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REVIEW

Hemophagocytic Lymphohistiocytosis: an update to diagnosis and management

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ABSTRACT

Hemophagocytic lymphohistiocytosis (HLH) is the uncontrolled reaction of the immune system against a triggering pathogen and inability of the immune system to elliminate this triggering factor, which ends up with hypercytokinemia and hemophagocytosis. Hemophagocytic lymphohistiocytosis is classified into two major groups as genetic (primary) and acquired (secondary). The condition has high mortality rates and specific tratment is required in most of the cases.

Key words: Hemophagocytic lymphohistiocytosis, HLH, perforin

Hemophagocytosis may accompany many conditions such as infections, malignancies and metabolic disorders, however hemophagocytic lymphohistiocytosis (HLH) is the clinical syndrome of an overstimulated but ineffective immune response associated with hypercytokinemia [1]. Killing of the infected cells or cancer cells are achieved with the help of the cytotoxic T lymphocytes and natural killer (NK) cells in a normal functioning immune system. There are secretory lysosomes inside these cytotoxic T lymphocytes and NK cells which contain perforin and granzymes. Perforin is a tetrameric protein which can produce pores on the lipid bilayer of the membrane of the target cell, whereas granzymes are serine proteases which trigger apoptosis of the target cell by initiation of caspase cascade after taken inside throughout the pores produced by the aid of perforins [2,3]. In patients with HLH, there occurs a defect in killing of the pathogens or cancer cells due to an underlying inherited defect in cytotoxicity in genetic forms of the disease, ending up with not only inability to kill the target cell, but also a hypercytokinemia and activation of macrophages. Histopathological evaluation reveals tissue infiltration by macrophages, hemophagocytosis and lymphohistiocytic infiltration. Moreover, serum cytokines such as IFN-γ, IL-6, IL-10, IL-12, IL-6 and TNF-α are highly elevated in addition to markers of immune activation such as soluble CD8, CD25 and CD163 [3,4].

Classification

Hemophagocytic lymphohistiocytosis is classified into two major groups as genetic (primary) and acquired (secondary):

1. Genetic (primary) HLH

This group includes the autosomal recessive, familial HLH cases, in addition to HLH associated to immune deficiency syndromes such as Griscelli syndrome, Chediak-Higashi syndrome, Hermansky-Pudlak syndrome and X-linked lymphoproliferative syndrome [4,5]. Familial HLH is a rare disease and the incidence in Sweden has been reported to be 1 in 50,000 births [5]; however the disease is higher in countries with higher rates of consanguineous marriages and higher birth rates, such as Turkey.

Familial HLH cases are diagnosed before one year of age in 70-80% of the cases [6,7]; however there are occasional patients who have been reported to have onset of manifestations as late as adolescence or event during adulthood [8,9]. The molecular spectrum of genetic causes of HLH are summarized in Table 1. The genes involved in familial HLH (FHL) forms are responsible from the cytotoxic granule exocytosis and function. The molecular defects of patients with HLH associated with primary immune-deficiencies are also related to granule exocytosis pathways, explaining the bleeding tendency, albinism and immune deficiency state of these patients related to granule exocytosis defects

Table 1. The molecular spectrum of genetic causes of HLH

Subtype	Gene	Locus	Protein
FHL1	Unknown	9q21.3–22	Unknown
FHL2	*PRF1*	9q21.3–q22	Perforin
FHL3	*UNC13D*	17q25.1	Munc13-4
FHL4	*STX11*	6q24	Syntaxin11
FHL5	*STXBP2*	19p13	Munc18-2
GS2	*RAB27A*	15q21	RAB27a
CHS	*LYST*	1q42.1–q42.2	LYST
HPS2	*ADTB3A*	5q14.1	AP-3
XLP-1	*SH2D1A*	Xp25	SAP
XLP-2	*XIAP*	Xp25	XIAP

FHL: Familial hemophagocytic lymphohistiocytosis, GS: Griscelli syndrome, CHS: Chédiak–Higashi syndrome, HPS: Hermansky–Pudlak syndrome, XLP: X-linked lymphoproliferative syndrome

in platelets, melanocytes and neutrophils, respectively [3,5].

