Original Research Article

CBC parameters and morphological alterations in peripheral blood cells in COVID-19 patients: Their significance and correlation with clinical course

Devajit Nath[1], Ujjwal Madan[2], Savitri Singh[1], Neema Tiwari[1], Jyotsna Madan[1*], Renu Agrawal[3]

[1]Department of Pathology, Super Speciality Pediatric Hospital & Postgraduate Teaching Institute, Sector 30, Noida , Uttar Pradesh, India
[2]University College of Medical Science and GTB Hospital, Delhi, India
[3]Combined District Hospital, Sector 30, Noida, Uttar Pradesh, India

Received: 23-09-2020 / Revised: 26-10-2020 / Accepted: 20-11-2020

Abstract

Background:SARS-CoV2 infection induces inflammatory responses and acute lung injury in human beings.CBC and its derivatives are important investigative tools in its prognosis. However very few studies highlight the importance of Peripheral blood cell morphology in this disease.**Aim:**To Analyze significance of the CBC, derived parameters and peripheral blood cells morphology in Covid-19 patients,and to study their correlation with its Clinical Status of the patients at admission and at subsequent assessment during hospital stay. **Material and methods:** A Single center retrospective study of laboratory-confirmed 26 COVID-19 patients admitted at SSPH & PGTI, from April to July 2020**.**, CBC, its derived parameters and Romanowsky stained peripheral blood smears were analysed at two points of time during clinical course. Data was tabulated and analyzed using SPSS22 software.**Results:**On admission, 42.3% cases were mildly symptomatic and 19.2% were moderate to severely symptomatic requiring oxygen/ventilatory support. No significant statistical findings noted in CBC and its derived parameters at the time of admission . Follow-up, revealed a significant change in WBC and platelet count. (p=0.002). 7 cases showed persistent changes in CBC parameters and blood cell morphology and 3 out of 7 had moderate to severe clinical course. Bizarre atypical mononuclear cells , 2-3 times the size of RBC with dense homogenous chromatin, nuclear membrane irregularity and deep blue cytoplasm(?covicytes) were the most significant morphological finding. **Conclusion:** It is evident from our studies that a relevant number of cases having moderate to severe symptoms showed persistence of morphological changes and alteration in CBC parameters.

Keywords: COVID-19,CBC,d-NLR,NLR,LMR,PLR,Peripheral Smear.

Introduction

Severe Acute Respiratory Syndrome coronavirus 2 (SARS-CoV-2), causative agent of Corona Virus Disease-19 (COVID-19) is a novel coronavirus, first reported in Wuhan, China in December 2019, which frequently induces fatal inflammatory responses and acute lung injury[1,2].

**Correspondence*
Dr. Jyotsna Madan
Department of Pathology,Super Specilaity Pediatric Hospital and Post Graduate Teaching Institute, Noida, Uttar Pradesh, India.
E-mail: drjyotsnamadan@yahoo.co.in

Patients suffering from COVID-19 experience a myriad of symptoms ranging from mild cough and myalgias to severe breathlessness caused by pneumonia and ARDS. CNS involvement, COVID-19 associated coagulopathy mimicking disseminated intravascular coagulopathy, anosmia, ageusia, autoimmune hypersensitivity reactions, severe diarrhea accompanied by nausea, are other systemic manifestations. Noticeably, some children have also suffered from an entity called COVID-19 great toe[3]. The gold standard test RT-PCR (Real-time reverse transcriptase-polymerase chain reaction) is the cornerstone of establishing the etiology with multiple other supporting modalities like radiological and

laboratory investigations, that are being used in COVID-19 patients during the course of illness. HRCT (high resolution computed tomography) Chest is a common radiological tool that is useful for monitoring patients. Laboratory parameters like CBC and its derivatives, NLR(Neutrophil Lymphocyte Ratio), PLR (Platelet Lymphocyte Ratio), LMR (Lymphocyte Monocyte Ratio), and dNLR (Derived Neutrophil Lymphocyte Ratio) are important investigative tools that provide an insight into the severity of inflammation, which can predict further clinical course and the associated systemic complications and thereby, help the clinician assess the clinical status, disease progression and plan the treatment for the patient[4]. CBC and its derived parameters are routinely being done in the laboratories, but the importance of peripheral blood smear examination to study the alteration in blood cell morphology in COVID-19, has been overlooked until now. To date, very few studies have been published where the morphological characteristics of peripheral blood smear in COVID-19 have been discussed. After extensive search on the Google database, we found few studies discussing the morphological findings in the peripheral smears[5,6]. A study of 15 cases documented normocytic normochromic changes in the majority of the cases, and hemolysis was not observed in any case[7]. The study also mentioned the presence of medium to large size atypical lymphocytes having loosely condensed chromatin with moderate to deep basophilic cytoplasm. Some atypical cells with plasmacytoid morphology with eccentric nuclei, perinuclear hoff and some mimicking immunoblasts were also noted in the study. They also correlated the clinical finding at the time of sample collection with the lymphocyte population in the smear and found that the percentage of atypical lymphocytes did not correlate with the severity of the disease.Few studies have discussed the CBC and peripheral blood cell morphology findings in COVID-19 cases where they have noted findings like leucocytosis with neutrophilia, relative lympho cytopenia, and monocytopenia initially, with subsequent improvement in the monocyte count on the fifth day onwards.

Few of these studies have also studies the usefulness of flowcytometric analysis in detection of COVID related changes in monocyte subset[4,6].However, study correlating the peripheral blood morphological findings, Complete blood count and it's derivatives with the clinical course of the disease at two different point of time, in COVID-19 is lacking and there is a clear need of research in this area in the Indian scenario.

Aim: The objectives of the study were: 1) To find the statistical significance of the CBC parameters and CBC derived parameters like NLR, LMR, d-NLR and their correlation with clinical status (asymptomatic, mild, moderate to severe symptoms) of the patients at admission and on subsequent assessment during hospital stay, 2) To compare the peripheral blood smear morphological findings of the cases on the day of admission and at follow up with the clinical status.

Material and methods

Study period

A single center, retrospective study of laboratory-confirmed COVID-19 patients, admitted at Super Speciality Pediatric Hospital & Post Graduate Teaching Institute, from April to July 2020.

Patient selection

Patients were divided clinically into asymptomatic, mild, and moderate-severe cases based on the Ministry of Health and Family Welfare (MoHFW) Government of India (GOI) Revised Guidelines on clinical management of COVID-19 (www.https://www.mohfw.gov.in).

Inclusion criteria

All pediatric and adult cases, where two CBC samples - one at admission and second at follow up - during the hospital stay were available were included in the study.

Exclusion criteria

Patients with chronic lung diseases, hematological disorders, liver disease, and malignancy on treatment were excluded from the study. All patients where no follow-up CBC sample was available, were excluded.

