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

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Research Article

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Abstract

A novel highly pathogenic human corona virus (COVID19) has been recently recognised 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. In our study we have categorized the covid positive patients into mild, moderate and severe based on the clinical criteria suggested by WHO. The coagulation parameters of the patients were analysed and documented. A peripheral smear was made for every patient and the morphological changes in blood cells were documented. The peripheral smear findings were then correlated with the disease stage and coagulation parameters. There were significant differences in the total WBC count and the differential WBC count between stages 1 &2 and stages 1 & 3 ($p<0.005$). Leucocytosis, neutrophilia and toxic changes in neutrophils were seen in severe stage of the disease and in covid coagulopathy suggesting these are important indicators of disease severity. Schistocytes an important finding in any other coagulopathy was not present in covid associated coagulopathy. Activated lymphocytes was found to be the most common morphological presentation seen in all covid patients irrespective of the disease stage whereas plasmacytoid lymphocytes was an important finding in severe stage disease. Monocyte cytoplasmic vacuoles, large/giant platelets were other morphological findings observed but these findings did not have any significant correlation with disease stage. Since follow up smears of the same patient were not analysed during disease progression and also post recovery, additional research in this field will provide further insights.

Introduction

A novel highly pathogenic human corona virus (COVID19) 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 an alarming morbidity and mortality occurring as a result of acute respiratory distress syndrome [1].

Covid 19, emerged in Wuhan China in 2019. The disease is caused by coronavirus which belongs to a group of RNA viruses that causes disease in mammals and birds. It belongs to the family coronaviridae in the order Nidovirales. They are positively charged RNA virus with the largest genome [2].

The mode of spread of this virus is by droplet infection and close contact[3]. The pathogenesis of Covid 19 seems to be a little complicated. The virus enters through the respiratory tract and attaches to the epithelial cells, the macrophages and the dendritic cells causing their activation. Their activation results in the activation of innate immune system and release of a variety of cytokines and chemokines.This leads to a dysregulated immune response activation resulting in a prothrombotic state[3].The prothrombotic states results in formation of microclots along the microvasculature. D dimer, a degradation product of fibrin (FDP) is increased in the peripheral blood of the Covid positive patients supporting the thrombotic state [4].Thus as a result the coagulation system and the hemostasis is deranged leading to abnormalities in the coagulation studies [5-7].

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 Ebstein Barr virus infection produces atypical lymphocytes whereas Dengue fever caused by Flaviviridae produces many activated plasmacytoid & monocytoid lymphocytes. Certain viral infections like HIV may not cause any significant morphological changes.

In a study by Aminder Singh the various morphological changes of RBC, WBC and Platelets in Covid 19 positive patients have been described and correlated with clinical findings [8].They have described that morphological changes in monocytes played a significant role in clinical presentation. In a study by Chun Tsu Lee the various morphological changes of lymphocytes in Covid 19 positive patients have been described [9].

Our 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 WHO[10, 11]. The various coagulation parameters of the patients like PT, APTT, D dimer and platelet count were estimated and documented simultaneously. Peripheral smears of the patients stained with Leishman stain were analysed to look for morphological changes. The peripheral smear findings were then correlated with the clinical stage of the disease and coagulation parameters. An analysis was done to identify whether the morphological changes in peripheral smears can be used as a parameter to indicate disease progression.

Materials And Methods

Study design and population

The study was planned as a prospective, multicenter study. A total of thousand Covid positive patients admitted in the tertiary care centre during the study period were taken for the study.

Inclusion criteria

All Covid RT PCR positive patients were taken into the study irrespective of the age, sex and pre existing health status.

Exclusion criteria

RT PCR negative patients were excluded from the study irrespective of the CT chest findings.

Data collection

The patients were classified as mild, moderate & severe based on the clinical criteria suggested by the WHO and the findings were documented.

The coagulation parameters like prothrombin time(PT), activated partial thromboplastin time (aPTT), D-dimer of the patients were analysed in blood collected in blue top vacutainers (3.2% sodium citrate). The

preanalytical procedures were strictly adhered to and the samples were run within two hours of collection in IL-ACL top semi-automated coagulation analyser. The values were documented simultaneously.

The EDTA sample of the Covid patients were used to prepare the peripheral smears. The smears were stained using Leishman stain following the standard operating protocol. The stained smears were then analysed by a trained pathologist.

