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Hemophagocytic histiocytosis in severe SARS-CoV-2 infection: A bone marrow study

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Abstract

Introduction: The clinical and laboratory features of severe COVID-19 infection overlap with those of hemophagocytic lymphohistiocytosis (HLH), a hyperinflammatory disorder often associated with several viral infections. The clinical syndrome of HLH encompasses fever, organomegaly, cytopenias, hyperferritinemia, hypertriglyceridemia, raised transaminases, hypofibrinogenemia, absent natural killer (NK) cell activity, increased soluble CD25 and hemophagocytic lymphohistiocytosis in bone marrow, spleen, and lymph nodes.

Methods: We analyzed clinicopathological and laboratory features of thirteen patients with severe COVID-19 infection suspected to have HLH and found to have hemophagocytic histiocytosis on bone marrow examination (BME).

Results: Five of thirteen (38.46%) patients fulfilled five of eight HLH 2004 criteria and/or had a H-score ≥169. Three (23.08%) satisfied four of eight and remainder five (38.46%) satisfied three of eight HLH 2004 criteria. Fever, raised serum ferritin (13/13, 100%), transaminases (9/13, 69.23%), triglycerides (4/13, 30.76%), cytopenias (5/13, 38.46%), hypofibrinogenemia (2/13, 15.38%), and organomegaly (1/13, 7.69%) were observed in our patients. BME showed hemophagocytic histiocytosis without lymphocytosis in all. Contrary to HLH, lymphocytopenia (11/13, 84.61%), leukocytosis (7/13, 53.84%), neutrophilia (7/13, 53.84%), and hyperfibrinogenemia (7/13, 53.84%) were observed. Serum CRP, LDH, and plasma D-dimer were elevated in all, while serum albumin was decreased in 12 of 13 (92.3%) patients. Five patients recovered with high-dose pulsed corticosteroid therapy.

Conclusion: The immune response associated with severe COVID-19 infection is similar to HLH with few differences. HLH should be suspected in severe COVID-19 infection although all patients may not fulfill required HLH diagnostic criteria. BME should be done in suspected cases so that appropriate therapy may be initiated early.

KEYWORDS

bone marrow examination, Hemophagocytic lymphohistiocytosis, hemophagocytosis, histiocytosis, SARS-CoV-2

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19), a disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a pandemic with massive disease burden. As of November 10, 2020, COVID-19 has been confirmed in 51.5 million people worldwide, with a mortality rate of approximately 3.4%. In India, 8.64 million confirmed cases have been recorded with an estimated mortality of 1.6% as of November 10, 2020.[1,2] It commonly presents with fever, cough, dyspnea, and myalgia. Although the majority of patients with COVID-19 have mild symptoms, some progress to serious outcomes including pneumonia, acute respiratory distress syndrome (ARDS), multiorgan failure and even death.[3] Some of the serious patients admitted in the intensive care unit (ICU) have clinical and laboratory features mimicking hemophagocytic lymphohistiocytosis (HLH) a condition characterized by a cytokine storm with severe life-threatening hyperinflammation.[4-6] The early identification of this HLH-like picture is crucial for the management of these patients. In this study, we evaluated clinicopathological and laboratory parameters in thirteen patients with serious SARS-CoV-2 infection who underwent bone marrow examination (BME) for suspected HLH based on clinical and laboratory parameters and were found to have hemophagocytic histiocytosis on BME.

2 | MATERIALS AND METHODS

This study included thirteen SARS-CoV-2 infected patients who turned out negative for SARS-CoV-2 with due course of time but still had severe respiratory distress and were in the ICU. All patients had clinical features and laboratory findings partially overlapping with HLH 2004 diagnostic criteria and/or the H-score which are used for the diagnosis of HLH (Table 1).[7,8] All patients underwent BME which included bone marrow aspiration (BMA) and bone marrow biopsy (BMB) and were found to have hemophagocytic histiocytosis on BMA and BMB.

The demographic details (age, sex), date of onset of symptoms and date of admission of each patient were recorded. Clinical findings of each patient including fever, sore throat, cough, organomegaly, and respiratory distress were recorded from history obtained from the patient's relatives and from hospital records. Laboratory investigations done in all patients included complete blood count, serum ferritin, C-reactive protein (CRP), aspartate transferase (AST), alanine transferase (ALT), lactate dehydrogenase (LDH), albumin, triglycerides and creatinine, plasma fibrinogen and D-dimer and screening for viral infections including hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and herpes simplex virus

TABLE 1 Diagnostic criteria for hemophagocytic lymphohistiocytosis (HLH). Adapted from Henter et al[7] and Fardet et al[8]

HLH 2004 criteria (5 of the 8 criteria below are required for a diagnosis of HLH)[7]

Clinical variables	Biochemical variables	Cytological variables	Other variables
• Fever ≥38.5°C	• Serum triglycerides >265 mg/dL and/or • Plasma fibrinogen <150 mg/dL	• Cytopenia in ≥2 series ○ Hemoglobin <9 g/dL ○ Platelet count $<100 \times 10^9$/L ○ Absolute neutrophil count $<1 \times 10^9$/L	• Low or absent NK cell activity
• Splenomegaly	• Serum ferritin >500 ng/mL	• Hemophagocytosis in bone marrow, spleen, lymph nodes, liver	• Soluble CD25 (soluble interleukin-2 receptor) > 2400 U/mL

