

EVALUATION OF BONE MARROW & PERIPHERAL BLOOD SMEAR FINDINGS IN SARS-COV-2 PATIENTS, CENTRAL INDIA

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ABSTRACT

This study was done to assess the morphological changes in peripheral blood smears during COVID-19 infection. We aimed to examine the characteristics of the cells detected in the peripheral blood smear and bone marrow at the time of diagnosis in COVID-19 patients. Clinical features, laboratory data, peripheral blood smear of 35 patients diagnosed with COVID-19 by PCR was evaluated at diagnosis. Peripheral smear samples of the patients were compared with the age and sex-matched 35 healthy controls. The relationship between the laboratory values of all patients and the duration of hospitalization was analyzed. Peripheral smear shows neutrophilic leucocytosis, lymphopenia and thrombocytopenia while Bone marrows were normocellular to hypercellular, most showing maturing trilineage hematopoiesis with myeloid left shift. In 09 out of 35 evaluable bone marrows, hemophagocytic histiocytes were identified.

Keywords: Covid-19, Bone marrow, Hemophagocytosis, Lymphopenia. Peripheral smear

INTRODUCTION:

Corona virus disease 2019 (COVID-19) is pandemic affecting millions of people caused by the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2). It is a RNA virus belonging to the family Coronaviridae. ^{1, 2, 3}Coronaviruses causes cytokine storm due to which bone marrow progenitor cells are destroyed resulting in abnormal hematopoiesis ⁴. Viral infection is well known to be associated with abnormal hematological parameters. Viral infections can change the cell numbers and morphology in the peripheral blood smear. In addition to changes

like leukocytosis, leucopenia, neutrophilia, neutropenia, monocytosis, monocytopenia, lymphocytosis, lymphopenia, thrombocytopenia, it can also change the morphology of the blood cells like atypical lymphocyte, inclusion bodies, vacuolization. Autopsy of patients who died of COVID-19 showed markedly shrunken spleen with reduced lymphocytes, macrophages proliferation, and phagocytosis⁵. In addition, studies have shown that due to cytokine storm caused by viruses' bone marrow progenitor cells are destroyed that causes decreased primary platelet production which leads to thrombocytopenia.⁶ Also increased formation of immune complexes causes platelet destruction which ultimately leads to thrombocytopenia. Also, lung injury causes platelet activation, aggregation, microthrombus formation due to this platelet consumption increased and there is decreased circulating platelet.

Evidence⁷ has shown that virus causes lung injury resulting in fibrotic changes in lungs, also pulmonary capillaries get damaged causes megakaryocyte rupture leads to reduced platelet synthesis in systemic circulation. Recent studies discussing the hyper inflammatory state of patients who test positive for COVID-19 have noted an over production of cytokines, which may correlate with secondary hemophagocytic lymphohistiocytosis (HLH).^{8,9} Understanding changes in the bone marrow of affected individuals is of particular importance given these clinical concerns about HLH. In this study, we describe the hematologic findings in the bone marrow and peripheral smear of 20 patients of COVID-19. Hemophagocytic lymphohistiocytosis (HLH) is a rare life-threatening syndrome that occurs secondary to severe systemic immune activation. Cytotoxic T-cell proliferation leads to increased cytokine production and activation of tissue resident macrophages.¹⁰ Hemophagocytic lymphohistiocytosis presents abruptly over a period of several days to weeks with a consistent pattern of fever, pancytopenia, and splenomegaly. Common laboratory abnormalities include hyperferritinemia, hypofibrinogenemia, hypertriglyceridemia, elevated soluble IL-2 receptor, and abnormal liver function tests.¹¹

MATERIAL AND METHOD

Type of study- Retrospective study

Setting- This study was done in Department of Pathology at tertiary care center.

Duration of Study- The total duration of study was 12 months from Feb 2020 to Feb 2021.

Inclusion criteria: All the confirmed covid positive cases admitted in the hospital during the study period were included in the study.

Exclusion criteria: Patients suspected of covid but reports not confirmed or inconclusive.