Data Collection and Methodology

The demographic data, the clinical status of the patients at the time of admission and at follow up during the hospital stay(duration for which the follow-up samples were considered ranged from 7-10 days) and hematological findings (Complete blood counts and peripheral blood smear examination for cell morphology) were recorded at admission and at follow up. A total of 26 patients were included in the study after fulfilling all the inclusion criteria. The cases were divided on the basis of their clinical presentation on the day of admission as asymptomatic(n=10), mildly symptomatic (n=11), and moderate to severely symptomatic (n=5).The samples were tested for complete blood count on 5-part hematology analyzer Abbott Cell Dyn Ruby, and peripheral blood smears were stained by Romanowsky stains. The findings and morphological changes of the peripheral blood smear were noted and, the values and changes in CBC parameters and its derived parameters were recorded on the day of admission and of the repeat sample. The

follow up time ranged from 7 to 10 days. The findings were correlated with the clinical course of the patients. The composite endpoint of the study was a clinical improvement, or stability, or deterioration.

Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS), version 22.0. Chi-square test, paired 't'-test and ANOVA were used to compare the data. A 'p' value < 0.05 indicated a significant association.

Results

Age of patients ranged from 3 to 67 years. Mean age of patients was 35.85 ± 17.69 years. The majority of patients were males (61.5%) with the sex ratio of the study being 1.6:1(M: F). At admission mean RBC, hemoglobin and WBC counts were 4.51±0.78 $x10^6$/ microL, 12.70±2.05 g/dl and 6.77±3.41 thousands/mm^3 ,respectively. Mean absolute neutrophil count, absolute lymphocyte count, absolute monocyte count and absolute eosinophil count, were 3.91±3.36, 2.21±1.30, 0.39±0.21 and 0.13±0.16$x10^9$/L, respectively. Mean platelet count was 151.31± 78.24 thousands/mm^3. At admission, the mean neutrophil-to-lymphocyte ratio (NLR) was 3.90±6.86, mean platelet-to-lymphocyte ratio (PLR)was 115.32±149.00, mean lymphocyte to monocyte ratio(LMR) was 8.84±12.93 and mean derived neutrophil to lymphocyte ratio (d-NLR) was 2.55±3.91. (Table 1).At admission, a total of 10 (38.5%) cases were asymptomatic while 11 (42.3%) were mildly symptomatic and 5 (19.2%) were moderate-severely symptomatic requiring oxygen/ ventilatory support. All the cases under study were RT-PCR positive. Upon follow up, a significant change was noted in the mean WBC count and, the mean platelet count. Mean WBC increased from 6.77±3.41 to 8.25±3.98 thousands/mm^3, thus showing an increase of 1.47±3.32 thousands/mm^3 (p=0.033). Platelet count also showed an increase from 151.31±78.24 to 201.36±89.63 thousands/mm^3, thus showing a significant increase of 50.05±74.21 thousands/mm^3 (p=0.002). No other significant change was observed in the status of CBC parameters. (Table 1).

At the time of the follow up, RT-PCR was done and only 1 (3.8%) patient was positive, thus showing a decline of 96.2% in positivity rate (p<0.001). On analyzing the CBC parameters of patients and correlating them with their clinical status at admission, only hemoglobin levels showed a significant association. Lymphocyte counts showed a decreasing trend from asymptomatic to moderate-severely symptomatic category, however, this difference was marginally non-significant (p=0.052). For other CBC parameters, no association with clinical status at admission, was seen (p>0.05) (Table 2).

Further, upon correlating the CBC parameters with the clinical status of the patients, at the time of repeat sample, we observed that, most of the patients (n=20/ 26; 76.9%) were clinically recovered (planned for discharge) with negative RTPCR, while 4 (15.4%) were recovering with negative follow up RTPCR and only 2 cases (7.7%) were in ICU but stable. Out of these two ICU cases, 1 case had negative follow up RT-PCR and second case had persistent positive RT-PCR. Statistically significant differences in the CBC parameters, of patients with different clinical status, were seen for WBC, absolute Neutrophil count , absolute Monocyte count, Platelet count and derivatives like NLR, PLR and d-NLR. Mean WBC count was significantly lower in clinically recovered patients (planned for discharge) (6.65±2.06) as compared to those who were in ICU (13.70±4.24), or those who were recovering (13.48±5.10). Absolute Neutrophil and monocyte count also showed similar trends. For platelet count, maximum value was observed for stable patients (in ICU care) followed by recovering patients and then clinically recovered (planned for discharge) patients. NLR was minimum in clinically recovered (planned for discharge) patients followed by stable patients (in ICU care) and, maximum in recovering patients. PLR was maximum in stable cases (in ICU care) followed by recovering cases and, minimum in recovered (planned for discharge) patients. Meanwhile, d-NLR was minimum in recovered patients (planned for discharge) followed by patients in ICU and maximum in recovering patients (Table 3).

On analyzing the morphology of peripheral blood cells, we noted the following findings:-

Red blood cells morphology

Peripheral blood smear (PBS) for the RBC morphology revealed predominantly Normocytic normochromic picture (69.2%, 18/26), followed by dimorphic (microcytic-macrocytic) (15.4%,4/26) ,Microcytic hypochromic (7.7%,2/26) and macrocytic (7.7%,2/26) in both the first sample and on follow up sample (Table 4).

White blood cells morphology

- Polymorphs having dyspoietic features like hypolobation, ring shaped polymorph nucleus, cytoplasmic vacuoles, coarse granules mimicking toxic granules and some hypogranular polymorphs (Fig 3-5)
- Apoptotic cells reminiscent of polymorphs with nuclear fragmentation were seen.(Fig 6)
- Atypical lymphoid cells, large granular lymphocytes and monocytoid and plasmacytoid lymphoid cells were seen routinely.(Fig 7)

- Bizarre looking mononuclear cells with cell size 2-3 times that of an RBC, high N:C Ratio, irregular nuclear membrane, basophilic cytoplasm (? Covicytes). which have not been mentioned in the available literature, could be due to the viral induced changes of COVID-19 (Fig 8).
- Eosinophils did not show any relevant morphological changes.
- Monocytes and monocytoid cells showed relevant morphological alterations and were included in the same category because of morphological overlapping

Platelet morphology

Platelets showing large, giant and hyperchromatic forms.

The number of cases showing the morphological changes in the first and on follow up sample are depicted in (Table 5).(Fig 3-8)

We also made an attempt to correlate values of NLR and LMR with peripheral blood cells at two points of time.