H-score (H-score values ≥169 favors a diagnosis of HLH[8]

Clinical variables	Biochemical variables	Cytological variables	Other variables
• Immunosuppression ○ Absent: 0 points ○ Present: 18 points	• Serum triglycerides (mmol/L) ○ <1.5: 0 points ○ 1.5-4.0: 44 points ○ 4.0: 64 points • or plasma fibrinogen (g/L) ○ 2.5: 0 points ○ ≤2.5: 30 points	• Cytopenia ○ Single series: 0 points ○ Two series: 24 points ○ Three series: 34 points	
• Fever (°C) ○ <38.4: 0 points ○ 38.4-39.4: 33 points ○ >39.4: 49 points	• Ferritin ng/mL ○ <2000: 0 points ○ 2000-6000: 35 points ○ >6000: 50 points	• Hemophagocytosis in the bone marrow ○ Absent: 0 points ○ Present: 35 points	
• Splenomegaly ○ Absent: 0 points ○ Hepatomegaly or splenomegaly: 23 points ○ Hepatomegaly and splenomegaly: 38 points	• Serum AST IU/L ○ <30: 0 points ○ ≥30: 19 points		

(HSV). Prior to BME, all patients had at least four of the following mentioned features (fever, organomegaly, hypertriglyceridemia, hypofibrinogenemia, hyperferritinemia, raised transaminases, and cytopenias). All patients had at least one chest X-ray and CT scan done during the period of admission in the ICU.

All patients underwent BME after obtaining written, informed consent from their close relatives. BMA and BMB were done from the posterior superior iliac spine or the anterior superior iliac spine, under aseptic precautions while using personal protective equipment including N-95 mask. 16-gauge Salah needle and 11-gauge Jamshidi needle were used for obtaining BMA and BMB specimens, respectively. Smears were made from BMA specimens and were stained with Leishman stain. BMB specimens were fixed in 10% buffered formalin; decalcified in EDTA for 48 hours, and paraffin embedded using standard procedures. Sections (4 μm thick) were made from paraffin-embedded BMB specimens and stained separately with hematoxylin and eosin to assess morphology and with immunohistochemical stain (monoclonal mouse anti-human anti-CD68 antibody (clone PG-M1, M0876, Dako, Agilent) at a dilution of 1:200) for identification of histiocytes.

We used the method described by Ho et al for quantification of hemophagocytosis in BMA smears.[9] For each case, two BMA smears were examined initially at low power magnification (40×) for the detection of histiocytes, followed by counting of hemophagocytes per slide at high power magnification (1000×). The average number of hemophagocytes per slide was estimated by dividing the total number of hemophagocytes observed in both the slides by two. Only those histiocytes showing engulfment of intact nucleated red cells, neutrophils, granulocytic precursors, lymphocytes, or plasma cells were counted. BMB sections in each patient were examined for cellularity, presence of hemopoietic precursors and hemophagocytes. The study was approved by the Institutional Ethics Committee.

Statistical analysis was done using Statistical Package for Social Sciences, version 23 (SPSS-23, IBM, Chicago, USA). The intergroup data were compared using the independent sample *t* test for parametric and the Mann-Whitney *U* test for nonparametric distributions.

3 | RESULTS

3.1 | Demography, baseline clinical characteristics and co-morbidities

The study included seven males and six females with age ranging from forty-one to seventy-four years. Fever was present in all thirteen patients. Organomegaly (hepatomegaly) was seen in only one patient. Nine (69.23%) patients had chronic underlying health conditions. Six (46.15%) patients had hypertension, four (30.76%) patients had diabetes and coronary artery disease and one patient had bronchial asthma. All patients had varying extent of bilateral ground glass opacities and/or consolidation in predominantly peripheral and basal locations on their chest radiographs and CT scans.