2. Acquired (secondary) HLH

Acquired forms of HLH are more common than genetic forms. Secondary HLH has been initially described related to viral infections following organ transplantations and was formerly decsribed as virus-associated HLH [10]; however in latter times bacterial, fungal and protozoal agents have been shown to be causative pathogens, as well. Among the common infectious agents causing secondary HLH, Epstein-Barr virus (EBV), cytomegalovirus (CMV), *Leishmania,* influenza viruses are the prominent causatives [1,3,5,11]. Sepsis/systemic inflammatory response syndrome (SIRS) share many common clinical findings and HLH may accompany severe sepsis cases in which the control of the infectious trigger is not easily achieved. In addition to infection-associated HLH, HLH may develop secondary to malignancies, autoinflammatory/autoimmune diseases and metabolic disorders [5,12-16]. The most common malignancies that may trigger HLH are lymphomas and leukemias mostly of T-cell phenotype including anaplastic large cell lymphoma and mature T-cell lymphomas [17]. Secondary HLH might be seen in any age. Of the autoinflammatory/autoimmune diseases that may initiate secondary HLH are systemic juvenile idiopathic arthritis, systemic lupus erythematosus, Kawasaki disease and this type of secondary HLH developing due to an underlying autoinflammatory/autoimmune disease is usually termed as macrophage activation syndrome (MAS). Macrophage activation syndrome usually develops during the early or active phase of these disorders.

Table 2. HLH-2004 Diagnostic criteria (At least five of the eight criteria below needs to be fulfilled for the diagnosis, except for the patients who have a known molecular defect which is by itself adequate to establish the diagnosis of familal HLH)

1. **Familial HLH or known molecular defect**
2. **Clinical and laboratory criteria**
   - Fever
   - Splenomegaly
   - Cytopenias (at least bicytopenia)
     Hemoglobin <9 g/dL (neonates <10 g/dl)
     Platelet <100×10^9/L
     Absolute neutrophil count <1×109/L
   - Hypertriglyceridemia and/or hypofibrinogenemia
     Fasting serum triglyceride ≥3 mmol/L (≥265 mg/dl)
     Plasma fibrinojen <1.5 g/L
   - Serum ferritin >500 µg/L
   - sCD25 (s IL-2 receptor) ≥2400 U/ml
   - NK cell activity decreased or absent
   - Hemopahagocytosis (bone marrow, other tissues such as lymph nodes, cerebrospinal fluid)

Clinical symptoms, laboratory findings and diagnostic criteria

Histiocyte Society proposed a guideline, namely HLH-2004 criteria, for diagnosis of patients with primary and secondary HLH (Table 2) [18]. If patient has a known molecular defect, the diagnosis of primary HLH is established. However, for patients without a known postive molecular defect, the diagnosis of either primary or secondary HLH could be established with the presence of at least five of the eight diagnostic criteria, including fever, bi- or pancytopenia, splenomegaly, hypertriglyceridemia or hypofibrinogenemia, hyperferritinemia, elevated sCD25 (sIL-2 receptor), low NK cell activity and demonstration of hemophagocytosis in tissues [18]. Hemophagocytosis may be demonstrated from the samples obtained from bone marrow (Figure 1) [19] or other tissues including lymph nodes, cerebrospinal fluid (CSF). The absence of hemophagocytosis in the earlier samples may necessitate re-sampling, if the diagnosis is highly suggested. About half of the primary HLH cases have findings of central nervous system involvement (CNS), including increased CSF

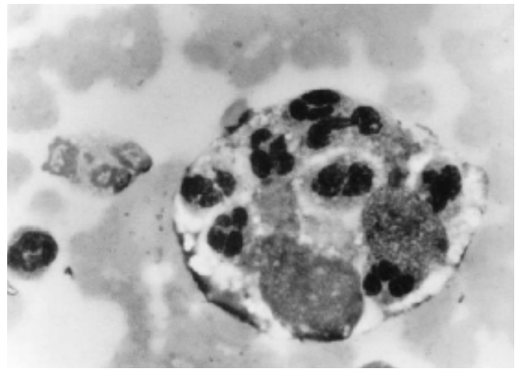

Figure 1A. Hemophagocytosis in bone marrow [Ref 19] .

protein, pleocytosis or MRI findings suggestive for CNS involvement of HLH [1,4,5].

Despite having normal number of NK cells (CD56+/16+), in most of the patients with primary HLH, NK cell activity is decreased or absent. However, it is not practical to use NK cell activity in order to discriminate primary and secondary forms of the disease.