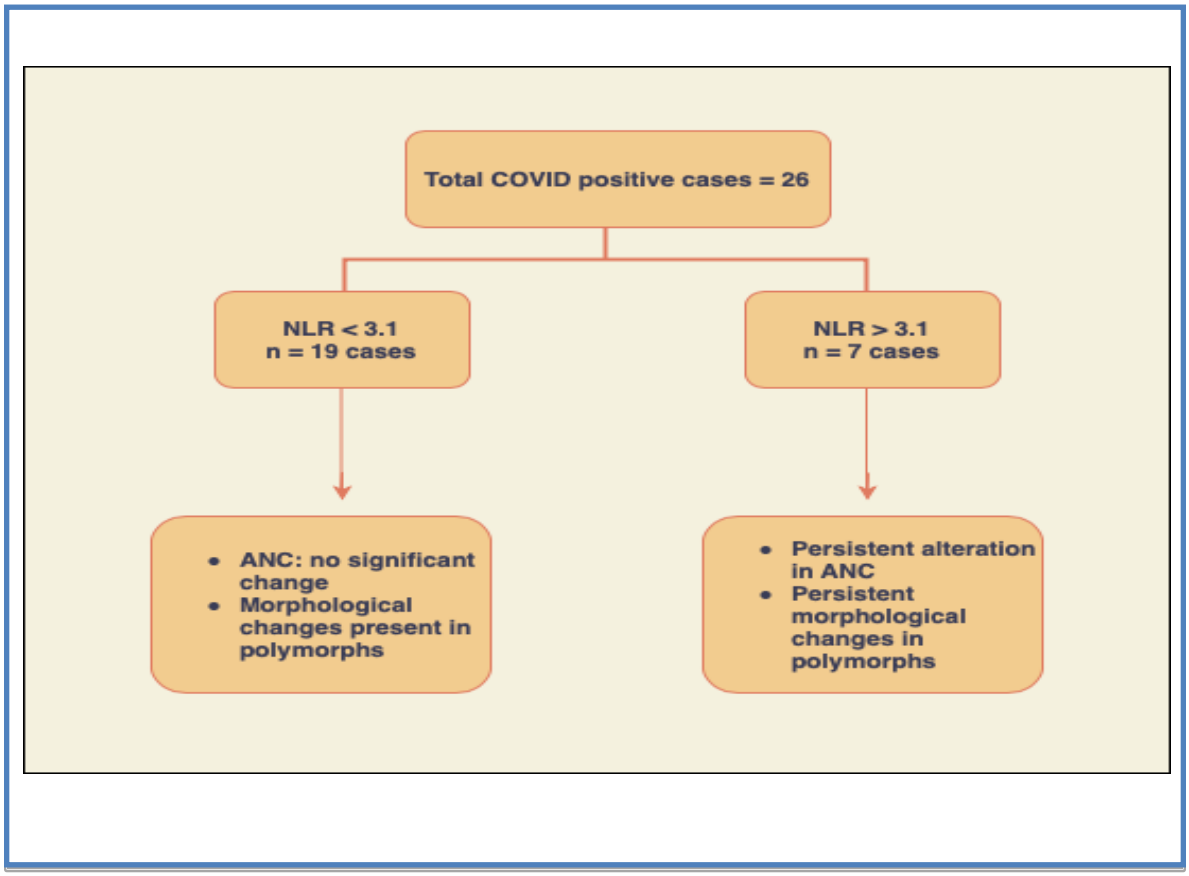

Fig 1:NLR and peripheral blood smear cells morphology at admission and on subsequent follow up

In the 19 cases despite a normal NLR (CBC parameter) which is used for monitoring therapy and disease progression, there were morphological changes in the smear in polymorphs. Hence importance of peripheral smear examination in cases where COVID is clinically suspected and the NLR is normal becomes important as a hint at possibility of existing infection even before RT-PCR results(NLR cut off >3.1)(Figure 1)[8].

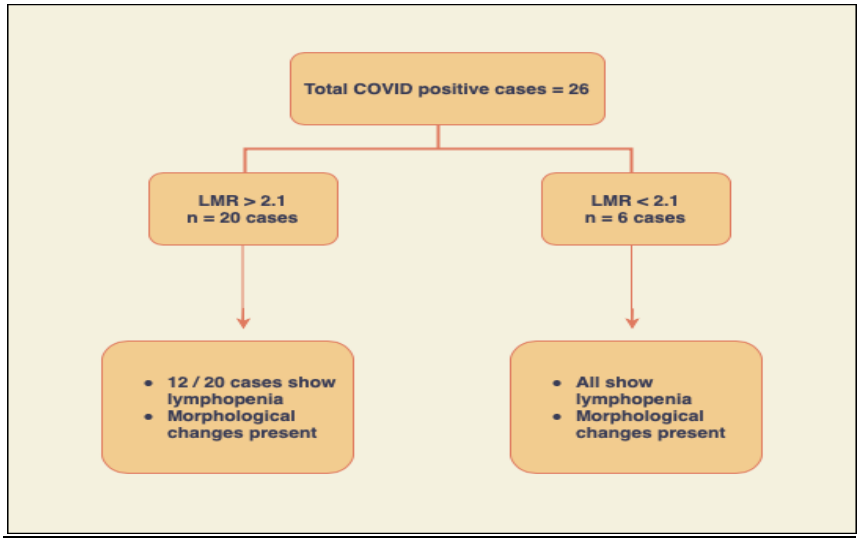

Fig 2: LMR and peripheral blood smear cells morphology at admission and on subsequent follow up

In the 20 cases despite a normal LMR(LMR>2.1) there were changes in lymphocyte and monocyte morphology present in peripheral smear. Hence importance of peripheral smear examination in cases where COVID is clinically suspected and the LMR is normal becomes important as a hint at possibility of existing infection even before RT-PCR results (significant LMR<2.1) (Fig 2) [9]

Discussion

Since the COVID-19 pandemic has hit the world, there have been various research activities to understand the etiology, pathogenesis, disease course and progression of the disease and to find a suitable antiviral drug, and vaccines to combat this virus. COVID-19 earlier thought of as a respiratory tract infection, is now considered a systemic disease involving respiratory, gastrointestinal, neurological, cardiovascular, hematopoietic and other systems[10,11]. Although the etiopathogenesis of the disease is still undergoing research, some studies have shown that, during the incubation period and the early phase of the disease, the virus causes changes in peripheral blood leukocyte and lymphocyte counts resulting in normal to low count[4-6].Viremia in the disease primarily affects the tissues expressing high levels of ACE2 receptors including lungs, heart and gastrointestinal tract[12]. In some patients who experienced severe symptoms, a "cytokine storm" set in, owing to the increase in systemic inflammatory response in the body. A number of studies have been published on the alterations in CBC and its derived parameters, in COVID-19 infection, however, very few studies have been published in the Indian population where the pandemic has spread with a fast pace [13,14].In the Indian perspective we published a study on 32 cases of COVID- 19, where the CBC findings were correlated with clinical presentation and outcome of the patient. It was observed that the majority of the patients were younger and had mild clinical presentation with female predominance[14]. Pediatric cases had mild symptomology. Baseline CBC findings showed mild neutrophilia, lymphopenia, eosinopenia and, normal to mild platelet count. An increase in NLR was also noted in some of the cases on follow up samples during hospital stay. In one of the studies the authors observed that majority of patients had lymphocytopenia (83.2%), whereas 36.2% had thrombocytopenia, and 33.7% showed leukopenia at presentation [15].They also correlated the findings with clinical status and noted that peripheral blood abnormalities were more prominent among severe as compared to the non-severe cases (96.1% versus 80.4% for lymphocytopenia, 57.7% versus 31.6% for thrombocytopenia and 61.1% versus 28.1% for leucopenia) [16]. Their results were consistent in few other descriptive studies that were conducted during the same period in China and included 41, 99, 138 and 201 confirmed cases with COVID-19, respectively. Huang et al. and Wang et al.specifically highlighted an association between lymphopenia and the need of ICU

