Systematic Review

Hemophagocytic lymphohistiocytosis: An unusual complication in disseminated *Mycobacterium tuberculosis*

Somanath Padhi, Kandasamy Ravichandran[1], Jayaprakash Sahoo[2], Renu G'Boy Varghese, Aneesh Basheer[3]

Departments of Pathology, [1]Biostatistics and [3]General Medicine, Pondicherry Institute of Medical Sciences, [2]Department of Endocrinology and Metabolism, Jawaharlal Institute of Postgraduate Medical Education and Research, Puducherry, India

ABSTRACT

Background: Hemophagocytic lymphohistiocytosis (HLH) is an uncommon, potentially fatal, hyperinflammatory syndrome that may rarely complicate the clinical course of disseminated *Mycobacterium tuberculosis* (MTB). The clinical course of tuberculosis-associated HLH (TB-HLH) has been reported to be unpredictable. **Materials and Methods:** Here we describe the clinicopathological features, laboratory parameters, management, and outcome data of a patient who satisfied the 2004 diagnostic criteria for HLH secondary to disseminated MTB; we also do a systematic review of the international literature on TB-HLH. The literature review (January 1975–March 2014) found that HLH complicated the clinical course of 63 tuberculosis patients (41 males, 22 females, mean age = 45 ± 23.5 years) with a high mortality rate of 49% (31/63 died). The mean serum ferritin level (n = 44/63) was 5963 ng/mL (range 500–38,539 ng/mL); and a higher proportion (54.2%) of patients had pancytopenia at presentation. On univariate analysis (n = 53/63), age >30 years [hazard ratio (HR): 2.79, 95% confidence interval (CI):1.03–7.56, P = 0.03], presence of comorbidities (HR 4.59, CI: 1.08–19.52, P = 0.04), marked hemophagocytosis in bone marrow (HR: 2.65, CI: 1.16–6.05, P = 0.02), and nonusage/delayed usage of antitubercular therapy (ATT) (HR: 3.44, CI: 1.51–7.87, P = 0.003) were associated with decreased survival, though none of these parameters attained statistical significance (P > 0.05) in multivariate analysis. Usage of corticosteroids and/or immunomodulator drugs (HR 1.00, CI: 0.66–3.22, P = 0.35) did not alter the outcome in these patients. **Conclusion:** HLH should be considered as a differential diagnosis in patients with tuberculosis who present with cytopenias, organomegaly, and coagulopathy. Strong clinical suspicion and early usage of ATT might be useful in reducing the morbidity and mortality. The utility of immunosuppressive/immunomodulator therapy lacks general concensus among treating physicians, and warrants further studies.

KEY WORDS: Antitubercular therapy, hemophagocytic lymphohistiocytosis, survival, tuberculosis

Address for correspondence: Dr. Somanath Padhi, Department of Pathology, Pondicherry Institute of Medical Sciences, Puducherry - 605 014, India.
E-mail: somanath.padhi@gmail.com

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is an often fatal syndrome of exacerbated but ineffective inflammatory responses, characterized by excessive macrophage and T-cell activation as well as impairment of the ability of natural killer (NK) cells and cytotoxic T lymphocytes to kill the target cells. This results in uncontrolled histiocytic phagocytosis of mature blood elements and their precursors throughout the reticuloendothelial organs, and associated cytokine-mediated multiorgan dysfunction.[1,2] Primary or familial HLH appears to have a genetic basis, whereas secondary or acquired HLH may be associated with infections (commonly the Epstein–Barr virus or EBV, bacteria, *Rickettsia*, etc.), hematological malignancies (mostly T/NK cell leukemias/lymphomas), rheumatological/autoimmune disorders (so-called macrophage activation syndrome), etc.[3,4] The diagnosis is established by fulfilling one of the following HLH 2004 criteria: i) Positive family history or molecular diagnosis consistent with HLH (mutations of PRF, SAP, or Munc13-4 genes), ii) any five out of the following eight criteria: Prolonged fever, unexplained progressive cytopenias involving at least two cell lines (hemoglobin ≤90 g/L, platelet count ≤100 × 10^9/L, absolute neutrophil

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count <1× 10^9/L); splenomegaly; hyperferritinemia (≥500 ng/mL); fasting hypertriglyceridemia (≥265 mg/dL) or hypofibrinogenemia (≤1.5 g/L); histiocytic hemophagocytosis in bone marrow, liver, spleen, or lymph nodes without evidence of malignancy; low or absent NK cell cytotoxicity; and elevated soluble CD25 levels (≥2400 IU/mL of interleukin-2Rα chain).[5]

Tuberculosis, being a chronic disease, remains a common health problem in Southeast Asian and other underdeveloped countries, with significant morbidity and mortality. The causative organism *Mycobacterium tuberculosis* (MTB) is known as a "great mimicker" and has a diverse range of clinical manifestations. Tuberculosis may rarely be complicated by HLH, which may be diagnostically challenging to the treating physicians, and in the absence of early and definitive therapy may lead to significant morbidity and mortality.[6]

In this manuscript, we describe a patient, with tuberculosis-associated HLH with a favorable outcome following early initiation of antitubercular therapy (ATT). We also present a brief, concise, systematic review of all relevant international literature regarding tuberculosis-associated HLH (TB-HLH) with regard to the clinicopathological characteristics, immunopathology, and therapeutic outcome.

