Proceedings S.Z.M.C. Vol: 36(1): pp. 8-13, 2022. PSZMC-826-36-1-2022

Frequency of Leukoerythroblastic Picture and Hematological Parameters in COVID-19 Patients and Association With Disease Severity

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ABSTRACT

Introduction: Patients infected by COVID-19 can present with severe lung damage and acute respiratory distress syndrome (ARDS) and have a significant mortality risk involving all body system such as cardiovascular (CVS), gastrointestinal (GIT), neurological (CNS), immune system and haemopoietic system.
Aims & Objectives: To determine frequency of Leukoerythroblastic picture, compare complete blood counts and cell ratios (NLR, PLR, LMR) of positive COVID-19 patients with suspected COVID-19 patients and their association with severity as stratified by mode of admission and clinical status.
Place and duration of study: King Edward Medical University, Mayo Hospital Lahore from December 2020 to January 2021.
Material &Methods: 75 RT-PCR confirmed COVID-19 and 75 suspected patients of both genders and aged above 18 years were included. Severity was classified by mode of admission and patient status. CBC samples of all patients were analyzed for counts, differential and LE picture on Sysmex XE-1000 automatic blood analyzer. NLR, LMR and PLR were calculated in both groups. Peripheral smear findings were noted for leukoerythroblastic picture in all patients. Data was analyzed using SPSS version 25, p value ≤0.05 was considered significant.
Results: Each group consist of equal (75) number of patients with male predominance. Mean age of patient in confirmed and suspected groups were 58±14 and 61±15 respectively in both groups. Majority were admitted in HDU and ICU compared to isolated wards (P: .000). Frequency of LE picture in both groups was (2.7%) (P:0.12) by Chi-square. Mean± SD were determined, of hematological parameters and ratios in confirmed and suspected groups. ANC (P:0.022), ALC (P:0.032), NLR (P:0.002) were significantly different when compared in both groups.
Median (IQR) of Hb (p: 0.05), WBC (P: 0.000), platelet (P: 0.008), ANC (P: 0.000), NLR (P: 0.000), LMR (P: 0.016) were significantly correlated with severity, when analyzed by independent-sample Kruskal Wallis Test.
Conclusion: Leukoerythroblastic picture is not associated with severity in COVID-19. Neutrophil to lymphocyte ratio is important prognostic factor in suspected and confirmed COVID-19 patients.

Key words: COVID-19, Neutrophil lymphocyte ratio (NLR), platelet lymphocyte ratio (PLR), lymphocyte monocyte ratio, (LMR), Leukoerythroblastic reaction.

INTRODUCTION

Corona virus disease starting in 2019 in Wuhan, Hubei Province, China involved all countries and became pandemic in 2020.
Patients infected by COVID-19 can present with severe lung damage and acute respiratory distress syndrome (ARDS) and have a significant mortality risk involving all body system such as cardiovascular (CVS), gastrointestinal (GIT), neurological (CNS), immune system and haemopoietic system.[1,2]
This infection is caused by the severe acute respiratory syndrome coronavirus 2 strain (SARS-CoV-2). Patient is labeled as COVID 19 positive when PCR report shows viral detection. Suspected is the term denoted to patients with positive contact history, any 2 clinical features (fever, respiratory symptoms and positive radiological findings) and awaited/negative PCR as per institution protocol.[3]
Clinical course of disease differs in patients; some patients develop mild symptoms with good prognosis while others present with difficult treatment and high mortality. Classification of disease severity is very important to guide the right treatment and care.[1,2]
Identification of routine laboratory parameters that can guide the disease categorization between mild and severe COVID-19 cases could help to predict patients at risk.

CBC parameters and ratios abnormalities in COVID-19 are correlated with disease advancement, stringency and mortality.[4]

Leukoerythroblastic (LE) picture is defined as nucleated red cells and left shift of myeloid series circulating in the blood. It can be seen in conditions like bone marrow fibrosis, myeloproliferative disorders, and infiltration of bone marrow by metastatic diseases. Viral infections such as parvovirus can be the rare cause. A case report published in 2020 showed presence of LE picture in COVID-19 patient.[5]

The case report was based on findings noted in start of pandemic and author described the LE picture in a COVID-19 patient which improved and disappeared with treatment. Author suggested further studies to see exact frequency and role of LE picture in a disease severity and progression.

