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Diagnosing and treating hemophagocytic lymphohistiocytosis in the tropics: Systematic review from the indian subcontinent

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Background. Hemophagocytic lymphohistiocytosis (HLH) is a catastrophic syndrome of unrestrained immune activation. Evaluation and management of HLH in the tropics is challenging. **Objectives.** To examine the reported etiologies and management of HLH reported from the sub-continent. **Methods.** Systematic review of all published cases from the Indian sub-continent. **Results.** We found only 156 published cases of HLH from the sub-continent. HLH was reported from the immediate perinatal period to 46 years of age. Infection-associated HLH (IAHS) constituted 46.8% of all cases of HLH (44% and 51% in children and adults respectively). In adults, tropical infections triggered 51% of these cases of IAHS. Steroids were used in 47% of children and 10% of adults. Etoposide and/or cyclosporine were used in 8% children and 8% of adults only. Intravenous immunoglobulin was used in another 30% of children and 4% of the adults. HLH-related mortality occurred in 31.8% and 28% of children and adults respectively. **Conclusions.** HLH is under-reported in the sub-continent and has high mortality. Cyclosporine and etoposide are seldom administered early despite diagnosis of HLH. Larger cohorts with IAHS triggered by tropical infections are urgently needed to understand its natural history and implications of this differing prescription pattern on mortality.

Key words: Hemophagocytic lymphohistiocytosis, Hemophagocytic syndrome, India, Indian sub-continent.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a potentially fatal syndrome secondary to immune hyperactivation (1). HLH may be genetic in origin or arise secondary to infectious, rheumatologic, malignant or metabolic disorders (Table 1).

Irrespective of cause, the unrestrained immune hyperactivation characteristic of this syndrome leads to host tissue damage (2). The diagnosis of this syndrome is cur-

Table 1 Etiology of Hemophagocytic lymphohistiocytosis

Category	Specific causes	Defect
Genetic HLH		
FHLH1	Unknown	Unknown
FHLH2	PRF1	Vesicle content
FHLH3	Munc13.4	Vesicle priming
FHLH4	STX11	Vesicle docking and fusion
FHLH5	STXBP2	Vesicle docking and fusion
Associated with other syndromes		
Chediak-Higashi I	Autosomal recessive, oculocutaneous albinism, easy bruising and frequent pyogenic infections, due to decreased chemotaxis and bactericidal activity; large neutrophil granules & abnormalities in LYST gene	
Griscelli II	Autosomal recessive syndrome; hypomelanosis with immunologic abnormalities with or without neurologic impairment, caused by mutation in the RAB27A gene; normal leukocyte pigmentation	
Hermansky-Pudlak II	Homozygous mutations of β3A subunit of the AP3 complex (AP3β1) on chromosome 5q14.1. Partial oculocutaneous albinism, platelets lacking dense bodies and storage of ceroid-like material in tissues. May include interstitial lung disease, renal abnormalities, cardiomyopathy	
XLPI (X-linked)	Mutation in the SH2D1A gene encoding SLAM-associated protein (SAP). Phenotype has severe or fatal mononucleosis, acquired hypogammaglobulinema, HLH and lymphoma. May include aplastic anemia, red cell aplasia, and lymphomatoid granulomatosis	
XLPII (X-linked)	Similar phenotype; mutation in X-linked inhibitor of apoptosis	
Metabolic syndromes		
Congenital lysinuric protein intolerance, Di- George's, Omenn's and Wiskott-Aldrich syndrome		
Infections		
Viral	EBV (most common); CMV, HIV, Avian influenza, Parvovirus B19, dengue shock syndrome, HHV-6,8, Varicella-Zoster, Herpes simplex	
Bacterial	Salmonella spp, leptospirosis, Rickettsia	
Protozoal	Plasmodium, leishmania	
Mycobacterial	*Mycobacterium tuberculosis* (disseminated tuberculosis)	
Fungal	Invasive aspergillosis, Penicillium marneffi infection	
Malignancy		
	T-cell lymphoblastic leukemia	
Rheumatologic disorders		
	Adult-onset still disease, juvenile-onset rheumatoid arthritis, systemic lupus erythematosus	

Abbreviations: FHL-Familial hemophagocytic lymphohistiocytosis, HLH- hemophagocytic lymphohistiocytosis, XLP-X-linked lymphoproliferative syndrome (Duncan's syndrome), EBV-Epstein Barr virus, CMV-cytomegalovirus, HHV-Human herpes virus, PRF1- pore forming protein 1, STX11 syntaxin 11, STXBP2-syntaxin binding protein 2, Munc-mammalian uncoordinated protein gene, LYST-lysosomal trafficking regulator gene, RAB27A- Ras-related protein Rab-27A gene.

rently based on meeting the HLH-2004 criteria (Table 2).

In the tropics, especially in adults, infections are common triggers of HLH (3) and the prevalence of perforin mutations is unknown. The clinical features of tropical infectious triggers can overlap with that of HLH and the diagnosis of the inciting etiology can be challenging (Table 3) (4).

Table 2 Revised diagnsotic criteria for hemophagocytic lymphohistiocytosis (1)

The diagnosis of hemophagocytic lymphohistiocytosis can be established if one of either one or two is fulfilled
1. A molecular diagnosis consistent with hemophagocytic lymphohistiocytosis OR
2. Diagnostic criteria for HLH fulfilled (At least 5 out of the 8 below to be fulfilled)
1. Fever
2. Splenomegaly
3. Cytopenias (affecting ≥2 of 3 lineages in the peripheral blood with Hemoglobin <10 g/dl, platelets <100 x 109/l and neutrophils <1.0x109/l)
4. Hypertriglyceredemia and/or hypofibrogenemia (fasting triglycerides > 265 mg/dl, fibrinogen ≤1.5 g/dl)
5. Hemophagocytosis in bone marrow or spleen or lymph nodes[1]
6. Low or absent NK-cell activity (according to local laboratory)[2]
7. Ferritin ≥500/ µg/dl
8. Soluble CD25 (Soluble Interleukin-2 receptor) ≥2400 U/ml[3]

[1] Hemophaogcytosis may be absent at initial evaluation; repeated marrow and/or tissue aspirates and biopsies may be needed;
[2] Sustained low NK cell activity suggests perforin and/or granzyme pathyway abnormality and screening for CD107a (LAMP-1 cell surface expression by cytometry) expression is recommended; NK-cell activity may be transiently depressed in IAHS. Persistent NK-cell activity depression even in the face of clinical resolution and absent known mutations should trigger referral for bone marrow transplantation.
[3] This is the most sensitive biochemical test and levels co-relate with response and prognosis; levels are age-dependent and local data needs to be ascertained.

