Received: 22 June 2021 | Revised: 19 August 2021 | Accepted: 9 September 2021

DOI: 10.1111/ijlh.13720

ISLH International Journal of Laboratory Hematology WILEY

ORIGINAL ARTICLE

Changes in peripheral blood in SARS CoV-2 patients and its clinico-pathological correlation: A prospective cross-sectional study

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Abstract

Introduction: Coronavirus disease 2019 (COVID-19) caused by SARS-CoV2 can present from mild flu-like symptoms to acute respiratory distress syndrome. There is multi-organ involvement; particularly, hematopoietic system can be associated with morphological changes in blood cells of COVID-19 patients.

Method: We conducted a cross-sectional study on a cohort of 50 COVID-19 patients, confirmed on RT-PCR with documented cycle threshold (Ct) value. Peripheral blood sample of these patients was collected and examined for complete blood counts (CBC) on automated haematological analyser as well as Leishman-stained blood smears to look for morphological changes in blood cells. Morphological changes were evaluated with reference to clinical severity and Ct value. Additionally, association between Ct value and clinical severity was also performed. Statistical tests were performed, and *P* value <.05 was considered significant.

Results: Mean age of our study group was 42.16 ± 15.55 years, with male preponderance. Most commonly observed peripheral blood changes were hypolobation (*P* value = .002) and toxic granules (*P* value = .005) in neutrophils, atypical granules with nucleolar prominence in lymphocytes, cytoplasmic granulation with clumped nuclear chromatin in monocytes, giant platelets and thrombocytopenia and normocytic normochromic anaemia.

Conclusion: No association was found between clinical severity and Ct value as well as peripheral blood morphological changes with Ct value. We conclude that examination of peripheral smear coupled with complete blood count (CBC) is only partially supportive of disease pathogenesis and to assess the viral load other parameters should be utilised instead of relying solely on Ct value.

KEYWORDS

clinical severity, COVID-19, cycle threshold value, morphological changes, peripheral blood smear

1 | INTRODUCTION

In December 2019, a cluster of cases of pneumonia of unknown cause was detected in Wuhan, capital of Hubei province in China.[1] It gained pandemic proportions within a span of few weeks and was attributed to an unknown virus genetically related to the corona virus that had resulted in the "severe acute respiratory syndrome" (SARS) outbreak in 2003. By February 2020, this novel virus was termed SARS-CoV-2 or Corona virus disease 2019 (COVID-19) by World Health Organization (WHO).[1,2]

At the outset of the novel corona virus outbreak, the challenge of containment of infection was paramount with efforts to enhance survival and ameliorate morbidity being the mainstay of therapy utilising drugs that limited viral replication or modified immune responses[3,4] The clinical course of COVID-19 varied. Patients experienced mild symptoms to severe acute respiratory distress syndrome, altered coagulation profiles resulting in coagulation disorders and hyperinflammation.[5] With multi-organ involvement particularly hematopoietic and immune system being affected, a variety of haematological findings were observed in patient blood samples. Neutrophilia, eosinopenia, lymphopenia, thrombocytopenia or thrombocytosis were commonly encountered.[6-9] Morphological alterations in granulocytes, agranulocytes and platelets have been reported via isolated case reports and observations in peripheral blood smear (PBS).[10-13]

Present study was undertaken to assess changes in peripheral blood of confirmed COVID-19 patients vis a vis Ct values and clinical severity.

2 | MATERIAL AND METHODS

A prospective cross-sectional study was undertaken at Government Institute of Medical Sciences, Greater Noida, on COVID-19 patients based on haematological changes encountered. 50 consecutive patients with confirmed COVID-19 infection following positive RT-PCR test were enrolled to the study after taking necessary approvals from the Institutional Ethics Committee.

RT-PCR was conducted on nasopharyngeal and oropharyngeal swab collected by a trained technician and cycle threshold (Ct) value noted. Ct value denotes the number of amplification cycles required for the specific targeted gene to exceed a given threshold level; hence, it is an indirect method to quantify the number of viral RNA present in a given sample, and thus it is presumed inversely proportional to viral load.[14] RNA extraction was done by Magna Max viral RNA extraction kit on Zybio extraction system using standard protocols. The extracted RNA was taken for real-time RT-PCR in Quant Studio 5 Applied Biosystems.