3.2 | Laboratory characteristics including BME findings

The mean serum triglycerides (mg/dL) was 248.30 ± 162.20 (median = 219, range = 79-634). Serum triglycerides was increased in 30.76% (4/13) patients. The mean plasma fibrinogen (mg/dL) was 389.07 ± 164.21 (median = 422, range = 46-673). Hypofibrinogenemia was seen in 15.38% (2/13) patients while hyperfibrinogenemia was observed in 53.84% (7/13) patients. The mean serum ferritin (ng/mL), CRP (mg/L), LDH (IU/L), and plasma D-dimer (ng/mL) were 6544.23 ± 16 157.66 (median = 1429, range = 795-60 000), 105.40 ± 109.89 (median = 50, range = 10.4-345), 1693.84 ± 1634.82 (median = 1319, range = 625-7046), and 2219.38 ± 995.75 (median = 1892, range = 826-4124), respectively. Serum ferritin, CRP, LDH, and plasma D-dimer were elevated in all patients. The mean serum AST (IU/L) and ALT (IU/L) were 557.24 ± 1265.09 (median = 63.59, range = 27-4556) and 395.11 ± 804.65 (median = 82.00, range = 22-2866), respectively. Liver transaminases were elevated in 69% (9/13) patients. The mean serum albumin (g/dL) was 2.79 ± 0.59 (median = 2.83, range = 1.32-3.7). Hypoalbuminemia was observed in 92.31% (12/13) patients. The mean serum creatinine (mg/dL) was 1.17 ± 0.53 (median = 1.0, range = 0.6-2.29). Serum creatinine was elevated in 23.07% (3/13) patients. The mean hemoglobin (g/dL) was 10.04 ± 2.63 (median = 10.5, range = 6.4-14.4). Hemoglobin was decreased in 76.92% (10/13) patients. The mean total leucocyte count (TLC) (/mm^3) and absolute neutrophil count (ANC) (/mm^3) were 11 560.84 ± 7206.63 (median = 12 600, range = 691-22 900) and 10 227.07 ± 6693.16 (median = 9108, range = 476-21 526), respectively. TLC and ANC were decreased in 15.38% (2/13) patients and increased in 53.84% (7/13) patients. The mean absolute lymphocyte count (ALC) (/mm^3) was 887.84 ± 938.29 (median = 600, range = 126-3588). ALC was decreased in 84.61% (11/13) patients. The mean Neutrophil/lymphocyte (N/L) ratio was 19.33 ± 24.30 (median = 12.6, range = 1.39-95). N/L ratio was increased in 84.61% (11/13) patients. The mean platelet count (/mm^3) was $1.5 \times 10^5 \pm 0.96 \times 10^5$ (median = 1.5×10^5, range = 0.10×10^5-3.5×10^5). Platelet counts were decreased in 46.15% (6/13) patients. Cytopenias in ≥2 lineages (chiefly anemia, thrombocytopenia, and neutropenia) were present in 38.46% (5/13) patients. Soluble CD25 assay and natural killer (NK) cell activity were not done in our cases as these tests were not available in our Institute. All patients were negative for viral markers for HBV, HCV, HIV, CMV, EBV, and HSV by real time polymerase chain reaction. Bone marrow was hypercellular in ten patients and paucicellular in three patients. Megakaryocytes were adequate in all patients. Increased granulocytic precursors were observed in eleven patients, and erythroid hyperplasia was observed in two patients. All patients had hemophagocytic histiocytosis with engulfment of intact erythroid precursors, neutrophils, granulocytic precursors and occasionally lymphocytes and plasma cells (Figure 1). The mean number of hemophagocytes per slide was 4.23 ± 1.64 (median = 4, range = 2-7).

Five of thirteen (38.46%) patients satisfied ≥5/8 HLH 2004 criteria and/or had H-score ≥169. Of these five patients, three patients

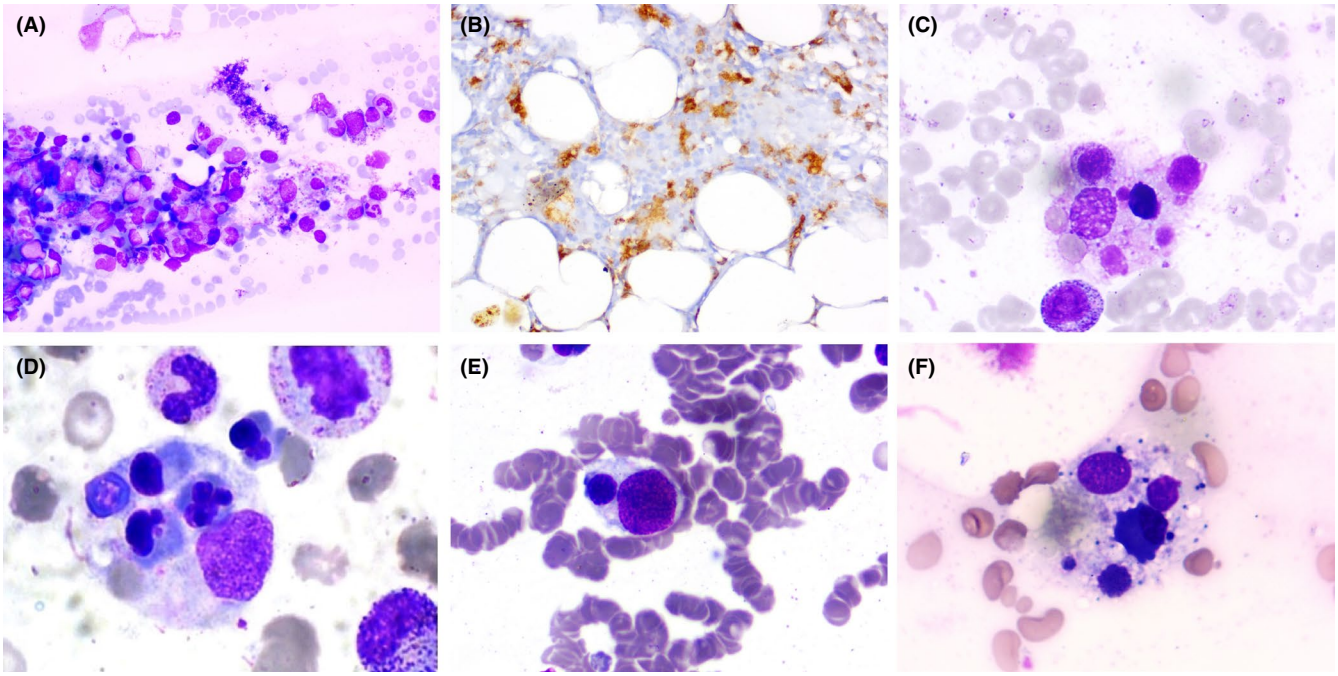

FIGURE 1 Bone marrow examination in a patient with severe COVID-19 infection. A, Bone marrow aspirate (BMA) smear showing histiocytic hyperplasia (Leishman stain 40×). B, CD68 Immunohistochemical staining on bone marrow biopsy highlighting increased number of histiocytes with engulfed nuclei (CD68, 400×). C, BMA smear showing a macrophage with engulfed myelocyte, platelet, and normoblast (Leishman stain, 1000×). D, BMA smear showing hemophagocytosis with engulfment of multiple normoblasts (Leishman stain 1000×). E, BMA smear showing a histiocyte with a well-engulfed normoblast (Leishman stain 1000×). F, BMA smear showing a histiocyte with hemophagocytosis of a lymphocyte and a plasma cell (Leishman stain 1000×)