MATERIALS AND METHODS

Presentation of a case

A 17-year-old Indian male, student by occupation, required admission into the intensive care unit of our Institute with persistent high-grade fever, cough with expectoration, worsening breathlessness, tachypnea, bilateral coarse crepitation, and hypoxemia suggestive of acute respiratory distress syndrome (ARDS), thus requiring ventilator support. Prior to the present admission he had been evaluated at an outside hospital for a 2-month history of persistent low-grade fever, night sweats, and weight loss (5 kg in the last month), possibly of tubercular origin. On examination he was found to be thin of build, febrile (oral temperature: 103°F) with significant conjunctival pallor, scleral icterus, bilateral subcentimeter cervical and axillary lymphadenopathy, hepatosplenomegaly (liver 4 cm, spleen 6 cm below the costal margin), bilateral coarse crepitation (anterior and midaxillary lines), and mild ascites. Radiological evaluation showed miliary shadows in bilateral lung fields, hepatosplenomegaly without focal lesions, and ascites. Routine laboratory evaluation on day 2 revealed microcytic hypochromic anemia [hemoglobin: 68 g/L (120–140 g/L), mean corpuscular volume (MCV): 70 fL (80–98 fL)], leukopenia [leukocyte count: 1.3 × 10^9/L (ref.; 4–11 × 10^9/L), absolute neutrophil count (ANC): 790/cmm], total platelet count: 160 × 10^9/L (150–450 × 10^9/L), total bilirubin: 3.4 mg/dL (direct 1.6 mg/dL), raised liver transaminases (>1.5 times the upper limit of normal), prolonged prothrombin time [27 s, control 12 s, international normalized ratio (INR) 1.2] and activated partial thromboplastin time (45 s, control 26 sec), and negative D-dimer. Plasma fibrinogen and serum triglyceride levels were within normal reference range. Microbiological and serological work-up were negative for human immunodeficiency virus (HIV), hepatitis B and C viruses, dengue, malaria, *Leptospira*, *Brucella*, and scrub typhus; and microbial cultures of blood, urine, sputum, and brochoalveolar lavage fluid were sterile. In view of persistent fever, worsening cytopenias, and organomegaly, bone marrow aspiration and trephine biopsy were performed on day 4 *post* admission, which showed multiple well-formed, caseating epithelioid granulomas, suggestive of tuberculosis. Besides these, there was evidence of a marked degree of histiocytic hemophagocytosis in the bone marrow aspirate smears. The assessment of histiocytic hemophagocytosis on bone marrow aspirate smears was done as per the method devised by Ho *et al*. (low/mild: 1–5 hemophagocytic cells per entire slide, moderate: 6–10 hemophagocytic cells per entire slide; and >10 hemophagocytic cells per entire slide).[7] Biochemical evaluation revealed hyperferritinemia (>2000 ng/mL), raised lactate dehydrogenase (903 IU/L), hypoalbuminemia (2.5 g/dL), and hyponatremia (125 meq/L). Thus, the clinicolaboaratory parameters satisfied five of six diagnostic criteria for HLH,[5] as NK cell activity and soluble CD25 levels were not tested due to lack of facilities. The patient was managed with broad-spectrum intravenous antibiotics and supportive measures. Furthermore, he was started on four-drug ATT with isoniazid, rifampicin, pyrazinamide, and ethambutol. He responded dramatically to ATT and was finally discharged in a stable condition on day 14 *post* admission with hemoglobin of 80 g/L, ANC of 1300/cmm, and platelet count of 170 × 10^9/L. On follow-up he was found to be responding well to ATT, and is presently on a two-drug continuation phase of ATT with isoniazid and rifampicin.

Collection of data on TB-HLH

A systematic search of HLH that complicated the clinical course of tuberculosis, over the last 39 years (January 1975–March 2014), was done by searching the PubMed, PubMed Central, Medline, and Directory of Open Access Journal databases. The following keywords were used for the literature search: Tuberculosis-associated HLH, tuberculosis-associated hemophagocytosis/hemophagocytic syndrome, macrophage activation and tuberculosis, and tuberculosis complicated with HLH. The references of all articles were cross-checked for relevant articles. In 2006, Brastianos *et al*. reported a case of TB-HLH and reviewed 36 similar cases reported around the world till that time.[7] A systematic search found 27 more articles from 2006 till date. A total of 55 articles describing nearly 70 cases of TB-HLH (including 37 reviewed by Brastianos *et al*.) were found in the world literature till March 2014.[7-34] The following data were collected for TB-HLH cases and placed in Microsoft office Excel 2007 for statistical analysis: Age, gender, duration of symptoms, associated

comorbidities, organomegaly, complete blood count, serum biochemical parameters (ferritin, triglyceride, fibrinogen, liver transaminases, alkaline phosphatase, total bilirubin, albumin, and lactate dehydrogenase), coagulation abnormalities, evidence of hemophagocytosis on bone marrow evaluation, source and method of isolation of the organism, usage of ATT and/or immunosuppressive/immunomodulators drugs, and final outcome (alive or dead). Finally, complete information was available in 63 out of 70 cases, and the remaining 7 were excluded due to inaccessibility of the complete articles and/or lack of adequate information.

Statistical analysis

Baseline characteristics were described using mean (±standard deviation) for continuous variables, and count (percentage) for nominal variables. Among 53 of 63 cases, the follow-up duration from the diagnosis was not reported; hence the symptom duration till diagnosis for each case was taken as the observation period for analysis and taken as overall survival for the patients. Cox proportional hazard models were used to estimate the hazard ratio (HR) and the 95% confidence interval (CI) for death due to TB. Factors that had *P* values less than 0.10 in univariate analysis were included in the multivariate analysis. In addition, the Kaplan–Meier method was used to determine survival patterns for variables identified as having a significant effect on survival. Differences between survival curves were evaluated using log-rank tests. All statistical tests were two-sided; *P* values of less than 0.05 were considered to be statistically significant. Analyses were performed using IBM SPSS statistics (version 20.0).