Table 3 Unique practice concerns during evaluation and treatment of HLH in the tropics (compared to the western world)

TROPICS	WESTERN WORLD
Age of onset is bimodal with a larger adolescent-young adult population	Most cases are infants and young children; smaller adolescent and adult group
Frequency of familial HLH not characterized in any major ethnic group in the tropics; familial HLH reported but mutation NOT characterized	
Screening studies of immunologic dysfunction1 not widely available	Defined mutations (5 in females and 7 in males part of the initial genetic evaluation2)
Confirmatory genetic tests for HLH not available and frequency undefined	
Extensive search needs to be focused on ruling out tuberculosis, leishmaniasis & malaria as causes of HLH	Similar considerations; however tropical infectious causes are extremely rare as triggers of HLH
HLH secondary to tropical infections may have a better prognosis	Viral (EBV)-related HLH similar prognosis and treatment; increased mortality if etoposide delayed
Case series indicate tuberculosis, leishmaniasis, malaria, rickettsia & dengue-related HLH recover with pathogen-specific treatment ± steroids alone	EBV-related HLH treated with similar (HLH-2004) regimen; relapses may occur. Antivirals also in conjunction
HLH recognition may be delayed, especially in adults with acute presentation because symptoms & signs mimic leishmaniasis, disseminated tuberculosis, malaria or sepsis syndrome	HLH is less common in this age group and these infections are uncommon except in immigrants
IVIG is used along with steroids as the main immunomodulatory therapy	Cyclosporine, etoposide and dexamethasone; methotrexate intrathecally in select cases
No large series or published experience as part of a protocol; case reports and small single-centre series only	Enrollment in HLH-2004 trials; established HLH-2004 or ATG-steroid-BMT protocol

[1] Includes perforin and granzyme assays and/or CD107a expression in all and SAP protein and XIAP protein expression in males only;
[2] Includes PRF1, MUNC13-14, STXBP2, STX11, RAB27A; (SH2D1A, BIRC4 in males only).
Abbreviations: HLH=Hemophagocytic lymphohistiocytosis, ATG=anti-thymocyte globulin, HCT=hematopoietic stem cell transplantation, EBV=Epstein Barr virus, ATG=Anti-thymocyte globulin, IVIG-Intravenous immunoglobulin, BMT=Bone marrow transplantation.

Intense immunosuppression administered for HLH without appropriate antimicrobial therapy can have disastrous consequences and the course of tropical infections associated with HLH may be different from other causes of HLH (5). We performed a systematic review to identify the common etiologic triggers of HLH and suggest an appropriate initial etiologic evaluation and management strategy for HLH in the sub-continent.

Methods

Literature search

Two of the authors (Dr. R.S and Dr. N.S) conducted a systematic search of English literature independently using the terms "hemophagocytosis", "hemophagocytic lymphohistiocytosis" "Macrophage activation" *and* "Asia" *or* "India" in the MEDLINE, OVID and CINAHL databases. This was further supplemented by search of IndMED, the internet search engine GOOGLE and a hand search of the references and our personal databases for published cases of hemophagocytic lymphohistiocytosis from the Indian sub-continent including India, Pakistan, Sri Lanka, Bangladesh, Nepal and Bhutan.

Only those articles were included for analysis which were reported in English literature and included patients with diagnosed HLH fulfilling the 2004 criteria of the Histiocyte society as evidenced by 1) genetic diagnosis of HLH *or* 2) At least 5 out of the 8 criteria for clinical diagnosis of HLH (Table 1).

Data extraction

Both the abstracts and full text articles, where available, were reviewed. Where neither was available, the authors were mailed for data on their published cases. Data was extracted in a pre-designed data extraction form regarding the age, sex, etiology of HLH, treatment of HLH administered and outcomes. Data was extracted and expressed in a descriptive fashion (Mean, SD; Table 4)

Results

Our search yielded 682 references. This included 156 cases of HLH (Table 4 A&B) in 56 published reports, including 63 adults (≥8 years of age) and 93 children.

Full text or abstracts were available for all 156 cases in the articles reviewed (4-59). An additional 24 reports were excluded because they reported cases from outside the subcontinent, they did not meet current criteria for HLH (60-64) or did not report any new case of HLH (65). Cases reported in duplicate were not included for the analysis (26).

HLH was reported from the immediate perinatal period (55) to 46 years of age (36). Adults formed 40.4% (N=63/156) of the total number of cases reported (Table 4 A&B). The male to female ratio was 2:1 in adults (N=15) and children (N=49). Most published cases were single case reports. Clinical series reported a confirmed infection as a triggering factor for HLH in 42-43% (5, 9, 56) of their cohorts.

In children, definite (known mutations) or possible familial HLH (FHLH), as suggested by family history, multiple relapsing courses or prominent neurologic disease at onset was reported as the cause in 24.7% (13/93) of the pediatric cohort. No series reported the results of genetic testing for HLH. Another 2.1% (2/93) were due to inherited causes such as Griscelli syndrome (17) and Chediak-Higaski syndrome (51). Infectious triggers were seen in 44% (41/93) overall; Viruses (56%, 23/41) and tropical infectious agents (32%, 13/41) were the agents recognized. Viruses that were found on evaluation included unknown agents 39% (9/23), Epstein-Barr virus (EBV) 17.3% (4/23), Dengue 26% (6/23), Cytomegalovirus and Parvovirus B19 (2/23, 8.7% each). Connective tissue disease, especially Still's disease, triggered another 18.3% (17/93) of cases of HLH.