fulfilled ≥5/8 HLH 2004 criteria, four patients had a H-score ≥169 and two patients satisfied both HLH diagnostic criteria. Of the eight patients who could not be classified to have HLH by either of the two criteria, three patients (23.08%, n = 3/13) fulfilled 4/8 HLH 2004 criteria and five patients (38.46%, n = 5/13) fulfilled 3/8 HLH 2004 criteria. All patients received high flow nasal oxygen (40 L/min to maintain a FiO_2 of 100), intravenous steroid therapy (Dexamethasone 6 mg/day), broad-spectrum antibiotics, and low molecular weight heparin as part of the institutional COVID-19 ICU treatment protocol. On finding hemophagocytic histiocytosis on BME, all patients were shifted to high-dose intravenous pulsed steroid therapy (Methyl Prednisolone 500 mg/day). Two patients were also administered intravenous immunoglobulin G. Eight patients required ventilatory support and succumbed to the illness. Of the five patients who survived, one patient required noninvasive ventilation. Demographic details, clinical features, laboratory findings, findings on BME, outcome and cause of death of patients in our study are enumerated in Table 2.

3.3 | Comparative data between patient groups (patients satisfying ≥4/8 vs patients satisfying <4/8 HLH 2004 criteria) and (patients with H-score ≥163 vs patients with H-score <163)

Although all patients in our study had bone marrow hemophagocytic histiocytosis, only five of thirteen patients satisfied ≥5/8 HLH 2004 criteria and/or had H-score ≥169. We compared the laboratory parameters of patients in our series who fulfilled ≥4/8 HLH 2004 criteria vs those who satisfied <4/8 HLH 2004 criteria and patients in our series who had H-score ≥163 vs those with H-score <163 to determine whether the association of hemophagocytic histiocytosis with these groups was incidental or not. No statistically significant differences were observed between the compared groups (Table 3).

4 | DISCUSSION

Hemophagocytic lymphohistiocytosis is a rapidly progressive often fatal hyperinflammatory systemic disorder characterized by excessive cytokine release and multisystem involvement. It can be primary or genetic and secondary or acquired.[10] Acquired HLH is associated with viral infections, malignancies, autoimmune disease, and allogenic hematopoietic stem cell transplantation.[11] The diagnosis of HLH is made when ≥5/8 HLH 2004 criteria are fulfilled, or the H-score is ≥169. Histopathological examination of reticuloendothelial organs (bone marrow, liver, spleen, lymph node) shows lymphocytosis and histiocytosis with hemophagocytosis.[12] The hyperinflammatory immune response in severe SARS-CoV-2 infection has the lung at its epicenter and is characterized by fever, ARDS, and systemic tissue damage involving particularly the liver, cardiovascular system, and kidneys.[13] A number of variables of the severe SARS-CoV-2 immune response overlap with HLH diagnostic criteria variables including fever, hyperferritinemia, raised transaminases and

TABLE 2 Demographic details, clinical features, laboratory and bone marrow examination findings, outcome and cause of death in patients with severe COVID-19 infection