DISCUSSION

The hallmark pathophysiologic mechanism of HLH is exacerbated but deregulated Th_1 cell-mediated immune response against an intracellular pathogen, macrophage hyperactivity, widespread hemophagocytosis, and hypercytokinemia leading to multiorgan dysfunction. This results due to impaired or suppressed function of cytotoxic T cells and NK cells to effectively clear the antigenic stimulus and thus turn off the inflammatory response.[1] Splenomegaly is the result of activation and proliferation of splenic macrophages, as evidenced by the increased expression of major histocompatibility complex (MHC)-I and MHC-II molecules, as well as macrophage colony-stimulating factor (M-CSF) receptors. The cytokine milieu in HLH is characterized by increased levels of interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), granulocyte monocytes colony-stimulating factor (GM-CSF), and interleukin-18 (IL-18). The bi/pancytopenia in HLH is likely the consequence of high TNF-α and IFN-γ leading to suppressed hematopoiesis and increased apoptosis. While TNF-α– and IFN-γ–mediated inhibition of lipoprotein lipase leads to hypertriglyceridemia, macrophage overactivity might explain excess release of ferritin (an acute-phase reactant) in response to inflammatory cytokines, tissue infiltration leading to organomegaly, and fever (secondary to IL-1, IL-6).[1] Therefore MTB, being an obligate intracellular pathogen, may exacerbate Th_1 cell-mediated cytotoxicity and macrophage overactivity, which may lead to HLH in susceptible individuals, as is evidenced by increased serum levels of IFN-γ, M-CSF, and TNF-α in patients with tuberculosis.[31]

Associated comorbidities in TB-HLH

Concise, systematic details of the clinicopathological characteristics of all the 63 cases of TB-HLH are presented in Tables 1 and 2. There were 41 males and 22 females, and the mean age was 45 years (±23.5 years) (range 14 days–83 years). A high proportion (41/63, 65%) of patients had underlying comorbidities; 11 of 41 (26.8%) patients had end-stage renal disease and were receiving either hemodialysis (n = 8) or had undergone renal transplant (n = 3); 4 had type 2 diabetes mellitus (2 with nephropathy), 6 had a past history of malignancy (2 Hodgkin lymphoma, 2 non-Hodgkin lymphoma, 1 myelodysplastic syndrome, 1 small-cell lung carcinoma), 5 had underlying autoimmune diseases (2 systemic lupus erythematosus, 1 ankylosing spondylitis, 1 Wegener's granulomatosis, 1 Crohn's disease), 1 had sarcoidosis, and 3 had HIV/acquired immune deficiency syndrome (AIDS) (1 with coexistent EBV infection). Prior history of tuberculosis was present in 3 adults, and 2 neonates contracted the infection from their mothers (perinatal tuberculosis). Chamsi-Pasha *et al*. reported the only case of Mycobacterium avium complex (MAC)-associated HLH in a sickle cell patient (homozygous) with a fatal outcome.[10] Naha *et al*., reported a case of disseminated tuberculosis with secondary HLH and the presence of tuberculosis-associated reactive arthritis (Poncet's disease), in an immunocompetent male.[12] Mancebo *et al*. reported the first case of familial HLH in an adult patient with PRF1 gene mutation and coexistent tuberculosis.[33] The mean duration of symptoms before diagnosis of TB-HLH was 51 days (±47.3 days) (median 45 days), and in 8 patients (12.7%) the diagnosis of tuberculosis was made at autopsy; this was an indirect reflection of disease chronicity, delay in diagnosis, and thus a delay in initiation of ATT. In 3 patients, HLH was reported to be exacerbated after initiation of ATT, which required stoppage of ATT or a change of drug regimen.[8,25]

HLH diagnostic features

HLH diagnosis was made in all cases by a constellation of fever, organomegaly, cytopenia (s), elevated serum ferritin and triglyceride levels with or without lower plasma fibrinogen, and demonstration of histiocytic hemophagocytosis on bone marrow examination. Fever was the most common clinical presentation and was present in all cases. Hepatosplenomegaly and lymphadenopathy were observed in 43 of 61 cases (70.5%), and 6 (9.8%) cases had isolated splenomegaly. A higher proportion [32/59 (54.2%)] of patients had pancytopenia, and 22/59 (37.2%) had bicytopenia, which was present at the time of initial presentation or developed during the course

Table 1: Clinicopathological characteristics of tuberculosis associated hemophagocytic lymphohistiocytosis published in the world literature (January 1975- March 2014) (*n*=63)