Table 4 A Systematic search of all published reports of HLH in adult patients published from South Asian subcontinent

Author	Age	No. of cases	Etiology of HLH	Treatment	Outcome	Comments
Kumar et al. [8]	NA	2	Histoplasmosis	Empiric ATT; No antifungals -Died at 48 hours		HLH: Node (1), spleen (1)
Bhutani et al. [10]	28/M	1	VL	AmB	Alive	VL diagnosis-serology alone
Pahwa et al. [12]	Bone marrow review (n=14)		VL (9), P. vivax (2), P. falciparum (3)		Died (3/14; all VL-HLH bleeding, SSG-related myocarditis)	
Saluja et al. [15]	43/M	1	Histoplasmosis	Itraconazole orally x 6 months		Alive
Karthik et al. [18]	50/M	1	? Tuberculosis	Empiric ATT; no immunomodulation-Alive		Clinical response to ATT alone
Pinto et al. [20]	Chart review; 8/13 adults-mean age 45.75 years			CTD (4); Nephrotic (1); CLD (1); Malignancy (2)		5/13 died; Rx details NA
Rajagopala et al. [4]	23/M	1	VL	Amphotericin B	Alive	rk-39 ELISA; LD negative
Singh et al. [27]	2/14 patients with Still's disease; mean 29.8 years			CSA, steroids, sulfasalazine		Died (1), alive (1)
Patel et al. [31]	NA	1	VL	NA		HIV co-infection
Prasad et al. [33]	NA	2/3 cases	VL	Amphotericin	Alive	LD positive; rk-39+
Premaratna et al. [34]	NA	2	Rickettsia	Antibiotics	Alive	Serology diagnosis
Aggarwal et al. [36]	46/F	1	B-cell lymphoma	Supportive; died < 48 hours after admission		BMA ante-mortem
Gopal et al. [39]	3 patients over a year		Scrub typhus	Antibiotics alone	Alive	Weil-Felix, ELISA+
Koul et al. [40]	40/M	1	Salmonella typhi	Antibiotics alone	Alive	Blood cultures positive; BMA HLH (Ferritin data missing for 3 patients)
	18/F	1				
	25/M	1				
	25/F	1	?VAHS	Methylprednisolone	Alive	No recurrence
	45/M	1	? FLH	HLH-94	Died; recurrent disease	Recurrence @ 4 months
Valsalan et al. [45]	22/M	1	Scrub typhus	Antibiotics alone	Alive	Weil-Felix, ELISA+
Ray et al. [50]	24/F	1	Dengue fever	Steroids; slow taper	Alive @ 6 months	NS1 antigen detection
Chandra et al. [53]	38/F	1	Histoplasmosis	Antifungals	NA	HIV co-infection; BMA+
John et al. [54]	28/M	1	? IAHS Acinetobacter super-infection	Steroids, CSA, antibiotics	Alive; etiologic evaluation poor	
Nayan et al. [57]	19/M	1	?IAHS	Steroids, etoposide	Died @ 96 hours	Shock, ARDS
Mishra et al. [56]	Retrospective review 14 cases 43% (6/14) IAHS; EBV (3/6) Parvovirus B19 (2/6) and CMV (1/6)					3/14 died

Table 4B Systematic search of all published reports of HLH in pediatric patients published from South Asian subcontinent

Author	Age	No. of cases	Etiology of HLH	Treatment	Outcome	Comments
Joseph et al. [6]	7/M	1	? Viral HLH	None	Died; S. aureus sepsis	Post-mortem Bx
Biswal et al. [7]	2 month/M	1	?FLH	None	Died @ 10 days	Evaluation limited
Mathew et al. [9]	N=7, Median 1 year	M (4); F(3)	IAHS (? 4); FLH (3)	Supportive	IAHS 2/4 died; FLH (3/3) died	Bone marrow (5/7); liver Bx HLH (2/7)
Kakkar et al. [11]	Autopsy series; antenatal diagnosis (1/4) All FLH with advanced HLH on autopsy; supportive Rx only					
Bakhshi et al. [13]	9/M	1	EBV	Alive; Supportive only. No antivirals or etoposide		
Dutta et al. [14]	13/F	1	Parvovirus B19	Antibiotics; antifungals	Died	IPA also at autopsy
Agarwal et al. [16]	6/M	1	VL	SSG	Alive	BMA-amastigotes, HLH
Malhotra et al. [17]	4 months		Griscelli syndrome	Supportive	Died	Superadded infection
Karthik et al. [18]	17/M	1	Salmonella	Ceftriaxone x 14 days	Alive	Resolution by 7th day
Mathur et al. [19]	4/M	1	VL	SSB; Later AmB-died DIC		LD bodies; rk-39+
Pinto et al. [20]	Chart review; 5/13 children-mean age 13.7 years			CTD (4); VL (1)		5/13 died; Rx details NA
Das et al. [21]	2/F	1	?Viral HLH	AmB for Candidemia; Alive		Marrow C/S-Candida
Jain et al. [22]	14/F	1	Dengue	Supportive care alone; Alive @ 1 month		IgM Dengue ELISA+
Medhi et al. [23]	11/M	1	T-cell lymphoma	Chemotherapy	Alive	Panniculitis
Rajam et al. [24]	13/F	1	Juvenile-onset R.A	Methylprednisolone	Alive; ARDS, shock and liver dysfunction	
	14/F	1	SLE	Methylprednisolone	Alive; cardiac tamponade	
Raka et al. [25]	1.5 mo/F	1	FLH	Supportive	Died	
Balasubramaniam [26]	52 days/M	1	Tuberculosis	ATT and IVIG	Died; ARDS, gastric aspirate C/S-M tuberculosis	
Gosh et al. [28]	3/M	1	?VAHS	Supportive (antibiotics)	Alive; Acinetobacter super-infection	
Gupta et al. [29]	17/M	1	Tuberculosis	ATT, steroids	Alive; Repeated negative Bx of nodes for AFB	
Juneja et al. [30]	12/F	1	soJRA	Steroids, CSA	Died	Long delay to Rx
Pramanik et al. [32]	8/M	1	? Viral	Steroids alone	Alive	Etiology unclear
	10/M	1	? FLH ?Viral	Steroids alone	Alive at 10 months	Seizures
	12/F	1	? Viral	Steroids, IVIG	Alive	Rx 8 weeks
	6/M	1	Salmonella	Antibiotics alone	Alive	Rx 4 weeks
	3/M	1	? FLH	Steroids alone	Died	Jaundice, ascites

Continuation of Table 4B Systematic search of all published reports of HLH in pediatric patients published from South Asian subcontinent

