15.4	20.4
Monocyte-cytoplasmic vacuoles	15.7	8.6	12.2
Large/giant platelets	40	42	53

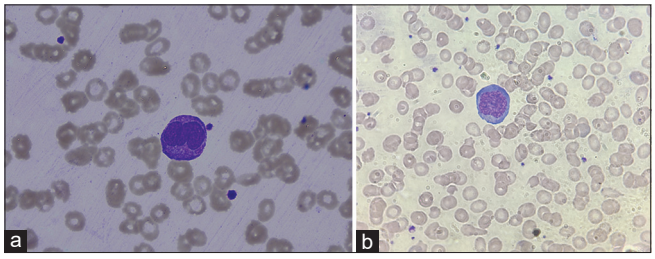

Figure 2: Activated lymphocyte - (a) Monocytoid lymphocyte with lobated nucleus and cytoplasmic granules - ×100 Leishman (b) plasmacytoid lymphocyte – with deep basophilic cytoplasm and peripherally placed round nucleus with perinuclear clearing resembling a plasma cell-×100 Leishman

false-positive cases. The mean PT, APTT, and D-dimer values observed in different stages of the disease are discussed in Table 3. There was an increased mean PT, APTT, and D-dimer values in Stage 3 disease. Although there were deranged coagulation parameters in these patients, they did not present with bleeding or thrombosis.

Peripheral smear findings of patients with prolonged prothrombin time

About 3.5% of cases in the study presented with prolonged PT. Sixty percentage of these cases belonged to Stage 2 and Stage 3 and 40% belonged to Stage 1. The peripheral smear findings of the patients with prolonged PT are discussed in Table 4.

Peripheral smear findings of patients with prolonged activated partial thromboplastin time

About 3.3% of cases in the study presented with prolonged APTT. Fifty-five percentage of these cases belonged to Stage 2 and Stage 3 and 45% belonged to Stage 1. The peripheral smear findings of the patients with prolonged APTT are discussed in Table 4.

Peripheral smear findings of patients with elevated D-dimer

About 19.5% of cases in the study presented with elevated D-dimer values. Fifty-five percentage of these cases belonged to Stage 2 and Stage 3 and 45% belonged to Stage 1. The peripheral smear findings of the patients with elevated D-dimer values are discussed in Table 4.

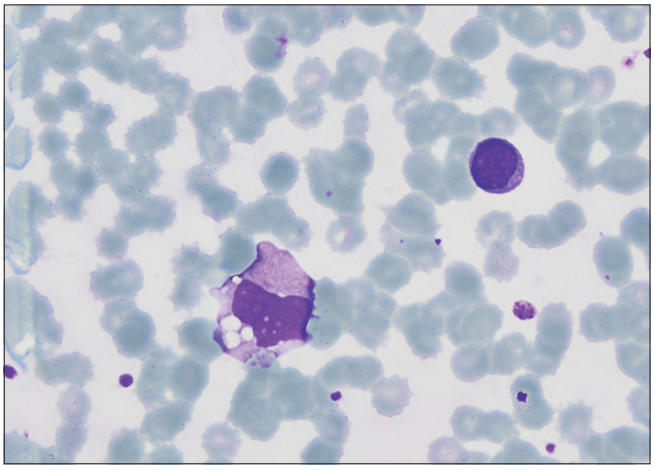

Figure 3: Monocyte exhibiting cytoplasmic vacuoles – ×100 Leishman

Peripheral smear findings of patients with prolonged prothrombin time, activated partial thromboplastin time, and elevated D-dimer values

1% of COVID patients in our study presented with derangement of all coagulation parameters (PT, APTT, and D-dimer). The peripheral smear of these patients showed neutrophilia, toxic changes in neutrophils, and activated lymphocytes as the most common presentation.

Discussion

The study demonstrates numerical and morphological changes in the blood cells in COVID-positive patients and significant variation in these parameters between different stages of the disease.

There was a significant variation in WBC counts between Stages 1 and 2 and between Stages 1 and 3. This was in correlation with the study by Pakos *et al*. who described an increase in WBC count as a significant factor to suggest disease progression.[12] Unlike other viral infections which are usually associated with lymphocytosis, COVID-19 was found to be associated with neutrophilia and lymphopenia in the severe stage. Neutrophilia can be attributed to secondary infections or intensive care unit-related infections in severe-stage disease. The lymphopenia can be a result of viral-induced apoptosis. Similarly, in the study, an increase in neutrophil count

and associated lymphopenia were noted in the severe stage of the disease which also was in correlation with the study by Lee *et al*.[9]

