Hemophagocytic Lymphohistiocytosis in Pediatric Patients: A Review

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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 hepatospleno-megaly 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
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, erythoderma, 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 (IFNγ, TNFα, 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].

<table>
<thead>
<tr>
<th>TYPE</th>
<th>Gene</th>
<th>Chromosome location</th>
<th>Protein</th>
<th>Affected function</th>
<th>Associated with</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHL 1</td>
<td>Unknown</td>
<td>9p21.3-q22</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Accounts for approximately 10% of cases of FHL [13]</td>
</tr>
<tr>
<td>FHL 2</td>
<td>PRF1</td>
<td>10q21-22</td>
<td>Perforin</td>
<td>Forming pores in the membrane of target cells (apoptosis and immune modulation) [11,13]</td>
<td>Prevalence among ethnic groups (Turkish, African/African American and Japanese origins)</td>
</tr>
<tr>
<td>FHL 3</td>
<td>UNC13D</td>
<td>17q25</td>
<td>Munc13-4</td>
<td>Fusion of cytolytic granules</td>
<td>More central nervous system involvement than the other subclasses [13,14]</td>
</tr>
<tr>
<td>FHL 4</td>
<td>STX11</td>
<td>6q24</td>
<td>Syntaxin 11</td>
<td>Control of granule exocytosis, membrane fusion, intracellular transport</td>
<td>Found almost exclusively in patients of Turkish/Kurdish descent [13,15,16]</td>
</tr>
<tr>
<td>FHL 5</td>
<td>STXBPF2</td>
<td>19p13.2-3</td>
<td>Munc18-2 (or Syntaxin binding protein 2) [17,18]</td>
<td>This protein binds to Syntaxin 11 and promotes the release of cytotoxic granules.</td>
<td></td>
</tr>
</tbody>
</table>

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

Table 1: FHL
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, autoimmune 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

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<thead>
<tr>
<th>Gene</th>
<th>Chromosome location</th>
<th>Protein</th>
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<tbody>
<tr>
<td>CHS</td>
<td>LYST</td>
<td>1q42.1-q42.2</td>
</tr>
<tr>
<td>GS2</td>
<td>RAB27A</td>
<td>15q21</td>
</tr>
<tr>
<td>XLP1</td>
<td>SH2D1A</td>
<td>Xp25</td>
</tr>
<tr>
<td>XLP2</td>
<td>XIAP</td>
<td>Xp25</td>
</tr>
<tr>
<td>HPS</td>
<td>ADTB3A</td>
<td>5q14.1</td>
</tr>
</tbody>
</table>

Abbreviations: Hemophagocytic Lymphohistiocytosis (HLH), Chediak-Higashi syndrome (CHS), Griscelli syndrome (GS) type 2, X-linked lymphoproliferative disease (XLP) Hermansky-Pudlak syndrome (HPS)
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
analyses 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
weeks 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 [45-46].

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-IFNγ
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-
IFNγ 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

Consensus 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.

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