Review Article

Hemophagocytic Lymphohistiocytosis – Recent Concept

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Abstract

Hemophagocytic lymphohistiocytosis is a rare condition characterized by highly stimulated but inactive immune response. The disease may be inherited or acquired due to infections, collagen vascular diseases and malignancies. The pathological hallmark of the syndrome is aggressive proliferation of macrophages and histiocytes. Decreased NK cell activity results in increased T cell activation resulting production of large quantities of interferon γ (IFNγ), tumor necrosis factor α (TNFα) and granulocyte macrophage colony stimulating factor (GM-CSF). This causes sustained macrophage activation and tissue infiltration as well as production of interleukin 1 (IL1) and interleukin 6 (IL6). The resulting inflammatory reaction causes extensive damage and associated symptoms. Patients with HLH commonly present with high fever, anemia and splenomegaly. Minimal diagnostic parameters are a complete hemogram, liver function test, serum triglycerides and ferritin, coagulation profile including fibrinogen and bone marrow aspiration. Two highly sensitive diagnostic marker are an increased plasma concentration of the α chain of soluble IL2 receptor (CD25) and impaired NK cell activity. Hyperinflammation can be treated with steroid, Cyclosporine prevents T lymphocytes and immunoglobulin infusion helps to control the infection. Etoposide may be life saving specially in case of HLH with Ebstein Barr Viruses infection. The Histiocyte Society in 1994 developed a common treatment protocol (HLH-94). In January 2004 a revised HLH treatment protocol was opened entitled HLH-2004, which is based on HLH-94 with minor modifications. There is a high remission rate on the HLH-94 and HLH-2004 treatment protocols.

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a rare but potentially life threatening condition characterized by uncontrolled activation of macrophages and lymphocytes. Pediatric age group is most commonly affected but it can occur at any age. It is not a single disease and can be encountered in association with a variety of underlying diseases that leads to highly stimulated but inactive immune response.

Classification:

1. Inherited HLH
   - Familial HLH (Farquhar disease)
     - Known genetic defects (perforin, munc 13-4, syntaxin 11)
     - Unknown genetic defects.
   - Immune deficiency syndrome
   - Chediak Higashi syndrome
   - Griscelli syndrome
   - X-linked lymphoproliferative syndrome

2. Acquired HLH
   - Exogenous agents (infectious organisms, toxins)
   - Infection – associated hemophagocytic syndrome (IAHS)
   - Endogenous products (tissue damage, metabolic products)
   - Rheumatic diseases
   - Macrophage activation syndrome (MAS)
   - Malignant disease

The syndrome, which has also been referred to as histiocytic medullary reticulosis, was first described in 1939.1 HLH was initially thought to be a sporadic disease caused by neoplastic proliferation of histiocytes. Subsequently, a familial form of the disease2 (now referred to as familial hemophagocytic lymphohistiocytosis (FHLH))3 was described. Familial HLH is an inherited autosomal recessive disorder first described by Farquhar and Claireaux in 1952,2 estimated to occur in a frequency of 1 in 50,000 births. The diagnosis of FHLH is made based on the presence of clinical criteria and is confirmed by molecular genetic testing. Four disease subtypes (FHL1, FHL2, FHL3, and FHL4) are described; three genes have been identified and characterized: PRF1 (FHL2), UNC13D (FHL3), and STX11 (FHL4). Molecular genetic testing of the PRF1 (FHL2), UNC13D (FHL3), and STX11 (FHL4) genes is available on a clinical basis (Table 1).

Secondary HLH (ie acquired HLH) occurs after...
strong immunologic activation such as that occur with a variety of viral, bacterial, fungal, and parasitic infections, as well as collagen-vascular diseases and malignancies, particularly T-cell lymphomas. The association between HLH and infection is important because 1) both sporadic and familial cases of HLH are often precipitated by acute infections; 2) HLH may mimic infectious illnesses, such as overwhelming bacterial sepsis and leptospirosis; 3) HLH may obscure the diagnosis of a precipitating, treatable infectious illness (as reported for visceral leishmaniasis); and 4) a better understanding of the pathophysiology of HLH may clarify the interactions between the immune system and infections.