RT-PCR, a gold standard test, is being used for detection of SARS-CoV2.[15,16] CoviSure Kit COVID-19 Real-Time PCR Kit (Single Tube Multiplex Assay for Qualitative Detection of Novel Coronavirus SARS Cov-2) provided by Indian Council of Medical Research (ICMR) for detection of RdRp, E gene and RNase P gene was used. Cut-off value of 36 cycles against the threshold was taken according to the kit (≤36 was considered to be positive and >36 negative).[17]

Peripheral blood samples of COVID-19 patients, before administration of antiviral and anti-inflammatory drugs, were collected in vacutainers containing ethylenediaminetetraacetic acid (K2-EDTA) as anticoagulant. To obtain complete blood counts (CBC), these samples were run on Medonic, semi-automatic three-part haematology analyser. PBS were prepared and stained by Romanowsky method using Leishman stain and studied as per recommendations of the International Council for Standardization in Haematology (ICSH)[18]

Based on symptoms and investigations, patients were categorised into mild, moderate and severe clinical category groups according to the guidelines laid by Ministry of Health and Family Welfare (MOHFW) utilising clinical severity and assessment parameters.[18] Patients with no evidence of breathlessness/hypoxia were categorised under mild clinical severity group. Patients with clinical features of dyspnoea, SpO2 < 94% on room air and respiratory rate (RR) ≥ 24/minute belonged to moderate clinical severity group, whereas severe clinical category group included patients with signs of severe pneumonia, SpO2 < 90% on room air and RR > 30/minute.[19]

2.1 | Statistical analysis

Information about demographic data, clinical signs and symptoms, CBC, morphological changes on peripheral smear and Ct values was collected. Continuous variables were expressed as mean with standard deviation. Categorical variables were expressed in percentages. Chi-square and Fischer exact tests were applied to find correlation

TABLE 1 Correlation of patient age with clinical severity

Serial. no.	Age (y.)	Mild	Moderate	Severe
1	21-30 (n = 12)	8 (66.6%)	03 (25%)	01 (8.3%)
2	31-40 (n = 15)	04 (26.6%)	11 (73.3%)	0
3	41-50 (n = 09)	02 (22.2%)	06 (66.6%)	01 (11.1%)
4	51-60 (n = 07)	01 (14.2%)	04 (57.1%)	02 (28.5%)
5	61-70 (n = 06)	0	03 (50%)	03 (50%)
6	71-80 (n = 00)	0	0	0
7	81-90 (n = 01)	0	0	01 (100%)
Total	50	15	27	08

