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RESEARCH ARTICLE

Hemophagocytic lymphohistiocytosis: critical reappraisal of a potentially under-recognized condition

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Abstract **Hemophagocytic lymphohistiocytosis (HLH) is an uncommon, potentially life threatening, hyper inflammatory syndrome of diverse etiologies. Cardinal signs include prolonged fever, organomegaly, and persistent unexplained cytopenias. In spite of the well known diagnostic criteria put forth by HLH society, this continues to pose great diagnostic challenge in both pediatric and adult intensive care settings. We describe 4 adult (2 males, 2 females, aged 19, 29, 40, and 17 years) and 3 pediatric (2 males, 1female, aged 1 month, 6 months, and 12 years) patients with secondary HLH who satisfied the HLH-2004 diagnostic criteria. Definite evidence of hemophagocytosis was noted in 4 patients on initial bone marrow examination. The underlying etiologies were as follows: *Rickettsia tsutsugamushi* (case 1), autoimmune disorder (case 2), systemic onset juvenile idiopathic arthritis (sJIA) (case 3), unknown bite (possibly a venomous snake) (case 4), *Plasmodium vivax* (case 5), Cytomegalo virus (case 6), and *Mycobacterium tuberculosis* (case 7). In one patient, hemophagocytosis was presumed to have been exacerbated by administration of granulocyte monocyte colony stimulating factor (GM-CSF) for severe neutropenia. Two patients died with disseminated intravascular coagulation (DIC) and multi organ failure within few days of HLH diagnosis. Immunosuppressive therapy was started in 3 patients, and etoposide was started in one patient only. Due to lack of specificity of diagnostic criteria, diagnosing and differentiating HLH from its closest mimickers like sepsis/septic shock may be quite challenging in critically ill patients. Therefore, increasing awareness among physicians is essential for early diagnosis and effective therapy to reduce the mortality.**

Keywords hemophagocytic lymphohistiocytosis; diagnosis; therapy; GM-CSF; bone marrow

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is an uncommon, potentially life-threatening hyperinflammatory syndrome caused by severe hypercytokinemia due to a highly stimulated but ineffective immune process. This is characterized by defect in the cytotoxicity of natural killer (NK) cells and cytotoxic T lymphocytes which results in over activation of benign macrophages resulting in the engulfment of various blood precursors (hemophagocytosis) in several organs. This dysfunctional immune system is activated by several factors which in turn trigger a cytokine storm leading to multi organ dysfunction [1,2]. HLH encompasses two forms such as (1) primary or familial HLH, frequently reported in neonates and children with inherited immunodeficiency (Chediak-Higashi syndrome, Griselli syndrome, Hermansky-Pudlak syndrome, X-linked lymphoproliferative syndromes), and often triggered by viral infections [Epstein-Barr virus (EBV), cytomegalo virus (CMV), human immunodeficiency virus (HIV), etc.]; and (2) secondary or acquired HLH, increasingly reported nowadays, in adults with diverse etiologies including infections (most common), autoimmune/connective tissue disorders (so called macrophage activation syndrome, MAS), as well as hematological malignancies [3]. Rarely, inadvertent use of growth factors may exacerbate hemophagocytosis in a subset of neutropenic patients, thus warranting their judicial use in emergency setting [4,5].

In the absence of verified gene mutations or a positive family history, the diagnosis of HLH is based upon the presence of at least five of eight HLH-2004 criteria which include (1) fever ($\geqslant$ 38°C) persisting for at least 1 week, (2) splenomegaly, (3) unexplained progressive peripheral

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blood cytopenias involving at least 2 cell lines (hemoglobin, < 90 g/L; platelet count, $< 100 \times 10^9$/L; absolute neutrophil count, $< 1 \times 10^9$/L), (4) fasting hypertriglyceridemia ($\geqslant 265$ mg/dl) and/or hypofibrinogenemia (fibrinogen $\leqslant$ 150 mg/L), (5) hyperferritinemia ($\geqslant 500$ μg/L), (6) evidence of histiocytic hemophagocytosis in the examination of bone marrow, spleen, liver, or lymph nodes, (7) low or absent natural killer (NK) cell activity, and (8) high levels of soluble CD25 ($\geqslant 2400$ U/ml). Other supportive criteria include presence of neurological symptoms, cerebrospinal fluid pleocytosis and increased protein, raised liver transaminases, hyperbilirubinemia, elevated lactate dehydrogenase, coagulopathy, and hyponatremia [6]. With additional experience, these criteria have been further modified in the proposed HLH-2009 criteria [7].