Patient No.	1	2	3	4	5	6	7	8	9	10	11	12	13	Mean ± SD Median (range)
Demographic parameters														
Age (years)	53	65	65	59	55	74	52	41	65	57	52	65	52	58.07 ± 8.58 57 (41-74)
Sex	M	M	M	M	F	M	F	M	F	F	F	M	F	-
Clinical Features														
Comorbidities	HTN	HTN, BA	DM, CAD	HTN	DM, HTN	None	None	None	CAD, HTN	DM, TB	DM, CAD, Hypo-thyroidism	DM, CAD	None	-
Fever	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Organomegaly	Nil	Nil	Nil	Nil	Hepatomegaly	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	-
O_2 support	HFNO, IV	IV	HFNO	IV	NIV	IV	IV	IV	HFNO	IV	HFNO	HFNO	IV	-
Laboratory Parameters (normal value)														
Serum Triglycerides (70-200 mg/dL)	98	512	99	294	228	129	634	252	284	187	213	79	219	248.30 ± 162.20 219 (79-634)
Plasma Fibrinogen (200-400 mg/dL)	210	168	484	395	422	439	509	371	459	499	673	383	46	389.07 ± 164.21 422 (46-634)
Serum Ferritin (11-336 ng/mL)	60 000	1200	2667	1543	795	1260	7283	1429	812	1322	3685	979	2100	6544.23 ± 16 157.66 1429 (795-60 000)
Serum AST (0-40 IU/L)	4556	58	216	27	63.59	56.56	1486	29	424	135	27	31	135	557.24 ± 1265.09 63.59 (27-4556)
Serum ALT (0-40 IU/L)	2866	82	412	37	91.88	128.55	1178	36	166	55	22	56	65	395.11 ± 804.65 82.00 (22-2866)
Hb (12-16 gm/dL)	12.7	13.7	10.8	14.4	7.3	8.8	10.5	11.9	6.5	10.5	8.2	8.9	6.4	10.04 ± 2.63 10.5 (6.4-14.4)
TLC (4000-11 000 / mm^3)	1400	12 600	8200	19 000	7500	6700	17 500	22 900	15 800	19 400	13 800	691	4800	11 560.84 ± 7206.63 12 600 (691-22 900)
ANC (1500-8000 / mm^3)	644	11 970	7708	17 290	6450	6200	16 100	21 526	14 378	16 878	9108	476	4224	10 227.07 ± 6693.16 9108 (476-21 526)
ALC (1000-4000 / mm^3)	462	126	610	950	600	300	525	916	948	1940	3588	145	432	887.84 ± 938.29 600.00 (126-3588)
Platelet count (1.5-4.0 lakhs/ mm^3)	0.10	1.6	3.5	2.0	2.4	0.79	1.9	2.6	1.5	0.50	0.95	1.2	0.50	1.5 ± 0.96 1.5 (0.10-3.5)
N/L ratio (1-3)	1.39	95	12.6	18.2	10.2	20.6	30.6	23.5	15.1	8.7	2.5	3.2	9.7	19.33 ± 24.30 12.6 (1.39-95)

(Continues)

TABLE 2 (Continued)

Patient No.	1	2	3	4	5	6	7	8	9	10	11	12	13	Mean ± SD Median (range)
Bone Marrow Examination														
Bone marrow cellularity	Pauci cellular	Hyper cellular	Hyper cellular	Pauci cellular	Hyper cellular	Hyper cellular	Hyper cellular	Hyper cellular	Hyper cellular	Hyper cellular	Hyper cellular	Hyper cellular	Hyper cellular	-
Average number of hemophagocytes per slide	2	3	5	4	3	5	5	7	2	5	4	3	7	4.23 ± 1.64 4 (2-7)
Number of HLH 2004 criteria fulfilled	5 of 8	3 of 8	3 of 8	3 of 8	3 of 8	3 of 8	4 of 8	4 of 8	4 of 8	3 of 8	5 of 8	4 of 8	5 of 8	-
H-score	177	158	138	147	170	127	197	128	128	128	163	108	198	-
Other parameters of inflammation/tissue injury evaluated														
Serum CRP (0-6 mg/L)	178	50	36	186	29	345	224	10.4	22.9	11.5	214	13.5	50	105.40 ± 109.89 50.00 (10.4-345.0)
Plasma D-dimer (<500 ng/mL)	2100	1892	1800	2726	1720	4124	1720	2899	1650	1075	4000	826	2320	2219.38 ± 995.75 1892 (826-4124)
Serum LDH (240-480 U/L)	7046	1500	625	1212	1143	1285	1319	1334	1645	1391	1104	784	1632	1693.84 ± 1634.82 1319.00 (625-7046)
Serum Albumin (3.5-5 gm/dL)	2.83	3.12	1.3	2.86	2.43	2.64	3.4	3.7	3.08	2.5	3.3	2.6	2.53	2.79 ± 0.59 2.83 (1.32-3.7)
Serum Creatinine (0.5-1.4 mg/dL)	0.74	0.81	0.6	1.34	1.19	1.36	0.74	0.85	2.14	0.78	2.29	1.42	1	1.17 ± 0.53 1.0 (0.60-2.29)
Outcome	D	D	S	D	S	D	D	D	S	D	S	S	D	-
Cause of death	MOF	RF	-	RF	-	RF	MOF	RF	-	RF	-	-	MOF	-

Abbreviations: ALC, Absolute lymphocyte count; ALT, Alanine transferase; ANC, Absolute neutrophil count; AST, aspartate transferase; BA, Bronchial asthma; CAD, Coronary artery disease, O_2, Oxygen; CRP, C-reactive protein; D, died; F, Female; Hb, Hemoglobin; HFNO, High flow nasal oxygen; HTN, Hypertension; IV, Invasive ventilation; LDH, Lactate dehydrogenase; M, Male; MOF, Multi organ failure; N/L ratio, Neutrophil/lymphocyte ratio; NIV, Noninvasive ventilation; RF, Respiratory failure; S, survived; SD, Standard deviation; TLC, Total leukocyte count.