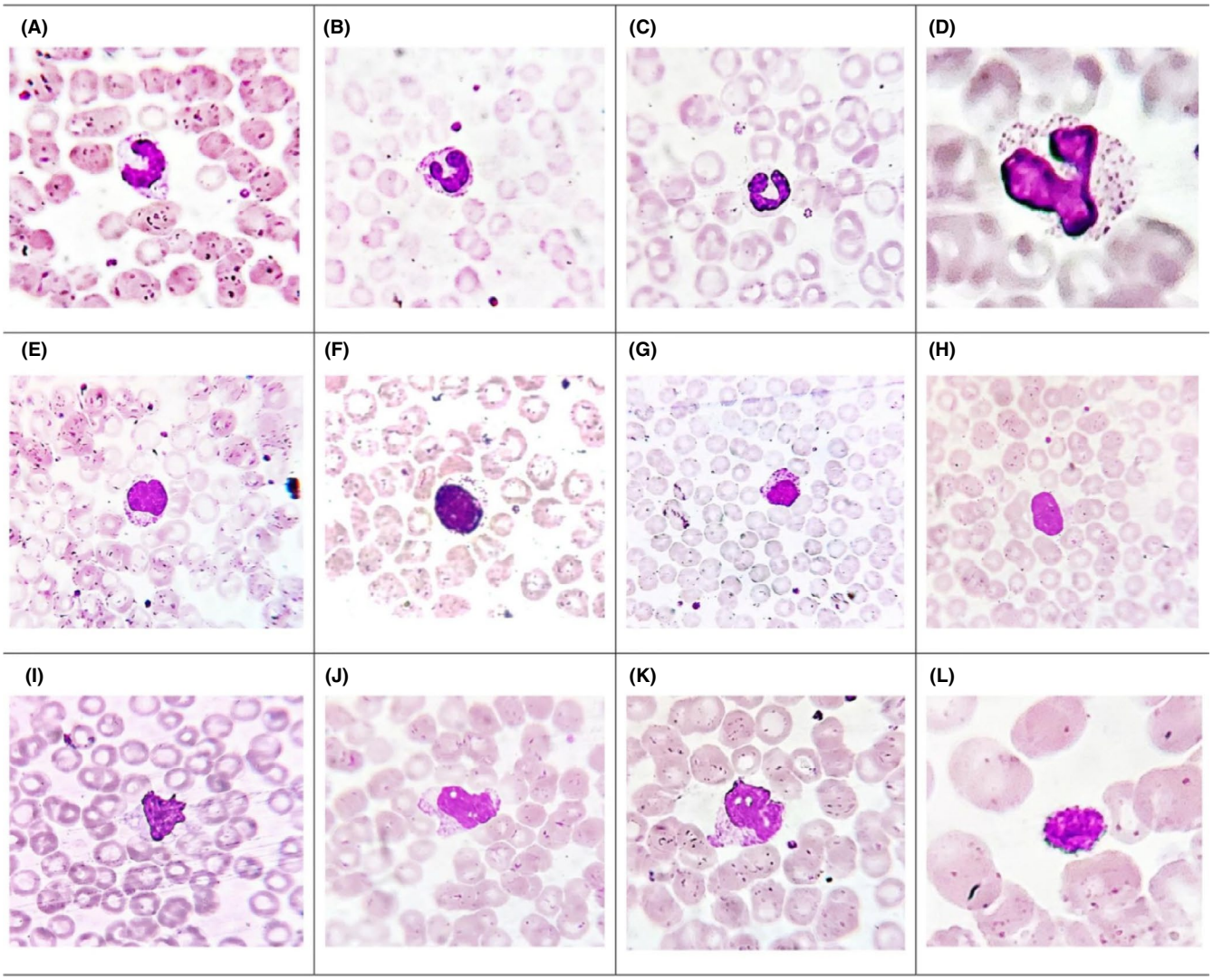

FIGURE 1 A-C, Neutrophils with hypolobated nuclei, azurophil granules and nuclear budding; Leishman stain (40×). D, Hypolobated neutrophil with toxic granules; Leishman stain (100×). E-H, Lymphocyte showing nuclear indentation, cytoplasmic granulations and hyper chromatic nuclei; Leishman stain (40×). I-K, Monocytes with clumped chromatin and nuclear vacuolation; Leishman stain, 40×). L, Giant platelet, normocytic normochromic red blood cells; Leishman stain (100×)

between morphological changes in blood cells with the severity of the COVID-19 infection as well as with the Ct value. *P* value of <.05 was taken as statistically significant. Statistical evaluation was carried out using SPSS 15 software (SPSS Inc).

3 | RESULTS

3.1 | Patient characteristics

The study group comprised 50 patients with RT-PCR-confirmed COVID-19. Sampling of all 50 was performed and analysed. The mean age was 42.16 ± 15.55 years. Majority of the patients were males (70%, n = 35).

Of the 50 patients included in the study group, maximum patients (n = 15) were aged 31 to 40 years followed by 21- to 30-year-olds (n = 12). Majority (n = 27) were categorised in the moderate clinical severity group with maximum patients belonging to age 31-40 years (73.3%), followed by 41-50 years (66.6%), 51-60 years (57.1%) and 61-70 (50%). 66.6% of patients aged 21-30 years belonged to the mild clinical severity group. 61-70-year-old patients were equally divided into moderate and severe clinical category, while single patient aged 81-90 years belonged to the severe clinical category group (Table 1).

3.2 | Alterations in complete blood counts

On hemogram, there was leucocytosis in 28% (n = 14) of the samples, with 16% (n = 08) showing neutrophilia, 22% (n = 11) lymphocytopenia, 20% (n = 10) monocytopenia and 48% (n = 24) thrombocytopenia. In 4% (n = 02) patients, thrombocytosis was encountered.

3.3 | Alterations in peripheral blood smears

Screening of PBS showed some distinct morphological alterations in red blood cells (RBC), leucocytes and platelets (Table 3). The most pronounced morphological change seen in neutrophils was hypolobation (n = 42; 84%) and toxic granulation (n = 25; 50%) with a statistically significant *P* value of .002 & .005, respectively, when compared between patients who didn't require ICU admission, ie mild clinical severity group, and patients who required ICU admission, ie moderate and severe clinical severity group [Figure 1A-D]. Azurophil granules [Figure 1E-G], clumped nuclear chromatin [Figure 1I] (12% each) and giant platelets (28%) [Figure 1L] were the most frequently encountered morphologic alterations in lymphocytes, monocytes and platelets, respectively. These findings were not statistically significant. Red blood cells were largely normocytic normochromic (76%) and not significant on statistical analysis [Table 2].