Morphological changes in various blood cells were analysed and documented. Morphological changes in neutrophils included toxic changes and shift to the left. Toxic change is defined as 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 like cytoplasmic vacuolations were documented. Presence of large/giant platelets were looked for and documented. Giant platelets are those platelets which are almost the size of RBC where as large platelets are somewhat smaller in size when compared to RBC's. Other parameters like blood hemoglobin level, WBC count(total count & differential count)and the platelet count were also documented.

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, dyspnoea, fast breathing) but no signs of severe pneumonia, including SpO2 ≥ 90% on room air

Severe:

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

DATA ANALYSIS

The peripheral smear findings documented were then correlated with the coagulation parameters and the disease severity. The data was analyzed using SPSS software. 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 cut-off point for differential morphological pattern between mild, moderate and severe disease stage.

Results

Thousand patients of Covid 19 admitted during the study period were taken into study. The patients were classified as mild(stage 1) moderate(stage 2)& 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 category.

Comparison of Peripheral blood counts and Clinical Stage

The clinical stage of the patient was correlated with total WBC count and differential count. The variation of total WBC count , differential neutrophil count, differential lymphocyte count and differential monocyte count between stages 1&2 and stages 1&3 was found to be significant ($p<0.005$). Whereas the correlation between other groups were not significant($p>0.005$).

The mean blood count values in different clinical stage of the disease is 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.

Totally 12% of cases (out of 1000 cases) showed leucocytosis. 8% 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 leucocytosis. Thus emphasizing the fact that an increase in WBC count was seen in the course of disease progression.

35% of cases (out of 1000 cases) showed neutrophilia. 25% cases in stage 1(out of 789 cases), 72% cases in stage 2 (out of 163 cases) & 89% (out of 48 cases) cases in stage 3 showed neutrophilia. Thus an increase in neutrophil count serves as a significant factor in determining disease progression.

14.7% of cases (out of 1000 cases) showed thrombocytopenia. There was no significant difference in platelet count between groups.

Comparison of Peripheral smear morphological changes and Clinical Stage

The morphological changes of the blood cells and their presentations in various disease stages are discussed in Table 2. From the findings it has been observed that presence of neutrophilia with toxic changes were more prevalent in stage 3 disease thus emphasizing the fact that an increase in neutrophil count and occurrence of toxic changes in neutrophils is an important indicator for disease progression[Fig. 1]. Activated lymphocytes and monocytoid lymphocytes were common in all stages signifying the fact that this is a common morphological parameter seen in all covid patients irrespective of the stage of the disease[Fig. 2]. Whereas occurrence of plasmacytoid lymphocytes was more common

in stage 3 disease emphasizing the fact this morphological finding can also be a factor indicating disease progression [Fig. 2].

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

Comparison of peripheral smear blood counts , morphological changes with coagulation parameters (PT, APTT & D dimer)

The mean prothrombin time, activated partial thromoboplastin time and D dimer values 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.

(i) Prolonged PT & peripheral smear findings

3.5% of cases in the study presented with prolonged PT. 60% 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 2

(ii) Prolonged APTT & peripheral smear findings

3.3% of cases in the study presented with prolonged APTT. 55% 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 2

(ii) Elevated D dimer & peripheral smear findings

19.5% of cases in the study presented with elevated D dimer values. 55% 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

(iv) Prolonged PT, APTT , elevated D dimer values & peripheral smear findings

1% of covid patients in our study presented with derangement of all coagulation parameters(PT, APTT & D dimer). The peripheral smear findings of these patients are described in table 5

The peripheral smear findings of patients with deranged coagulation parameters (prolonged PT , APTT & elevated Dimer) showed neutrophilia, toxic changes in neutrophils & activated lymphocytes as the most common presentation. Therefore presence or development of these morphological parameters in covid positive patients may suggest that the patient is progressing into a state of coagulopathy.

Discussion

Our study demonstrates significant numerical and morphological changes in the blood cells in Covid positive patients.It shows that there is significant variation in these parameters between different stages of the diseases.