TABLE 3 Comparison of clinical and laboratory parameters of patients in our study who satisfied ≥4/8 HLH 2004 criteria vs those who satisfied <4/8 HLH 2004 criteria and patients who had H-score ≥163 vs those with H-score <163

	Comparison of laboratory parameters in COVID-19 patients satisfying ≥4/8 HLH 2004 criteria vs those satisfying <4/8 HLH 2004 criteria			Comparison of laboratory parameters in COVID-19 patients with H-score ≥163 vs those with H-Score <163		
Parameter	Cases satisfying ≥4/8 HLH 2004 criteria (n = 7) Mean ± SD Median (range)	Cases satisfying <4/8 HLH 2004 criteria (n = 6) Mean ± SD Median (range)	*P*	Cases with H-Score ≥163 (n = 5) Mean ± SD Median (range)	Cases with H-score <163 (n = 8) Mean ± SD Median (range)	*P*
Serum Triglyceride (mg/dL)	254.14 ± 184.02 219 (79-634)	241.5 ± 149.69 207.5 (99-512)	0.945	278.40 ± 205.75 219 (98-634)	229.5 ± 140.93 219.5 (79-512)	0.943
Plasma Fibrinogen (mg/dL)	378.71 ± 203.82 383 (46-509)	401.16 ± 120.58 430.5 (168-499)	0.818	372 ± 247.17 422 (46-673)	399.75 ± 104.77 417 (168-484)	0.781
Serum Ferritin (ng/mL)	10 898 ± 21 768.80 2100 (812-60 000)	1464 ± 637.57 1291 (795-2667)	0.295	14 773 ± 25 399.38 3685 (795-60 000)	1401.5 ± 562.55 1291 (812-2667)	0.127
Serum AST (IU/L)	955 ± 1672.12 135 (27-4556)	92.69 ± 70.23 60.80 (27-216)	0.628	1253.51 ± 1944.98 135 (27-4556)	122.07 ± 138.63 57.28 (27-424)	0.354
Serum ALT (IU/L)	627 ± 1071.32 65 (22-2866)	124.57 ± 116.26 86.94 (37-353)	1	844.58 ± 1229.66 91.88 (22-2866)	114.19 ± 106.85 69.0 (36-353)	0.524
Hemoglobin (gm/dL)	9.30 ± 2.50 8.9 (6.4-12.7)	10.9 ± 2.74 10.65 (7.3-14.4)	0.29	9.02 ± 2.56 8.2 (6.4-12.7)	10.69 ± 2.63 10.65 (6.4-14.4)	0.286
TLC (cells/mm^3)	10 984 ± 8675.76 13 800 (691-22 900)	12 233 ± 5771.88 10 400 (6700-19 400)	0.77	9000 ± 6575.33 7500 (1400-17 500)	13 161.38 ± 7530.91 14 200 (691-19 400)	0.332
ANC (cells/mm^3)	9494 ± 8163.24 9108 (476-21 526)	11 083 ± 5091.06 9839 (6200-17 290)	0.688	7305.20 ± 5813.67 6450 (644-16 100)	12 053.25 ± 6897.42 13 174 (476-21 526)	0.228
ALC (cells/mm^3)	1002 ± 1174.46 525 (145-3588)	754 ± 646.52 605 (126-1940)	1	1121.40 ± 1380.37 525 (432-3588)	741.88 ± 597.59 763 (126-1940)	1
Platelet count (× 10^5/mm^3)	1.25 ± 0.84 1.2 (0.10-2.60)	1.79 ± 1.10 1.8 (0.50-3.50)	0.332	1.17 ± 0.96 0.96 (0.10-2.40)	1.71 ± 0.98 1.55 (0.50-3.50)	0.351
N/L ratio	12.28 ± 11.35 9.7 (1.39-30.6)	27.55 ± 33.36 15.4 (8.7-95)	0.366	10.88 ± 11.74 9.7 (1.39-30.6)	24.61 ± 29.18 16.65 (3.2-95)	0.222
Serum CRP (mg/L)	101.82 ± 98.64 50 (10.4-224)	109.58 ± 131.41 43 (11.5-345)	0.836	139 ± 92.72 178 (29-224)	84.41 ± 120.33 29.45 (10.4-186)	0.171
Plasma D- dimer (ng/mL)	2216.42 ± 1014.22 2100 (826-2899)	2222.83 ± 1070.16 1846 (1075-4124)	0.991	2372 ± 945.68 2100 (1720-4000)	2124 ± 1077.81 1846 (826-4124)	0.681
Serum LDH (U/L)	2123.43 ± 2191.10 1334 (784-7046)	1192.67 ± 305.74 1248.5 (625-1500)	0.366	2448.8 ± 2578.34 1319 (1104-7046)	1222 ± 348.39 1309.5 (625-1645)	0.724
Serum Albumin (gm/dL)	3.06 ± 0.43 3.08 (2.53-3.70)	2.48 ± 0.63 2.59 (1.30-3.12)	0.101	2.89 ± 0.44 2.83 (2.43-3.40)	2.720 ± 0.69 2.75 (1.3-3.12)	0.943
Serum Creatinine (mg/dL)	1.310.66 ± 0.66 1.0 (0.74-2.29)	1.01 ± 0.33 1.0 (0.6-1.36)	0.338	1.19 ± 0.64 1.0 (0.74-2.29)	1.16 ± 0.50 1.10 (0.60-2.14)	0.928

Abbreviations: ALC, Absolute lymphocyte count; ALT, Alanine transferase; ANC, Absolute neutrophil count; AST, Aspartate transferase; CRP, C-reactive protein; Hb, Hemoglobin; LDH, Lactate dehydrogenase; N/L ratio, Neutrophil/lymphocyte ratio; SD, Standard deviation; TLC, Total leukocyte count.