3.4 | Cyclical threshold values

In (<20) Ct value group, 66.66% of patients were of moderate severity group, followed by 16.66% of mild & severe clinical severity group each. In (<20-30) Ct value group, 52.27% of the patients were of moderate severity group, followed by mild severity group 31.81% and 15.9% of severe clinical severity group. No patient was found to have Ct value more than 30. [Table 3].

In an attempt to relate frequency of morphological changes observed in blood smear with the Ct values, no statistically significant results were obtained. [Table 4].

4 | DISCUSSION

The coronavirus disease 2019 emerged as a challenge globally not just in terms of medical therapies to counteract its onslaught but also due to its socioeconomic impact.[5] As documented, COVID-19 resulted in a markedly contagious disease caused by SARS-CoV-2.[6] It affected nearly 220 countries across the world with more than an overwhelming 3.4 million deaths thus reported. [1]

In the present study, the findings observed in CBC were lymphopenia (22%), monocytopenia (20%) and thrombocytopenia (48%) with neutrophilia (16%). These corroborated with findings reported by Singh S et al, Luke F, et al and Singh A.[5,20,21] Data by Luke F et al[5] were also in concert with present findings albeit with higher percentages of reported lympho-/mono-cytopenia, thrombocytopenia and leucocytosis. In a solitary case report by Singh A et al on morphology of COVID-19-affected cells in PBS, similar findings of neutrophilia with relative lymphocytopenia and monocytopenia have been documented.[21]

TABLE 2 Frequency of morphological changes in blood smear of COVID-19 patients

Morphological changes in blood cells	Frequency N = 50 (%)	Didn't require ICU admission Mild (n = 15)	Required ICU admission (moderate + severe) (n = 35)	*P* value
Neutrophils				
Hypolobation	42 (84%)	09	33	**.002**
Toxic granules	25 (50%)	03	22	**.005**
Nuclear projections	9 (18%)	01	08	.172
Ring nuclei	07 (14%)	01	06	.327
Lymphocytes				
Indented nuclei	04 (8%)	01	03	.820
Prominent nucleoli	05 (10%)	01	04	.607
Atypical granules/azurophilic granules	06 (12%)	01	05	.447
Cytoplasmic pods	04 (8%)	01	03	.820
Monocytes				
Vacuolizations	05 (10%)	01	04	.607
Cytoplasmic granules	04 (8%)	01	03	.820
Clumped nuclear chromatin	06 (12%)	01	05	.447
Platelets				
Platelets aggregates	20 (40%)	06	14	1.00
Giant platelets	28 (56%)	08	20	.803
Red blood cells				
Microcytic hypochromic RBC'S	5 (10%)	02	03	.607
Normocytic normochromic RBCs	38 (76%)	12	26	.664
Macrocytes	7 (14%)	01	06	.327

TABLE 3 Frequency of Ct value and clinical severity

S. no.	Ct value	Viral load	Mild (n = 15)	Moderate (n = 27)	Severe (n = 08)	*P* value
1	<20 (n = 06) (12%)	High	01 (16.66%)	04 (66.66%)	01 (16.66%)	Mild vs moderate + severe (.707)
2	>20-30 (n = 44) (88%)	Medium	14 (31.81%)	23 (52.27%)	07 (15.9%)	Moderate vs mild + severe (.506)
3	>30 (n = 00)	Low	0	0	0	Severe vs mild + moderate (.962)

TABLE 4 Frequency of morphological changes in blood smear of COVID-19 patients in relation to CT value

Morphological changes in blood cells	Frequency N = 50 (%)	Ct Value <20 (n = 06)	Ct Value >20-30 (n = 44)	*P* value
Neutrophils				
Hypolobation	42 (84%)	04	38	.216
Toxic granules	25 (50%)	04	21	.384
Nuclear projections	9 (18%)	02	07	.297
Ring nuclei	07 (14%)	02	05	.145
Lymphocytes				
Indented nuclei	04 (8%)	01	03	.404
Prominent nucleoli	05 (10%)	02	03	0.422
Atypical granules/ azurophilic granules	06 (12%)	02	04	.086
Cytoplasmic pods	04 (8%)	00	04	.589
Monocytes				
Vacuolizations	05 (10%)	02	03	.422
Cytoplasmic granules	04 (8%)	00	04	.589
Clumped nuclear chromatin	06 (12%)	01	05	.707
Platelets				
Platelets aggregates	20 (40%)	02	18	.722
Giant platelets	28 (56%)	03	25	.752
Red blood cells				
Microcytic hypochromic RBCs	5 (10%)	01	04	.561
Normocytic normochromic RBCs	45 (90%)	05	33	.653
Macrocytes	7 (14%)	00	07	.512