The exact incidence of HLH is not known though the studies have reported that it may range from 0.8% to 4% among critically ill patients presenting with cytopenias [8]. The clinical signs and symptoms of HLH, sepsis, septic shock, systemic inflammatory response syndrome (SIRS), multiorgan dysfunction syndrome (MODS) may overlap in some patients, especially in intensive care unit (ICU), making the distinction quite challenging. Inspite of well known criteria put forth by HLH society, the condition may still pose diagnostic challenges to treating physicians due mainly to lack of awareness as well as poor reproducibility of these criteria in severely ill patients. Bone marrow examination is the most useful diagnostic test, but may yield false negative result on initial evaluation for which serial marrow examination may be necessary [1,8,9].

In this manuscript, we describe the clinicopathological, hematological, biochemical, and therapeutic outcome data of seven patients with secondary HLH with a brief review of the literature, and create awareness among physicians regarding a potentially underrecognized condition. Furthermore, the potential complication of hemophagocytosis following growth factor therapy for severe cytopenias in critical care set up is also briefly highlighted.

Description of cases

We evaluated seven patients over a period of 2 years (January 2011–April 2013) in the Department of Pathology of a tertiary care center. These included 3 pediatric (2 males, 1 female) (age: 1 month, 6 months, and 12 years) and 4 adult patients (2 males, 2 females) (age: 19, 29, 40, and 17 years). All patients were diagnosed as per the HLH-2004 criteria [6]. The detailed clinicopathological features, management, and outcome of these seven patients are presented in Table 1.

Clinical presentation

Six patients (cases 1, 2, and 4 to 7) required admission into ICU whereas one (case 3) was managed as an indoor patient. Six of the ICU patients presented with persistent high grade fever (more than 1 week), with altered sensorium, hypotension, hepatosplenomegaly, and nonlocalizing constitutional symptoms suggestive of underlying sepsis, SIRS, or a hematological malignancy. In addition, the possibility of an underlying storage disorder was suspected in one pediatric patient (case 5). One of six ICU patients (case 4) had signs and symptoms of severe sepsis/SIRS following cellulitis at the site of an unknown bite (possibly a venomous snake). The indoor patient (case 3) presented with a 6-month history of high grade fever (on and off), pain and swelling around major joints (knee, elbow, ankle, and temperomandibular joints) with restricted movement, hepatosplenomegaly, and bicytopenia, suggestive of acute leukemia, musculo-skeletal tuberculosis, or juvenile idiopathic arthritis (JIA). Over a period ranging from 2 to 4 weeks, two of six ICU patients (cases 1 and 4) developed persistent unexplained pancytopenia, whereas 4 patients (cases 2, 5, 6, and 7) had bicytopenia. Three patients had hyperbilirubinemia with raised liver transaminases and elevated lactate dehydrogenase (LDH, > 500 IU/L) whereas 4 developed coagulation abnormalities (prolonged prothrombin time and activated partial thromboplastin time), hypoalbuminemia (< 3 g/dl), and hyponatremia (< 130 mmol/L). Two patients (cases 2 and 4) progressed to disseminated intravascular coagulation (DIC), and multi organ failure (MOF) eventually resulting in death. Baring one patient (case 5), the clinical diagnosis of HLH was not considered or even suspected in any of these patients.

Hematological and biochemical evaluation

Six patients underwent once bone marrow evaluation for prolonged fever, cytopenias, and organomegaly; and in one (case 6) bone marrow procedure was not performed due to poor patient compliance. In all these 6 patients, bone marrow examination revealed moderate to marked hypocellularity (for age) with prominence of reticuloendothelial cells/benign histiocytes; though definite evidence of hemophagocytosis was demonstrated in four patients (cases 1, 2, 3, 4); and caseating epithelioid granuloma suggestive of tuberculosis was seen in one patient on trephine biopsy (case 7) (Figs. 1, 2, 3, 4A, 4B show morphological patterns of hemophagocytosis in bone marrow). There was no evidence of malignancy, hemoparasites, storage disorder or any other specific pathology demonstrated on routine examination of marrow supplemented with Per-iodic acid Schiff, Gomori's methenamine silver, and Zhiel-Neelsen staining. Subsequently, fasting hypertriglyceridemia ($\geqslant 265$ mg/dl) and hypofibrinogenemia (< 150 mg/L) were documented in 4 and 2 patients, respectively. All seven patients had elevated ferritin levels ($\geqslant 500$ μg/L); and a "sky high" ferritin level ($> 30\,000$ μg/L) was documented in one patient (case 1). Six patients satisfied the five of eight diagnostic HLH-2004 criteria (six of 8 were evaluated in all 7 patients). One patient with bone marrow

Table 1 Clinicopathological features, management, and subsequent follow-up data of seven patients with hemophagocytic lymphohistiocytosis (HLH) diagnosed as per HLH-2004 criteria [6]