The clinical picture of HLH can be induced by a variety of infectious organisms. The patient in the original report by Risdull and colleagues were mostly associated with viral infection following organ transplantation. Subsequently it was clear that it may be associated with viruses as well as a no of bacteria, fungi, mycobacteria and parasite and the term Viral Associated Hemophagocytic Syndrome (VAHS) was redesigned as Infection Associated Hemophagocytic Syndrome (IAHS). A review of published cases in children diagnosed with IAHS before 1996 reported that more than half of them were from Far East. Èbstein Barr Virus (EBV) was the triggering virus in 74% of the children in whom an infectious agent could be identified.

HLH in association with malignant disease is well known entity in adults but rare in children. Most common malignancy associated with HLH is Lymphoma. In a recent review of patient with lymphoma associated hemophagocytic syndrome (LAHS) from Japan EBV genome was detected from more than 80% of T/NK cell lymphoma but rarely from B cell lymphoma.

HLH has also been described in association with inborn error of metabolism like Lysinuric Protein Intolerance (LPI).

### Pathophysiology

The pathological hallmark of the syndrome is aggressive proliferation of macrophages and histiocytes which phagocytose other blood cells leading to the clinical symptoms. This uncontrolled growth is non-malignant and does not appear clonal in contrast to the lineage of cells in Langerhans cells histiocytosis. The spleen, lymph nodes, bone marrow, liver, skin and membranes that surrounds the spinal cord are preferential site of involvement.

Over the past 2 decades, the underlying pathophysiology of HLH has been characterized, although the processes are not entirely understood. A current accepted theory involves an inappropriate immune reaction caused by activated T cells associated with macrophage activation and inadequate apoptosis of immunogenic cells. Although the precise mechanism is unclear many research team propose convincing pictures for the role of perforin or NK cells in the HLH subtypes. NK and cytotoxic T cells kill their targets through cytolytic granules containing perforins and granzyme. When activated by challenges NK cells release granules containing granzymes and perforins which forms pores in the target cell membrane and causes osmotic lysis and protein degradation respectively. Patients with HLH have severe impairment of the cytotoxic function of NK cells and cytotoxic T lymphocytes. Decreased NK cell activity results in increased T cell activation and expansion with resulting production of large quantities of cytokines, including interferon γ (IFNγ), tumor necrosis factor α (TNFα) and granulocyte macrophage colony stimulating factor (GM-CSF). This causes sustained macrophage activation and tissue infiltration as well as production of interleukine1 (IL1) and interleukine 6 (IL6). The resulting inflammatory reaction causes extensive damage and associated symptoms (Fig. 1). Inflammatory cytokines are responsible for the characteristic disease markers such as cytopenias, coagulopathy and high triglycerides.

Upon contact between the effector killer cell and the target cell, an immunological synapses is formed and cytolytic granules have to traffic the contact site, dock and fuse with the plasma membrane and release their content. All known defects in HLH seems to be involved in this processes. LYST mutations impair granule secretion, RAB27α deficiency leads to impaired docking at the membrane, mutations in UNC13 causes defective granule priming at the immunologic synapses. Finally lack of PRF1 leads to loss of cytolytic activity. In XLP, granule mediated cytotoxicity is defective through impaired lymphocyte activation.

Factors leading to cytolytic defects in acquired HLH is less clear. Viruses may interfere with T cell activity

### Table 1: Locus name and genes associated with familial hemophagocytic lymphohistiocytosis

<table>
<thead>
<tr>
<th>Locus Name</th>
<th>% of FHLH</th>
<th>Mutations</th>
<th>Chromosomal Locus</th>
<th>Protein name</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHL1</td>
<td>4 inbred Pakistani Families</td>
<td>N/A</td>
<td>9q21.3–q22</td>
<td>Unknown Perforin-1</td>
</tr>
<tr>
<td>FHL2</td>
<td>20-30% worldwide, 50% in African American families</td>
<td>&gt;50 distinctive mutations throughout the entire coding region</td>
<td>10q22</td>
<td>Unc-13 homolog D</td>
</tr>
<tr>
<td>FHL3</td>
<td>20-30% world wide</td>
<td>&gt;10 mutations throughout the entire coding region and splicing sites</td>
<td>17q25.1</td>
<td>Syntaxin-11</td>
</tr>
<tr>
<td>FHL4</td>
<td>~ 20% of Turkish/Kurdish families</td>
<td>3 recurrent mutations</td>
<td>6q24</td>
<td></td>
</tr>
</tbody>
</table>