Age/gender, symptom duration	Co-morbidity	Fever/ liver/spleen	Hb (g/L)	ANC (/cmm)	TPC (x 10^9/L)	Ferritin (ng/ml)	TG (mg/dl)	Fibrinogen (g/L)	HP in BM	Therapy, outcome, days from diagnosis	Number of sites of isolation of organism (*n*)	Year, [reference]
22y/male, 21d	ESRD on HD	Yes/NA/NA	Low	Low	Low	33, 324	NA	NA	Present	ATT + IT, alive, 540	2	2014, [8]
2m/male, 15d	Nil	Yes/yes/yes	83	553	150	5269	297	Normal	BM not done	ATT + IT, death, 3	1	2014, [9]
22y/female, 60d	Sickle cell disease	Yes/no/yes	104	WNL	45	38, 539	566	Low	Marked	ATT + IT, death, NA	3 (MAC)	2013, [10]
18y/female, 60d	Nil	Yes/yes/yes	49	1070	7	2575	256	Low	Marked	ATT + IT, alive, 60	-	2013, [11]
27/male, 180d	Poncet disease	Yes/yes/yes	59	500	15	2500	499	Normal	Mild	ATT, death, 3	2	2013, [12]
29y/male, 30d	ARDS	Yes/yes/yes	130	970	56	4675	376	Normal	BM not done	NA, death, 21	2	2013, [13]
34y/male, 30d	Pleural effusion	Yes/yes/yes	76	840	60	4164	466	<1.5	Marked	ATT + IT, death, 15	1	2012, [14]
21d/female, 15d	Perinatal tuberculosis	Yes/yes/yes	84	3000	67	4000	367	< 0.3	Moderate	ATT + IT, alive, 270	1	2012, [15]
25y/male, 30d	Nil	Yes/yes/yes	67	870	66	3000	298	Normal	Mild	ATT, alive, 180	1	2012, [16]
47y/female, 50d	SLE (no flare up)	Yes/yes/yes	130	1055	58	6800	470	Normal	Moderate	ATT, death, 45	2	2012, [17]
78y/female, 7d	Hypertension	Yes/no/no	86	776	37	33,000	280	1.18	Moderate	ATT, alive, 540	3	2012, [18]
80y/male, 90d	Atrial fibrillation	Yes/no/no	80	680	29	1530	NA	3.38	Marked	ATT, death, 30	3	2012, [18]
NA/4 males, 3 females, NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	2 alive, 5 dead	NA	2011, [19]
60d/male, 15d	Nil	Yes/yes/yes	56	1020	118	583	285	1.2	Mild	ATT + IT, alive, 30	1	2010, [20]
60y/female, 10d	Hypertension	Yes/yes/yes	81	370	10	8317	557	Normal	Mild	ATT + IT, alive, 21	2 (*M kansasii*)	2010, [21]
44y/male, 35d	Ankylosing spondylitis on Infliximab	Yes/yes/yes	NA	NA	NA	NA	NA	NA	Moderate	ATT + IT, alive, 30		2010, [22]
30y/male, 45d	Nil	Yes/yes/yes	70	2500	65	3000	225	Normal	Mild	ATT + IT, alive, NA	1	2010, [23]
48y/female, 37d	Crohn ds on Infliximab	Yes/yes/yes	75	975	37	5324	267	0.7	Mild	ATT + IT, alive, NA	2	2010, [24]
41y/male, 15d	Wegner's granulomatosis	Yes/yes/yes	78	1310	5	3510	NA	Normal	Moderate	ATT + IT, alive, 180	3	2009, [25]
58y/male, 35d	Diabetic nephropathy on HD	Yes/no/yes	99	2110	77	1954	261	NA	Marked	ATT + IT, death, 30	2	2009, [26]
17y/male, 15d	Nil	Yes/yes/yes	70	1234	70	960	280	Normal	Moderate	ATT + IT, alive, 45	1	2009, [27]
70y/male, 60d	HD	Yes/no/no	56	870	56	1678	384	NA	Marked	Nil, death, NA	>3 (autopsy)	2009, [28]
52d/male, 25d	Seborrhoic dermatitis	Yes/yes/yes	69	17,500	75	1885	218	Low	Moderate	ATT + IT, death, 18	2	2008, [29]
63y/male, 35d	Diabetes mellitus	Yes/yes/yes	70	1345	78	1770	216	Normal	Moderate	ATT + IT, death, 20	2	2008, [30]
46y/male, 28d	HIV on HART, EBV	Yes/-/-	Low	Low	Low	-	-	-	Present	ATT + IT, death, 35	1 (L5 spine)	2007, [31]
28y/male, 180d	HSV, EBV, CMV	Yes/yes/yes	50	675	46	996	117	3.77	Marked	ATT + IT, death, NA	1	2006, [32]
49y/male, NA	TB spondylitis,	Yes/NA/NA	104	800	14	7000	277	NA	Present	ATT + IT (HLH-2004), death, 10	1 (sputum culture)	2006, [33]
60y/female, 15d	Diabetes mellitus	Yes/no/no	95	2673	95	NA	NA	Normal	Marked	ATT, death, 28	2	2006, [7]
36y/female, NA	SLE on steroids	Yes/NA/NA	89	374	92	NA	NA	Normal	Moderate	ATT + IT, death, NA	2	2005, [7]
67y/female, NA	Nil	Yes/no/no	NA	NA	114	NA	NA	NA	NA	ATT + IT, death, NA	2	2005, [7]
50y/male, 15d	Nil	Yes/yes/yes	106	1100	142	>2000	NA	Normal	Moderate	ATT + IT, death, 26	1	2004, [7]
78y/male, 180d	ESRD on HD	Yes/no/no	85	150	9	2438	338	Normal	Marked	ATT + IT, death, 3	2	2004, [7]
22y/male, 30d	Nil	Yes/yes/yes	69	1340	10	2785	290	Normal	Marked	ATT, alive, NA	1	2004, [7]
44y/male, NA	Renal transplant, HD	Yes/yes/yes	61	605	34	>2000	467	NA	Moderate	ATT + IT, death, NA	-	2004, [7]
45y/male, NA	B-NHL, renal transplant, HD	Yes/yes/yes	56	2500	129	3423	200	Normal	Marked	ATT, alive, NA	-	2004, [7]
83y/female, NA	ESRD, HD	Yes/no/yes	55	1200	37	1750	167	Low	Marked	Nil, death, 30	1	2004, [7]
60y/female, NA	Nil	Yes/yes/no	77	3450	120	500	175	Normal	Mild	ATT, alive, NA	2	2003, [7]
14d/female, 21d	Perinatal tuberculosis	Yes/yes/yes	113	2000	58	NA	NA	Low	BM study not done	ATT + IT, alive, 360	2	2003, [7]
76y/female, NA	Nil	Yes/no/no	76	2575	64	1876	NA	NA	Mild	ATT + IT, alive, NA	2	2003, [7]
9y/female, NA	Nil	Yes/yes/yes	67	2500	47	780	289	Low	Moderate	ATT + IT, alive, NA	>3	2002, [7]

Contd...

Table 1: Contd...