Author	Age	No. of cases	Etiology of HLH	Treatment	Outcome	Comments
Puliyel et al. [35]	12/M	1	FLH	HLH-2004	Remission; relapse 8 months and died	
Dass et al. [37]	16/M	1	Falciparum malaria	Methylprednisolone	ARDS, Shock; steroids taper over 14 days	
Deshpande et al. [38]	2 month/M	1	Miliary tuberculosis	ATT and steroids	Alive; AFB+, granulomas BMA	
Kumar et al. [41]	6/M	1	soJRA	Steroids	Alive	
Mondal et al. [42]	2.5 month/M	1	Brucellosis	Antibiotics alone	Bone marrow HLH; C/S-Brucella	
Ramesh et al. [43]	9/M	1	soJRA	Antibiotics, CSA, steroid	Alive; urinary tract infection	
	10/M	1	Hodgkin's lymphoma	Chemotherapy	Better; malignancy on 2..nd BMA only	
Suresh et al. [44]	3/F	1	Kawasaki's disease	Aspirin, IVIG, steroids	Better; BMA negative; no response to IVIG	
Ali et al. [46]	NA	1	FLH	HLH protocol and BMT	Alive; report from Pakistan	
Gupta et al. [47]	2 month/M	1	CMV	Ganciclovir alone	Better; IgM, PCR+ BMA-negative, FNA Node+	
Jayakrishnan et al. [48]	5/F	1	Scrub typhus	Antibiotics alone	NA	
Kumar et al. [49]	6/F	1	SoJRA	Steroids, cyclosporine	Alive; PRES during steroid therapy	
Ramachandran et al. [5]	N=32*; Age mean 46 months; 33/43 over 2 years fulfilled HLH criteria		Dengue (5), EBV (3), CMV, leptospira and bacterial (5)	Steroids (67%), IVIG (64%), CSA (33%), Etoposide (15%)	Died (8, 26%); BMA-86% HLH; 2/33 only treated with HLH-2004 protocol x 8 weeks	
Roy et al. [51]	1/5 of CHS with HLH 8months/F			Supportive	Died <48 hours	
Vinoth et al. [52]	11 month/M	1	Falciparum malaria	Artesunate alone	Better; peripheral smears negative; BMA parasites	
Maheshwari et al. [55]	Perinatal	1	Tuberculosis	NA		
Sood et al. [59]	16/M	1	Parvovirus B19	Antibiotics alone	Klebsiella bacteremia;	
Singh et al. [58]	6 patients (5 M; 1 F)-13 years		soJRA	Steroids; IVIG (2/6)	1/6 died; BMA (4); Nodes (1)	

Abbreviations: Male (M), Female (F); NA- not available; + present; - absent; Bx – Biopsy; ATT- anti-tuberculosis therapy; HLH - Histo-pathologic evidence of hemophagocytic lymphohistiocytosis; VL - Visceral leishmaniasis; AmB - Amphotericin B desoxycholate: P. vivax - Plasmodium vivax; SSG - Sodium stibogluconate; CTD - connective tissue disease: CLD - Chronic liver disease; Rx – Treatment; LD - Leishman-Donovan bodies; CSA - cyclosporin A; HIV - Human immunodeficiency virus; BMA-Bone marrow aspiration; ELISA-Enzyme - linked immunosorbent assay; FLH - Familial HLH; IAHS - Infection associated HLH; ARDS - Acute respiratory distress syndrome; EBV - Epstein Barr virus; CMV – Cytomegalovirus; C/S - culture & sensitivity; RA - Rheumatoid arthritis; soJRA - systemic onset Juvenile rheumatoid arthritis (Still's disease); IVIG – immunoglobulin; BMT - Bone marrow transplantation; PRES - Posterior reversible encephalopathy syndrome. *Duplicate reporting of one case of tuberculosis removed.

In adults, no confirmed or probable case of FLH has been reported from the sub-continent. Infections were reported as the most common triggers for HLH. In adults, tropical infectious diseases were reported to have triggered 51% (32/63) of the cases of HLH [Visceral leishmaniasis (VL) 40.6% (13/32), Rickettsia 18.8% (6/32), Malaria 15.6% (5/32), Histoplasmosis 12.5% (4/32), Enteric fever 9.4% (3/32), Tuberculosis 1/32]. Viral agents were reported as possible triggers in another 30% (19/63) cases of HLH, but the etiologic agent was unrecognized in 68.5% of cases (13/18). Where an etiologic agent was reported, EBV 16.7% (3/18) and Parvovirus B19 (11.1%) were the triggers most often. Connective-tissue disease and malignancy were other important recognized triggers in adults (9.5%, 6/63 and 4.8%, 3/63 respectively).

Data on the use of immunomodulatory treatments for HLH were available in 93.5% (87/93) and 79.4% (50/63) of adults. Steroids were the most common immunomodulatory agents used in 47% (41/87) of children and 10% (5/50) of adults. Etoposide and/or cyclosporine were used in 8% (7/87) children and 4/50 (8%) of adults only. Intravenous immunoglobulin (IVIG) was used in another 30% of children (26/87) and 2/63 (4%) of the adult cases reviewed. HLH-related mortality occurred in 31.8% (29/91) and 28% (17/61) of children and adults respectively.

Discussion

Hemophagocytic lymphohistiocytosis (HLH) is an entity which presents major diagnostic and therapeutic challenges (1). HLH is a clinical syndrome secondary to hyper-cytokinemia and organ infiltration by phagocytizing histiocytes (Figure 1) resulting from defects in critical regulatory pathways responsible for the termination of inflammatory responses (1, 2).

This entity is often under-recognized, especially in adults, and specific therapy is not considered early in the disease course. Our systematic review found less than 160 cases in a population of 1.2 billion, indicating under-recognition of this entity. Most cases were single reports and most of these reports clustered around the same centers across the sub-continent (Table 4 A&B). Given the prevalence of the tropical triggers of HLH in the sub-continent, the possibility of under-diagnosis remains highly likely, especially in adults. One of the largest series and a seminal report of HLH from India was not included as it did not fulfill the current requirements for diagnosis of HLH (60).