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by specific proteins or cytokines.\textsuperscript{31,32}

Macrophage activation syndrome (MAS) occurs in children and adults with autoimmune diseases most commonly seen in association with systemic onset juvenile rheumatoid arthritis (sJRA) or adult onset Still’s disease but also occur rarely with systemic lupus erythematosus or other entities.\textsuperscript{33,34} Clinical picture and laboratory findings are similar to HLH. Patients of sJRA was found to have low NK cell function and perforin expression compared to other form of rheumatoid arthritis.\textsuperscript{35} MAS is a grave disorder with a mortality of about 10-20%. It has been suggested by some rheumatologists that MAS be classified as a form of secondary HLH.\textsuperscript{36,37}

Clinical Features

Patients with HLH commonly present with high fever, anemia and splenomegaly. The fever often fluctuates with complete remission and recurrences. Severe fulminant liver failure with coagulopathy or neurological symptoms may dominate the clinical picture and thereby delay the diagnosis of HLH. Nearly 75% of patients will show some form of CNS involvement – including seizures, ataxia, hemiplegia, altered sensorium. Patient may have even only neurological manifestations.\textsuperscript{38,39} About 65% of patients will have skin rashes\textsuperscript{40} (Table 2).

The diagnostic criteria set forth by the Histiocytic Society for inclusion in the international registry\textsuperscript{41} for HLH is as follow –

I) Fever as high as 38.5°C for 7 days or more.
II) Splenomegaly – 3 cm below left costal margin
III) Cytopenias –
   - Absolute Neutrophils < 1000/µL
   - Platelets less than 100,000/µL
   - Hemoglobin < 9 g/dL
IV) Fibrinogen < 1.5 g/L or fasting triglyceride > 3 mmol/L
V) Hemophagocytosis in spleen, lymph node or Bone Marrow.
VI) Serum Ferritin > 500 µg/L
VII) sCD25 > 2400 U/L
VIII) Decreased or altered NK cell activity.

Laboratory investigations

In every patient with prolonged fever, hepatosplenomegaly and pancytopenia, the diagnosis of HLH should be considered. Minimal diagnostic parameters are a complete hemogram, liver function test, serum triglycerides and ferritin, coagulation profile including fibrinogen. Ferritin has been observed as a marker of HLH with the serum level paralleling the course of the disease.\textsuperscript{36} Altered hepatic function including hyperbilirubinemia, increased hepatic enzymes and low albumin has also been reported.\textsuperscript{42} All patients should undergo bone marrow aspiration, however, hemophagocytosis may not be present in the initial bone marrow smears, only increased monocytes and monohistiocytic cells may be present. In about 50% patients there may be elevated cell count, protein or both in the CSF even in the absence of clinical

\begin{table}
\centering
\caption{Clinical signs and laboratory abnormalities associated with hemophagocytic lymphohistiocytosis}
\begin{tabular}{|l|c|l|}
\hline
Clinical sign & % of patients affected & Reference \\
\hline
Fever & 60-100 & 3,46 \\
Splenomegaly$^*$ & 35-100 & 3, 46 \\
Hepatomegaly & 39-97 & 3,46 \\
Lymphadenopathy & 17-52 & 3,46 \\
Rash & 3-65 & 3,46 \\
Neurologic signs & 7-47 & 3,46 \\
Laboratory abnormality & % of patients affected & Reference \\
Anemia$^*$ & 89-100 & 3,44,45 \\
Thrombocytopenia$^*$ & 82-100 & 3,44, 45 \\
Neutropenia$^*$ & 58-87 & 3,44,46 \\
Hypertriglyceridemia$^*$ & 59-100 & 3,44,46 \\
Hypofibrinogenemia$^*$ & 19-85 & 3,44,46 \\
Hyperbilirubinemia & 74 & 44 \\
\hline
\end{tabular}
\end{table}

* Proposed diagnostic criterion for HLH

Fig. 1: Schematic representation of possible immunopathologic mechanisms in infection-associated hemophagocytic lymphohistiocytosis (HLH). TNF-α = tumor necrosis factor-α; IFN-γ = interferon-gamma; IL-1 = interleukin-1; IL-2 = interleukin-2; IL-6 = interleukin-6; IL-18 = interleukin-18; sFasL = soluble Fas ligand; T = T-lymphocyte; M = macrophage; EBV = Epstein-Barr virus.

