

Hemophagocytic Lymphohistiocytosis in Pediatric Patients: A Review

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Contino A¹, Trombatore G² and Timeus F^{3*}

¹Specialization School in Pediatrics, University of Turin, Italy

²Division of Pediatric Onco-Hematology, Catholic University of Rome, Italy

³Pediatric Onco-Hematology, Regina Margherita Children Hospital, Turin, Italy

Abstract

Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening disease due to a highly stimulated but ineffective immune response, with uncontrolled proliferation of activated lymphocytes and macrophages that may lead to multisystem failure. HLH can be caused by underlying genetic diseases (primary HLH or familial hemophagocytic lymphohistiocytosis, FHL), or it can be acquired, resulting after strong immunologic activation, such as that which can occur with infections, autoimmune/rheumatologic diseases, malignancies or others conditions. FHL is more frequent in children. Clinical signs include prolonged high-grade fever, multi-organ involvement including cytopenias, hepatosplenomegaly and liver dysfunction, skin rash, coagulopathy and variable neurologic symptoms. Diagnosis of HLH requires high level of suspicion and applying the diagnostic criteria as accepted by HLH Society. The aim of therapy is the suppression of the hyperactive T cells and histiocytes using chemotherapy and immunosuppressive drugs and, in FHL, if donor and resources are available, subsequent hematopoietic stem cell transplantation (HSCT). Biologic therapy is a promising therapeutic approach.

Keywords: Hemophagocytic lymphohistiocytosis, HLH, FLH

Introduction

HLH is a rare disease characterized by severe hypercytokinemia due to a highly stimulated but ineffective immune response that rapidly evolves into multisystem organ failure [1]. The term hemophagocytosis describes the pathologic finding of activated macrophages, engulfing host blood cells and their precursors [2]. This can be seen in bone marrow aspirates/biopsies and in biopsies of lymph nodes, spleen, liver, skin. However, the presence of hemophagocytosis is not mandatory for diagnosis (see HLH Society diagnostic criteria), especially in the bone marrow, and repeat bone marrow may be needed in the diagnostic work up. Often the greatest barrier to a successful outcome is the delay in diagnosis because of the rarity of this syndrome and the variable clinical presentation. High level of suspicion, knowledge of clinical features and accepted diagnostic criteria allow early diagnosis and prompt initiation of treatment.

Epidemiology

An HLH incidence of 1-2 per million was reported in Europe and Japan, but probably it is underestimated due to the difficulties in diagnosis. The incidence of FHL in Sweden was estimated to be 0.12/100,000 children under age 15 per year [3-5]. HLH is more common in infants, but is diagnosed in patients of all ages. The male to female ratio is close to 1:1 [6].

Clinical signs

FHL and secondary HLH share similar clinical pictures, however FHL is more frequent in infants. In 70–80 % of the cases the onset of FHL disease is below 1 year of age [7]. Despite the variety of underlying genetic defects, patients with HLH present fairly similar clinical features. HLH presents as a prolonged high-grade fever associated with multiple organ involvement. The patients can show cytopenias, progressive hepatosplenomegaly with liver dysfunction, skin rash, coagulopathy and variable neurologic symptoms.

Usually fever is high grade and prolonged, but not always seen in neonates. In the initial stage of the disease cytopenias (affecting ≥ 2 of three lineages in the peripheral

*Corresponding Author: Fabio Timeus, MD, PhD
Pediatric Onco-Hematology, Regina Margherita
Children Hospital, piazza Polonia, 94-10126 Torino-
Italy, Tel: +390113135791, Email: fabio.timeus@unito.it

blood) and hemophagocytosis (in bone marrow aspirate or in spleen and lymph nodes samples) may not be present [8]. Other laboratory findings include hypertriglyceridemia and/or hypofibrinogenemia, liver dysfunction with high LDH and hyperferritinemia.