TABLE 4 Comparison of recent COVID-19 studies associated with HLH where antemortem bone marrow examination was done

	Debliquis et al[19] (June 2020)		Prieto-Perez et al[5] (July 2020)		Present study	
	3		3[a]		13	
Number of patients	(Mean ± SD)	Median (range)	(Mean ± SD)	Median (range)	(Mean ± SD)	Median (range)
Demographic and clinical characteristics						
Age (years)	69.33 ± 7.76	67 (63-78)	58.33 ± 12.58	60 (45-70)	58.07 ± 8.58	57 (41-74)
Male: Female ratio	2:1		1:2		7:6	
Fever (% of patients)	33.33%		100%		100%	
Splenomegaly (% of patients)	Absent		33.33%		Absent	
Hepatomegaly (% of patients)	Absent		No data		7.69%	
Laboratory parameters						
Serum Triglyceride (mg/dL)	228.81 ± 204.99	119.42 (101.72-465.29)	No data		248.30 ± 162.20	219 (79-634)
Plasma Fibrinogen (mg/dL)	343.33 ± 275.38	210 (160-660)	No data		389.07 ± 164.21	422 (46-673)
Serum Ferritin (ng/mL)	2047 ± 2469.32	624 (620-4899)	8775.33 ± 7031.96	7790 (2288-7790)	6544.23 ± 16 157.66	1429 (795-60 000)
Serum AST (IU/L)	79.66 ± 51.39	87 (25-127)	No data		557.24 ± 1265.09	63.59 (27-4556)
Serum ALT (IU/L)	No data		No data		395.11 ± 804.65	82.00 (22-2866)
Hemoglobin (gm/dL)	10.4 ± 1.95	10.4 (8.5-12.4)	7.25 ± 0.21	7.25 (7.1-7.4)	10.04 ± 2.63	10.5 (6.4-14.4)
TLC (cells/mm^3)	14 046.66 ± 8986.76	12 650 (5840-23 650)	No data		11 560.84 ± 7206.63	12 600 (691-22 900)
ALC (cells/mm^3)	1167.33 ± 522.12	950 (800-1770)	No data		887.84 ± 938.29	600 (126-3588)
Platelet count (× 10^5/mm^3)	0.40 ± 0.43	0.26 (0.05-0.89)	0.38 ± 0.22	0.39 (0.23 - 0.54)	1.50 ± 0.96	1.5 (0.10-3.5)
Serum CRP (mg/L)	224.3 ± 172.86	204 (12-357)	No data		105.40 ± 109.89	50.00 (10.4-345)
Plasma D- dimer (ng/mL)	15 057 ± 11 691.52	17 994 (2177-25 000)	No data		2219.38 ± 995.75	1892 (826-4124)
Serum LDH (U/L)	463.66 ± 211.89	584 (219-588)	No data		1693.84 ± 1634.82	1319 (625-7046)
Serum Albumin (gm/dL)	No data		No data		2.79 ± 0.59	2.83 (1.3-3.7)
Serum Creatinine (mg/dL)	No data		No data		1.17 ± 0.53	1.0 (0.6-2.29)
Soluble CD25 (U/mL)	No data		1869.33 ± 936.22	1512 (1165-2932)	No data	
Bone marrow Hemophagocytosis (% of patients)	100%		100%		100%	
HLH diagnostic criteria						
Number of patients satisfying ≥5/8 HLH 2004 criteria	1		1		3	
Number of patients with H-score ≥169	1		0		4	

(Continues)

TABLE 4 (Continued)

	Debliquis et al[19] (June 2020)		Prieto-Perez et al[5] (July 2020)		Present study	
	3		3[a]		13	
Number of patients	(Mean ± SD)	Median (range)	(Mean ± SD)	Median (range)	(Mean ± SD)	Median (range)
Number of patients who satisfied ≥5/8 HLH 2004 criteria and also had H-score ≥169	1		0		2	
Number of patients satisfying ≥5/8 HLH 2004 criteria and/or having H-score ≥169	1		1		5	

Abbreviations: ALC, Absolute lymphocyte count; ALT, Alanine transferase; AST, Aspartate transferase; CRP, C-reactive protein; Hb, Hemoglobin; LDH, Lactate dehydrogenase; SD, Standard deviation; TLC, Total leukocyte count.

[a]The study by Prieto-Perez et al[5] included 3 patients who underwent antemortem BME and 33 cases of postmortem lung (n = 22) and bone marrow (n = 17) biopsy. Clinicopathological data of n = 17 cases of bone marrow biopsy were not separately described in their study.

hypertriglyceridemia, raising the suspicion that severe SARS-CoV-2 infection is associated with a HLH like cytokine storm.[4,6,11,14] Few clinical studies have observed that the immune response in severe SARS-CoV-2 infection is unlike that in HLH as it is not associated with organomegaly, cytopenias, or hypofibrinogenemia. On the contrary, it is associated with hyperfibrinogenemia, neutrophilia, and lymphopenia.[15-17]