Our study showed significant variation in WBC counts between stages 1 &2 and between stages 1&3. This was in correlation with the study by Irene S Pakos et al who described an increase in WBC count as a significant factor to suggest disease progression[12]. Similarly an increase in neutrophil count and associated lymphopenia were noted in severe stage of the disease which also was in correlation with the study by Chen H et al[13].

There was significant changes in the morphology of WBC seen in the peripheral smear. The most common morphological change observed in our study is activated lymphocytes which was seen in all Covid 19 patients irrespective of the disease stage, this was in correlation with a study by Florian Luke et al[14]. Yue Ping Lee et al reports lower incidence of plasmacytoid lymphocytes when compared to monocytoid lymphocytes which was in correlation with our study[15]. However our study showed increased incidence of plasmacytoid lymphocytes in stage 3 disease which was also in correlation with a study by Chun Tsu lee[9].

The neutrophils also showed significant morphological changes. The presence of toxic changes in the cytoplasm of neutrophils was found in higher percentage in stage 3 disease, which was in correlation with the study by Olga Pozdnyalova et al[16]. However Pozdnyalova et al described a higher percentage of shift to 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 Ilhami Berber et al but in our study these changes were found only in insignificant numbers [17].

The morphological changes observed in monocytes in our study were cytoplasmic vacuolations and granulations. These changes were also described by Amindersingh et al[1]. However in our study there was no correlation between the monocyte morphology and disease stage but a study by Ilhami Berber et al described the occurrence of monocyte cytoplasmic vacuoles in higher percentage in severe disease.

Thrombocytopenia was observed in 14.5% of cases in our study but there was no significant correlation between platelet count and disease stage. A study by Julie Brogaard Larsen et al described mild thrombocytopenia as a common presentation in stage 3 disease [18]. Large /giant platelets were found in 50% of patients in our study which was in correlation with a study by Maryame Abnach et al [19].

Only limited studies have compared coagulation parameters with the peripheral smear findings. A study by Charles et al suggested toxic changes in neutrophils as the common morphological findings in patients with Covid associated coagulopathy [20].This was in correlation with our study which showed neutrophils with toxic changes and also activated lymphocytes as the most common presentation.

The most common finding, presence of schistocytes (fragmented RBC's) seen in any other coagulopathy was almost absent in Covid coagulopathy. The patients with deranged coagulation parameters in our study also did not show any significant association with presence of schistocytes [Fig.5].

This study has some limitations. Even though the study population was high (1000 patients) the percentage of patients in stage 3 severe disease was comparatively low (5%). Second, follow up smears of the same patient was not analysed during disease progression and also post recovery. So any occurrence or disappearance of the preexsisting morphological changes were not studied.

Conclusion

Our study demonstrated significant morphological changes in blood cells during the course of disease progression. Since other investigations like coagulation parameters and CT evaluation are expensive, the morphological changes of blood cells in peripheral smear can be used as an early indicator of disease progression to suggest further evaluation of the patient. Since follow up and post covid morphological examination were not done further studies in this aspect can give a clear criteria for clinical categorization of Covid patients based on the morphological findings in peripheral smear.

Declarations

Funding

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Conflicts of interest/Competing interests

The authors declare no conflict of interest.

Variability of data and material

Not applicable

Code availability

Not applicable

Authors' contributions

Concept and design- T M Karthikeyan, Data collection, Data collection monitoring Data interpretation, preparation and maintenance of master chart- Abinaya Sundari A, Laboratory report interpretation – Shivapriya , Veennaa

Ethics approval

Our study was supported with the approval of the Institutional Human Ethics Committee, KMCH Institution Of Health Sciences And Research

Consent to participate

Not applicable

Consent for publication

Consent was obtained from the Institutional Human Ethics Committee, KMCH Institution Of Health Sciences And Research

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Tables

Table 1 Mean blood cell counts in different stages of Covid 19

	STAGE 1	STAGE 2	STAGE 3
Hemoglobin (g/dl)	13.2	12.2	11.8
Total WBC count (*10^3cells/microlitre)	6905	9953	10036
Differential Neutrophil count (%)	67	79	84
Differential lymphocyte count (%)	26	15	11
Differential monocyte count(%)	4	3	2
Platelet count (*10^3cells/microlitre)	238400	238500	223700

Table 2 Morphological changes of blood cells in different clinical stages of Covid 19 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 vaculoes	15.7%	8.6%	12.2%
Large/giant platelets	40%	42%	53%

Table 3 Mean value of coagulation parameters in different clinical stages of Covid 19.