In around 35-40 % of cases patients may have generalized skin rashes, as transient maculopapular, nodular or purpuric lesions, erythroderma, edema or petechiae [9]. A lymphadenopathy is observed in less than half of the patients [10]. Bleeding can be seen, due to altered coagulation from liver failure, thrombocytopenia from bone marrow failure or defects in platelets function [11]. Neurological manifestations can be seen in around 35% of the patients with FHL2 and 60 % of the patients with FHL3 [12]. They include irritability, depressed consciousness level, seizures, hypotonia, cranial nerve (VI-VII) palsies, blindness, unconsciousness, ataxia, hemiplegia/tetraplegia and signs of raised intracranial pressure. CNS abnormalities represent a late stage of disease and are the major cause of morbidity in long term survivors. Pulmonary and renal involvements have also been reported [9].

Pathophysiology

Several cell types are involved in the pathophysiology of HLH, including macrophages, NK-cells, and cytotoxic T-lymphocytes (CTLs). Activated but ineffective NK cells and/or CTLs fail to eliminate activated macrophages. This dysfunction results in excessive macrophage activity with elevated circulating proinflammatory cytokines (IFN γ , TNF α , IL-6, and M-CSF) causing a cytokine storm that is responsible for the multiorgan failure and the high mortality of the syndrome [11].

Classification

HLH may be genetic or acquired under a variety of conditions. This distinction may be important to identify the various mechanisms underlying HLH, but in the acute setting is not clinically helpful. The genetic causes of HLH can be divided into familial HLH and primary immunodeficiencies.

Familial HLH

Based upon functional protein anomalies and the prerequisite gene mutations responsible, FHL syndromes are sub-classified into FHL1 through FHL5. The first genetic defect described in FHL was a mutation in the perforin gene (PRF1). Subsequently, mutations in other genes that regulate granule-dependent lymphocyte activity have been identified in patients

with inherited HLH [13-18]. Patients with PRF1 null mutations typically present within the first year of life, whereas those with variable degrees of perforin expression and missense mutations have a more variable age of presentation [11]. In a study of 76 HLH patients, those with PRF1 mutations had higher risk of early disease onset (<6 months) than those with STX11 mutations (adjusted odds ratio 8.2; 95% CI 1.2-56) [19].

HLH in Primary Immunodeficiencies

Several congenital immunodeficiency syndromes are also associated with an increased incidence of HLH. Among them Chediak-Higashi syndrome (CHS), Griscelli syndrome (GS) type 2, Hermansky-Pudlak syndrome (HPS) type 2, and X-linked lymphoproliferative disease. CHS and GS have both anomalies of cellular granules. CHS typically features albinism and recurrent pyogenic infections; white blood cells present decreased chemotaxis and contain giant lysosomal inclusion bodies. Albinism/hypopigmentation and neutrophil dysfunction are also seen in GS. In XLPS, a defect of T-lymphocytes function is associated with a marked vulnerability to EBV infection with subsequent viral-associated HLH in up to 60% of patients. Moreover a predisposition to lymphomas and dysgammaglobulinemia has been observed in these patients [20]. More recently, mutations involved in T-cell function including IL-2-inducible T-cell kinase (ITK), CD27, and magnesium transporter gene (MAGT) have also been reported in association with EBV infection and HLH [1]. Other conditions associated with a predisposition to HLH and reported in the literature include common variable immunodeficiency, renal transplant patients, Hermansky-Pudlak syndrome and others [13] (Tables 1 and 2).

Monoallelic mutations in FHL genes and HLH

The possibility of a clinical picture of HLH in the presence of a monoallelic mutation in a FHL gene is an interesting field of investigation. Fifty-four out 600 patients (9%) enrolled in Italian HLH database showed a monoallelic mutation in a FHL gene. In these patients the clinical manifestations were milder and the onset later than in the omozygous patients [21].

Secondary HLH

A number of conditions are associated with secondary HLH. These include viral infections (29%), other infections (20%), malignancies (27%) and autoimmune and rheumatologic disorders (7%) [13]. In Table 3 the more frequent associations. Distinction between severe sepsis/systemic inflammatory response syndrome (SIRS) and HLH can be difficult [22,23].