Neutrophils, lymphocytes, thrombocytes have important role in regulation of infection, inflammation so ratios of these parameters are important as early inflammatory markers. Neutrophil lymphocyte ratio (NLR) is ratio of absolute neutrophil to absolute lymphocyte count. Normally it is below 3, but ratio above 3 is noted especially in stress, sepsis according to research studies.[2]

Lymphocyte to monocyte ratio was calculated by absolute lymphocyte to absolute monocyte count. The range is 3-9 according to research studies.[2]

Platelet to lymphocyte ratio is calculated by absolute lymphocyte count (%) to absolute platelet count (%). Normal value is between 50-150.[2]

Disseminated intravascular coagulation (DIC) is reported in COVID-19 patients characterized by lymphopenia, thrombocytopenia, coagulopathy.[4]

Cell ratios including Neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR), lymphocyte to monocyte ratio (LMR) can be easily determined through a CBC report and have been shown to be useful prognostic marker. Raised NLR, Raised PLR and decreased value of LMR are indicators of severe disease and bad prognosis.[6-11]

In this study we wanted to correlate CBC and peripheral smear findings with this disease. Our aim was to determine frequency of LE picture in COVID-19 patients and its relation with severity of disease. We desired to compare complete blood counts and cell ratios (NLR, PLR, LMR) of positive confirmed COVID-19 patients with suspected COVID-19 patients to see whether these can be beneficial in adding to diagnostic and treatment criteria. We also wanted to study the association of these parameters with severity on basis of severity stratification by clinical status of patients in both groups.

MATERIAL AND METHODS

A Retrospective Cross sectional was carried out at King Edward Medical University, Mayo Hospital Lahore from December 2020 to January 2021. Approval was obtained from the Ethical Review Board of King Edward Medical University (Institutional Review Board Approval letter no: 196/RC/KEMU).

During this study, 150 patients in total were included in study, 75 COVID-19 PCR positive and 75 suspected patients. Patients above 18 years of age and of either sex were included. 75 COVID-19 PCR positive patients and 75 suspected patients were studied. Patients with known Hematological disorders (Acute or chronic Leukemia, Myeloproliferative neoplasms) were excluded from the study.

Epidemiological data of both groups including age and sex, clinical data including symptoms at presentation and severity status of COVID-19 was classified by mode of admission as patients with mild disease in wards/rooms, moderate in high dependency units (HDU), severe/critical in Intensive care unit (ICU) and the choice of oxygen depend on patient status and its availability (whether on oxygen, nasal catheter, NRM, at CPAP or at ventilator) was collected. CBC samples of all patients of both groups was taken and analyzed for counts, differential and LE picture on Sysmex XE-1000 automatic blood analyzer. NLR, LMR and PLR in confirmed and suspected patients is included. Peripheral smear was stained with field Giemsa stain and examined under microscope. Frequency of leukoerythroblastic picture in confirmed, suspected patients and its association with severity of disease was noted.

Data was stratified according to age, gender, severity of disease, suspected and confirmed cases for COVID-19. Haematological parameters were compared between confirmed and suspected cases as well as in subgroups on basis of clinical status. Quantitative variables WBC, HB, platelets, ANC (absolute neutrophil count), ALC (absolute lymphocyte count), NLR and LMR were analyzed by mean and standard deviation in confirmed and suspected cases, while median (IQR) interquartile range is calculated mild, moderate and severely ill groups. Categorical variables like gender, status and severity of patients, frequency of leukoerythroblastic (LE) picture in each group by frequency and percentage. Continuous variables were analyzed by independent t-test in both groups and Kruskal Wallis test in severity based groups, while categorical variables by Chi-square test.

Statistical analysis:
Data was analyzed in SPSS version 25. p-value ≤ 0.05 is significant.

RESULTS

Each group consisted of (75) patients, with 75% male predominance as compared to 25% females.
Mean age of patients(yrs) in confirmed and suspected groups were 55±11, 58±14 and 61±15 respectively in both groups. Frequency (%) of patients on the basis of clinical status, indoor location and treatment instituted in confirmed and suspected cases is presented in Table-1. Mean±SD of hematological parameters were determined in confirmed and suspected groups in Table-2. These were analyzed by independent t-test for significance.