Patient characteristics	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Age (years)/gender	19, female	29, female	12, male	40, male	1, male	6/12, female	17, male
Managing departments	ICU[a]	ICU	Orthopaedics	ICU	ICU	ICU	ICU
Presumptive diagnosis	?Sepsis	?Sepsis	sJIA[b]/leukemia/ tuberculosis	?Sepsis/SIRS[c]	?HLH	?Storage disorder	?Sepsis, atypical pneumonia
HLH-2004 criteria							
Fever	+[d] (3 weeks)	+ (3weeks)	+ (6 months)	+ (2 weeks)	+ (1 week)	+ (2 weeks)	+ (2 months)
Splenomegaly±hepatomegaly	+	+	+	+	+	+	+
Cytopenia (⩾2 cell lines)	+	+	+	+	+	+	+
• Hemoglobin⩽90 g/L	+ (66 g/L)	+ (90 g/L)	+ (88 g/L)	+ (66 g/L)	+ (90 g/L)	+ (39 g/L)	+ (68 g/L)
• Platelet count⩽100×10^9/L	+ (80×10^9/L)	+ (30×10^9/L)	–[e]	+ (40×10^9/L)	+ (90×10^9/L)	+ (68×10^9/L)	–
• Absolute neutrophil count (<1000/L)	+ (675/L)	–	+ (688/L)	+ (160/L)	–	–	+ (792/L)
Fasting serum triglyceride (>265 mg/dl)	+(760)	+(503)	–	Not done	+(277)	+(660)	–
Hypofibrinogenemia (<150 mg/L)	–	+	–	+	–	Not done	–
Hyperferritinemia (>500 μg/L)	+ (>30 000)	+ (>2000)	589	+ (>1000)	793	840	+ (>2000)
Hemophagocytosis in bone marrow	+	+ (marked)	+	+	Not evident[f]	(g)	Not evident
Natural Killer cell activity [h]	Not done	Not done	Not done	Not done	Not done	Not done	Not done
Soluble CD25[i]	Not done	Not done	Not done	Not done	Not done	Not done	Not done
Molecular testing[j]	Not done	Not done	Not done	Not done	Not done	Not done	Not done
Supportive laboratory features[k]							
Liver transaminases (⩾200 IU/L)	+	+ (>1000)	–	+	–	–	–
Hyperbilirubinemia (mg/dl) (T/D)	+(22.5/17.5)	+(4.5/2.8)	–	+(4.0/2.8)	–	–	–
Prolonged PT±aPTT	+	+	–	+	–	–	+
Lactate dehydrogenase (IU/L)	3000	Not done	267	889	Not done	Not done	903
Hypoalbuminemia (<3.5 g/dl)	+(2.5)	+(2.2)	–	+(2.8)	–	–	+(2.2)
Hyponatremia (<135 meq/L)	+(126)	+(125)	–	+(128)	–	–	+(125)
D-dimer (semi-quantitative)	–	+(800)	–	+	–	Not done	–
Antinuclear antibody	–	+	–	–	–	Not done	–
Procalcitonin (ng/dl)	+ (>100)	+ (>100)	Not done	+ (>100)	Not done	Not done	+ (>100)
Etiology screen	*Rickettsia tsutsuga-mushi* (IgG ELISA) and secondary bacterial infection	Autoimmune disease with sepsis (MAS)[l]	sJIA (MAS) HLA-B27 & IgM rheumatoid factor negative	? Viper snake bite, SIRS, GM-CSF[m] aggravated	*Plasmodium vivax* (card test +)	CMV[n] (IgG ELISA +)	Miliary TB[o] and bone marrow caseating granulomas
Management	IV antibiotics, doxy-cyclin, ventilator sup-port, vasopressor, steroids, etoposide	IV antibiotics, vaso-pressor, steroids, ventilator support, FFP[p]	IV antibiotic + tablet naproxen (10 mg/ kg) + low dose steroid	IV antibiotics, vaso-pressor, steroids, ventilator support, FFP	Syrup chloroquine + primaquine + IV antibiotic	Supportive care + IV antibiotics	Ventilator support + IV antibiotic + ATT[q]
Outcome	Alive, on follow-up	DIC[r], MOF[s], death	Alive, on follow-up	DIC, MOF, death	Alive	Alive, on follow-up	On follow-up

(a) intensive care unit; (b) systemic onset juvenile idiopathic arthritis; (c) systematic inflammatory response syndrome; (d) present; (e) not present; (f) reticuloendothelial cells/histiocytes were increased, but hemophagocytosis was not evident at the time of bone marrow evaluation; (g) bone marrow examination was not performed in this case due to poor patient compliance, though the required HLH-2004 criteria (5 of 8) was fulfilled in this case; (h, i, j) these tests were not performed due to unavailability and patients' financial constraint; (k) T/D, total/direct bilirubin; PT, prothrombin time; aPTT, activated partial thromboplastin time; (l) macrophase activation syndrome (same as hemophagocytic syndrome associated with autoimmune disorders); (m) granulocyte monocytes colony stimulating factor; (n) cytomegalo virus; (o) suggestive of *Mycobacterium tuberculosis* by bone marrow morphology and dramatic response to antitubercular therapy (ATT); (p) fresh frozen plasma; (q) four-drug antitubercular therapy (rifampin, isoniazid, ethambutol, pyrizinamide); (r) disseminated intravascular coagulation; (s) multi organ failure.