Unlike HLH, we observed hyperfibrinogenemia in 53.84%, peripheral blood neutrophilia in 53.84%, peripheral blood lymphopenia in 84.61%, and absence of bone marrow lymphocytosis in 100% of our COVID-19 patients. However, a small number of our patients had hypofibrinogenemia (15.8%), cytopenias (38.46%), and organomegaly (7.69%) similar to HLH. All patients in our study had hemophagocytic histiocytosis on BME. Although the clinical picture in our patients showed similarities to HLH, only five of thirteen patients fulfilled HLH diagnostic criteria. It is possible that a few more of our patients could have fulfilled HLH diagnostic criteria, had the estimation of soluble CD25 and NK cell activity also been done. Although the mere finding of hemophagocytosis on BME may not be sufficient to diagnose HLH, it is an important criteria to diagnose HLH.[10] Gars et al observed that 23% of their HLH patients needed the finding of hemophagocytosis on BME to satisfy 5/8 HLH 2004 criteria and it was the only HLH 2004 criteria variable that was observed in all their HLH patients.[12] BME may be even more necessary in patients with severe COVID-19 infection with clinically suspected HLH as the frequent occurrence of hyperfibrinogenemia, neutrophilia, and infrequent occurrence of cytopenias in severe COVID-19 infection may contribute to lesser number of HLH diagnostic criteria points. A standardized method for quantification of hemophagocytosis is not well described in literature. Ho et al counted the number of hemophagocytes over the entire smear in two slides and after averaging, obtained the number of hemophagocytes per smear.[9] Singh et al considered hemophagocytosis to be significant if there were ≥2 hemophagocytes per slide.[18] Gars et al suggested that the presence of ≥1 hemophagocyte with an ingested granulocyte, ≥2 hemophagocytes with ingested nucleated red cells and ≥1 hemophagocyte with ingested lymphocytes together in the bone marrow has a 100% accuracy for predicting HLH.[12]

Table 4 compares our study with two recent COVID-19 studies with fewer patients (n = 3, in both studies) where antemortem BME was done. All patients in our study and the study by Prieto-Perez et al[5] presented with fever, while only one patient had fever in the study by Debliquis et al.[19] Splenomegaly was observed in one patient in the study by Prieto-Perez et al[5] while we observed hepatomegaly in one patient. Hyperferritinemia and bone marrow hemophagocytic histiocytosis were observed in all patients in all three studies. Elevated mean serum AST, LDH and CRP, mean plasma fibrinogen and D-dimer, mean TLC along with decreased mean ALC were observed in our study and in the study by Debliquis et al.[19] Data on these parameters were not available in the study by Prieto-Perez et al.[5] Bicytopenia was observed in five of thirteen patients in our study, two of three patients in the study by Prieto-Perez et al[5]

and in one of three patients in the study by Debliquis et al.[19] Soluble CD25 was elevated in one patient in the study by Prieto-Perez et al[5] Soluble CD25 was not evaluated in our study and in the study by Debliquis et al.[19] NK cell activity was not evaluated in all three studies. Five of thirteen patients in our study satisfied 5/8 HLH 2004 criteria and/or had H-score ≥169. In the study by Debliquis et al one of three patients satisfied 5/8 HLH 2004 criteria and had a H-score of 207 and two patients satisfied 2/8 HLH 2004 criteria and had H-score <169.[19] In the study by Prieto-Perez et al one patient satisfied 5/8 HLH 2004 criteria and two patients fulfilled 4/8 HLH 2004 criteria.[5]

The clinical and laboratory manifestations of severe COVID-19 infection are caused by a cytokine storm associated with increased interleukin-6 levels. Increased interleukin-6 levels induce a persistent inflammatory state which is responsible for the increased levels of acute phase reactants including plasma fibrinogen.[20,21] Increased levels of pro-inflammatory cytokines interleukin-6, interleukin-10 and tumor necrosis factor–alpha (TNF-α) induce apoptosis of cytotoxic T cells and NK cells and are thought to responsible for the lymphopenia observed in in severe COVID-19 infection.[22,23] Sequestration of lymphocytes in lungs, gastrointestinal tract, and lymphoid tissues has also been proposed as a cause of lymphopenia in severe COVID-19 infection although autopsy studies do not show excessive lymphocytic infiltration in these organs, rather lymphocyte depletion has occasionally been documented.[22,24] Along with reduced numbers, functional defects and exhaustion of cytotoxic T cells and NK cells have also been reported in COVID-19 infection as suggested by increased expression of inhibitory receptors (PD1, NKG2A) on cytotoxic T cells and NK cells and reduced levels of intracellular cytokines (CD107a, interferon-γ, interleukin-2, granzyme B, and TNF-α).[25-27] NKG2A receptor overexpression in NK cells is also seen in HLH leading to reduced activity of NK cells in HLH.[28]

5 | CONCLUSION

The immune response associated with severe COVID-19 infection is similar to HLH with a few differences. It is associated with lymphopenia in the peripheral blood and hemophagocytic histiocytosis without lymphocytosis in the bone marrow. Cytopenia, organomegaly, and hypofibrinogenemia are rare though not unseen in the severe COVID-19 immune response. On the contrary, hyperfibrinogenemia and neutrophilic leukocytosis are frequently observed. All patients with severe COVID-19 infection may not fulfill HLH 2004 criteria or have a H-score >169. We suggest that HLH should be suspected in patients with severe COVID-19 infection or worsening of COVID-19 infection. BME should be done where the diagnosis is in doubt so that appropriate therapy may be initiated as early as possible.

CONFLICT OF INTEREST

The authors have no competing interests.

AUTHOR CONTRIBUTION

All authors participated substantially so as to be considered authors in this manuscript. All authors read and approved the final manuscript. H. Dandu and G. Yadav designed the research study. S. Pandey, R. Sachu and K. Dubey collected the data and performed the research. HS Malhotra analyzed the data. H. Dandu, G. Yadav, and HS Malhotra wrote the paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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