Groups		Status					total	P value
CON	severity	FM	Oxy	CPAP	NRM	Ven	75	.000
	ICU	0	16	4	1	6	27	
	HDU	5	35	0	1	1	41	
	WARD	5	2	0	0	0	7	
SUS	ICU	0	23	7	5	3	17	.000
	HDU	2	33	0	5	0	40	
	WARD	8	12	0	0	0	18	

P: (.000) significant
Table-1: Subgrouping of Confirmed/Suspected COVID 19 cases as per Clinical *severity *status

Mild: In ward/isolated group Moderate: In HDU (high dependency unit) Severe/critical: ICUFM: facemask, Oxy: oxygen, ven: ventilator

Groups	Parameters	Values	p-value
Confirmed	Age	55±11	0.4
suspected		58±14	
Confirmed	HB	13.8±7.5	
Suspected		13.4±7.09	0.75
Confirmed	WBC	16.1±6.4	
Suspected		14.9±8.2	.31
Confirmed	PLR	232.2±127	.16
Suspected		260±121	
Confirmed		17±17	
Suspected	ANC	12.1±6.5	.022*
Confirmed	ALC	6.2±19	
Suspected		1.3±.1.7	.032*
mono confirmed	Mono	3.7±2.1	.61
Suspected		2.6±2	
NLR confirmed	NLR	35.4±43	.002*
Suspected		18±14.7	
Confirmed	LMR	3.3±11	
Suspected		2.6±2.5	.07
Confirmed	PLR	80±135	.07
Suspected		49±52	

Table-2: Hematological parameters according in confirmed and suspected groups.

Haematological parameters and ratios (NLR, LMR, PLR) according to severity stratification are shown in Table-3. Median (IQR) of hematological parameters were determined according to severity stratification Their association were analyzed by independent-sample Kruskal Wallis Test, while frequency of leukoerythroblastic picture in suspected and confirmed group was observed 1 (1%) in confirmed and 3 (2%) in suspected cases (p-value: 0.12) was found to be insignificant by Chi-square test.

Severity	Wards/ isolated Rooms (mild)	HDU (moderate)	ICU Sever/ critical)	p-value
Hb (g/dl) Median (IQR)	9.5(15.2)	12(3.8)	13.9(2.6)	.055*
WBC (X 109/L) Median (IQR)	14(7.9)	14.9(10.2)	18.5(7.2)	.000*
PLT X (109/L) Median (IQR)	304(252)	206(111)	204(168)	.008*
ANC X 109/L) Median (IQR)	11(5.7)	13(10)	14.7(9.93)	.000*
ALC X 10^3/UL) Median(IQR)	1.4(1.05)	0.6(0.7)	0.6(.8)	.695*
NLR X 10^3/UL) Median (IQR)	8.6(16.8)	18(43.8)	30(23.6)	.000*
LMR Median (IQR)	2(0.6)	1(1.2)	1.3(0.84)	.016*
PLR Median (IQR)	45(80)	35(96)	35(110)	.094*
Total	25	81	44	.028^

The significance level is 0.05
* (Krusal independent test), ^ (Chi square test)
Table-3: Hematological parameters according to severity of COVID-19

DISCUSSION

In our study on 150 patients, majority of patients were males (72% in confirmed and 65% in suspected group). Male predominance in COVID-19 patients has also been seen in studies by Asgher, Usul etal.[12] No significant difference in severity for gender difference was found in our study however studies by Taj etal,[13] Terpos etal[4] showed male gender a risk factor for severity of disease. This difference can be due to different time period as their study was based on data obtained in first wave whereas our data is based on 3rd wave.
Mean age was above 50 years in mild, moderate and severe condition in both groups, and age was not risk factor for severity in our study (P value:0.19). The study by Usul etal also had similar findings while

studies by Taj S. etal [13] and Terpos etal [4] showed that older age is risk factor for severity of disease. Old age was associated with increased mortality in first wave while in second wave patients of all age groups are seen.[12,13,4]

Mode of admission in our study was mainly HDU and ICU (78.7% in confirmed and 89% in suspected group (p-value .000). In contrast to our study, Asgher etal showed in their study that most of the patients presented with mild to moderate symptoms so admitted in isolation wards (60%) while rest of patients (30%) were admitted in ICU. Since Mayo hospital is biggest hospital in government setup with extensive ICU facilities and patients are referred here so mode of admission in our study was HDU and ICU predominantly.