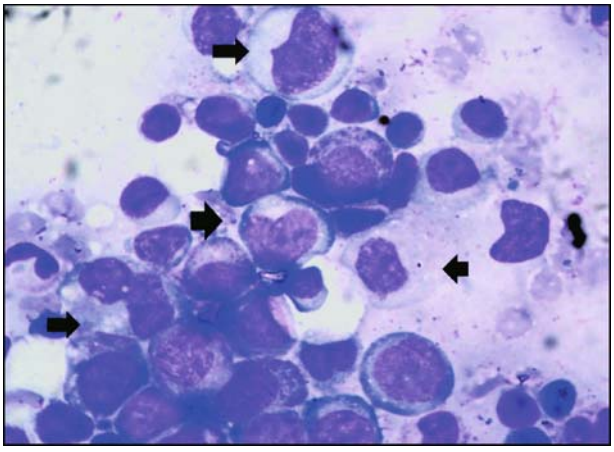

Fig. 1 Bone marrow aspirate smears from a patient with miliary tuberculosis (case 7, 17 years, male) showing increased reticuloendothelial cells/histiocytes with engulfed debris and without evidence of hemophagocytosis (Black arrow) (May Grunwald Giemsa stain, 400 ×).

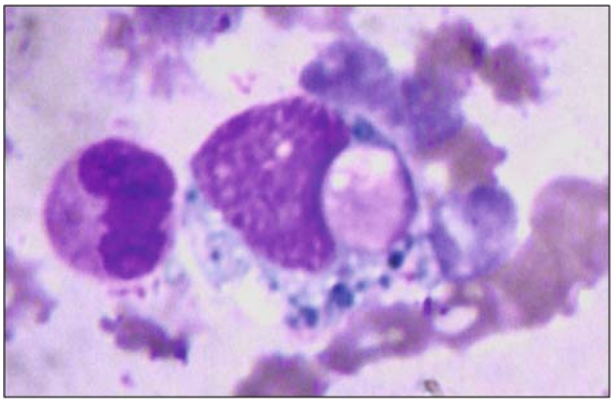

Fig. 2 Aspirate smears from another patient with scrub typhus (*Rickettsia tsutsugamushi*) (case 1, 19 years, female) with evidence of erythrophagocytosis.

tuberculosis (case 7) fulfilled four of six evaluated criteria, and 3 supportive diagnostic criteria such as elevated LDH, hypoalbuminemia, coagulation abnormality, and hyponatremia for which a presumptive diagnosis of HLH was made. Advanced diagnostic tests like molecular testing, NK cell activity, and soluble CD25 assay were not performed in any of our patients due to unavailability of these tests and financial constraints.

Etiology screen

As per institutional protocol for evaluation of all patients with pyrexia, samples (blood, CSF, ascitic/pleural fluid, urine, bone marrow aspirate, etc.) were subjected to infectious disease screen by microbiological culture, serological, and

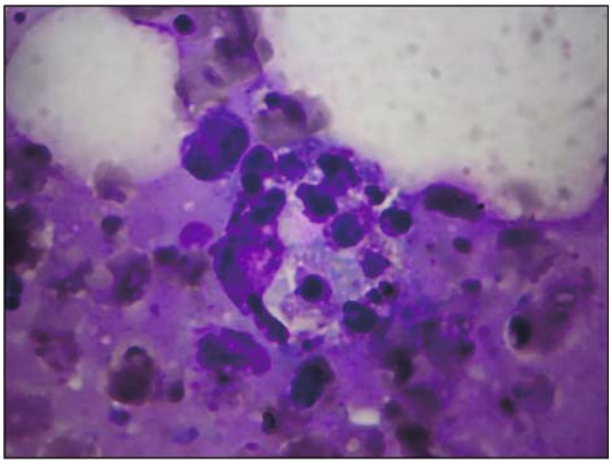

Fig. 3 Aspirate smears from case 2 with autoimmune disorder (29 years, female) with marked hemophagocytosis of all three elements (May Grunwald Giemsa stain, 400 ×).