Age/gender, symptom duration	Co-morbidity	Fever/ liver/spleen	Hb (g/L)	ANC (/cmm)	TPC (x 10^9/L)	Ferritin (ng/ml)	TG (mg/dl)	Fibrinogen (g/L)	HP in BM	Therapy, outcome, days from diagnosis	Number of sites of isolation of organism (*n*)	Year, [reference]
59y/female, NA	Nil	Yes/yes/yes	65	low	68	NA	NA	NA	Moderate	ATT + IT, death, NA	1	2002, [7]
29y/female, 180d	Nil	Yes/yes/yes	69	3040	43	NA	NA	NA	Mild	ATT, alive, NA	1	2001, [7]
73y/female, 15d	ESRD	Yes/yes/yes	67	568	39	NA	NA	Low	Mild	ATT, alive, NA	1	2000, [7]
40y/male, 21d	Nil	Yes/yes/yes	45	3456	55	776	NA	Normal	Moderate	ATT, alive, NA	1	2000, [7]
46y/female, 40d	MDS, CGN, HD	Yes/yes/yes	89	1455	45	>2000	NA	NA	Moderate	ATT + IT, alive, NA	1	1999, [7]
53y/female, 15d	AIDS	Yes/yes/no	67	700	163	770	347	Low	Marked	ATT + IT, death, NA	1	1998, [7]
31y/female, 35d	Stage IV Hodgkin lymphoma	Yes/no/yes	47	3000	70	790	460	Normal	Moderate	ATT + IT, alive, NA	3	1998, [7]
48y/male, 35d	Nil	Yes/yes/yes	45	650	57	1267	NA	Low	Marked	ATT + IT, alive, NA	3	1998, [7]
63y/female, 10d	Aplastic anemia, HSV	Yes/yes/yes	65	1200	49	3467	NA	NA	Marked	IT, death, NA	5 (PM diagnosis)	1998, [7]
37y/male, 180d	Renal transplant, HD	Yes/yes/yes	76	1000	37	NA	NA	NA	Marked	Nil, death, NA	1	1996, [7]
40y/male, 25d	Candidal esophagitis	Yes/yes/yes	69	2200	30	867	NA	NA	Mild	ATT + IT, alive, NA	1	1996, [7]
46y/male, 120d	Nil	Yes/yes/yes	100	2550	303	1000	260	Normal	Moderate	ATT, alive, NA	2	1996, [7]
43y/male, 25d	AIDS	Yes/no/yes	65	4000	19	10,000	NA	Low	Marked	ATT, death, NA	1	1995, [7]
56y/female, 30d	Diabetic nephropathy	Yes/yes/yes	57	654	29	NA	NA	Low	Moderate	Nil, death, NA	1 (PM diagnosis)	1995, [7]
83y/male, 15d	Nil	Yes/no/yes	87	1350	120	NA	NA	Normal	Moderate	ATT, alive, NA	3	1995, [7]
70y/male, 60d	Nil	Yes/no/yes	65	2365	37	650	225	Normal	Mild	ATT + IT, alive, NA	2	1995, [7]
42y/male, 90d	Sarcoidosis	Yes/no/yes	NA	NA	96	NA	NA	NA	Moderate	ATT + IT, death, NA	5 (PM diagnosis)	1994, [7]
82y/female, 45d	Malignant lymphoma	Yes/yes/no	66	760	39	NA	NA	Low	Mild	ATT, death, NA	5 (PM diagnosis)	1990, [7]
49y/male, 10d	Nil	Yes/no/yes	130	600	5	2378	NA	Normal	Marked	ATT + IT, alive, NA	2	1988, [7]
68y/female, 7d	Miliary tuberculosis, adrenal tuberculosis	Yes/yes/yes	120	4500	64	NA	NA	Very low	Marked	Nil, death, NA	>5 (PM diagnosis)	1986, [7]
59y/male, 40d	Disseminated tuberculosis	Yes/yes/yes	83	860	200	NA	NA	NA	Mild	Nil, death, NA	5 (PM diagnosis)	1986, [7]
76y/male, 35d	Small cell lung cancer, prior tuberculosis	Yes/yes/yes	120	780	70	NA	NA	NA	Mild	Nil, death, NA	5 (PM diagnosis)	1986, [7]
19y/male, 7d	Nil	Yes/yes/yes	122	3500	75	NA	High	Very low	Marked	ATT + IT, alive, NA	3	1975, [34]

y: Year, d: Days, NA: Not available, ESRD: End stage renal disease, HD: Hemodialysis, ARDS: Acute respiratory distress syndrome, SLE: Systemic lupus erythematosus, HIV: Human immunodeficiency virus, HAART: Highly active antiretroviral therapy, EBV: Epstein Barr virus, HSV: Herpes Simplex virus, CMV: Cytomegalo virus, B-NHL: B cell non Hodgkin lymphoma, MDS: Myelodysplastic syndrome, CGN: Chronic glomerulonephritis, AIDS: Acquired immunodeficiency syndrome, Hb: Hemoglobin, ANC: Absolute neutrophil count, TPC: Total platelet count, TG: Serum triglyceride, HP: Hemophagocytosis, BM: Bone marrow, ATT: Antituberculous therapy, IT: Immunotherapy which includes immunosuppressive drugs such as steroids, immunomodulators such as etoposide, cyclosporine, intravenous immunoglobulin, antithymocyte globulin, interleukin-1 receptor antagonists, etc., in varying combination, PM: Post mortem diagnosis