Our study was aimed to find frequency of LE picture in COVID-19 (confirmed and suspected) patients. It was seen in 4 patients (2.7%). Precursors of leukocytes are reported in a study in Covid -19 patients and seen mostly in patients developing DIC. LE picture has not been reported elsewhere and is found to be insignificant in our study also. This negative association is important as it rules out LE picture association with COVID-19 infection and when present is mostly due to causes other than this infection. Since according to exclusion criteria all other causes have been excluded, presence of LE picture in few patients indicated stress on bone marrow and was not associated specifically with this virus infection.

Current study showed no significant difference for HB, WBC, platelet and monocyte counts on CBC in comparison of suspected with confirmed group.

Decreased Lymphocyte count (lymphopenia) was observed in all patients as depicted by low ALC. It was significant when compared in confirmed group and suspected groups. LMR is not clinically significant different in suspected and confirmed groups. The probable reason can be that lymphopenia develops progressively in suspected group and becomes stable in confirmed group.

In this study on suspected and confirmed COVID-19 has not showed significant difference on PLR, it can be due to observation that platelet count remained in normal range and was not associated with disease severity. S. Blomme showed no relation of thrombocytopenia with disease and it remained normal in range in COVID-19. [17]

This is similar to study conducted by Taj S etal. In contrast to our study Asgher M S etal, showed high PLR in severe cases as compared to recovered patients and isolation ward.[13,14] Another study conducted by Liao D etal revealed significant thrombocytopenia in severe disease.[16] So the platelet can remain normal, low and high in COVID-19 as different strains of virus are evolving in different waves. However PLR is not good prognostic marker.

Our data showed leukocytosis, neutrophilia and increased NLR in confirmed group as compared to suspected group. As it is hypothesized that Covid -19 has effect on T-cell lymphocytes and leads to reduction of lymphocytes resulting in high NLR in confirmed group. As evidence suggest that PCR test remaining negative within 4-5 days after symptoms is due to low viral load, it can be the cause of above findings.[15]

In summary the hematological parameters do not differ much in confirmed and suspected group except for NLR. So management on strong clinical suspicion of Covid 19 disease will be beneficial and is being practiced.

Our study showed, hemoglobin was slightly high (≥13 g/dl) in severe/critical cases of COVID-19 positive patients than moderate and mild cases (≤13 g/dl) (p value 0 .05). This difference may be due to presence of co morbidities, dehydration, smoking habits and varies from patient to patient or due to hypoxia induced compensated erythrocytosis. Similar findings were seen in study by Usul E etal.[12] In contrast to our results a study was by S Blomme etal showed mild anemia but no statically significant difference in sub groups on the basis of severity.[17] Taj etal showed no association of anemia Hb, MCV, HCt parameters with severity of COVID-19. [13].Whereas Fagihdinvari etal found an association of anaemia and outcome of COVID 19. [18] In the present study LMR was significantly correlated for disease severity (p value 0.016). And it is poor prognostic marker as shown by Asgher MS.[14] In addition to above ratios the most significant poor prognostic marker was NLR due to leukocytosis with neutrophilia on differential. This fact has been proved by number of studies. Ahmed MAS et al showed leukocytosis, high neutrophil to lymphocyte ratio in critically ill (ICU) patients when compared with patients in isolation wards. Taj S et al also observed leukocytosis, high Neutrophil to lymphocyte ratio in critical COVID-19 patients. Pervaiz A, Pasha U, et al observed that that NLR in patients with COVID-19 is predictor of mechanical ventilation.[19]

CONCLUSION

Leukoerythroblastic picture is not associated with severity in COVID-19. NLR is important independent prognostic factor in suspected and confirmed COVID-19 patients.

Limitations:

This study had limitations. Firstly, this was a retrospective study, secondly, we had no data of death and recovery. Finally, radiological findings, and
comorbidities like hypertension, diabetes were not included.

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