

Hemophagocytic lymphohistiocytosis: A concise review for the practicing physician

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Question

Of the following, which consolidation of laboratory findings is most consistent with the hemophagocytic lymphohistiocytosis syndrome?

- A. Leukocytosis, anemia, and hyperferritinemia
- B. Uremia, metabolic acidosis, and hyperkalemia
- C. Hypertriglyceridemia, anemia, and hyperferritinemia
- D. Thrombocytopenia, cerebral spinal fluid pleocytosis, and elevated lactate dehydrogenase
- E. Leukocytosis, transaminitis, and hypofibrinogenemia

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a syndrome of systemic immune dysregulation due to excessive cytokine release from aberrant cytotoxic T cells, natural killer (NK) cells, and macrophages. The resulting hyperinflammatory state, characterized by fever, cytopenia, and hepatosplenomegaly, can be rapidly fatal if not recognized in time.¹ Furthermore, HLH can be the first manifestation of another primary immunodeficiency syndrome.² Therefore, even though HLH is a rare disease, the basic knowledge of its presentation and treatment is vital for not only practicing immunologists but also for hematologists and critical care physicians who may be involved in the care of these patients. Early diagnosis and rapid initiation of treatment is essential for patients' improved survival.

HLH is a heterogeneous disorder categorized into genetic and acquired etiologies. The genetic causes of HLH can be subcategorized into familial HLH (FHL) and into that associated with another primary immunodeficiency syndrome. FHL is further subcategorized

into five specific loss-of-function mutations that lead to a defect in cytotoxic granule exocytosis of NK cells and CD8 T cells. These mutations are designated FHL 1–5.³

HLH is also associated with other primary immunodeficiency syndromes and can sometimes be the presenting manifestation of these diseases. In acquired HLH, patients present with the same clinical syndrome but lack any known genetic etiology. This syndrome is thought to occur in the setting of underlying immune dysregulation triggered by an immunogenic condition such as infection, autoimmune disease (known as macrophage activation syndrome [MAS]), or malignancy.⁴ Overall, the incidence of HLH is low. FHL is estimated to have an incidence of 1 per 50,000.⁵ There are incomplete data to accurately estimate the incidence of acquired HLH, but acquired HLH is presumed to be largely underrecognized.⁶

CLINICAL CHARACTERISTICS AND PATHOPHYSIOLOGY

Patients with HLH present on a spectrum of clinical signs and symptoms that vary from persistent fevers to a fulminant sepsis picture. Typically, patients are critically ill and nonresponsive to antimicrobial therapy. A significant portion of these patients also have neurologic symptoms, the most common of which is seizure.⁴ Radiographic findings are commonly seen but are neither sensitive nor specific. A chest radiograph may show consolidations, effusions, or mediastinal lymphadenopathy. Abdominal imaging by computed tomography or ultrasound likely will show hepatosplenomegaly, lymphadenopathy, and, possibly, ascites. Neurologic imaging may show nonspecific white-matter signal changes, hydrocephalus, or distinct lesions.⁷ The most common clinical findings in all patients with genetic HLH are fever, cytopenia, and splenomegaly.⁴ In acquired HLH, the three most common features are fever, anemia, and hypertriglyceridemia.⁴ It is important to note that no single clinical or laboratory sign is universally seen in this syndrome.

The pathophysiology of HLH is incompletely understood, and, therefore, the purpose of this overview is to help the reader conceptualize the disease. In brief, failure of cytotoxic T cells and/or NK cells to com-

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pletely eradicate an immune-triggering antigen results in overstimulation of the cytotoxic immune response. In genetic HLH, this failure is due to a defect in the perforin-granzyme-induced apoptotic pathway. Recently, animal models that use genetically modified knockout mice have begun to shed light on the intricacies of this pathway and the pathophysiology of HLH. The best-studied model to date is in perforin-deficient transgenic mice. Perforin is a tetrameric protein that is able to form pores across lipid bilayers. In cytotoxic T and NK cells, secretory lysosomes are manufactured with perforin and a serine protease called granzyme. On activation by an antigen-presenting cell, the cytotoxic lysosome docks with the cell wall and releases its contents at the immunologic synapse. Perforin forms a pore in the target cell and granzyme enters, which triggers apoptosis of the antigen-presenting cell by initiation of the caspase cascade.²

Researchers showed that, in pfp^{-/-} mice, infection with viral trigger induces fulminant HLH syndrome.³ Failure of pore formation in a select population of dendritic cells leads to hypersecretion of inflammatory cytokines (interferon γ and tumor necrosis factor α). These cytokines activate native macrophages, which feed back to continue overactivation of the cytotoxic cell.⁸ The activated T cells go on to infiltrate multiple organs, including the spleen, liver, and lymphoid system.

All five of the familial causes of HLH have been found to have a mutation involved in this key immunologic pathway, although the exact gene and encoded protein in FHL1 is yet to be identified. FHL2 is caused by mutation in the perforin 1 gene, which encodes perforin. It is the most common familial form and has the highest prevalence in North America.⁹

Similar to that of FHL, impaired NK-cytotoxic T cells mediated apoptosis has been identified in the primary immunodeficiencies that are associated with HLH. These include Chediak-Higashi syndrome, Griscelli syndrome type 2, Hermansky-Pudlak syndrome type 2, X-linked proliferative syndrome, CD27 deficiency, and interleukin-2 tyrosine kinase deficiency.²

The pathogenesis of acquired HLH is not as well understood as that of genetic HLH. The syndrome has been seen in a wide range of clinical states. Case reports continue to reveal previously unknown clinical associations with the HLH syndrome.