of disease evolution. The mean hemoglobin (Hb) (n = 56), absolute neutrophil count (ANC) (n = 55), and total platelet count (TPC) (n = 58) of cases (where data were available) were 78.45 ± 22.36 g/L, 1548 ± 1052/cmm, and 67 ± 51.9 × 10^9/L respectively. Similarly, the median and interquartile range of Hb, ANC, and TPC among cases were 72.5 g/L (65.0–88.75), 1100/cmm (760–2500), and 57.5 × 10^9/L (37.0–77.25), respectively. The mean and median serum ferritin values (n = 44) among cases were 5963 ng/mL and 1955 ng/mL (interquartile range 960–3510) respectively. Serum triglyceride levels were measured in 31 cases; the mean and median values were found to be 307.5 ± 108.6 mg/dL, and 285 mg/dL (interquartile range: 225–376), respectively. Furthermore, among 45 cases where data were available, plasma fibrinogen was reported as low (<1.5 g/L) among 22 (49%) cases; and it was within the normal reference range among the remaining 23 cases (51%). Bone marrow examination performed in all 63 cases revealed the presence of histiocytic hemophagocytosis in 58 (92%), which was reported as moderate to marked in intensity among 40 (69%) cases. This was possibly an indirect reflection of the persistent disease activity reflected by cytopenia (s), hyperferritinemia (mean: 5963 ng/mL), hepatic dysfunction, and consequent coagulation abnormality.

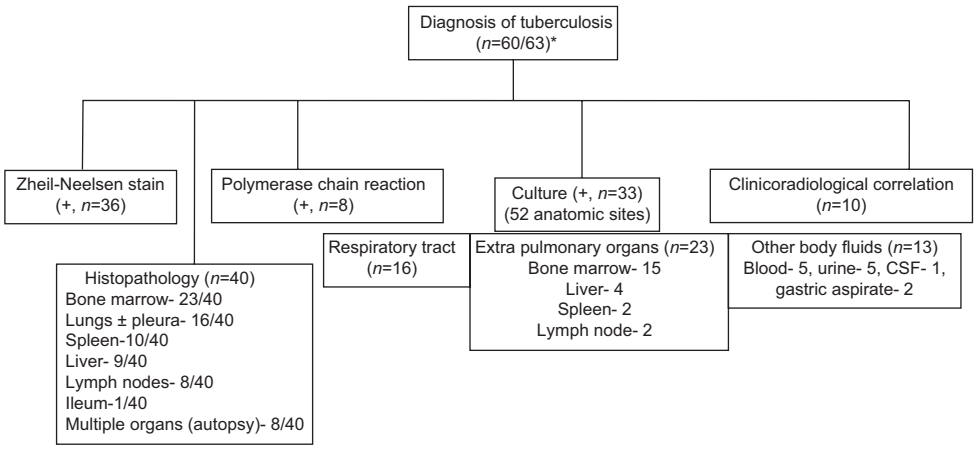
Table 2: Clinicopathological characteristics of patients with tuberculosis associated hemophagocytic lymphohistiocytosis (January 1975 till March 2014)

Parameters	Values
Number of cases	63
Male/female (n, %)	41/22 (65/35)
Mean age (range)	45 (14 days-83 years)
Associated co-morbidity (n, %)	41/63 (65%)[a]
Mean duration of symptom before diagnosis (range)	51 days (7-180)
Fever (n, %)	63/63 (100)
Splenomegaly ± hepatomegaly (n, %)	43/61 (70.5)[b,c]
Pancytopenia (n, %)	32/59 (54.2)[d]
Bicytopenia (n, %)	22/59 (37.2)
Median hemoglobin (g/L) (n=56)	72.5 (65.0-88.75)
Median absolute neutrophil count (n=55) (per cmm)	1100 (760-2500)
Median total platelet count (n=58) (x10^9/L)	57.5 (37.0-77.25)
Hemophagocytosis in bone marrow	58/63 (92%)[e] (marked in 20, moderate in 20, mild in 18)
Serum ferritin (ng/mL) (n=44)	
Mean (range)	5963 (500-38,539)[f]
Median (range)	1955 (960-3510)
Median serum triglyceride (mg/dl) (n=31)	285 (225-376)
Plasma fibrinogen (g/L) (n=45)	
Low (<1.5 g/L) (n, %)	22 (49)
Normal (n, %)	23 (51)
Management	16/62; ATT[g] alone 37/62;ATT + immunosuppresive/ immunomodulator 1/62; only immunotherapy 8/62; no therapy
Outcome	31/63 (49%); death

a: End stage renal disease was the most common (11/41), b: Data not available in 2 cases, c: 6 cases had isolated splenomegaly, d: In 4 cases, data were not available, e: In 5 cases, bone marrow assessment was not done, histiocytic hemophagocytosis was demonstrated in other tissues, f) data available from 44 of 63 case, g: Antitubercular therapy

Diagnosis of tuberculosis

A schematic representation of diagnostic algorithm in all cases with tuberculosis is presented in Flow chart 1. Overall, among 40 of 60 cases where complete data were available (66.6%), the diagnosis of tuberculosis was made at ≥2 different anatomic sites by using either histopathological evaluation (n = 40, 66.6%) or by microbiological methods [demonstration of acid-fast bacilli by the Ziehl–Neelsen stain (n = 36, 60%), isolation by microbial culture (n = 33, 55%), or polymerase chain reaction (PCR) (n = 8, 13.3%)]. On the contrary, clinicoradiological features consistent with tuberculosis were present in only 10 cases (16.7%). On histopathological examination, granulomatous inflammations were demonstrated most commonly in the bone marrow (n = 23), lungs and/or pleura (n = 16),



Flow Chart 1: Diagnostic algorithm of all cases of tuberculosis who presented with hemophagocytic lymphohistiocytosis. +; positive, *; in 3 cases, no information was available. Note: in 33 of 60 cases, the disease was evident in ≥ 2 anatomic sites. *Mycobacterium kansasii*[21] and *Mycobacterium avium intracellulare* complex (MAC)[10] were isolated in one case each; and rest cases were attributable to Mycobacterium tuberculosis

spleen (n = 10), liver (n = 8), and lymph nodes (n = 8). Similarly, among 33 cases where the culture results were positive, the organisms were isolated from 52 different anatomic samples such as the respiratory tract (n = 16), extrapulmonary sites (n = 23) [bone marrow (n = 15), liver (n = 2), spleen (n = 2), and lymph node (n = 4)], and body fluids (n = 13). The isolate was reported as MAC in a sickle cell patient,[10] *Mycobacterium kansasii* in another case;[21] in the remaining cases it was reported as MTB. Therefore, it can be postulated that cases with disseminated tuberculosis were complicated by the process of HLH.