TYPE	Gene	Chromosome location	Protein	Affected function	
FHL 1	Unknown	9p21.3-q22	Unknown	Unknown	Accounts for approximately 10% of cases of FHL [13]
FHL 2	PRF1	10q21-22	Perforin	Forming pores in the membrane of target cells (apoptosis and immune modulation) [11,13]	Prevalence among ethnic groups (Turkish, African/African American and Japanese origins)
FHL 3	UNC13D	17q25	Munc13-4	Fusion of cytolytic granules	More central nervous system involvement than the other subclasses [13,14]
FHL 4	STX11	6q24	Syntaxin 11	Control of granule exocytosis, membrane fusion, intracellular transport	Found almost exclusively in patients of Turkish/Kurdish descent [13,15,16]
FHL 5	STXB2	19p13.2-3	Munc18-2 (or Syntaxin binding protein 2) [17,18]	This protein binds to Syntaxin 11 and promotes the release of cytotoxic granules.	

Abbreviations: Hemophagocytic Lymphohistiocytosis (HLH), Familial HLH (FHL)
Table 1: FHL

	Gene	Chromosome location	Protein
CHS	LYST	1q42.1-q42.2	LYST
GS2	RAB27A	15q21	RAB27a
XLP1	SH2D1A	Xp25	SAP
XLP2	XIAP	Xp25	XIAP
HPS	ADTB3A	5q14.1	AP-3

Abbreviations: Hemophagocytic Lymphohistiocytosis (HLH), Chediak-Higashi syndrome (CHS), Griscelli syndrome (GS) type 2, X-linked lymphoproliferative disease (XLP) Hermansky-Pudlak syndrome (HPS)

Table 2: HLH In Primary Immunodeficiencies [6, 11]

Infections [9,11,13,22]	Viruses: EBV, CMV parvovirus, HSV, HZV, HHV-6, HHV-8, H1N1 influenza virus, HIV Bacteria: Mycobacterium spp, Mycoplasma pneumoniae, Brucella spp Fungi: Histoplasma spp, Candida spp Parasites: Leishmania spp, Plasmodium falciparum, Plasmodium vivax
Malignancies [11,13,22]	Lymphoid cancers (including T, NK, and anaplastic large cell lymphomas), B cell lymphoblastic leukemia, myeloid malignancies, solid tumors
Autoimmune diseases [11,13]	Juvenile idiopathic arthritis, dermatomyositis, systemic sclerosis, mixed connective disease, polyarteritis nodosa, pulmonary sarcoidosis, antiphospholipid syndrome, Sjögren's syndrome, ankylosing spondylitis, vasculitis
Acquired immunodeficiencies [11]	HIV/AIDS, hematopoietic stem cell transplantation, liver or kidney transplant

Abbreviations: Hemophagocytic Lymphohistiocytosis (HLH)

Table 3: Secondary HLH

A molecular diagnosis of HLH
and/or
Five out of the eight here below criteria Fever Splenomegaly Cytopenia (affecting at least two of three lines in the peripheral blood): Hemoglobin < 9 g/dl (infant < 4 wk: Hb < 10 g/dl) Platelets < 100.000/mm ³ Neutrophils < 1000/mm ³ Hypertriglyceridemia (fasting > 265 mg/100 ml) and/or hypofibrinogemia (< 150 mg/100 ml) Hyperferritinemia (> 500 ng/ml) Hemophagocytosis (bone marrow, spleen, lymph nodes) Low or absent Natural Killer cell activity Elevated soluble IL-2 receptor (CD25) (> 2400 U/ml)
Patients with HLH could present other biochemical parameters: - Elevated transaminases, bilirubin - Elevated LDH - Elevated D-Dimers - Elevated CSF cells or CSF protein

Abbreviations: Hemophagocytic Lymphohistiocytosis (HLH)

Table 4: HLH Diagnostic Criteria

They share high levels of pro and anti-inflammatory molecules and many laboratory findings such as cytopenias, elevation of triglycerides, ferritin, and soluble interleukin-2 receptor and low fibrinogen.

The association between malignancies and HLH may be related to direct immune activation by transformed lymphocytes and/or loss of inhibitory immune function. Many HLH-associated genes are also associated with increased risks of malignancy. Therefore, malignancy-associated HLH should not preclude a complete genetic evaluation [24]. The term macrophage activation syndrome (MAS) describes a serious complication of rheumatologic disorders and should be considered as a type of secondary HLH. It may develop at any time during the course of the disease.