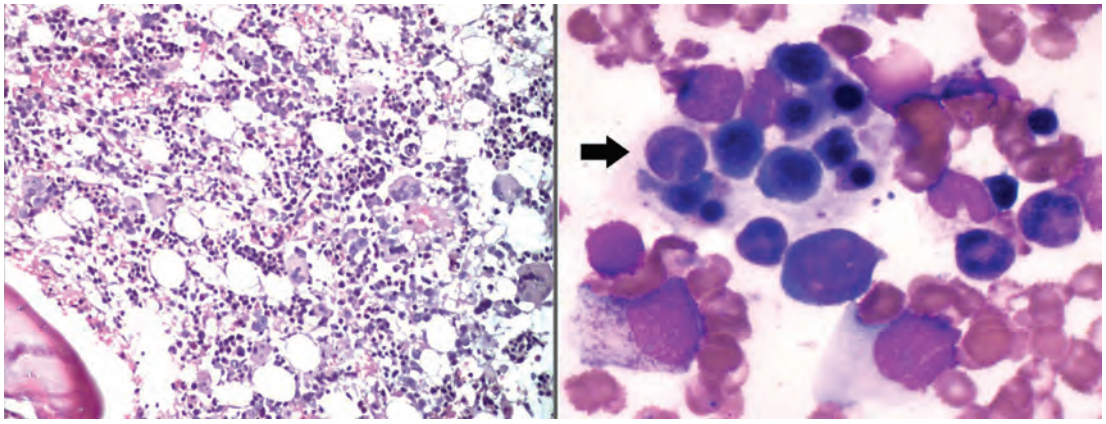

Figure 1 Composite figure showing low-power photomicrograph (x 100 hematoxylin stain) of bone marrow in a patient with HLH. The oil immersion image (right x 400) of the same section shows histiocytes with hemophagocytosis and leukophagocytosis.

Genetic HLH may be: familial (FHL), autosomal recessive], sporadic (sHLH) or complicate certain well characterized syndromes involving defects in the cytolytic T-cell pathway (Table 1). FHL has an estimated incidence of around 1:50,000 and is invariably fatal if untreated, with a median survival of two months. The presentation is often in infancy, though onset in adolescence and adults is well known. The family history may be non-contributory, given the recessive nature of inheritance and the absence of large families (66); furthermore, FHL may often be triggered by infections. Infections, notably Epstein-Barr virus, are another leading cause of HLH (67). Salmonella, tuberculosis, malaria and leishmaniasis are tropical infections that are well-recognized triggers of infection-associated hemophagocytosis (IAHS) (Table 1). In the tropics, infections are overwhelmingly the most common triggers of HLH. In our systematic review, age was not a predictor of etiology of HLH. Tuberculosis has been reported in the neonatal period (55) when FLH is usually prevalent in the Western world. Caution is however required in interpreting our results. The authors did not use a common etiologic evaluation panel, evaluation was often limited, unknown triggers constitute a large sub-group and genetic tests for FLH were seldom done, even in children.

The clinical features of HLH, secondary to such unrestrained immune activation, are not specific and mimic tropical infections (VL, disseminated tuberculosis, and severe malaria), hematological malignancy and auto-immune disease in adults. The diagnosis of HLH is made by fulfilling the revised HLH criteria (1) (Table 2) which were primarily designed to select enrollment into clinical trials. The sensitivity of these criteria for early HLH is unknown given the lack of a gold standard test. Importantly, the clinical picture might be aggressive and the diagnostic criteria might not be fulfilled at onset, making management extremely challenging. In particular, the finding of bone marrow hemophagocytosis is not sensitive for the diagnosis of HLH or the underlying trigger (4, 43). Also, the finding of isolated marrow hemophagocytosis in the absence of the clinical syndrome does not qualify for the diagnosis of HLH (Table 2). In the West and South-East Asia, IAHS is usually viral (EBV)-triggered and a distinction between FHL and IAHS at onset is *not* made; indeed, delay in administration of etoposide to cases of EBV-related HLH is associated with increased mortality. In contrast, tropical HLH may be triggered by *tuberculosis, VL, Salmonella, Plasmodium, dengue or Parvovirus B19*. Several case reports and small series suggest that the natural history of IAHS may be different from EBV-triggered HLH (3, 5, 18).

The HLH-2004 protocol uses upfront cyclosporine [with etoposide, dexamethasone] and intrathecal methotrexate for patients with neurological signs, persistent active CNS disease and CNS reactivation of HLH. All children with familial disease, known mutations, severe and persistent non-familial disease and relapsed HLH are treated with continuation phase etoposide, dexamethasone, and CSA. Stem-cell transplantation is performed as early as possible, when an acceptable donor is available. Therapy is discontinued otherwise at remission (8 weeks) as the completed regimen for patients with possible sHLH and viral-triggered HLH. Patients with refractory disease are treated with ATG, rituximab or alemtuzumab for remission induction. Our systematic review shows that steroids and IVIG (in children) are the common regimens reported; the use of CSA and/or etoposide was very low. The reasons for this may include fulminant presentation, late recognition, the inability to rule out tropical-triggers of HLH, physician perception on the differing profiles of non-viral infection-triggered HLH and severe cytopenia (4). Data from systematic reviews

suggest that HLH secondary to VL, tuberculosis, malaria and dengue may recover with *early* anti-microbial therapy and steroids alone. Indeed, the major correlate with mortality is the time to diagnosis (and treatment) of the offending pathogen. Further, the co-existing organ dysfunction due to HLH may also complicate drug administration [e.g. anti-tubercular drugs and HLH-related liver dysfunction, cytopenia and etoposide dose]. Our adult series also highlights these difficulties; infections were the most common triggers (80%; 10% unknown), short presentation was (median 11 days, IQR 9.25-30) and HLH criteria not being fulfilled at ICU admission median of 4 (IQR 2-4.25) (3).

A uniform protocol for rapid early evaluation of suspected HLH and initiation of therapy (Table 5, Figure 2) is important.

Such a protocol, especially in adults, should balance the exhaustive search for tropical triggers and early initiation of HLH-2004 (including etoposide) in patients with viral-triggered HLH and patients with tropical infection-triggered HLH not responding to steroids alone (Figure 2).