Management and outcome of TB-HLH

The biological behavior of cases with TB-HLH was unpredictable, with a mortality rate of 49% (31/63 died); the overall survival at 3 months was 45% [Figure 1a]. Fifty-four of the 62 cases where data were available received treatment: either ATT alone (n = 16; 10 survived, 62.5%) or in combination with immunosuppressive (steroids and/or intravenous immunoglobulin) and/or immunomodulators (n = 37; 20 survived, 54%). The following immunomodulator drugs were used: Cyclosporine in 3, etoposide in 2, cyclosporine and etoposide in 1, vincristine in 1, chlorambucil and fludarabine in 1, and IL-1 receptor antagonist in 2 cases. None of the 8 cases who did not receive any therapy survived, and 1 who received only immunotherapy also did not survive. In most cases, the failure of therapy was attributed to delayed diagnosis and/or initiation of therapy late in the course of the illness. On univariate analysis, age >30 years (HR: 2.79, 95% CI: 1.03–7.56, P = 0.03), presence of comorbidities (HR: 4.59, CI: 1.08–19.52, P = 0.04), presence of marked hemophagocytosis in bone marrow (HR: 2.65, CI: 1.16–6.05, P = 0.02), and delayed/nonusage of ATT (HR: 3.44, CI: 1.51–7.87, P = 0.003) were significantly associated with decreased survival [Table 3, Figure 1b-g].

Highly elevated ferritin is strongly associated with HLH and its levels may provide a prognostic marker. Lin *et al*. suggested that a rapid rate of fall in ferritin levels following therapy initiation was associated with decreased mortality.[35] However, Park *et al*. in their cohort of 23 patients with secondary HLH found that the rate of decline in ferritin was not associated with survival, and that high fibrinogen at the time of diagnosis was significantly associated with survival.[36] We found that high serum ferritin (>1000 ng/mL) was an important indicator of disease severity and was associated with decreased survival, though the impact was statistically insignificant on univariate analysis (HR: 2.26, CI: 0.51–10.02, P = 0.25) [Figure 1g]. Paradoxically, the literature review also showed that patients with low hemoglobin (≤90 g/L) (P = 0.15) and low platelet count (≤100 × 10^9/L) (P = 0.12) did not affect the outcome in a Cox regression model [Table 3]. Similarly, the presence of disseminated disease, coagulation abnormalities, and usage of immunosuppressive and/or immunomodulators in therapy [Figure 1f] were not significantly associated with survival. However, the interpretation of outcomes from univariate analysis must be interpreted with great caution, as the cohort of patients reported in the literature is small and heterogenous, and the therapeutic strategies differed from case to case.

Comparative analysis of intensive chemotherapy and conventional combination therapy, in the management of HLH, has not shown any difference in outcomes. However, there exists general agreement that early treatment may improve outcomes. Imashuku *et al*.[37] have suggested that young adult patients receiving early etoposide treatment have a better prognosis than those not treated with etoposide or treated late. However, a recent Korean study by Park *et al*.[36] did not find any differences in patient outcomes between the two groups based on therapy (HLH protocol vs immunosuppressive therapy). Furthermore, as rightly pointed out by Park *et al*.,[36] in the patients with severe disease and/or associated sepsis or multiple organ failure at the time of diagnosis, it may be difficult to use cytotoxic agents such as etoposide. In such circumstances, immunosuppression with corticosteroids and/or cyclosporine remains the foundation of early management as it can control systemic inflammation. At present, there are no guidelines as to when and which patients with secondary HLH require the complete HLH 2004 regimen, although targeted therapy for the underlying disease is crucial in determining outcome.

Table 3: Cox proportional Hazard model result in tuberculosis associated hemophagocytic lymphohistiocytosis (n=53/63)

Variables	Univariate			Multivariate		
	HR†	95% CI#	*P* value*	HR	95% CI	*P* value
Age; >30 years	2.79	1.03-7.56	0.03	1.92	0.51-7.23	0.34
Presence of co- morbidity	4.59	1.08-19.52	0.04	5.18	0.63-42.29	0.13
Hemoglobin; ≤90 g/L	0.53	0.22-1.30	0.15	-	-	-
Platelet; ≤100 x 10^9/L	0.43	0.14-1.29	0.12	-	-	-
Ferritin; >1000 ng/mL	2.26	0.51-10.02	0.25	-	-	-
Triglyceride; >265 mg/dL	0.82	0.26-2.56	0.73	-	-	-
Coagulation abnormality; yes	1.41	0.62-3.21	0.41	-	-	-
Marked hemophagocytosis in bone marrow	2.65	1.16-6.05	0.02	1.80	0.74-4.36	0.19
Non-usage/delayed usage of ATT$	3.44	1.51-7.87	0.003	1.66	0.64-4.27	0.30
Immunosuppressive therapy; no	1.46	0.66-3.22	0.35	-	-	-
Disseminated disease	0.82	0.30-2.27	0.70	-	-	-

†: Hazard ratio, #: Confidence interval, *: *P* value <0.05 is considered as statistically significant, $: Antitubercular therapy