Evaluation of PBS of COVID-19 patients in the present study cohort revealed multi-lineage changes in practically all the cellular blood components. Changes were more pronounced and uniform in neutrophils but also observed to a lesser extent in lymphocytes, monocytes, platelets and red blood cells. Aberrant granulopoiesis with the presence of hypergranulation and hypolobated (pseudo-Pelger Huet) neutrophils was also reported by Luke F et al[5] albeit in higher percentages. Above-mentioned findings along with giant platelets are features which overlap with some haematological malignancies, namely myelodysplastic syndrome (MDS) and myelodysplastic/myeloproliferative syndrome (MDS/MPN), which warrant caution when encountered in PBS.[22] Singh A et al have reported certain findings in neutrophils such as clumped chromatin, toxic granules, cytoplasmic vacuoles, c-shaped (hypolobated/foetus-like) nuclei and ring-shaped nuclei; features common to the present study were the presence of granular lymphocytes with indented nuclei and prominent nucleoli, however in fewer proportions of cases. "Aberrant lymphocytes" with multi-lobulated nuclei, plasmacytoid appearance, enlarged lymphocytes with cytoplasmic basophilia and apoptosis were not encountered in patient's smears evaluated as reported by Luke F et al[5] Viral infections namely infectious mononucleosis and dengue fever are known to present with reactive lymphocyte morphology and should be kept on radar while screening smears.[23,24] Monocytes with vacuolation (10%), cytoplasmic granulation (8%) and clumped chromatin (12%) were reported in very few subjects of present cohort compared to 91% "aberrant monocyte" morphology reported by Luke F et al.[5]

Ct values signify the number of amplification cycles mandatory to achieve threshold level by a target gene. Role of Ct value in reflecting viral load or ability to relate to the clinical severity has garnered support as well as opposition by many researchers who opine that Ct value for any given specimen is subject to variability on a number of counts, namely kits used, techniques utilised, method of sample collection as well as timing of sample collection from onset of symptoms. It has also been documented that Ct values have been known to fluctuate for the same sample between different runs.[25-28] Low Ct values are generally indicative of higher viral loads; however, it needs to be kept in mind that log viral load and Ct value may not necessarily be directly proportional due to assays linear dynamic range and presence within the clinical sample itself of inhibitory factors.[25] Rao SN et al in a systematic review documented that eight of eleven studies reporting correlation between Ct value (on viral load) and disease severity found an association; six of these were statistically significant.[25] This feature was not seen in the present study. An association of lower Ct value with reduced lymphocytes and raised neutrophil count has also been reported which was not reflected in findings of the present study.[29-31]

5 | LIMITATIONS

Absence of control group and small sample size were the limitation in our study. Further studies with follow-up of the COVID-19-recovered patients for the next 3-6 months are advisable to validate persistence or resolution of the morphological changes in the blood picture.

6 | CONCLUSION

In the present study, hypolobation and toxic granules in neutrophils were found statistically significant with clinical severity. Atypical granules with nucleolar prominence in lymphocytes, cytoplasmic granulation with clumped nuclear chromatin in monocytes, giant platelets and thrombocytopenia in platelets and normocytic normochromic anaemia in red blood cells were observed in PBS of COVID-19 patients. These findings can be confused with viral infections (eg infectious mononucleosis, dengue) and haematological malignancies (eg MDS, MDS/MPN); hence, one needs to be more vigilant. Occurrence of morphologic changes in multilineage cells in peripheral blood possibly raises questions regarding involvement of haematopoietic progenitor cell in COVID-19 pathogenesis and thus warrants a systematic long-term follow-up.

No association of Ct value was found with clinical severity as well as morphological changes in PBS. It is recommended that other parameters be utilised to assess clinical severity.

CONFLICT OF INTEREST

The authors have no competing interest.

AUTHORS CONTRIBUTION

1st Author (Dr Shalini Bahadur): Manuscript writing. 2nd author (Dr Tushar Kalonia): Contributed Photo micrograph. 3rd Author (Dr Kanchan Kamini): Data Collection. 4th/Corresponding Author (Dr Bhumika Gupta): Statistical analysis and Manuscript Writing. 5th Author (Dr Shivani Kalhan): Proof reading of manuscript. 6th Author (Dr Mansi Jain): Data collection.

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How to cite this article: Bahadur S, Kalonia T, Kamini K, Gupta B, Kalhan S, Jain M. Changes in peripheral blood in SARS CoV-2 patients and its clinico-pathological correlation: A prospective cross-sectional study. *Int J Lab Hematol*. 2021;43:1334–1340. https://doi.org/10.1111/ijlh.13720