Table 5 Summary of suggested evaluation of a suspected patient with hemophagocytic lymphohistiocytosis in the Indian sub-continent

1. Tests to confirm the diagnosis of hemophagocytic lymphohistiocytosis (HLH)
Complete blood counts, peripheral smear, reticulocyte counts
Liver function tests
Serum creatinine, bicarbonate
Prothrombin time, activated partial thromboplastin time, fibrinogen, D-dimers
CSF (if symptomatic): Total counts, protein levels and cytology
Serum triglycerides, serum ferritin levels
Bone marrow aspirate or tissue aspirates (where involved)
Soluble CD25 levels
NK-cell activity (if possible)
2. Etiological work-up for proven HLH
Review of marrow for *Leishman-donovan* bodies, granulomas, *Histoplasma* inclusions, malignancy, *Plasmodium* inclusions or normoblasts; *Candida* and CMV in neonates; Request mycobacterial and bacterial cultures
Peripheral smears for malaria
Blood cultures, Widal test where applicable
Anti-EBV VCA IgM (PCR if available)
CMV PP65; Qualitative PCR in neonates, immunosuppressed and neutropenia
Human immunodeficiency virus ELISA
rk-39 ELISA for leishmanisis
IgM Parvovirus ELISA
IgM Dengue ELISA or Macro agglutination assay for dengue and leptospirosis
IgM ELISA or Macro agglutination assay for leptospirosis
Weil-Felix test, IgM immunochromotographic test for scrub typhus
Anti-nuclear antibody ELISA or Immunofluorescence for anti-nuclear antibodies
Lymph node biopsy (If prominent lymphadenopathy and sub-acute course suggests lymphoma)
Assay for perforin expression by flow cytometry and/or CD107a expression (*if FLH suspected and all assays for IAHS negative*)
3. Search for complications
Echocardiography (for pericardial effusions), ejection fraction
Contrast-enhanced CT-Head or MRI-Brain (if neurological symptoms)
4. Research in proven FLH in the tropics
Perforin mutations (in association with research centers or western centers)

Abbreviations: FHL - Familial hemophagocytic lymphohistiocytosis; HLH - hemophagocytic lymphohistiocytosis; CD - Cluster of differentiation; EBV - Epstein Barr Virus; CMV - Cytomegalovirus; C/S - culture & sensitivity; ELISA - Enzyme-linked Immunosorbent assay; CT - computed tomography; MRI - Magnetic resonance imaging; FLH - Familial HLH; IAHS - Infection associated HLH; CSF - Cerebrospinal fluid analysis; PCR - Polymerase chain reaction.

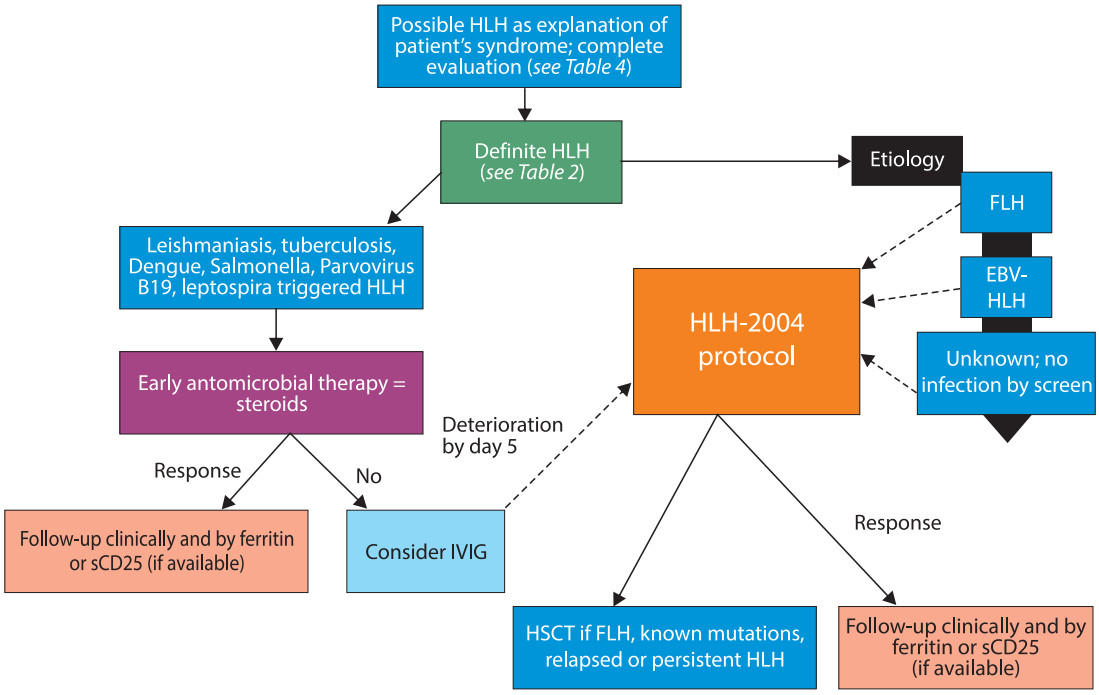

Figure 2 Suggested flow-chart for evaluation of an adult patient with suspected HLH in the sub-continent based on our systematic review.

Lastly, multi-centric prospective data from the sub-continent with such a common evaluation protocol and longitudinal outcomes in cohorts of patients with IAHS will clarify the etiology, management and outcomes of HLH in the sub-continent and whether the prescription patterns in the sub-continent merit reconsideration.

Conclusions

In conclusion, HLH is a catastrophic and fulminant clinical syndrome of immune activation. Heightened clinical recognition of this entity and early evaluation with rapid initiation of treatment may help in better outcomes. More data and multi-centric prospective studies from the tropics are urgently required.

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References

1. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48(2):124-31.
2. Creput C, Galicier L, Buyse S, Azoulay E. Understanding organ dysfunction in hemophagocytic lymphohistiocytosis. Intensive Care Med. 2008;34(7):1177-87.
3. Rajagopala S, Singh N, Agarwal R, Gupta D, Dass R. Severe hemophagocytic lymphohistiocytosis in adults-experience from an intensive care unit from North India. Indian J Critical Care Medicine. 2012. in press.
4. Rajagopala S, Dutta U, Chandra KS, Bhatia P, Varma N, Kochhar R. Visceral leishmaniasis as-