care, whereas Wu et al. showed an association between lymphopenia and acute respiratory distress syndrome (ARDS) development[15,17,18]. Wu et al retrospectively analyzed possible risk factors for developing ARDS and death among 201 patients with COVID-19 pneumonia in Wuhan, China[16]. Increased risk of ARDS during the disease course was significantly associated with increased neutrophils, regulatory T-cells that promote the initial hyperactivation which is followed by rapid exhaustion of cytotoxic CD8+ T-cells[19].In another study in Singapore, Fan et al. also found that patients requiring ICU support had significantly lower lymphocyte counts[20].In the current study, the authors observed lymphopenia in total 18/26 (69%) cases where 10 out of these 18 samples (38.5%) had lymphopenia at the baseline, while 8/18 (30.7%) samples had it in the follow up sample, however these findings did not always correlate with the severity of the clinical symptoms in our case, as described in some papers.Neutrophilia was reported in 3 (11.5%) cases for baseline sample and 1(3.84%) case in follow up sample and the single case progressed to severe clinical symptomatology and was on BiPAP(Bilevel Positive Airway Pressure) for more than 2 weeks. Eosinopenia was seen in 10(38.5%) cases with the baseline sample and 6(23.1%) samples in follow up time. Two of these cases showing persistent eosinopenia were on ventilatory support and one of these cases was positive on RT PCR on follow up repeatedly, over a period of 1 month. Rest CBC changes were not clinically significant.Morphology of cells of the peripheral blood gets altered in COVID-19 as is evident by few studies published from China and Italy[4,6]. In a study of 28 patients with confirmed COVID-19 infection with varying degrees of severity, morphological and functional changes of monocytes/ macrophages were noted using wright stained smears and flow cytometry, that was predictive of the severity of disease, likelihood of ICU admission, length of hospital stay and full recovery[4].Few Indian studies describing various morphological changes in RBCs, WBCs and platelets has been published, however no study has dealt in detail, with the impact of these changes on patient outcome or, whether these changes hold any clinical significance at the time of diagnosis or, during the patient's disease course[5,21]. In the present study, we have made an attempt to highlight the importance of careful examination of peripheral smear cell morphology apart from studying the CBC parameters, as well as its clinical correlation with the patient's condition on the day of admission and along the course of the disease.We also correlated these morphological changes with the NLR and LMR derived from the CBC and with the clinical and molecular status (RT-PCR) of the patient at two points of time, to see if any relevant information could be gathered. The hypothesis was devised to look for the following research questions:

-What are the morphological changes in the peripheral blood smear cells in COVID-19 cases?

-For how long the various morphological changes persisted in each cell lineage?

-Whether the correlation of CBC parameters, smear morphology and, clinical-molecular profile helps to prove the utility of peripheral blood smear examination as an adjunct tool for the pathologist, which could indicate presence of virus induced changes in non-infective or asymptomatic stage of the disease before the RT-PCR results are available .

On analyzing the morphology of peripheral blood cells, we observed a range of morphological changes occurring in the disease, especially in the WBC series and in platelets. Although all the cells of the WBC series showed some changes, the most pronounced finding was of large bizarre atypical looking cells with basophilic cytoplasm, probably a virocyte labelled as a "covicyte".Regarding persistence of morphological changes in blood cells, we observed that three cases of severe clinical symptoms, requiring respiratory support(2 cases of the 3 had continued respiratory support on follow up also)showed persistent changes in all the 3- cell lineage for the follow up period ranging from 7-10 days.Further, on correlating these morphological changes with clinical status and CBC parameters, we noticed that out of the 7 cases of raised NLR and 6 Cases of decreased LMR, 2 cases had both raised NLR and low LMR, were on ventilatory support. 1 of these 2 cases showed significant morphological alterations in all the three-cell lineage (Table 6)In a study by Singh et al. the authors observed that the morphological abnormalities in peripheral smear included many crowded, dark granules in the cytoplasm of polymorphs (similar to "toxic" granules) and of peripheral light blue agranular area. Cytoplasmic hypogranularity of polymorphs was seen in a few cases. Abnormalities of nuclear shape were striking, with increased frequency of band forms and dyspoeitic features, with total absence of nuclear segmentation, consistent with pseudo-PelgerHeut abnormality [5]. Apoptotic cells were easily noted in many peripheral blood films and they appeared with liquefied nuclear chromatin and granular or deep blue cytoplasm, suggesting possible derivation from different types of cells (i.e., neutrophils and lymphocytes, respectively). Immature granulocytes,

especially small myelocytes and metamyelocytes, sometimes showing immature nuclei and small azurophilic granules, were observed. However, upon analysing the smears after therapy, the authors noted a disappearance of changes in the polymorphs but persistent changes in the lymphoid cells[6]. In our analysis, the changes in polymorphs persisted in 3 cases on follow up (Table 6). Hence, this finding could become a significant predictor for post COVID prodrome.In the Indian scenario two studies, one case report of a 55 year old female with respiratory symptoms and positive RT-PCR and one short commentary published by the authors explained the morphological findings in peripheral smear of COVID-19 cases which include RBC with mainly non-specific changes, neutrophils with toxic granules, vacuoles, hypolobation, dyspoiesis, lymphocyte with Lympho-cytopenia, Large granular lymphocyte, variant reactive monocytoid and plasmacytoid forms and blastoid forms, monocytes with large bizarre forms, vacuoles, granules, platelets with agglutination and thrombo cytopenia [5,21].It is a known phenomenon with other viral infections, that virus - induced changes are persistent and disappear gradually, especially from the lymphoid cell population. In our analysis the RBC changes persisted in the follow up CBC in all these cases, while the WBC findings were variable on follow up, however changes in lymphoid and monocytoid cells persisted in 2 cases requiring continuous ICU care. Hence, it can be hypothesized that in cases where a post COVID severe prodrome persists or, in cases where a re-infection of COVID is occurring, it could be due to persistence of viral genome in the infected cells and, these morphological changes are not just a byproduct of cytokine storm but, they are actually danger signs in patients where they persist, alerting the clinicians to closely follow up these recovered patients, something that only CBC analysis or RT-PCR will not accomplish.It is also evident that the peripheral smear in any other viral illness does not match that of COVID-19 as shown in an analysis of monocyte subset by Zhang et al. in his study[4].Another study highlighting the morphological changes in COVID-19 in the peripheral smear cells documents presence of lympho - plasmacytoid cells as seen in our study. We also noted plasmacytoid cells in the cases examined. Another significant finding that we noted, was the presence of apoptotic cells in the peripheral smear[22]. According to recent updates, such quantitative and qualitative abnormalities can be related to the cytokine storm and hyperinflammation which is a fundamental pathogenic factor in the evolution of COVID-19 pneumonia, possibly in the form of secondary hemophagocytic lymphohis-tiocytosis, leading to an often-fatal multi-organ failure or possibility of relapse.