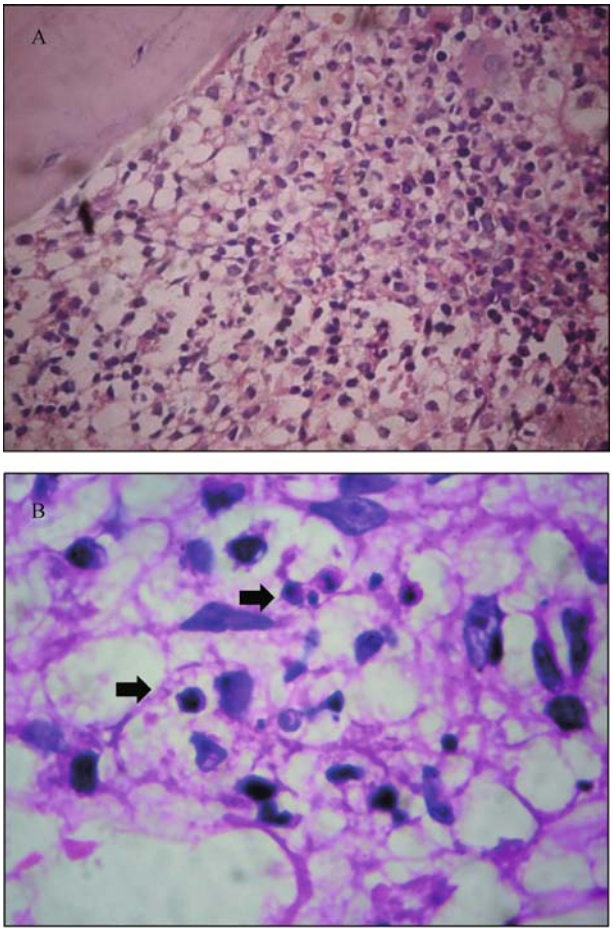

Fig. 4 Bone marrow trephine biopsy of a patient with systemic onset juvenile idiopathic arthritis (sJIA) showing sheets of foamy histiocytes (A, 200 ×) with hemophagocytosis (B, black arrow, 400 ×) (Hematoxylin and eosin). Note that histiocytic hemophagocytosis may not be detected on initial bone marrow aspirate smears; and may be inapparent on trephine sections compared to the aspirate smears [1].

pathological methods. Following diseases were screened routinely in our patients: HIV, HBV, HCV, dengue, leptospira, brucella, malaria, *Mycobacterium tuberculosis*, scrub typhus, salmonella, fungi, and EBV/CMV/human herpes viruses. The nutritional deficiency such as that of vitamin B12 and folate as a cause of cytopenias were ruled out by appropriate biochemical assays. The underlying etiologies were scrub typhus (*Rickettsia tsutsugamushi*) (raised IgG antibody titer, ELISA) with secondary bacterial sepsis in case 1, autoimmune disorder with sepsis (anti nuclear antibody +) in case 2, systemic onset JIA (sJIA) in case 3, sepsis/SIRS secondary to venomous snake bite in case 4, *Plasmodium vivax* (card test for antigen +) in case 5, CMV (raised IgG antibody titer, ELISA) in case 6, and *Mycobacterium tuberculosis* [necrotizing caseating epithelioid granulomas and favorable clinical response following initiation of four-drug antituberculous therapy (ATT)] in case 7. The diagnosis of sJIA in case 3 was based upon following criteria: (1) age < 16 years, (2) fever > 2 weeks, (3) polyarticular arthritis, (4) hepatosplenomegaly, (5) lack of positive family history of psoriasis in a first degree relative, and (6) HLA-B27 and IgM rheumatoid factor negativity [10]. Furthermore bone marrow examination ruled out the possibility of tuberculosis and leukemia in this patient. Serum procalcitonin (marker of sepsis) was found to be increased (> 100 ng/dl) in 4/7 patients.

Management and outcome

Broad spectrum intravenous antibiotics were administered in all patients for a presumptive diagnosis of sepsis. Four patients required ventilator support, 3 required vasopressor drugs for hypotension, 3 were managed with steroids, and one patient (case 1) received cytotoxic therapy (etoposide) as per protocol. Two patients (cases 2 and 4) progressed to DIC, multi organ failure (MOF), and death. One of these two patients (case 4) received GM-CSF for severe neutropenia (prior to bone marrow examination), following which, the patient's condition deteriorated with worsening laboratory parameters, increasing spleen size, and coagulopathy eventually resulting in death. It was presumed that GM-CSF therapy probably exacerbated hemophagocytosis in bone marrow, liver, and spleen. In addition to supportive care, the etiology based definitive therapy was instituted in case 1 (tablet doxycyclin), case 3 [tablet naproxen (10 mg/kg) and low dose steroid], case 5 (chloroquine), and case 7 [rifampicin, isoniazid, ethambutol, and pyrizinamide]. One patient (case 6) received supportive therapy only and finally left against medical advice.

Discussion

In this report, we presented the clinicopathological characteristics of 4 adult and 3 pediatric patients with secondary HLH diagnosed as per the HLH-2004 criteria.