sociated hemophagocytic lymphohistiocytosis--case report and systematic review. J Infect. 2008;56(5):381-8.
5. Ramachandran B, Balasubramanian S, Abhishek N, Ravikumar KG, Ramanan AV. Profile of hemophagocytic lymphohistiocytosis in children in a tertiary care hospital in India. Indian Pediatr. 2011;48(1):31-5.
6. Joseph VJ, Sunny AO, Pandit N, Yeshwanth M. Hemophagocytic syndrome. Indian Pediatr. 1992;29(7):897-900.
7. Biswal N, Shareef S, Nalini P, Sreenisasan S, Basu D. Hemophagocytic lymphohistiocytosis. Ind J Pediatrics. 1999;66:632-5.
8. Kumar N, Jain S, Singh ZN. Disseminated histoplasmosis with reactive hemophagocytosis: aspiration cytology findings in two cases. Diagn Cytopathol. 2000;23(6):422-4.
9. Mathew LG, Cherian T, Sudarshanam A, Korah I, Kumar NKS, Raghupathy P. Hemophagocytic Lymphohistiocytosis: A Case Series. Indian Pediatrics. 2000;37:526-31.
10. Bhutani V, Dutta U, Das R, Singh K. Hemophagocytic syndrome as the presenting manifestation of visceral leishmaniasis. J Assoc Physicians India. 2002;50:838-9.
11. Kakkar N, Vasishta RK, Banerjee AK, Marwaha RK, Thapa BR. Familial hemophagocytic lymphohistiocytosis: an autopsy study. Pediatr Pathol Mol Med. 2003;22(3):229-42.
12. Pahwa R, Singh T, Khurana N. Hemophagocytic syndrome in malaria and kala-azar. Indian J Pathol Microbiol. 2004;47(3):348-50.
13. Bakhshi S, Pautu JL. EBV-associated Hemophagocytic Lymphohistiocytosis with Spontaneous Regression Indian Pediatrics. 2005;42:1253-5.
14. Dutta U, Mittal S, Ratho RK, Das A. Acute liver failure and severe hemophagocytosis secondary to parvovirus B19 infection. Indian J Gastroenterol. 2005;24(3):118-9.
15. Saluja S, Sunita, S Bhasin, D K Gupta, B Gupta, S P Kataria, et al. Disseminated Histoplasmosis with Reactive Haemophagocytosis Presenting as PUO in an Immunocompetent Host. J Assoc Physicians India. 2005;53:906-8.
16. Agarwal S, Narayan S, Sharma S, Kahkashan E, Patwari AK. Hemophagocytic syndrome associated with visceral leishmaniasis. Indian J Pediatr. 2006;73(5):445-6.
17. Malhotra AK, Bhaskar G, Nanda M, Kabra M, Singh MK, Ramam M. Griscelli syndrome. J Am Acad Dermatol. 2006;55(2):337-40.
18. Karthik R. Infectious Causes of Macrophage Activation Syndrome. J Assoc Physicians India. 2007;55:877.
19. Mathur P, Samantaray JC, Samanta P. Fatal haemophagocytic syndrome and hepatitis associated with visceral leishmaniasis. Indian J Med Microbiol. 2007;25:416-8.
20. Pinto L, Kagalwala F, Singh S, Balakrishnan C, Prabhu S, Khodaiji S. Macrophage Activation Syndrome: Experience from a Tertiary Referral Centre. J Assoc Physicians India. 2007;55:185-7.
21. Das S, Kalyani R. Hemophagocytic syndrome. Ind J Pathol Microbiol. 2008;51(1):125-6.
22. Jain D, Singh T. Dengue virus related hemophagocytosis: a rare case report. Hematology. 2008;13(5):286-8.
23. Medhi K, Kumar R, Rishi A, Kumar L, Bakhshi S. Subcutaneous panniculitis like T-cell lymphoma with hemophagocytosis: complete remission with BFM-90 protocol. J Pediatr Hematol Oncol. 2008;30(7):558-61.
24. Rajam L, Prasad V, Yatheesha BL. Reactive hemophagocytic syndrome. Indian J Pediatr. 2008;75(12):1261-3.
25. Raka S, Nayar P, Godbole R, Manchanda R. Familial hemophagocytic lymphohistiocytosis. Indian J Hematol Blood Transfus. 2008;25(2):78-80.
26. Balasubramanian S, Kaarthigeyan K, Aparna V, Srinivas S. Tuberculosis associated hemophagocytic syndrome in infancy. Indian Pediatr. 2008;45:593-5.
27. Singh S, Samant R, Joshi VR. Adult onset Still's disease: a study of 14 cases. Clin Rheumatol. 2008;27(1):35-9.
28. Gosh JB, Roy M, Bala A. Infection Associated with Hemophagocytic Lymphohisticytosis triggered by nosocomial Infection. OMJ. 2009;24:223-5.
29. Gupta AP, Parate SN, Bobhate SK, Anupriya. Hemophagocytic syndrome: A cause for fatal outcome in tuberculosis. Ind J Pathol Microbiol. 2009;52(2):260-2.
30. Juneja M, Jain R, Mishra D. Macrophage activation syndrome in an inadequately treated patient with systemic onset juvenile idiopathic arthritis. Kathmandu Univ Med J (KUMJ). 2009;7(28):411-3.
31. Patel KK, Patel AK, Sarda P, Shah BA, Ranjan R. Immune reconstitution visceral leishmaniasis presented as hemophagocytic syndrome in a patient with AIDS from a nonendemic area: a case report. J Int Assoc Physicians AIDS Care (Chic). 2009;8(4):217-20.
32. Pramanik S, Pal P, Das PK, Chakrabarty S, Bhattacharya A, Banerjee S. Reactive Haemophagocytic Lymphohistiocytosis. Indian Journal of Pediatrics. 2009;76:643-5.