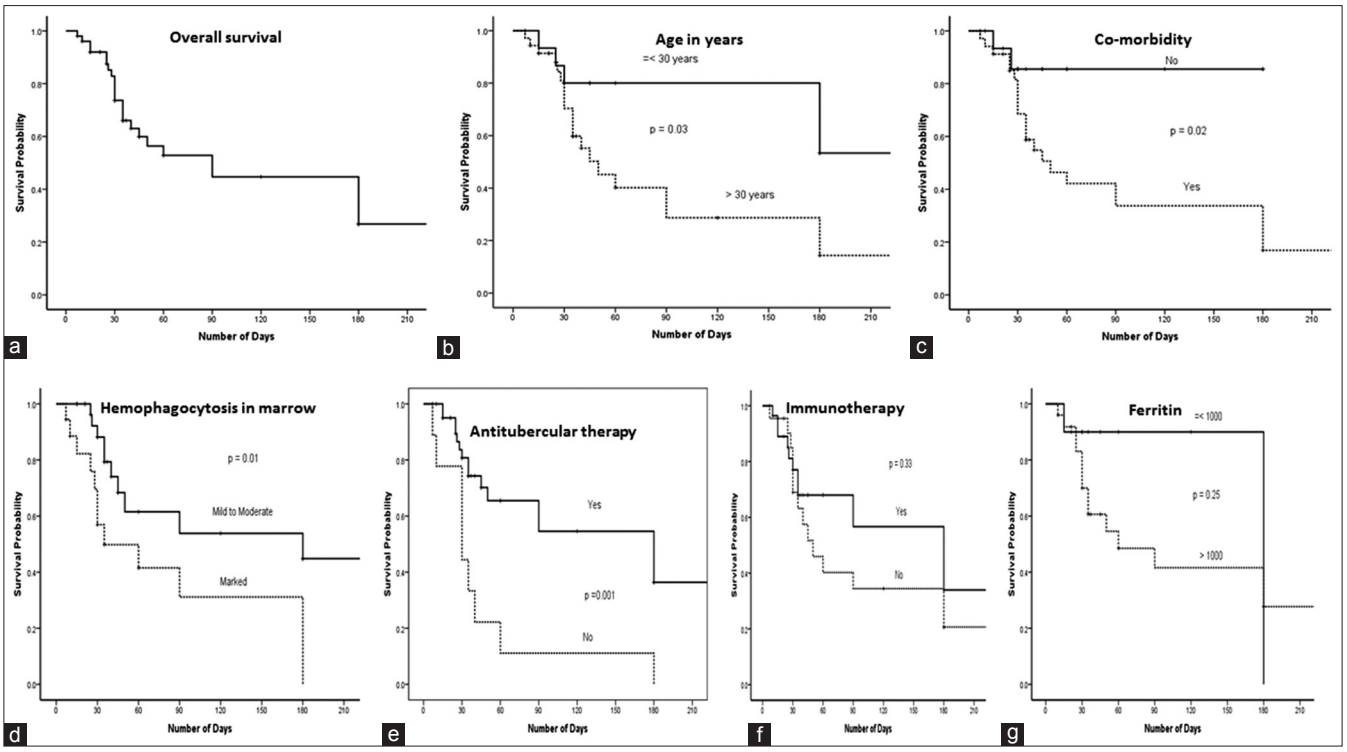


Figure 1: (Survival patterns in patients with tuberculosis associated hemophagocytic lymphohistiocytosis (TB-HLH) in relation to different parameters by Kaplan-Meier analysis using log-rank test. (a) Overall survival in patients with TB-HLH was approximately 45% after 3 months. On univariate analysis, (b) age > 30 years (P = 0.03); (c) presence of co-morbidity (P = 0.02); (d) evidence of moderate to marked degree of bone marrow hemophagocytosis (P = 0.01); and (e) non usage/delayed usage of antitubercular therapy (P = 0.001) were significantly associated with decreased survival. Usage of immunomodulators and/or immunosuppressive drugs (f) did not contribute significantly (P = 0.33) to the improved survival. High ferritin (>1000 ng/ml) was associated with poor survival; though it was not statistically significant (P = 0.25) (g)

CONCLUSION AND FUTURE PERSPECTIVE

HLH should be considered as a differential diagnosis in patients with tuberculosis who present with cytopenia(s), organomegaly, and coagulopathy. The existing literature points to the fact that TB-HLH may have an unpredictable and/or unfavorable outcome with or without ATT. Early diagnosis and initiation of ATT, even in the presence of disseminated disease, might alter the final outcome in these cases. First-line antituberculous drugs such as rifampicin have enzyme-inducing activity, which can lower the efficacy of drugs such as cyclosporine and etoposide used in the HLH 2004 protocol. Besides, HLH *per se* leads to significant derangement of liver functions, making the administration of ATT as well as etoposide difficult. Moreover, as was evident in 3 cases, HLH may even be exacerbated after initiation of ATT, which may be challenging to treat.[8,25] Therefore, it is open to speculation whether HLH *per se* or the delay in initiation of ATT is the predictor of unfavorable outcome in these cases. The best approach would be determination of treatment priorities based on the clinical condition of the patient and individualization of the treatment plan according to clinicolaboratory parameters, as was evident in our case. Finally, the utility of the HLH 2004 protocol in patients with TB-HLH seems to be controversial at present, and warrants larger future prospective studies.

Author contribution

SP did the conceptual design, collection, acquisition, and interpretation of the data, writing, and editing of the final manuscript. KR did the statistical analysis. JPS and AB did the collection and acquisition of data, and reviewed the literature. RGV reviewed and analyzed the manuscript for the intellectual content. All authors agreed as to the final content of the manuscript.

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