Diagnosis

FHL should be suspected in case of positive family history, parental consanguinity, recurrence of HLH, partial albinism (in GS2, CHS, and HPS2), enteropathy with chronic diarrhea (in early-onset FHL5) and colitis (in XIAP deficiency) [25,26].

The diagnosis of HLH (FHL or secondary HLH) is defined by

the following criteria, suggested by the Histiocyte Society (Table 4) [27]. A high level of suspicion and a careful evaluation and follow up of a patient not yet showing the complete picture of HLH allows early diagnosis.

It is important to consider that infections, autoinflammatory diseases and malignant triggers have been associated not only with secondary HLH, but also with FHL [28-30]. In the workup of HLH, blood-PCR for viral triggers should be performed, as well as PCR for Leishmania in bone marrow, more sensitive than cytology [31]. Lymphoma, leukemia and other malignancies must be excluded. Oncologic patients in remission could present HLH triggered by infections favored by immunosuppression [32].

MAS in sJIA has specific diagnostic criteria [33]. Some inborn errors of metabolism may present similar HLH features. In these cases enzyme dosage and a bone marrow aspirate can help diagnosis [34-36]. All patients with HLH should be evaluated for the presence of a genetic defect. Before molecular evaluation, the first screening test is flow cytometry which can evaluate perforin, SAP (XLP1), and XIAP (XLP2) expression [37,38]. Another rapid test based on flow cytometry is "granule release assay" (GRA), that estimates the pathway of granule exocytosis and gives

indications about which genes should be sequenced (when degranulation is absent or reduced FHL3-5, CHS, GS2, and HPS2 may be suspected) [1,39,40].

Treatment

The aim of HLH therapy is the suppression of the hyperactive T cells and histiocytes using chemotherapy and immunosuppressive drugs and, in FHL, if donor and resources are available, subsequent HSCT.

The protocol usually utilized to treat patients with HLH (except MAS) provides chemotherapy regimen (etoposide and dexamethasone) in conjunction with cyclosporine.

The HLH-94 trial, consisted of 8 weeks of etoposide and dexamethasone therapy following by maintenance therapy with cyclosporine and pulses of etoposide and dexamethasone. The analysis of results showed a reduction of HLH mortality from 95% to <30 % [41]. Because of the majority of deaths in HLH 94 occurred for active disease during induction therapy, in HLH-2004 trial, cyclosporine was utilized starting from the first 8 week of therapy, without a significant improvement in outcome [42]. Patients with complete resolution after therapy, without familial history, do not require further treatment and should be only closely monitored. HSCT is recommended for the patients with FHL (familial history, known genetic diagnosis) or persistent or reactivated HLH after adequate therapy. In these cases a continuation therapy is a bridge to transplant.

One alternative schedule treatment provides the use of ATG in combination with corticosteroids. In 2007 Mahlaoui and Chardin have published a single center retrospective study based on the use of the ATG instead of etoposide. According to this study ATG can be considered an alternative to etoposide in selected patients [43]. In the American-European Euro-Hit Study ATG was associated with etoposide, desametasone and cyclosporine. Results are under evaluation [44].

For patients with refractory disease, alemtuzumab (anti CD52) has been suggested as salvage therapy. In addition, various studies evaluated the use of monoclonal antibodies as daclizumab (anti CD25) and infliximab (anti-TNF) in the treatment of refractory HLH [45-48].

Recently, Jordan et al. have published first promising results from a pilot phase 2 study in children with refractory primary HLH. In this study the treatment with human monoclonal anti-IFN γ antibody combined with dexamethasone was well tolerated and 9 out of 13 patients achieved a satisfactory response [49]. A clinical trial to investigate the safety and efficacy of an anti-IFN γ mAb in children affected by primary HLH is ongoing [50]. Regarding infection-related HLH, the treatment of the causative agent alone is not sufficient and standard HLH protocols should be employed. Only Leishmania-associated HLH requires exclusively treatment with amphotericin [51-53]. Instead, in the EBV-HLH related etoposide appears interfering with EBV induced lymphocyte transformation [13].