Table 1: Clinico-hematological profile of patients at admission and at follow-up (n=26)

SN	Characteristic	At admission		At follow-up		Change		Significance of change (Paired 't'-test)	
		Mean	SD	Mean	SD	Mean	SD	't'	'p'
1.	Mean Age±SD (Range) in years	35.85±17.69 (3-67)						-	
2.	Male:Female	16 (61.5%) : 10 (38.5%)						-	
3.	RBC (10^6/mm^3)	4.51	0.78	4.59	0.70	0.08	0.79	-0.494	0.626
4.	Hb (gm/dl)	12.70	2.05	12.58	2.20	-0.12	1.85	0.321	0.751
5.	WBC (10^3/microL)	6.77	3.41	8.25	3.98	1.47	3.32	-2.261	0.033
6.	Neutrophil (10^3/microL)	3.91	3.36	5.10	3.72	1.19	3.00	-2.021	0.054
7.	Lymphocyte (10^3/microL)	2.21	1.39	2.37	1.07	0.16	1.16	-0.686	0.499
8.	Monocyte (10^3/microL)	0.39	0.21	0.48	0.36	0.09	0.32	-1.411	0.171
9.	Eosinophil (10^3/microL)	0.13	0.16	0.16	0.18	0.03	0.17	-0.973	0.340
10.	Platelet (10^3/microL)	151.31	78.24	201.36	89.63	50.05	74.21	-3.439	0.002
11.	NLR	3.90	6.86	2.89	3.02	-1.01	5.73	0.896	0.379
12.	PLR	115.32	149.00	99.76	60.54	-15.56	143.36	0.553	0.585
13.	LMR	8.84	12.93	36.88	107.19	28.04	107.87	-1.325	0.197
14.	d-NLR	2.55	3.91	1.84	1.56	-0.71	3.24	1.118	0.274
15.	RT-PCR Positive	26 (100%)		1 (3.8%)		-96.2%		χ^2=48.1; p<0.001	
16.	At admission status							-	

	Asymptomatic Mildly Symptomatic Mod to Severe Symptoms.(2 in ICU)	10 (38.5%) 11 (42.3%) 5 (19.2%)	
17.	Status at follow-up Recovered(planned for discharge) Recovering In ICU	 29 (76.9%) 4 (15.4%) 2 (7.7%)	

Table 2: Association of CBC parameters with Clinical Status at admission

SN	Characteristic	Asymptomatic (n=10)		Symptomatic (n=11)		Mod/Severe Symptomatic (n=5)		Significance of change (ANOVA)	
		Mean	SD	Mean	SD	Mean	SD	'F'	'p'
1.	RBC (10^6/mm^3)	4.17	0.93	4.77	0.66	4.61	0.57	1.680	0.208
2.	Hb (gm/dl)	11.35	2.12	14.04	1.34	12.46	1.48	6.533	0.006
3.	WBC (10^3/microL)	7.44	4.40	6.47	2.42	6.11	3.51	0.310	0.737
4.	Neutrophil(10^3/microL)	3.77	4.38	3.89	2.40	4.24	3.57	0.030	0.971
5.	Lymphocyte(10^3/microL)	2.96	1.67	1.98	1.00	1.23	0.78	3.366	0.052
6.	Monocyte(10^3/microL)	0.45	0.26	0.38	0.16	0.31	0.23	0.717	0.499
7.	Eosinophil(10^3/microL)	0.21	0.21	0.11	0.09	0.01	0.02	3.007	0.067
8.	Platelet(10^3/microL)	161.14	55.92	158.62	104.62	115.56	43.25	0.630	0.542
9.	NLR	1.84	2.53	4.05	5.30	7.68	13.45	1.237	0.309
10.	PLR	69.48	48.60	150.92	215.09	128.69	93.90	0.794	0.464
11.	LMR	14.73	19.62	5.61	3.56	4.17	1.95	1.820	0.185
12.	d-NLR	1.81	1.85	2.30	2.48	4.58	8.13	0.862	0.435

Table 3: Association of CBC parameters with Clinical Status at follow-up

SN	Characteristic	ICU but Stable (n=2)		Recovering (n=3)		Recovered (Planned for Discharged (n=20)		Significance of change (ANOVA)	
		Mean	SD	Mean	SD	Mean	SD	'F'	'p'
1.	RBC(10^6/microL)	4.60	0.42	4.52	0.66	4.60	0.75	0.019	0.981
2.	Hb (gm/dl)	12.25	1.91	11.88	2.24	12.76	2.28	0.277	0.760
3.	WBC(10^3/microL)	13.70	4.24	13.48	5.10	6.65	2.06	14.30	<0.001
4.	Neutrophil(10^3/microL)	9.80	1.41	10.85	4.97	3.48	1.46	22.56	<0.001
5.	Lymphocyte(10^3/microL)	2.20	1.41	1.49	0.31	2.56	1.08	1.84	0.182
6.	Monocyte(10^3/microL)	1.03	0.67	0.88	0.33	0.35	0.21	11.26	<0.001
7.	Eosinophil(10^3/microL)	0.03	0.03	0.15	0.27	0.17	0.18	0.545	0.587
8.	Platelet(10^3/microL)	348.00	114.55	192.00	79.33	188.57	80.20	3.477	0.048
9.	NLR	5.35	2.80	7.82	4.01	1.66	1.29	18.34	<0.001
10.	PLR	178.28	62.53	139.20	72.79	84.02	50.42	3.968	0.033
11.	LMR	2.16	0.03	2.01	1.33	47.33	120.92	0.391	0.680
12.	d-NLR	3.23	1.98	4.21	2.07	1.23	0.74	14.31	<0.001

Inference

Only WBC and Platelet count showed a significant change during the period. As far as clinical status is concerned, follow-up CBC parameters correlate with the clinical status, however given the few number of in ICU but stable or recovering as against recovered and planned for discharge patients, these results showed be interpreted with caution.

Limitation

In view of the small sample size, the results showed be inferred as indicative only.

Table 4: RBC morphology at Admission and at follow-up

RBCs Morphology	Number of cases showing Changes at admission	Morphological changes at the time of repeat sample
Normocytic normochromic	18(69.2%)	Persisted
Microcytic hypochromic	2(7.7%)	Persisted
Macrocytic	2(7.7%)	Persisted
Mixed picture	4(15.4%)	persisted

Table 5: WBC and Platelet morphology at admission and at follow-up

Morphology of Cells	Number of cases showing Changes at admission	Number of cases showing persistence of Changes at follow-up
Polymorphs		
Coarse granules	25	13
Vacuoles	12	2
Dyspoiesis	3	3
Left shift	3	0
Apoptotic cells	5	0
Mononuclear(lymphoid/monocyte) cells		
Activated/atypical lymphocyte	23	20
Plasmacytoid lymphocyte	4	1
Intranuclear Inclusions	1	0
Bizarre atypical mononuclear cell ?covicytes	3	0
Monocytoid/monocyte with vacuoles	3	0
Platelets		
Large/giant	2	0
hyperchromatic	2	0

We didn't find any relevant change in eosinophils morphology; left shift was present without any relevant dyspoietic features and no other significant WBC abnormality was noted apart from the findings tabulated above. We included monocytes with monocytoid lymphoid cells as it was difficult to ascertain their type morphologically.