The pathological hallmark of HLH is histiocytic accumulation and hemophagocytosis in the reticuloendothelial organs of the body, especially the bone marrow. It is important to note that this pathological finding is neither necessary nor pathognomonic for the diagnosis of HLH. Furthermore, bone marrow examination, though very useful, lacks sensitivity for demonstration of hemophagocytosis, and serial evaluation may be necessary [1]. In case of primary or familial HLH, mutation of the gene encoding perforin (a lytic protein essential in the apoptosis of T lymphocytes) is often present. This results in uncontrolled proliferation of cytotoxic T lymphocytes, hypercytokinemia, and enhanced hemophagocytosis by activated macrophages leading to fever, shock, coagulopathy, and MODS. It is presumed that both familial and adult HLH are triggered by diverse immunological challenges brought about by infections, autoimmune/connective tissue disorders, as well as hematological malignancies [1,11].

The fundamental pathophysiological mechanism of HLH and sepsis/septic shock/SIRS is closely interlinked. Cytotoxic NK cell activity may be suppressed, and proinflammatory cytokines and soluble CD25 are significantly increased in patients with sepsis [8,9]. Studies have shown that the incidence of histiocytic hemophagocytosis may be higher among ICU patients as bone marrow examinations are frequently being asked, nowadays, for evaluation of cytopenias [12]. Furthermore, studies have also shown that demonstration of histiocytic hemophagocytosis may be an independent predictor of MOF and death in critical care setup [13]. Most recently, HLH has been implicated in the pathogenesis of 2009 influenza A pandemic (H1N1) [14].

A recent study from Taiwan of China [3] described 96 adult ($\geqslant 16$ years) patients with secondary HLH, of which, 61 cases were attributed to underlying hematological malignancies, 30 were associated with infections, 3 had underlying rheumatological disorders, and 2 were related to nosocomial infections (*Burkholderia cepacia* and *Acinetobacter baumannii*). Infection associated HLH had a lower mortality rate compared to non-infectious etiologies. Viruses ($n = 12$), *Mycobacterium tuberculosis* ($n = 7$), bacteria ($n = 7$), and fungi ($n = 4$) were the most common isolates among the patients. Compared to other infections, *M. tuberculosis* related HLH had atypical presentations which was associated with higher mortality rate and longer duration of symptoms before diagnosis. Three of 7 patients with tuberculosis presented initially with DIC, 5 died, and three of these 5 patients received ATT before their death. Overall, age > 50 years, fever not subsiding within 3 days after HLH diagnosis, and presence of DIC were independent predictors of high mortality in all patients. Similarly, advanced age, underlying co-morbidities, use of immunosuppressive agents, presence of thrombocytopenia, anemia, DIC, and high serum ferritin level were also found to be important predictors of high

mortality in other studies [15–17]. A recent review on tuberculosis associated HLH (n = 48, 26 males, 22 females; age group: 14 to 83 years) by Shea *et al.* [18] reported a significantly higher mortality (44%) among these patients. All nine patients who did not receive ATT died. In contrast, those who received ATT with or without immunotherapy had a better survival [68% (27/40)]. These data suggest the fact that under diagnosis and delay in initiation of ATT are the two most important determinants of morbidity and mortality related to tuberculosis associated HLH. Two of our patients (cases 2 and 4) had evidence of DIC at the time of bone marrow evaluation; marked hyperferritinemia ($>$ 10 000 μg/L) was evident in one patient (case 1), and ferritin levels $>$ 1000 μg/L were documented in 3 patients (cases 2, 4, and 7). Immunosuppresive therapy was instituted in 3 patients, and cytotoxic therapy (etoposide) was administered in one patient (case 1). Both the patients with DIC died within few days of their diagnosis.

The HLH-2004 diagnostic criteria are considered to be the gold standard in primary/familial HLH [1,2]. The proposed HLH-2009 criteria are a modification of the previous one. As per this updated criteria, in the absence of molecular diagnosis, the diagnosis of HLH requires at least 3 of 4 features (fever, splenomegaly, bicytopenia, hepatitis) and minimum one of 4 parameters (hemophagocytosis, increased ferritin, absent/decreased NK cell activity, increased soluble IL2Rα). Other results supportive of HLH diagnosis include hypertriglyceridemia, hypofibrinogenemia, and hyponatremia [7]. All of our patients satisfied the both 2004 and 2009 criteria.

The utility of these criteria has been questioned in adult patients with differential diagnosis of septic shock/SIRS/MODS. Many of the HLH-2004 criteria such as fever, cytopenias, hypofibrinogenemia, hypertriglyceridemia are common features of both syndromes [19]. Studies have shown that hyperferritinemia ($>$ 500 μg/L) consistent with the diagnosis of HLH may be present in adult patients with septic shock [19,20]. In a cohort of patients with hyperferritinemia, levels $>$ 10 000 μg/L ("sky high") were found to be highly specific for diagnosis of HLH; and levels $>$ 30 000 μg/L were nearly 100% specific for the HLH diagnosis in the absence of inborn errors of metabolism. On the contrary, patients with proven HLH may have ferritin levels only slightly above normal [21]. In this context, only molecular testing (mutation analysis), test for NK cell activity, and CD 25 assay may help in definitive diagnosis [1]; but these tests are not readily available in most of the laboratories.