Conclusions

Consensuses in the definition of diagnostic criteria and selective use of chemotherapy and HSCT have dramatically improved the prognosis of HLH. As in all rare diseases, the

knowledge of the disease existence by the Colleagues operating in small Centers remains a central point. The use of biologic therapies might modify our therapeutic approach in the future.

References

- Janka Gritta E, Lehmborg Kai. Hemophagocytic syndromes - An update. *Blood Reviews*. 2014;28(4):135-142.
- Madkaikar M, Shabrish S, Desai M. Current Updates on Classification, Diagnosis and Treatment of Hemophagocytic Lymphohistiocytosis (HLH). *Indian J Pediatr*. 2016;83(5):434-443.
- Aricò M, Imashuku S, Clementi R, et al. Hemophagocytic lymphohistiocytosis due to germline mutations in SH2D1A, the X-linked lymphoproliferative disease gene. *Blood*. 2001;15:97(4):1131-1133.
- Ishii E, Ohga S, Imashuku S, et al. Nationwide survey of hemophagocytic lymphohistiocytosis in Japan. *Int J Hematol*. 2007;86(1):58-65.
- Meeths M, Horne A, Sabel M, et al. Incidence and clinical presentation of primary hemophagocytic lymphohistiocytosis in Sweden. *Pediatr Blood Cancer*. 2015;62(2):346-352.
- Henter JI, Elinder G, Söder O, Ost A. Incidence in Sweden and clinical features of familial hemophagocytic lymphohistiocytosis. *Acta Paediatr Scand*. 1991;80(4):428-435.
- Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. *Annu Rev Med*. 2012;63:233-246.
- Ishii E, Ohga S, Imashuku S, Yamamoto K, Yasukawa M. Review of familial hemophagocytic lymphohistiocytosis (FHL) in children with focus on Japanese experiences. *Crit Rev Oncol Hematol*. 2005;53(3):209-223.
- Ramos-Casals M, Brito-Zerón P, López-Guillermo A, Khamashta MA, Bosch X. Adult haemophagocytic syndrome. *Lancet*. 2014;383(9927):1503-1516.
- Ishii E, Ohga S, Tanimura M, et al. Clinical and epidemiologic studies of familial hemophagocytic lymphohistiocytosis in Japan. Japan LCH study group. *Med Pediatr Oncol*. 1998;30(5):276-283.
- McClain K, Eckstein O. Clinical features and diagnosis of hemophagocytic lymphohistiocytosis. Up to date 2017.
- Chandrakasan S, Filipovich AH. Hemophagocytic lymphohistiocytosis: advances in pathophysiology, diagnosis, and treatment. *J Pediatr*. 2013;163(5):1253-1259.
- George MR. Hemophagocytic lymphohistiocytosis: review of etiologies and management. *J Blood Med*. 2014;5:69-86.
- Meeths M, Chiang SC, Wood SM, et al. Familial hemophagocytic lymphohistiocytosis type 3 (FHL3) caused by deep intronic mutation and inversion in UNC13D. *Blood*. 2011;118(22):57835793.
- Zur Stadt U, Schmidt S, Kasper B, et al. Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. *Hum Mol Genet*. 2005;14(6):827-834.
- Rudd E, Goransdotter Ericson K, Zheng C, et al. Spectrum and clinical implications of syntaxin 11 gene mutations in familial haemophagocytic lymphohistiocytosis: association with disease-free remissions and haematopoietic malignancies. *J Med Genet*. 2006;43(4):e14.
- Côte M, Ménager MM, Burgess A, et al. Munc18-2 deficiency causes familial hemophagocytic lymphohistiocytosis type 5 and impairs cytotoxic granule exocytosis in patient NK cells. *J Clin Invest*. 2009;119(12):3765-3773.
- Horne A, Ramme KG, Rudd E, et al. Characterization of PRF1, STX11 and UNC13D genotype-phenotype correlations in familial hemophagocytic lymphohistiocytosis. *Br J Haematol*. 2008; 143(1):75-83.
- Janka G, zur Stadt U. Familial and acquired hemophagocytic lymphohistiocytosis. *Hematology Am Soc Hematol Educ Program*. 2005:82-88.
- Congilio ML, Micalizzi C, Todesco A, et al. Varianti monoalleliche nei geni correlati a linfocitocitosi emofagocitica familiare: report dal database nazionale. *Hematology Reports*. 2017;9:s1;C003:6-7.
- Huang DB, Wu JJ, Hamill RJ. Reactive hemophagocytosis associated with the initiation of highly active antiretroviral therapy (HAART) in a patient with AIDS. *Scand J Infect Dis*. 2004;36(6-7):516-519.