Fig. 2: Overview of the treatment protocol HLH-94. Dexa = dexamethasone daily [pulses are 10 mg/m² for 3 days]; VP-16 = etoposide 150 mg/m² intravenously; CSA = cyclosporin A; I.T. therapy = intrathecal methotrexate [if progressive neurological symptoms or if an abnormal CSF has not improved].
features. Two highly sensitive diagnostic marker are an increased plasma concentration of the α chain of soluble IL2 receptor (CD25) and impaired NK cell activity. In October 2002, the Arico group proposed an approach to the diagnostic work up of a patient with suspected HLH. The laboratory work-up should involve perforin expression by NK cell by using flow cytometry. Patients lacking perforin expression should be analyzed for the PRFI gene mutation, NK cell activity helps to differentiate between reactive form of HLH from familial type. Normal activity is suggestive of the reactive form rather than familial type. Search for a triggering infectious agent is important, however it should be emphasized that with the possible exception of leishmaniasis, anti-infectious therapy alone is not sufficient to control HLH (Table 2).

Therapy

The aim of treatment of HLH is to suppress the severe hyperinflammation and also to control the infection which has triggered the syndrome. In familial HLH the ultimate aim should be stem cell transplantation to replace the defective immune system with normal functioning immune system.

Hyperinflammation can be treated with steroid, which is cytotoxic for lymphocytes and inhibit secretion of various cytokines. Dexamethasone is the drug of choice since its CSF penetration is better than prednisolone. Cyclosporine prevents T lymphocytes and immunoglobulin infusion helps to control the infection . Emmenegger and others have evidence that IVIG is effective in the treatment of HLH. A key finding of their analysis is the efficacy of IVIG if it is administered at the beginning (within hours) of the macrophage activation process and its failure in later stages of the disease. Etoposide has high activity in monocyte and histiocytic diseases. Patients who are at low risk may be treated with only cyclosporine, corticosteroid or IVIG, histiocytic diseases. Patients who are at low risk may be treated with only cyclosporine, corticosteroid or IVIG, only high risk group required etoposide. Etoposide may be life saving especially in case of HLH with Ebstein Barr Viruses infection. CNS reactivation may occur during treatment, intrathecal Methotrexate plus Corticosteroid has been found to be beneficial in some cases. Serum ferritin seems to be a useful emergency marker for monitoring macrophage activation.

Although symptoms and laboratory features improve within 2 to 3 weeks in some cases cytopenia may persist. In these cases bone marrow examination should be repeated to differentiate between non-response and myelosuppression due to etoposide. If there is no response, unlikely to have benefit with treatment. There is no established salvage regimen. Isolated patient has responded to Daclizumab, Alemtuzumab or to stem cell transplantation. Hasegawa et al reported remission of HLH after syngenic bone marrow transplantation. A recent study has found favorable long –term disease control in patients who received a reduced-intensity conditioning regimen instead of conventional stem cell transplant.

The Histiocyte Society in 1994 developed a common treatment protocol (HLH-94), primarily designed for the primary, inherited disease FHL. In January 2004 a revised HLH treatment protocol was opened entitled HLH-2004, which is based on HLH-94 with minor modifications. Cyclosporin A, that in HLH-94 was introduced at week 9, is instead initiated upfront, and corticosteroids is now combined with methotrexate in the intrathecal therapy. The aim is first to achieve a clinically stable resolution and ultimately to cure by BMT. The protocols have been widely accepted internationally and used in more than 25 countries and on all continents.

There is a high remission rate on the HLH-94 and HLH-2004 treatment protocols, during which time a BMT donor usually can be identified. The overall prognosis with regard to survival improved dramatically during the last decade. One major problem is that many children still are diagnosed late, at a stage when they may have severe and irreversible brain damage. With regard to long-term survival, the prognosis will be dependent upon the results of BMT, which also are increasingly rewarding.

REFERENCES