Viral infections usually produce activation of lymphocytes with respective morphological changes in the smear. The most common morphological change observed in many viral infections is activated lymphocytes. While some viral infections such as HIV does not produce any significant morphological changes, the patients with COVID-19 demonstrated activated lymphocytes which were seen in most of the patients irrespective of the disease stage, this was in correlation with a study by Lüke *et al*.[13] Lee *et al*. report a lower incidence of plasmacytoid lymphocytes when compared to monocytoid lymphocytes which were in correlation with this study.[14] However, the study showed an increased incidence of plasmacytoid lymphocytes in Stage 3 disease which was also in correlation with a study by Lee *et al*.[9]

The neutrophils showed significant morphological changes. The presence of toxic changes in the cytoplasm of neutrophils was found in a higher percentage in Stage 3 disease, which was in correlation with the study by Pozdnyakova *et al*.[15] The toxic changes in neutrophils are usually observed in bacterial infection. The toxic changes seen in COVID patients in Stage 3 disease can be attributed to secondary or superadded bacterial infection and sepsis seen in severe stage disease. Pozdnyakova *et al*. described a higher percentage of the shift to the left of neutrophils in Stage 3 disease, whereas in our study, the percentage of occurrence was almost equal in all stages. Pseudo Pelger–Huet anomaly and hypogranulation were described as a common morphological change in neutrophils in a study by Berber *et al*. but in the study, these changes were found only in insignificant numbers.[16]

Despite the significant role played by monocytes in sustaining a hyperinflammatory response, it is observed that not all patients with COVID-19 display morphological changes in monocytes. The morphological changes observed in monocytes in the study were cytoplasmic vacuolations and granulations. These changes were also described by Singh *et al*.[8] However, in the study, there was no correlation between the monocyte morphology and disease stage, but a study by Berber *et al*. described the occurrence of monocyte cytoplasmic vacuoles in higher percentages in the severe stage of the disease.

Thrombocytopenia usually observed in viral infections such as dengue was also observed in COVID patients but not in significant numbers. Thrombocytopenia was observed in 14.5% of cases in the study but there was no significant correlation between platelet count and disease stage. A study by Larsen *et al*. described mild thrombocytopenia as a common presentation in Stage

Table 3: Mean value of coagulation parameters in different clinical stages of COVID-19 disease

	Stage 1	Stage 2	Stage 3
PT (s)	13	13	16
APTT (s)	30	30	33
D-dimer (mg/L)	1	1.5	2.0

PT=Prothrombin time; APTT=Activated partial thromoboplastin time

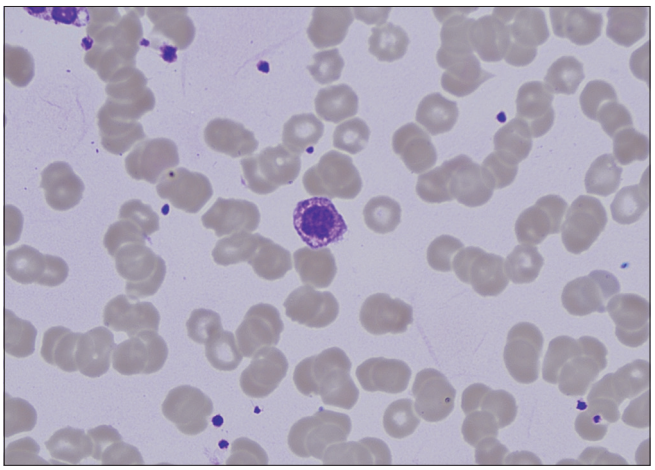

Figure 4: Giant platelet – platelet almost the size of an RBC-×100 Leishman. RBC = Red blood cells

Table 4: Morphological changes of blood cells in patients with varied derangement in coagulation parameters

Peripheral smear findings	Percentage of occurrence in patients with		
	Prolonged PT (%)	Prolonged APTT (%)	Elevated D-dimer (%)
Leukocytosis	42	31	21
Neutrophilia	71	71	50
Toxic changes in neutrophils	60	50	61
Shift to left of neutrophils	15	15	9
Activated lymphocytes	71	60	68
Monocytoid lymphocytes	57	50	61
Plasmacytoid lymphocytes	17	15	15
Monocyte cytoplasmic vacuoles	11	18	13
Thrombocytopenia	22	15	43
Giant/large platelets	42	46	50