Of the infection-associated subtypes, the most reported and best studied is the association between Epstein-Barr virus and HLH. Results of mice studies showed that in Epstein-Barr virus-associated HLH, infected CD8⁺ T cells predominate, which is not the case in most primary and chronic-persistent Epstein-Barr virus infections.¹⁰ The virus is thought to have properties that suppress antigen recognition that leads to persistent antigenemia. Non-antigen-spe-

Table 1 **The revised diagnostic guidelines for HLH***

The diagnosis of HLH can be established if one of either (1) or (2) is fulfilled	
(1) A molecular diagnosis consistent with HLH	
(2) Diagnostic criteria for HLH fulfilled (five of the eight criteria below)	
I. Fever	
II. Splenomegaly	
III. Cytopenias (affecting ≥ 2 of 3 lineages in the peripheral blood):	
a. Hemoglobin level < 9 g/dL (in infants < 4 wk of age: hemoglobin < 10 g/dL)	
b. Platelets $< 100 \times 10^3/\mu\text{L}$	
c. Neutrophils $< 1.0 \times 10^3/\mu\text{L}$	
IV. Hypertriglyceridemia and/or hypofibrinogenemia:	
a. Fasting triglyceride level of ≥ 3.0 mmol/L (<i>i.e.</i> , 265 mg/dL)	
b. Fibrinogen level of ≤ 1.5 g/L	
V. Hemophagocytosis in bone marrow or spleen or lymph nodes, and no evidence of malignancy	
VI. Low or absent NK-cell activity (according to local laboratory reference)	
VII. Ferritin level of ≥ 500 $\mu\text{g/L}$	
VIII. Soluble CD25 level (<i>i.e.</i> , soluble IL-2 receptor) of ≥ 2400 U/mL	

HLH = hemophagocytic lymphohistiocytosis; NK = natural killer; IL = interleukin.
 *From Ref. 12.

cific triggering of the innate immune system is thought to play a significant role in the pathophysiology of some autoinflammatory and/or autoimmune associated HLH. Mouse models have been able to induce HLH in immunologically normal mice by repeatedly injecting cytosine-phosphodiester-guanine oligodeoxynucleotide, a synthetic immunostimulant which activates toll-like receptors.³

Decreased NK- and T-cell cytotoxicity have been noted in some reviews of patients with MAS, which supports a pathophysiologic connection between MAS and HLH.¹¹ Other reviews reported polymorphisms and heterozygous mutations in the genes known to code proteins involved in cytotoxic granule formation and trafficking further blurring the distinction between MAS and HLH.¹²

DIAGNOSIS

Early diagnosis of HLH is challenging because the manifestations of HLH are diverse and multifactorial. Furthermore, laboratory tests are nonspecific. Both a high index of suspicion and knowledge of the typical constellation of clinical and laboratory findings are

critical for timely diagnosis. The current diagnostic guidelines for HLH are displayed in Table 1.

MANAGEMENT

Before current strategies, the prognosis of HLH was dismal. In 1983, survival was estimated at 4%.¹³ The Histiocyte Society designed a treatment protocol in 1991 for prospective study which has since been revised. This protocol utilizes etoposide, cyclosporine A, and dexamethasone as first line therapy. Cases identified as familial, persistent or relapsing then qualify for hematopoietic stem cell transplantation. Additional measures are taken in patients with cerebral spinal fluid disease by the administration of intrathecal methotrexate. In 2011, results of the protocol were published with 5-year survival rates reaching 54%.¹⁵

More recent studies^{16,17} indicate that there may be a benefit in diagnosing FHL versus acquired HLH irrespective of the need for hematopoietic stem cell transplantation in the former. A Turkish study with a small sample size found that therapeutic plasma exchange and intravenous immunoglobulin with methylprednisone was found to be effective in patients diagnosed with acquired HLH and was less toxic than the chemotherapeutic regimen outlined by the Histiocyte Society.¹⁶ As the pathophysiology of HLH becomes clearer, the role of biologics in treatment may expand. Currently, there are no sufficient studies that show efficacy. The interleukin-1 inhibitor, anakinra, was associated with a decrease in inflammatory markers in a small cohort of pediatric intensive care patients with presumed secondary HLH, but results were confounded by additional therapies.¹⁷ More outcomes studies are needed to elucidate which treatment protocols have the best survival and safety profiles.

Answer: C

Clinical Pearls

- In pediatric patients, ferritin levels of $>10,000 \mu\text{g/L}$ have a 90% sensitivity and 96% specificity for HLH¹⁸
- Decreased NK cell activity is an important diagnostic marker in HLH²

Clinical Pitfalls

- Fewer than 40% of patients with HLH will have hemophagocytosis on bone marrow biopsy at the time of presentation²
- Patients without hemophagocytosis on bone marrow biopsy can still have cytopenias due to bone

marrow suppression of tumor necrosis factor α and interferon γ ⁶

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