Macrophage activation syndrome (MAS) is a life threatening complication of a subset of patients with JIA (up to 12%) with a significant morbidity and mortality (8%–22%). The pathogenesis of MAS/HLH in the context of JIA is complex and poorly understood. The oligoarticular and polyarticular presentation of JIA are regarded as T helper 1 (Th_1) cell-mediated inflammatory disorders, mainly based on the abundance of activated Th_1 cells in the inflamed synovium and the pathogenetic role of proinflammatory cytokines that are mainly produced by Th_1 cell-stimulated monocytes. In contrast, the pathogenesis of sJIA differs from that of other types of JIA in several respects, including the lack of association with human leukocyte antigen type and the absence of autoantibodies or autoreactive T cells [10,22]. One of our patients (case 3) fulfilled the required diagnostic criteria for sJIA as well as 5 of 8 HLH-2004 diagnostic criteria (fever, organomegaly, bicytopenia, hyperferritinemia, and histiocytic hemophagocytosis in bone marrow).

There have been sporadic reports of florid histiocytic hemophagocytosis following the use of myeloid growth factors (G-CSF/GM-CSF) in patients with myelodysplasia [4,5]. In both cases described by the authors, use of growth factors resulted in worsening cytopenias, increasing spleen size, multi organ dysfunction, eventually resulting in death. Autopsy studies confirmed hemophagocytosis in spleen and the liver. It is postulated that the long acting growth factors like G-CSF (pegfilgrastim) may provide a continuous stimulus for the monocytes/macrophage system resulting in florid histiocytic hemophagocytosis. In one of our critically ill patients (case 4), GM-CSF was administered for severe neutropenia prior to bone marrow evaluation, which resulted in worsening of clinical scenario eventually resulting in death. We presume the sudden exacerbation of patient's condition might have been due to similar mechanism. These cases reinforce the fact that growth factor therapy should be judiciously used in neutropenic critically ill patients, and follow-up bone marrow evaluation may be necessary.

The HLH-2004 treatment protocol was initially developed for the children who had primary HLH [1]. Its main components are dexamethasone, cyclosporine, etoposide, and IV immunoglobulin. Though some of the hyperinflammatory mechanism is shared by both HLH and sepsis/SIRS/MODS, there are no prospective experimental data to support or define specific immunosuppressive therapy for secondary HLH in adults. Although widely recommended in the literature, these immunosuppresive drugs are of unproven benefit in adults, with the possible exception of etoposide [9]. Further studies in near future on patients with HLH and sepsis might throw more light into the best possible way to manage these groups of patients.

To summarize, we presented the clinicopathological characteristics of seven patients with secondary HLH based upon HLH-2004 diagnostic criteria. In our view, this number may be artifactually low in view of lack of suspicion, poor reproducibility of the diagnostic criteria, as well as non-performance of molecular testing in other cases. High index of suspicion, increasing awareness among physicians regarding the diagnostic criteria (especially persistent fever, unexplained cytopenias, organomegaly, a very high ferritin, coagulopathy, and unexplained liver function tests) in a critical care setting are essential for early diagnosis and reducing the morbidity and/or mortality. Larger prospective studies with detailed genetic studies could be interesting in

future to characterize this particular group of patients who developed the clinical and laboratory picture of HLH.

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Compliance with ethics guidelines

Somanath Padhi, Renu G' Boy Varghese, Anita Ramdas, Manjiri Dilip Phansalkar and RajLaxmi Sarangi declare that they have no conflict of interest. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from next of kin of all patients for being included in the study.