Other possible and common laboratory findings of HLH are elevated serum transaminases, bilirubin, lactate dehydrogenase [5]. Hypertransaminasemia is a common finding accompanying HLH. The liver biopsies obtained from patients with HLH usually reveal findings compatible with chronic hepatitis and occasional hemophagocytosis might be seen in the periportal infiltrate [1,4,5]. These findings may delay the diagnosis of HLH and patients might initially be diagnosed to have a primary hepatic disorder or a metabolic disease rather than HLH.

All of the findings of HLH are related to hypercytokinemia and infiltration of organs with activated lymphocytes and histiocytes. Fever is related to high IL-1 and IL-6 levels, whereas pancytopenia is related to high levels of TNF-α and IFN-γ, in addition to hemophagocytosis [1-5]. The inhibition of lipoprotein lipase by TNF-α results in hypertriglyceridemia. On the other hand, activated macrophages release plasminogen activators leading to increased plasmin and hyperfibrinolysis [1-5]. Hepatosplenomegaly, hypertransaminasemia and neurological findings are consequences of the tissue infiltration by activated lymphocytes and macrophages.

Any patient with prolonged fever, unresponsive to antibiotics, accompanied by cytopenias or hepatosplenomegaly are suggested to be screened for diagnostic criteria of HLH-2004, including serum ferritin, triglyceride, plasma fibrinogen and bone marrow aspiration. The bone marrow aspirations should be thoroughly screened for amastigotes of *Leishmania*, since specific treatment for this pathogen will rapidly remit the dramatic picture of HLH. Additionally, patients fulfilling the diagnostic criteria for HLH are suggested to be tested for EBV, CMV, adenovirus and parvovirus B19 in order to aid for the differentiation of primary and seconday cases. However, it is crucial to recognize that demonstration of a pathogen may not exclude the primary HLH causes, since the infectious agents may trigger the onset of primary HLH manifestations [20]. The personel history, physical examination and laboratory investigations directed for a search for a malignant, autoimmune/autoinflammatory disease or primary immune deficiencies such as Chediak-Higashi, Griscelli or X-linked lymphoproliferative syndrome should be obtained. As pointed out previously, it is sometimes difficult to make an accurate distinction between primary and secondary HLH cases. A detailed family history and molecular testing in patients with a suspicion of a primary disease are highly suggested for distinction, although not sufficient in some of the cases.

The genetic diversity in familial HLH

PRF1 mutaions explain almost 20-50% of the familial HLH cases depending on the reported cohort [21-24]. *PRF1* gene has three exons and over 120 mutations have been described to date [3]. Some of these mutations present with a very severe phenotype of extremely high serum ferritin levels and neonatal-onset of disease, like c.1122G >A (p.W374X) [25,26]. Additionally some of the mutations have been reported to be more common among some ethnic populations such as c.1122G >A (p.W374X) which is more prevelant among Turkish patients [23].

UNC13D gene mutations cover almost 30-40% of the familial HLH cases [3]. Clinically, patients with *PRF1* and *UNC13D* mutations are indistinguishable, however flow cytometry may help to differentiate two molecular types prior to accurate diagnosis by molecular testing. Patients with *PRF1* mutations have no perforin expression but normal degranulation, whereas patients with *UNC13D* mutations have normal perforin expression, but a degranulation defect in flow cytometric analyses [27]. Additionally, *UNC13D* mutations have been reported to be more commonly associated with CNS involvement compared to *PRF1* mutations [28]. *UNC13D* gene consists of 32 coding exona and more than 100 mutations have been reported to date [3].

STX11 gene has 2 exons [3]. Although, initially have been reported solely among patients of Turkish/ Kurdish ethnicity, there are reports from other populations [3]. STX11 encodes a SNARE protein syntaxin, which is important in intracellular vesicular trafficking [29].

A more recently defined gene responsible in the development of familial HLH cases is *STXBP2* and was found to be responsible from 20% of familal cases [30]. Patients with *STXBP2* gene mutations may have some additional findings such as gastrointestinal symptoms, including chronic diarrhea, gastroesophageal reflux in addition to bleeding diathesis [3,30].

Genetic remarks in some of the secondary HLH cases

It is of interest why some of the patients with an underlying infectious or autoimmune / autoinflammatory disease develop secondary HLH, whereas others do not. It has previously been reported that carrying a heterozygous A91V mutation of *PRF1* gene has been reported to be 2.8 times more common among patients with secondary HLH compared to healthy controls [31]. Additionally, there are reports of macrophage activation syndrome recurrence among patients with rheumatological disorders who are carriers for HLH mutations [32,33].