Table 6: Clinical ,hematological and molecular profiles of 7 patients with persistent changes in the peripheral blood smear morphology. Table depicts the profile on the day of admission(D1) and on follow up (F)

NLR>3.1(n-07)														
Morphology	**Case1**		**Case 2**		**Case 3**		**Case 4**		**Case 5**		**Case6**		**Case 7**	
	D1	F	D1	F	D1	F	D1	F	D1	F	D1	F	D1	F
Tg	+	-	+	-	+	-	+	+	+	-	+	+	+	-
Vacuole	-	-	+	-	+	-	+	+	-	-	+	+	+	-
Dyspoiesis	-	-	+	+	-	-	-	-	-	-	-	-	-	-
Left Shift	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Apoptotic cell	+	-	-	-	-	-	+	-	+	-	+	-	-	-
LMR>2.1 (n-06)														
Morphology	D1	F	D1	F	D1	F	D1	F	D1	F	D1	F	-	
Atypical Lymphocytes	+	-	+	-	+	-	+	+	+	-	+	-		
LGL	+	+	+	-	+	-	+	+	+	-	+	-		
Reactive lymphoid cells	+	+	+	+	+	+	+	+	+	+	+	+		
Bizzare cells ?covicytes	-	-	-	-	-	-	+	+	-	-	+	+		
Molecular analysis														
	D1	F	D1	F	D1	F	D1	F	D1	F	D1	F	D1	F
Real Time RT-PCR	+	-	+	-	+	-	+	+	+	-	+	-	+	-
Clinical status of the patient														
	D1	F	D1	F	D1	F	D1	F	D1	F	D1	F	D1	F
Symptoms	Sy	R	Sy	R	Sy	R	Sy*	S	Sy	R	Sy*	S	Sy	R

Sy-Symptomatic
Sy*-Symptomatic with ventilatory support
S-Stable(ICU care)
R-Recovered(planned for discharge)
LGL-Large granular lymphocyte
Tg-Toxic granules

Important points to be noted in the table 7 is that 2 cases in ICU had persistent morphological changes and significant CBC findings and one case remained RT-PCR positive.

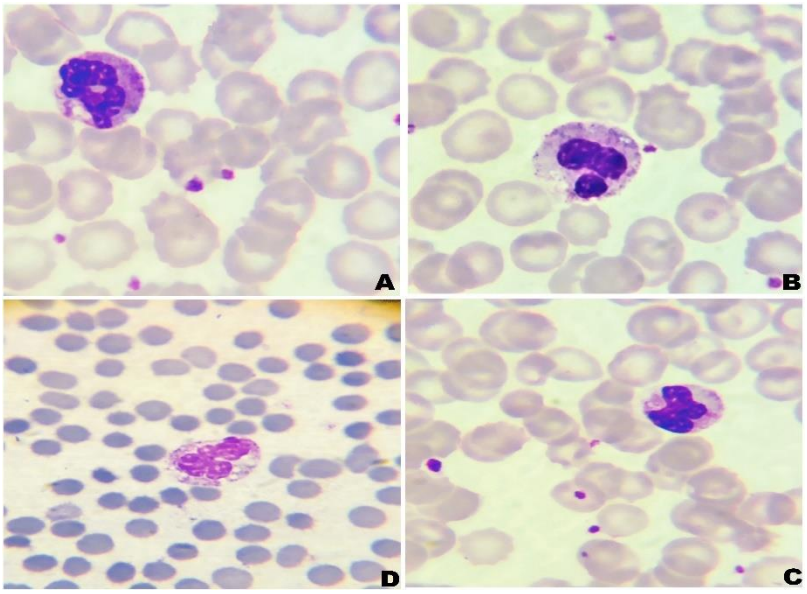

Fig 3: A-D Leishman-Giemsa stained smears showing morphological changes in the peripheral blood film of COVID-19 cases; A-Dyspoietic, Hypolobated polymorph with cytoplasmic vacuoles and dense cytoplasm(400X),B-Hypolobated polymorph with cytoplasmic vacuoles and coarse sparse granules sticking to the periphery(400x),C-Atypical bizarre cell with marked nuclear membrane irregularity (400x),D-Leishman stained smear shows dyspoietic polymorph with coarse granules and vacuoles(400x)

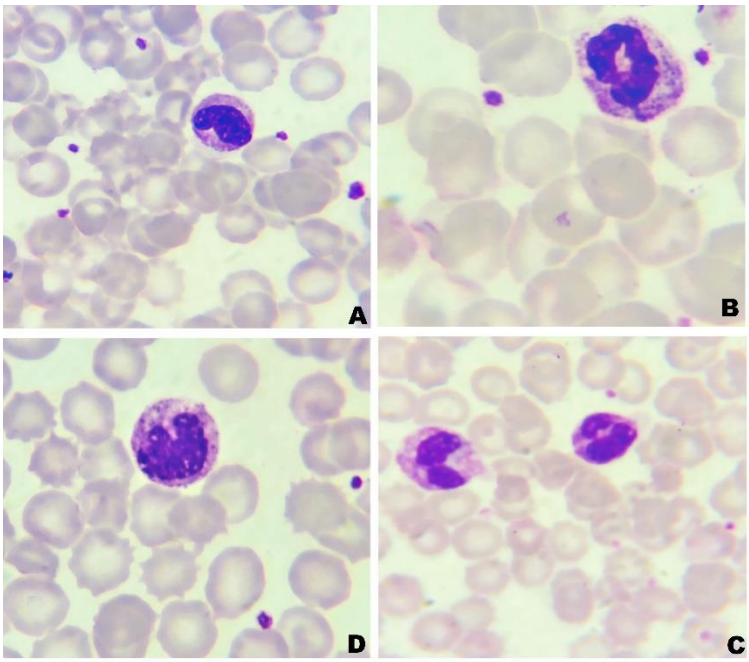

Fig 4:A-D Leishman-Giemsa stained peripheral smears showing spectrum of dyspoietic polymorphs (A-100X,B&C-400X,D-100X)