	STAGE 1	STAGE 2	STAGE 3
PT (in seconds)	13	13	16
APTT (in seconds)	30	30	33
D DIMER	1	1.5	2.0

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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
Leucocytosis	42%	31%	21%
Neutrophilia	71%	71%	50%
Toxic changes in neutrophils	60%	50%	61%
Shif 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%

Table 5 Morphological changes of blood cells in patients with Covid associated coagulopathy

PERIPHERAL SMEAR FINDINGS IN PATIENTS WITH DERANGED COAGULATION PARAMETERS (prolonged PT, prolonged APTT & elevated D dimer)	
Leucocytosis	66%
Neutrophilia	88%
Toxic changes in neutrophils	77%
Shif to left of neutrophils	22%
Activated lymphocytes	77%
Monocytoid lymphocytes	55%
Plasmacytoid lymphocytes	30%
Monocyte cytoplasmic vacuoles	33%
Thrombocytopenia	55%
Giant/large platelets	44%

Figures

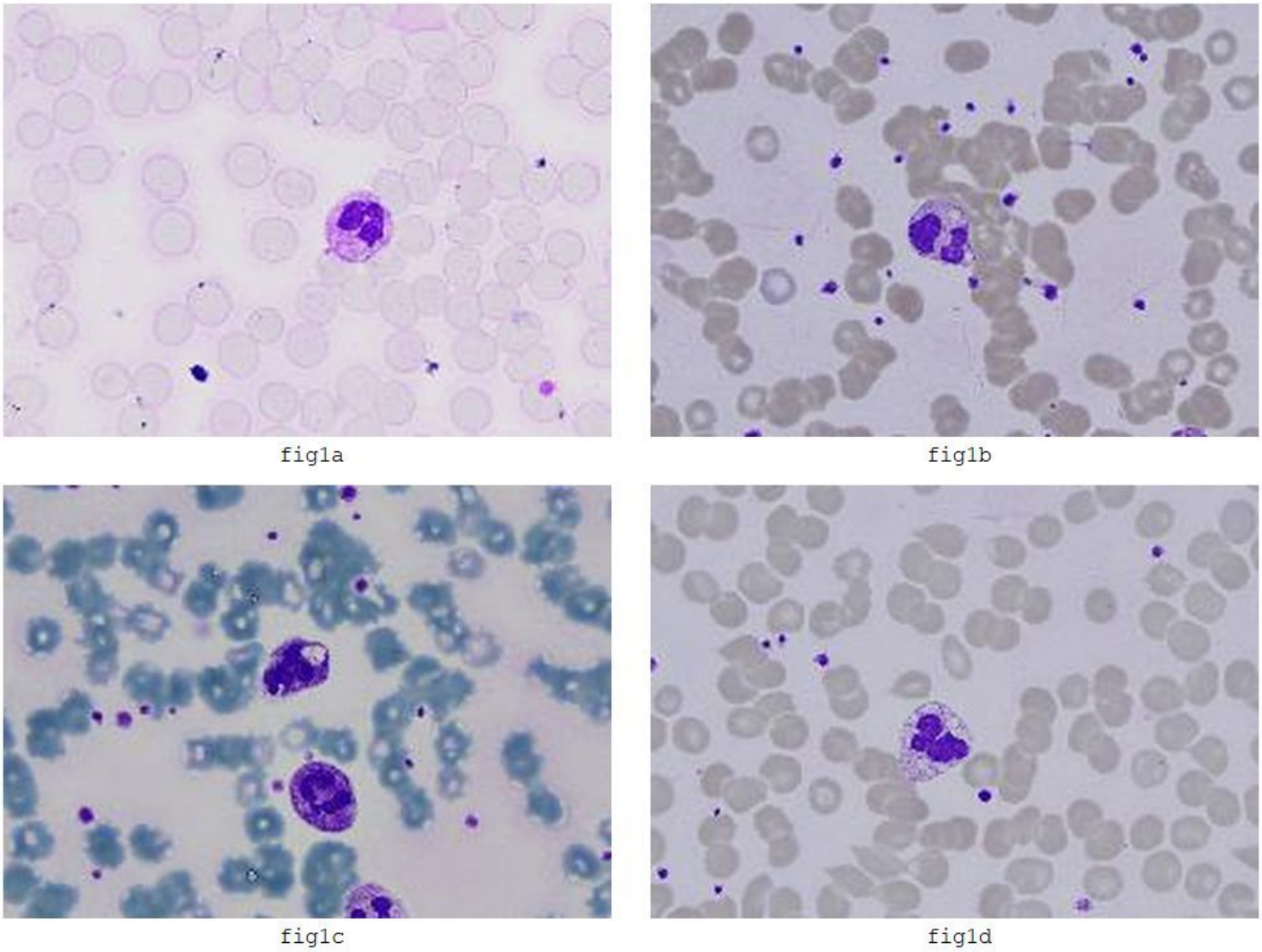

Figure 1