Treatment and prognosis

Primary HLH

Primary HLH cases have high mortality rates unless hematopoetic stem cell transplantation (HSCT) has been established. However, the patients who have no suitable donor are treated with HLH-2004 protocol, which includes etoposide, dexamethasone, cyclosporine A. The patients with CNS involvement receive intrathecal methotrexate. The results of HLH-2004 protocol have not been published yet and the patient recruitment has finished [5]. However, the results of the previous study protocol, namely HLH-94 has been reported to have a 5-year survival rate of 54% [34]. The HLH-94 and HLH-2004 protocols differ in terms of the presence of cyclosporine A in the first 8 weeks of HLH-2004 protocol. The patients who have a donor for HSCT have been reported to have better transplant outcomes if they were transplanted after achievement of remission in disease activation criteria after initiation of HLH-94 [34]. There is one report from a single-center that reported 55% survival with treatment with anti-thymocyte globulin (ATG) and methylprednisolone with subsequent HSCT [35]. The patients who are refractory to standard treatments of either HLH-94 or HLH-2004 have been reproetd to be treated with alemtuzumab (anti-CD52) prior to HSCT with 64% partial response, 77% survival until HSCT [36]. The ultimate curative treatment is HSCT in patients with familial HLH. This type of treatment is donor-dependent. The survival rates after HSCT have been reported as 53-71% with myeloablative conditioning regimens including busulfan, cyclophosphamide, etoposide and ATG [5, 37-39].

Secondary HLH

The association of HLH to an infection increases the mortality significantly and usually it is mandatory to control hyperinflammation concomitant to the treatment of the triggering infection. Among the viral triggers of HLH, the worst prognosis is seen among the EBV related HLH group. In a series of 78 patients with EBV related HLH from Japan survival rate has been reported as 75% for 43 months of follow-up. During treatment of these patients 85% required addition of etoposide [40]. Addition of aciclovir to treatment of EBV related HLH usually has no additional impact. In EBV related HLH, the patients who recceived no etoposide within 4 weeks of diagnosis of HLH have been reported to have 14 times more increased mortality rate compared to those who have received etoposide [41]. In a recent study, addition of rituximab (anti-CD20) to standard treatment has been reported to induce decrease in EBV viral load and serum ferritin levels in almost half of the patients with EBV related HLH [42]. However, in patients with EBV related HLH, it is not uncommon for EBV to infect T cells, as well; in contrast to classical EBV infection which has propensity to infect specifically B cells [43], which might be the situation in refractory patients.

Leishmania donovani may both trigger HLH, in addition to mimicking HLH with the presentational findings of fever, splenomegaly and cytopenias. Administration of liposomal amphotericin B is the treatment of choice [44].

In patients with HLH accompanying sepsis, the standard treatment approach is antibiotics and supportive treatment; however a course of corticosteroids and/or intravenous immunoglobulin may be suggested to control the hyperinflammation and hypercytokinemia in patients who do not improve with antibiotics and supportive measures and progress to organ failures. However, cytotoxic treatment with etoposide may not be beneficial an deven may

have deleterious effects on the already impaired immune system.

Patients with MAS may be treated with pulse corticosteroid treatment with or without the addition of cyclosporine A [5].

Plasma exchange may be used in patients who do not respond to standard treatments of primary or secondary HLH [45].

In patients with an underlying metabolic disease that triggered HLH, the correction of the metabolic defect is usually adequate to control HLH manifestations [46].

Patients who were initiated HLH 94 or HLH 2004 treatment protocols when a differentiation of primary or secondary HLH could not be established clearly, are usually suggested to have cessation at 8th week of treatment protocol, if the HLH diagnostic criteria have remitted. On the other hand, patients with primary HLH should continue HLH 94 or HLH 2004 protocols upto HSCT. Patients who were initiated these protocols with a diagnosis of secondary HLH, may cease treatment whenever the diagnostic criteria remits. Patients whose primary or secondary HLH discrimination could not be done with absolute clarity are suggested to have cessation of treatment at 8th weeks if the disease activity criteria have remitted. During the follow-up of these patients, if relapse of HLH occurs after cessation of HLH specific treatment, patients are usually considered as primary HLH and treated accordingly [1,5,6].

In conclusion, although the diagnostic criteria of HLH are well established, it is sometimes challanging to differ between primary and secondary HLH cases. Additionally, both conditions have high mortality rates.

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