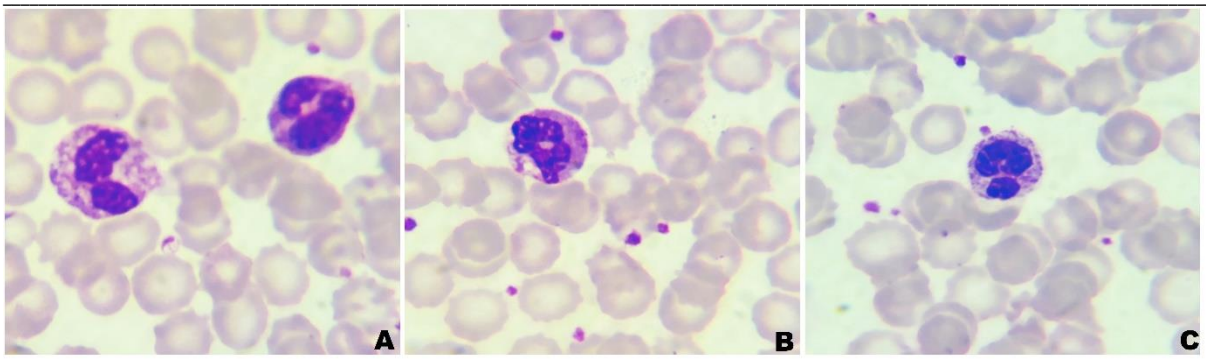

Fig 5:A-C: Leishman-Giemsa stained smear shows spectrum of dyspoietic changes in polymorphs with accompanying apoptosis(A,B,C-400X)

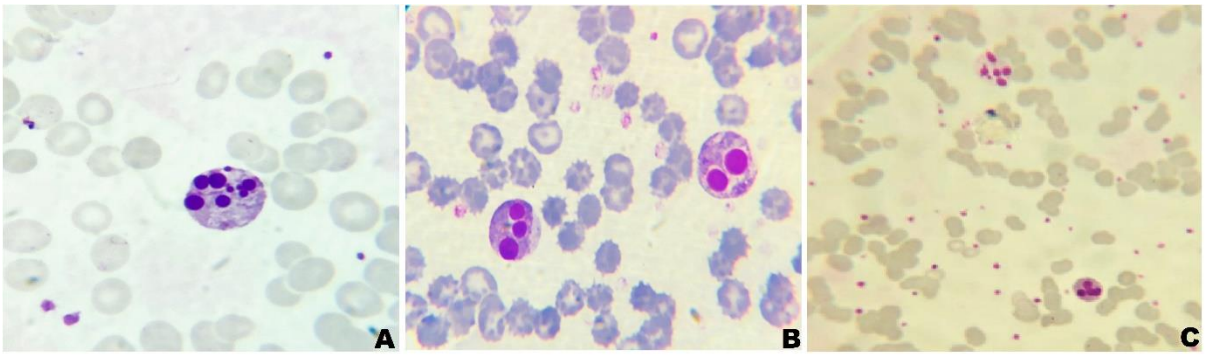

Fig 6:A-C Leishman Giemsa stained smears showing apoptotic/karyorrhectic cells(A,B-400X;C-100X)

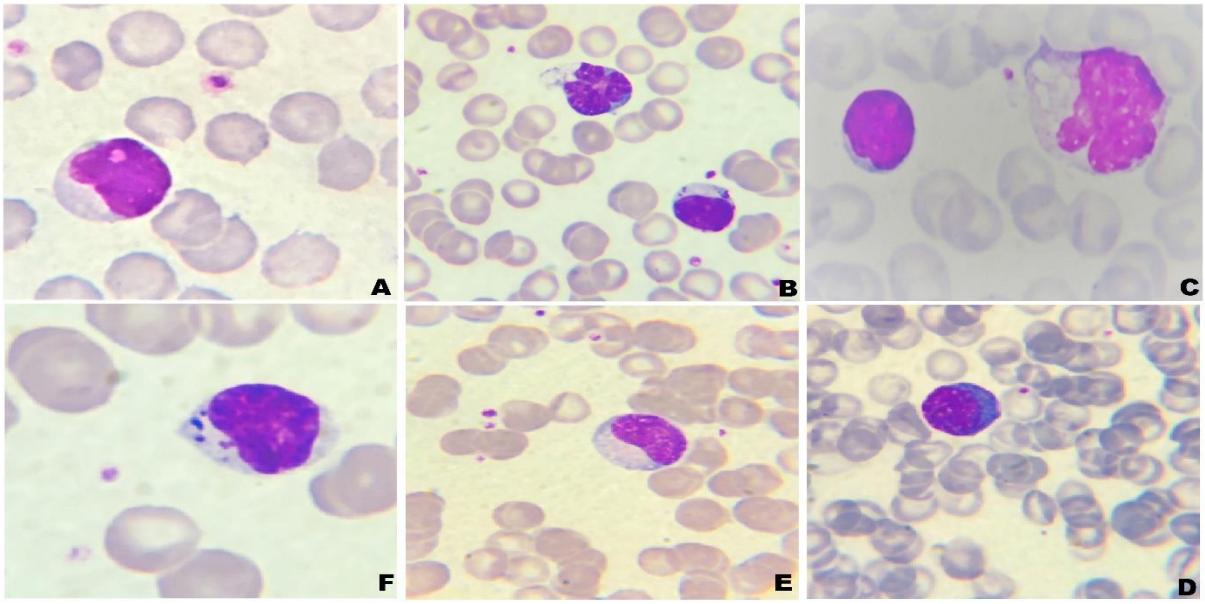

Fig 7:A-D Leishman-Giemsa stain smears showing atypical and variant lymphoid and monocytoid cells at 400x.