Data collection procedure: peripheral blood reports and bone marrow examination reports of all the Covid confirmed case was retrieved from the records without revealing the identity of the patient.

The peripheral smears and bone marrow smears examined by the pathologist was observed and their findings are recorded in Microsoft excel sheet. The peripheral smear was evaluated for hemoglobin, total WBC count, differential count, platelet count and morphology.

The bone marrow was evaluated for overall cellularity; myeloid-erythroid ratio; overall appearance of myeloid cells, erythroid cells, and megakaryocytes; presence and extent of hemophagocytosis; and any additional findings such as the presence of lymphoid aggregates.

Data Analysis- Data was analyzed with SPSS statistical package for social science version16 and different parameters of measurements were derived.

Ethical Consideration and Permission: Ethical approval was obtained from institutional ethics committee. The confidentiality of the patient was not breached at any point as only reports of all covid cases were collected and patient identity was not known to either investigator.

RESULTS

Peripheral blood and bone marrow from 35 patients who tested positive for COVID-19 were evaluated. There were 16 men and 19 women, with an age range of 40-80 (66.4 ± 11.5) The cohort showed the following comorbidities: Severe pneumonia in 74.2%, 80% with hypertension, 50% with diabetes, 25% with asthma, and 15% with immunosuppression (renal transplant, methotrexate use for psoriasis, and chronic prednisone use for sarcoidosis).

In peripheral blood examination: 17 out of 35 patients showing Leucocytosis (48.5%), 16 out of 35 patients showing Thrombocytopenia (45.7%), 20 out of 35 patients showing Lymphopenia (57.14%), 18 out of 35 patients showing neutrophilia (51.4%) . Bone marrow of these 35 patients are normoblastic to megaloblastic, M:E ratio is within normal range, all myeloid , erythroid, megakaryocyte series is within normal range, plasma cells are within normal limits. No significant findings seen in bone marrow.

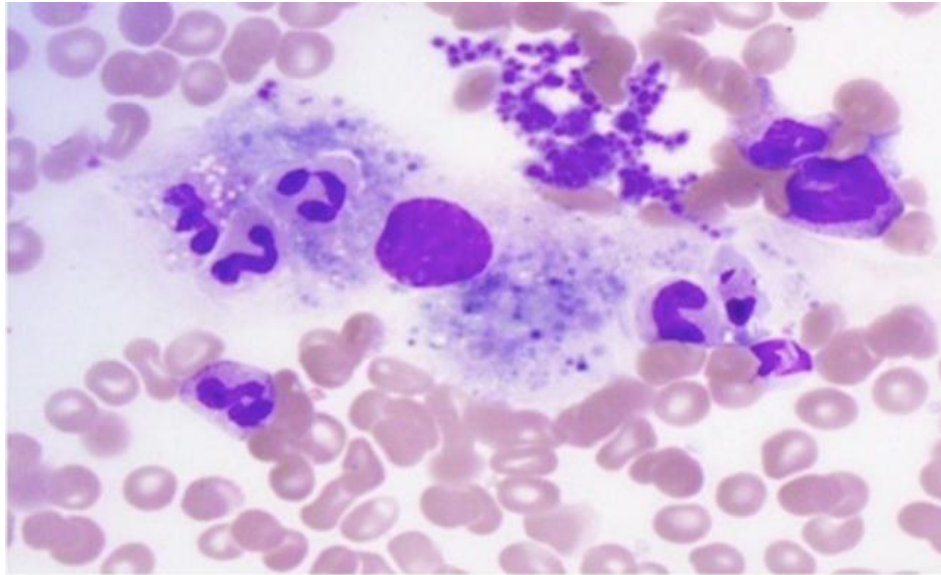
Table 1: Showing peripheral smear findings of covid-19 patients

Peripheral smear findings	Total patients (35)	Frequency (%)
Leucocytosis	17	48.5%
Thrombocytopenia	16	45.7%
Lymphopenia	8	51.4%
Neutrophilia	18	51.4%
Hemophagocytosis	09	25.7%