References

1. Janka G. Hemophagocytic lymphohistiocytosis: when the immune system runs amok. Klin Padiatr 2009; 221(5): 278–285
2. Machaczka M. Genetic and acquired hemophagocytic lymphohistiocytosis. Int Rev Allergol Clin Immunol 2011; 17(3–4): 63–69
3. Tseng YT, Sheng WH, Lin BH, Lin CW, Wang JT, Chen YC, Chang SC. Causes, clinical symptoms, and outcomes of infectious diseases associated with hemophagocytic lymphohistiocytosis in Taiwanese adults. J Microbiol Immunol Infect 2011; 44(3): 191–197
4. Glasser L, Legolvan M, Horwitz HM. Florid histiocytic hemophagocytosis following therapy with long acting G-CSF (pegfilgrastim). Am J Hematol 2007; 82(8): 753–757
5. Wang S, Degar BA, Zieske A, Shafi NQ, Rose MG. Hemophagocytosis exacerbated by G-CSF/GM-CSF treatment in a patient with myelodysplasia. Am J Hematol 2004; 77(4): 391–396
6. Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, Ladisch S, McClain K, Webb D, Winiarski J, Janka G. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007; 48(2): 124–131
7. Filipovich AH. Hemophagocytic lymphohistiocytosis (HLH) and related disorders. Hematology (Am Soc Hematol Educ Program) 2009; 2009(1): 127–131
8. Créput C, Galicier L, Buyse S, Azoulay E. Understanding organ dysfunction in hemophagocytic lymphohistiocytosis. Intensive Care Med 2008; 34(7): 1177–1187
9. Raschke RA, Garcia-Orr R. Hemophagocytic lymphohistiocytosis: a potentially underrecognized association with systemic inflammatory response syndrome, severe sepsis, and septic shock in adults. Chest 2011; 140(4): 933–938
10. Gurion R, Lehman TJ, Moorthy LN. Systemic arthritis in children: a review of clinical presentation and treatment. Int J Inflam 2012; 2012:271569
11. Gupta S, Weitzman S. Primary and secondary hemophagocytic lymphohistiocytosis: clinical features, pathogenesis and therapy. Expert Rev Clin Immunol 2010; 6(1): 137–154
12. Buyse S, Teixeira L, Galicier L, Mariotte E, Lemiale V, Seguin A, Bertheau P, Canet E, de Labarthe A, Darmon M, Rybojad M, Schlemmer B, Azoulay E. Critical care management of patients with hemophagocytic lymphohistiocytosis. Intensive Care Med 2010; 36 (10): 1695–1702
13. Kuwata K, Yamada S, Kinuwaki E, Naito M, Mitsuya H. Peripheral hemophagocytosis: An early indicator of advanced systemic inflammatory response syndrome/hemophagocytic syndrome. Shock 2006; 25(4): 344–350
14. To KK, Hung IF, Li IW, Lee KL, Koo CK, Yan WW, Liu R, Ho KY, Chu KH, Watt CL, Luk WK, Lai KY, Chow FL, Mok T, Buckley T, Chan JF, Wong SS, Zheng B, Chen H, Lau CC, Tse H, Cheng VC, Chan KH, Yuen KY. Delayed clearance of viral load and marked cytokine activation in severe cases of pandemic H1N1 2009 influenza virus infection. Clin Infect Dis 2010; 50(6): 850–859
15. Takahashi N, Chubachi A, Kume M, Hatano Y, Komatsuda A, Kawabata Y, Yanagiya N, Ichikawa Y, Miura AB, Miura I. A clinical analysis of 52 adult patients with hemophagocytic syndrome: the prognostic significance of the underlying diseases. Int J Hematol 2001; 74(2): 209–213
16. Dhote R, Simon J, Papo T, Detournay B, Sailler L, Andre MH, Dupond JL, Larroche C, Piette AM, Mechenstock D, Ziza JM, Arlaud J, Labussiere AS, Desvaux A, Baty V, Blanche P, Schaeffer A, Piette JC, Guillevin L, Boissonnas A, Christoforov B. Reactive hemophagocytic syndrome in adult systemic disease: report of twenty-six cases and literature review. Arthritis Rheum 2003; 49(5): 633–639
17. Kaito K, Kobayashi M, Katayama T, Otsubo H, Ogasawara Y, Sekita T, Saeki A, Sakamoto M, Nishiwaki K, Masuoka H, Shimada T, Yoshida M, Hosoya T. Prognostic factors of hemophagocytic syndrome in adults: analysis of 34 cases. Eur J Haematol 1997; 59 (4): 247–253
18. Shea YF, Chan JFW, Kwok WC, Hwang YY, Chan TC, Ni MYX, Li IWS, Chiu PKC, Luk JKH, Chu LW. Haemophagocytic lymphohistiocytosis: an uncommon clinical presentation of tuberculosis. Hong Kong Med J 2012; 18(6): 517–525
19. Castillo L, Carcillo J. Secondary hemophagocytic lymphohistiocytosis and severe sepsis/ systemic inflammatory response syndrome/ multiorgan dysfunction syndrome/macrophage activation syndrome share common intermediate phenotypes on a spectrum of inflammation. Pediatr Crit Care Med 2009; 10(3): 387–392
20. Castillo L. High elevated ferritin levels and the diagnosis of HLH/ Sepsis/SIRS/MODS/MAS. Pediatr Blood Cancer 2008; 51(5): 710–711, author reply 710–711
21. Allen CE, Yu X, Kozinetz CA, McClain KL. Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2008; 50(6): 1227–1235
22. Hahn YS, Kim JG. Pathogenesis and clinical manifestations of juvenile rheumatoid arthritis. Korean J Pediatr 2010; 53(11): 921–930