33. Prasad R, Muthusami S, Pandey N, Tilak V, Shukla J, Mishra OP. Unusual presentations of Visceral leishmaniasis. Indian J Pediatr. 2009;76(8):843-5.
34. Premaratna R, Williams HS, Chandrasena TG, Rajapakse RP, Kularatna SA, de Silva HJ. Unusual pancytopenia secondary to haemophagocytosis syndrome in rickettsioses. Trans R Soc Trop Med Hyg. 2009;103(9):961-3.
35. Puliyel MM, Rose W, Kumar S, Moses PD, Gibikote S. Prolonged neurologic course of familial hemophagocytic lymphohistiocytosis. Pediatr Neurol. 2009;41(3):207-10.
36. Aggarwal D, Gupta R, Singh S, Gupta K, Kudesia M. Hemophagocytic Lymphohistiocytosis in B-Cell Lymphoproliferative Disorder: Report of a Rare Association. Indian Journal of Hematology and Blood Transfusion. 2010;26(2):74-6.
37. Dass R, Barman H, Duwarah SG, Choudhury V, Jain P, Deka NM, et al. Macrophage activation syndrome in malaria. Rheumatol Int. 2010;30(8):1099-101.
38. 38. Deshpande A, Nayar PS, Pradhan AM, Manchanda RV. Miliary tuberculosis with hemophagocytosis in a two months old infant. Indian J Hematol Blood Transfus. 2010;26(3):115-7.
39. Gopal GK, Anugrah C, Boorugu H. Scrub typhus associated macrophage activation syndrome. Trop Doct. 2010;40(4):249-50.
40. Koul PA, Khan U, Shah S, Jan MR, Wani DA, Abdul D, et al. Hemophagocytic Lymphohistiocytosis: A 25-Year Experience at a Tertiary Care Hospital. WebmedCentral INFECTIOUS DISEASES [serial on the Internet]. 2010;1(9):Available from: http://www.webmedcentral.com/wmcpdf/Article_WMC00674.pdf.
41. Kumar S, Vaidyanathan B, Gayathri S, Rajam L. Systemic onset juvenile idiopathic arthritis with macrophage activation syndrome misdiagnosed as Kawasaki disease: case report and literature review. Rheumatol Int. 2010. Available from: https://springerlink3.metapress.com/content/84213931j616105v/resource-secured/?target=fulltext.pdf&sid=ceyydymehif4ssuffc14q4o3&sh=www.springerlink.com
42. Mondal N, Suresh R, Acharya NS, Praharaj I, Harish BN, Mahadevan S. Hemophagocytic syndrome in a child with brucellosis. Indian J Pediatr. 2010;77(12):1434-6.
43. Ramesh M, Singh V, Ghuliani R, Kapur BN, Singh J, Shankar S. Histiocytosis Syndromes of Childhood: A report of four cases. J Nepal Paediatr Soc. 2010;30(3):171-4.
44. Suresh N, Sankar J. Macrophage activation syndrome: a rare complication of incomplete Kawasaki disease. Ann Trop Paediatr. 2010;30(1):61-4.
45. Valsalan R, Kosaraju K, Sohanlal T, Kumar PSP. Hemophagocytosis in scrub typhus. J Postgrad Med. 2010;56:301-2.
46. Ali N, Fadoo Z, Masood N, Adil SN. Successful Engraftment and Survival Following Allogeneic Hematopoietic Stem Cell Transplant in a Child with Familial Hemophagocytic Lymphohistiocytosis. Indian J Pediatr. 2011. Available from: http://www.springerlink.com/content/9272744w8016562t/
47. Gupta A, Sen R, Batra C, Banerjee D, Jain M. Hemophagocytic syndrome secondary to cytomegalovirus infection in an infant. J Cytol. 2011;28(1):36-8.
48. Jayakrishnan MP, Veny J, Feroze M. Rickettsial infection with hemophagocytosis. Trop Doct. 2011;41(2):111-2.
49. Kumar S, Rajam L. Posterior reversible encephalopathy syndrome (PRES/RPLS) during pulse steroid therapy in macrophage activation syndrome. Indian J Pediatr. 2011;78(8):1002-4.
50. Ray S, Kundu S, Saha M, Chakrabarti P. Hemophagocytic Syndrome in Classic Dengue Fever. J Glob Infect Dis. 2011;3(4):399-401.
51. Roy A, Kar R, Basu D, Srivani S, Badhe BA. Clinico-hematological profile of Chediak-Higashi syndrome: Experience from a tertiary care center in south India. Ind J Pathol Microbiol. 2011;54(3):547-9.
52. Vinoth PN, Thomas KA, Selvan SM, Suman DF, Scott JX. Hemophagocytic syndrome associated with Plasmodium falciparum infection. Indian J Pathol Microbiol. 2011;54(3):594-6.
53. Chandra H, Chandra S, Sharma A. Histoplasmosis on bone marrow aspirate cytological examination associated with hemophagocytosis and pancytopenia in an AIDS patient. Korean J Hematol. 2012;47(1):77-9.
54. John TM, Jacob CN, Ittycheria CC, George AM, Jacob AG, Subramaniyam S, et al. Macrophage activation syndrome following Acinetobacter baumannii sepsis. Int J Infect Dis. 2012;16(3):e223-4.
55. Maheshwari P, Chhabra R, Yadav P. Perinatal Tuberculosis associated Hemophagocytic Lymphohistiocytosis. Indian J Pediatr. 2012:;79(9):1228-9.
56. Mishra B, Varma N, Appannanavar S, Malhotra P, Sharma M, Bhatnagar A, et al. Viral markers in patients with hemophagocytosis: A prospective study in a tertiary care hospital. Indian J Pathol Microbiol. 2012;55:215-7.
57. Nayan D, Bhagyalakshmi S, Nitin K, Farah J, Chandrakala S. Hemophagocytic Lymphohistiocytosis in a 19 Year Old Critically Ill Patient. Indian Journal of Hematology and Blood Transfusion. 2012;28(2):117-20.

58. Singh S, Chandrakasan S, Ahluwalia J, Suri D, Rawat A, Ahmed N, et al. Macrophage activation syndrome in children with systemic onset juvenile idiopathic arthritis: clinical experience from northwest India. Rheumatol Int. 2012;32(4):881-6.
59. Sood N, Yadav P. Hemophagocytic syndrome associated with concomitant Klebsiella and Parvovirus B-19 infection. Indian J Pathol Microbiol 2012;55:124-5.
60. Currimbhoy Z. An outbreak of an infection associated with circulating activated monocytes and hemophagocytes in children in Bombay, India. Am J Pediatr Hematol Oncol. 1991;13(3):274-9.
61. Vijayalakshmi AM, Ganesh VRR. Hemophagocytic Syndrome Associated with Dengue Hemorrhagic Fever. Indian Pediatrics. 2009;46:545.
62. Sharma M, Dass J, Tyagi S. ATRA Induced Reactive Hemophagocytosis: a Case Report. Mediterr J Hematol Infect Dis. 2011;3(1):e2011034.
63. Gupta A, Modi CJ, Gujral S. Hemophagocytosis by leukemic cells in biphenotypic acute leukaemia: a rare case. Indian J Pathol Microbiol. 2010;53(2):370-1.
64. Avasthi R, Mohanty D, Chaudhary S, Mishra K. Disseminated Tuberculosis: Interesting Hematological Observations. J Assoc Physicians India. 2010;58:243-4.
65. Bhattacharyya M, Ghosh MK. Hemophagoctic lymphohistiocytosis--recent concept. J Assoc Physicians India. 2008;56:453-7.
66. Henter JI. Biology and treatment of familial hemophagocytic lymphohistiocytosis: importance of perforin in lymphocyte-mediated cytotoxicity and triggering of apoptosis. Med Pediatr Oncol. 2002;38(5):305-9.
67. Imashuku S, Tabata Y, Teramura T, Hibi S. Treatment strategies for Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH). Leuk Lymphoma. 2000;39(1-2):37-49.