22. Garcia C, Haye K, Gabig T. Hemophagocytic Lymphohistiocytosis (HLH): A Case Series and Review. *American Journal of Medical Case Reports*. 2016;(3-4):374-79.
23. Allen CE, McClain KL. Pathophysiology and epidemiology of hemophagocytic lymphohistiocytosis. *Hematology Am Soc Hematol Educ Program*. 2015;2015:177-182.
24. Sieni E, Cetica V, Piccin A, et al. Familial hemophagocytic lymphohistiocytosis may present during adulthood: clinical and genetic features of a small series. *PLoS ONE*. 2012;7(9):e44649.
25. Pagel J, Beutel K, Lehmborg K, et al. Distinct mutations in STXP2 are associated with variable clinical presentations in patients with familial hemophagocytic lymphohistiocytosis type 5 (FHL5). *Blood*. 2012;119(25):6016-6024.
26. Speckmann C, Lehmborg K, Albert MH, et al. X-linked inhibitor of apoptosis (XIAP) deficiency: the spectrum of presenting manifestations beyond hemophagocytic lymphohistiocytosis. *Clin Immunol*. 2013;149(1):133-141.
27. Henter JI, Horne A, Aricó M, E et al. HLH-2004. Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48(2):124-31.
28. Menard F, Besson C, Rince P, et al. Hodgkin lymphoma-associated hemophagocytic syndrome: a disorder strongly correlated with Epstein-Barr virus. *Clin Infect Dis*. 2008; 47(4):531-533.
29. Balta G, Azik FM, Gurgey A. Defective UNC13D Gene-associated Familial Hemophagocytic Lymphohistiocytosis Triggered by Visceral Leishmaniasis: A Diagnostic Challenge. *J Pediatr Hematol Oncol*. 2014;36(1):e42-e45.
30. Hazen MM, Woodward AL, Hofmann I, et al. Mutations of the hemophagocytic lymphohistiocytosis-associated gene UNC13D in a patient with systemic juvenile idiopathic arthritis. *Arthritis Rheum*. 2008;58(2):567-570.
31. Scalzone M, Ruggiero A, Mastrangelo S, et al. Hemophagocytic lymphohistiocytosis and visceral leishmaniasis in children: case report and systematic review of literature. *J Infect Dev Ctries*. 2016;10(1):103-118.
32. Lehmborg K, Nichols KE, Henter JI, et al. Study Group on Hemophagocytic Lymphohistiocytosis Subtypes of the Histiocyte Society. Consensus recommendations for the diagnosis and management of hemophagocytic lymphohistiocytosis associated with malignancies. *Haematologica*. 2015;100(8):997-1004.
33. Ravelli A, Grom AA, Behrens EM, Cron RQ. Macrophage activation syndrome as part of systemic juvenile idiopathic arthritis: diagnosis, genetics, pathophysiology and treatment. *Genes Immun*. 2012;13(4):289-298.
34. Stark Z, Savarirayan R. Osteopetrosis. *Orphanet J Rare Dis*. 2009;4:5.
35. Rudman Spergel A, Walkovich K, et al. Autoimmune lymphoproliferative syndrome misdiagnosed as hemophagocytic lymphohistiocytosis. *Pediatrics*. 2013;132(5):e1440-1444.
36. Sharpe LR, Ancliff P, Amroia P, Gilmour KC, Vellodi A. Type II Gaucher disease manifesting as haemophagocytic lymphohistiocytosis. *J Inherit Metab Dis*. 2009;32:S107-110.
37. Kogawa K, Lee SM, Villanueva J, Marmer D, Sumegi J, Filipovich AH. Perforin expression in cytotoxic lymphocytes from patients with hemophagocytic lymphohistiocytosis and their family members. *Blood*. 2002;99(1):61-66.