PT=Prothrombin time; APTT=Activated partial thromoboplastin time

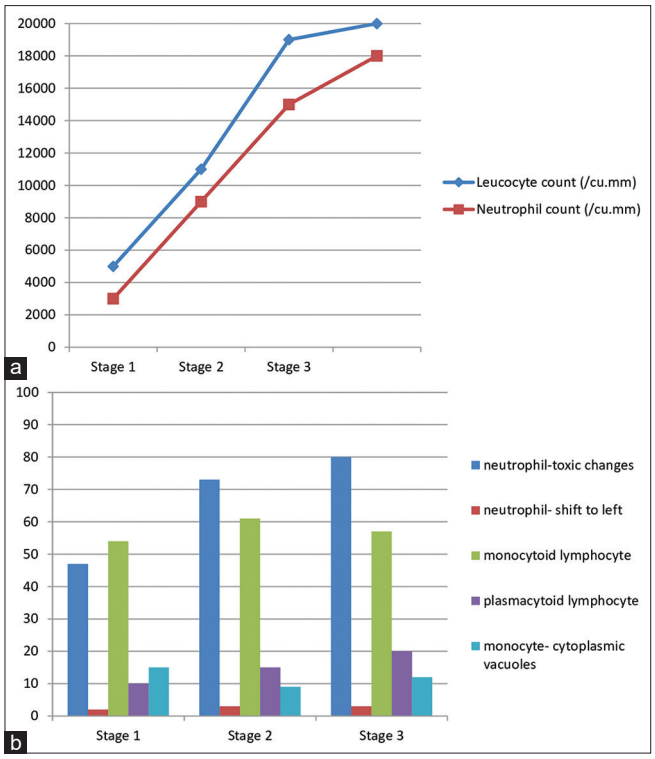

Figure 5: (a) Histogram representing the variation in leukocyte count and absolute neutrophil count in different stages of COVID-19 (b) Morphological changes of leukocytes in various clinical stages of COVID-19

3 disease.[17] Large/giant platelets are usually seen as a bone marrow response to thrombocytopenia. The large/giant platelets were found in 50% of patients in the study which was in correlation with a study by Ahnach *et al*.[18]

Only limited studies have compared coagulation parameters with the peripheral smear findings. A study by Agbuduwe *et al*. suggested toxic changes in neutrophils as the common morphological findings in patients with COVID-associated coagulopathy which can be attributed to the sepsis-related complications occurring in Stage 3 disease.[19] This was in correlation with the study which showed neutrophils with toxic changes and also activated lymphocytes as the most common presentation.

The most common finding, the presence of schistocytes (fragmented RBCs) seen in any coagulopathy, was present only in an insignificant percentage in COVID coagulopathy. Schistocytes are usually associated with microangiopathic hemolytic anemia, the lower incidence of schistocytes in COVID can be attributed to the fact that the underlying mechanism for coagulopathy is multifactorial and variable. The patients with deranged coagulation parameters in our study did not show any significant association with the presence of schistocytes and only 1% of these cases showed schistocytes [Figure 6].

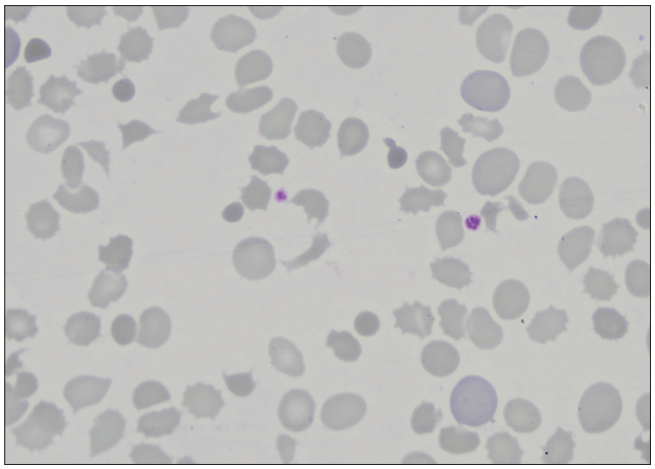

Figure 6: Schistocytes (fragmented RBCs) and microsherocytes in a patient with COVID-associated coagulopathy – ×100 Leishman. RBS = Red blood cells

Conclusions

The study demonstrated significant morphological changes in the blood cells of COVID-positive patients during the course of disease progression. Comprehensive daily complete blood count and peripheral smear examination should be undertaken in patients hospitalized with COVID-19 to predict potential clinical deterioration and signs of disease progression. Blood cell counts such as thrombocytopenia even though is not a common finding but when identified should be treated promptly. Occurrence of leukocytosis with neutrophilia, toxic changes in the neutrophils, and plasmacytoid lymphocytes can alert the clinicians indicating a deterioration of the clinical condition. These morphological changes in peripheral smear can be used as one of the factors indicating disease progression which can formulate for further evaluation of the patient. Since follow-up and post-COVID morphological examination were not done, additional research in this aspect can shed light on the clinical categorization of COVID patients based on the morphological findings.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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