Table 2: Baseline Characteristics of Covid-19 Cases (N=35):

Variables		Findings
Age, (Mean \pm 2SD) in years		66.4 \pm 11.5
Sex	Male, no. (%)	16 (45.7%)
	Female, no. (%)	19 (54.2%)
Comorbidities, no. (%)	Present	29 (82.8%)
	Absent	6 (17.1%)
Pneumonia, no. (%)	Mild	0 (0 %)
	Moderate	9(25.7%)
	Severe	26 (74.2%)
CT Score, no. (%)	1-10	4 (11.4%)
	11-20	19 (54.2%)
	>20	12(34.2%)
Inflammatory Markers (Mean \pm 2SD)	IL-6 pg/mL	37 \pm 2
	D-dimer, mcg/ml	5.4 \pm 2.4
	Hs-CRP, mg/L	4.2 \pm 1.2
	Ferritin, μ g/L	457 \pm 13
Blood Picture (Mean \pm 2SD)	Haemoglobin, g/dl	11.8 \pm 2.4
	WBC \times 103/ml	16.2 \pm 2.45
	Neutrophils \times 103/ml	11.89 \pm 2.6
	Lymphocytes \times 103/ml	1.62 \pm 6.7
	Platelets \times 103/ml	258.8 \pm 94.55

Figure 1: Peripheral smear showing lymphopenia and thrombocytopenia

Figure 2: Bone marrow smear showing hemophagocytosis**DISCUSSION**

In this study of the peripheral blood report and bone marrow report of 35 patients who was COVID-19 positive was observed. It was found that 17 out of 35 patients showing Leucocytosis (35%), 16 out of 35 patients showing Thrombocytopenia (45.7%), 20 out of 35 patients showing Lymphopenia (57.14%), 18 out of 35 patients showing neutrophilia (51.4%). Similar observations were made by Mo P, Xing Y, Xiao Y, et al. Arentz M, Yim E, Klaff L, et al, Huang C, Wang Y, Li X, et al. and Guan WJ, Ni ZY, Hu Y, et al.¹³⁻¹⁶ in their studies. Common hematologic abnormalities included neutrophilic leukocytosis, lymphopenia and thrombocytopenia; the anemia and thrombocytopenia and Lymphopenia is commonly associated with COVID-19,¹³⁻¹⁶ with more pronounced lymphopenia associated with more severe COVID-19 disease.^{17,18} The lymphopenia may be secondary to the cytokine storm induced by SARS-CoV-2. In addition, lymphocytes are reported to express ACE2 receptors on their surface and may become directly infected and killed by SARS-CoV-2.^{19,20}

In a study of US lymphopenia was present in around 60% of patients on initial laboratory tests³¹. Lymphopenia was observed in studies done in on Singapore and Korea^{32,33}. In this study 45.7% patients showing thrombocytopenia with is also seen in the multicentric study by Guan et al.²⁸⁻³⁰

In this study we examined the bone marrow findings of covid positive patients, We looked for Hemophagocytosis in bone marrow of patients infected by covid 19 as found in studies done by Andrey Prilutskiy, Eric Gars, Khaldoun Kuteifan^{21,22,23} But in this study we didn't get the Hemophagocytosis in bone marrow of covid positive patients. In this study bone marrow of all 35 patients are Normoblastic to megaloblastic, having normal M: E ratio, no hemophagocytosis seen. Our findings are similar to the study done by Andrey Prilutskiy,²⁴ In their study the Hemophagocytosis was found in spleen and liver. No hemophagocytosis was found in Bone marrow.

CONCLUSION

Peripheral blood with COVID-19 frequently showed neutrophilic leukocytosis, lymphopenia, and thrombocytopenia. Hemophagocytosis is found in bone marrow; Consistent with other studies, we find histopathological evidence of HLH development in patients with covid19. From this study it is concluded that the covid 19 infection affects the peripheral blood findings, it causes leukocytosis, lymphopenia and thrombocytopenia, and hemophagocytosis in bone marrow.

Source of finding: none

Conflicts of interest: none

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