38. Marsh RA, Bleesing JJ, Filipovich AH. Using flow cytometry to screen patients for X-linked lymphoproliferative disease due to SAP deficiency and XIAP deficiency. *J Immunol Methods*. 2010;362(12):1-9.
39. Kuriyama T, Takenaka K, Kohno K, et al. Engulfment of hematopoietic stem cells caused by down-regulation of CD47 is critical in the pathogenesis of hemophagocytic lymphohistiocytosis. *Blood*. 2012;120(19):4058-4067.
40. Bryceson YT, Pende D, Maul-Pavicic A, et al. A prospective evaluation of degranulation assays in the rapid diagnosis of familial hemophagocytic syndromes. *Blood*. 2012;119(12):2754-2763.
41. Trottestam H, Horne A, Aricó M, et al. Histiocyte Society. Chemoimmunotherapy for hemophagocytic lymphohistiocytosis: long-term results of the HLH-94 treatment protocol. *Blood*. 2011;118(17):4577-4584.
42. Bergsten E, Horne A, Aricó M, et al. Confirmed Efficacy of Etoposide and Dexamethasone in HLH Treatment: Long-Term Results of the Cooperative HLH-2004 Study. *Blood*. 2017;130(25):2728-2738.
43. Mahlaoui N, Ouachée-Chardin M, de Saint BG, et al. Immunotherapy of familial hemophagocytic lymphohistiocytosis with antithymocyte globulins: a single-center retrospective report of 38 patients. *Pediatrics*. 2007;120(3):e622-e628.
44. Hybrid Immunotherapy for Hemophagocytic Lymphohistiocytosis. *ClinicalTrials.gov*. 2017.
45. Marsh RA, Allen CE, McClain KL, et al. Salvage therapy of refractory hemophagocytic lymphohistiocytosis with alemtuzumab. *Pediatr Blood Cancer*. 2013;60(1):101-119.
46. Henzan T, Nagafuji K, Tsukamoto H, et al. Success with infliximab in treating refractory hemophagocytic lymphohistiocytosis. *Am J Hematology*. 2006;81(1):59-61.
47. Oda Y, Urushidani Y, Ooi S, et al. Hemophagocytic lymphohistiocytosis in a rheumatoid arthritis patient treated with infliximab. *Intern Med*. 2012;51(6):655-657.
48. Olin RL, Nichols KE, Naghashpour M, et al. Successful use of the anti-CD25 antibody daclizumab in an adult patient with hemophagocytic lymphohistiocytosis. *Am J Hematology*. 2008; 83(9):747-749.
49. Jordan M, Locatelli F, Allen C, et al. NI0501-04 Study Group & De Min C. A novel targeted approach to the treatment of hemophagocytic lymphohistiocytosis (HLH) with an anti-interferon gamma (IFN c) monoclonal antibody (mAb), NI-0501: first results from a pilot phase 2 study in children with primary HLH. *Blood*. 2015;126:LBA-3.
50. A Study to Investigate the Safety and Efficacy of an Anti-IFN-γ mAb in children affected by Primary Haemophagocytic Lymphohistiocytosis. *ClinicalTrials.gov*. 2017.
51. Rajagopala S, Dutta U, Chandra KS, Bhatia P, Varma N, Kochhar R. Visceral leishmaniasis associated hemophagocytic lymphohistiocytosis -case report and systematic review. *J Infect*. 2008;56:381-388.
52. Ay Y, Yildiz B, Unver H, Karapinar DY, Vardar F. Hemophagocytic lymphohistiocytosis associated with H1N1 virus infection and visceral leishmaniasis in a 4.5-month-old infant. *Rev Soc Bras Med Trop*. 2012;45(3):407-409.
53. Cancado GG, Freitas GG, Faria FH, de Macedo AV, Nobre V. Hemophagocytic lymphohistiocytosis associated with visceral leishmaniasis in late adulthood. *Am J Trop Med Hyg*. 2013;88(3):575-577.