Neutrophils exihibiting toxic granules and cytoplasmic vacuoles

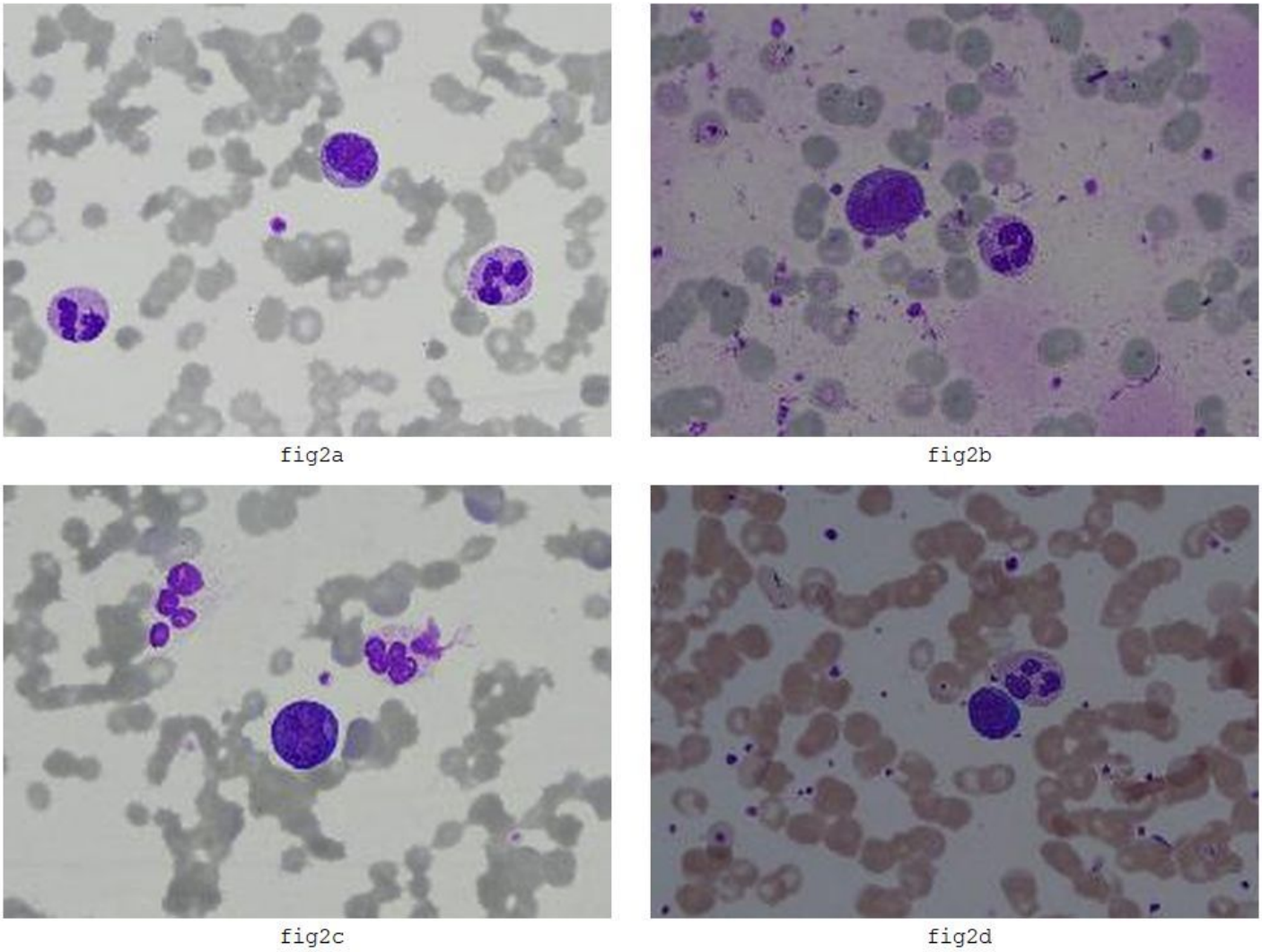

Figure 2

(a) Large granular lymphocyte (b) Monocytoid lymphocyte (c & d) Plasmacytoid lymphocyte

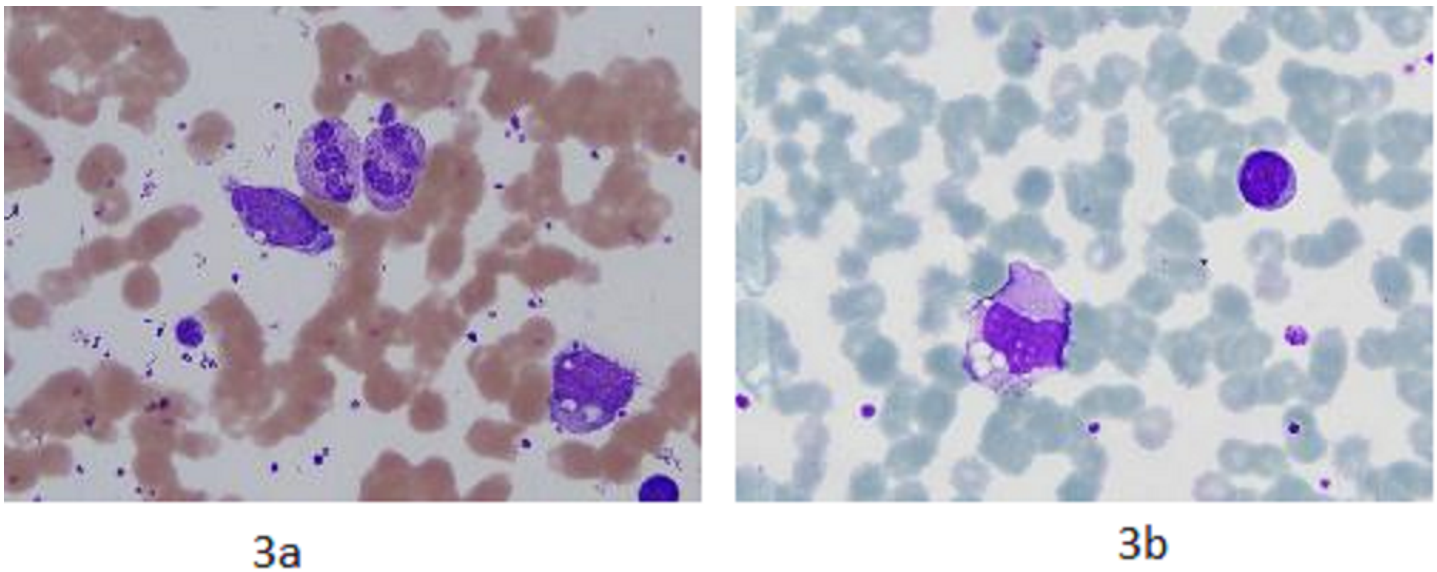

Figure 3

Monocytes with cytoplasmic vacuoles

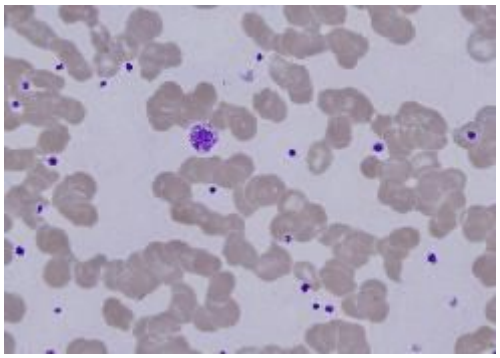

Figure 4

Giant platelet

Figure 5

Schistocytes and microsherocytes in a patient with covid associated coagulopathy