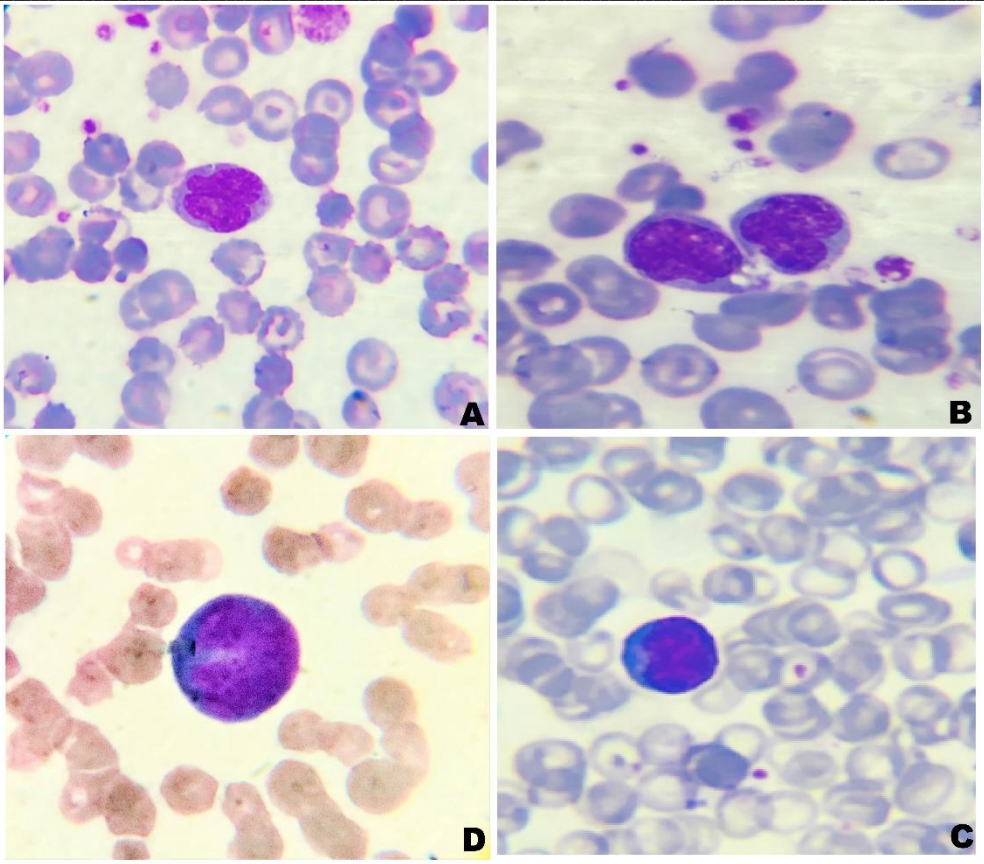

Fig 8:. A-D. Leishman-Giemsa stained smears show large atypical, bizarre looking mononuclear cells ,2-3 times the size of RBC having irregular nuclear membrane, dense chromatin, scant to moderate cytoplasm and few with cytoplasmic granules and vacuoles(?Virocyte?Covicyte)

Limitation

A small sample size with a large proportion of asymptomatic to mildly symptomatic cases, is the limitation of the study and should be inferred with caution. A large study with inclusion of more severe cases in the cohort, as well as longer follow up duration will give a better representation of the clinical and morphological profile of the patient.

Conclusion

The study highlights the importance of CBC parameters and morphological examination of peripheral blood smear at baseline and during subsequent assessment. It is evident from our study that a relevant number of cases having moderate to severe symptoms, showed persistence of morphological changes or presence of bizarre monocytoid cells (covicytes). Hence it may be assumed from the study that monitoring of CBC parameters with peripheral blood cell morphology may predict the severity of post COVID syndrome.

Acknowledgement

We thank the Department of Pediatric Medicine, SSPHPGTI, NOIDA, India, Mr Varun Arora, Visiting faculty, Department of Biostatistics, Lucknow University and the technical staff, Department of Pathology, SSPHPGTI, NOIDA for their support in carrying out the study . We also greatly appreciate the efforts of healthcare workers and the support of their families during this outbreak.

References

1. Organization WH. Laboratory testing for coronavirus disease 2019 (COVID-19) in suspected human cases: interim guidance, 2 March 2020. World Health Organization, 2020.
2. Lin Ling, Li Taisheng. Interpretation of the new health coronary virus infection pneumonia diagnosis and treatment program (Trial Version 5)

[J]. Chinese Medical Journal. 2020;100(00):E001-E001
3. Chan JF-W, Yuan S, Kok K-H, To KK-W, Chu H, Yang J, et al. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. The Lancet. 2020;395(10223):514-23.
4. Zhang D, Guo R, Lei L, Liu H, Wang Y, Wang Y, et al. COVID-19 infection induces readily detectable morphological and inflammation-related phenotypic changes in peripheral blood monocytes, the severity of which correlate with patient outcome. medRxiv. 2020
5. Singh A, Sood N, Narang V, Goyal A. Morphology of COVID-19–affected cells in peripheral blood film. BMJ Case Reports CP. 2020;13(5):e236117
6. Zini G, Bellesi S, Ramundo F, d'Onofrio G. Morphological anomalies of circulating blood cells in COVID-19. Am J Hematol. 2020;95:870–872
7. Weinberg SE, Behdad A, Ji P. Atypical lymphocytes in peripheral blood of patients with COVID-19. British Journal of Haematology. 2020;190:24-3
8. Liu, J., Liu, Y., Xiang, P. *et al.* Neutrophil-to-lymphocyte ratio predicts critical illness patients with 2019 coronavirus disease in the early stage. J Transl Med .2020;18:206
9. Lissoni P, Rovelli F, Monzon A, Privitera C, Messina G, Porro G. Evidence of Abnormally Low Lymphocyte-To-Monocyte Ratio In COVID-19-Induced Severe Acute Respiratory Syndrome. J Immuno Allerg. 2020;1(2):1-6.
10. Guan W-j, Ni Z-y, Hu Y, Liang W-h, Ou C-q, He J-x, et al. Clinical characteristics of coronavirus disease 2019 in China. New England journal of medicine. 2020;382(18):1708-20.
11. Qian G-Q, Yang N-B, Ding F, Ma AHY, Wang Z-Y, Shen Y-F, et al. Epidemiologic and Clinical Characteristics of 91 Hospitalized Patients with COVID-19 in Zhejiang, China: A retrospective, multi-centre case series. QJM: An Int J Med. 2020;113(7): 474–481.
12. McLachlan CS. The angiotensin-converting enzyme 2 (ACE2) receptor in the prevention and treatment of COVID-19 are distinctly different paradigms. Clinical Hypertension. 2020;26(1):1-3
13. Mannaerts D, Heyvaert S, De Cordt C, Macken C, Loos C, Jacquemyn Y. Are neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and/or mean platelet volume (MPV) clinically useful as predictive parameters for preeclampsia? The Journal of Maternal-Fetal & Neonatal Medicine. 2019;32(9):1412-9
14. Tiwari N, Nath D, Madan J, Bajpai P, Madan U, Singh S. Novel Insights into the Hematological Parameter Abnormalities in Pediatric COVID-19 Cases: Observation from A Preliminary Study of 11 Pediatric COVID-19 Cases in A Tertiary Care Center of North India. Saudi J Pathol Microbiol.2020; 5(5): 276-28
15. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. The lancet. 2020;395(10223):497-506
16. Bai Y, Yao L, Wei T, Tian F, Jin D-Y, Chen L, et al. Presumed asymptomatic carrier transmission of COVID-19. Jama. 2020;323(14):1406-7
17. Xu X-W, Wu X-X, Jiang X-G, Xu K-J, Ying L-J, Ma C-L, et al. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. BMJ. 2020;368
18. Yang X, Yu Y, Xu J, Shu H, Liu H, Wu Y, et al. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single- centered, retrospective, observational study. The Lancet Respiratory Medicine. 2020;8(5):475-481
19. Lippi G, Plebani M. Procalcitonin in patients with severe coronavirus disease 2019 (COVID-19): a meta-analysis. Clinica chimica acta; international journal of clinical chemistry. 2020;505:190
20. Fan BE, Chong VCL, Chan SSW, Lim GH, Lim KGE, Tan GB, et al. Hematologic parameters in patients with COVID-19 infection. American journal of hematology. 2020;95(6):E131-E4.
21. Singh, S., Madan, J., Nath, D., & Tiwari, N. (2020). Peripheral Blood Smear Morphology- A Red Flag in COVID-19. IJTDH;*2020; 41*(8), 54-58.
22. Wang F, Nie J, Wang H, Zhao Q, Xiong Y, Deng L, et al. Characteristics of peripheral lymphocyte subset alteration in COVID-19 pneumonia. The Journal of infectious diseases. 2020;221(11):1762-9.

Conflict of Interest: Nil
Source of support:Nil