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Secondary hemophagocytic lymphohistiocytosis has a high mortality rate among adults despite recent advances in treatment.

Hereditary and Acquired Hemophagocytic Lymphohistiocytosis

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare but life-threatening hyperinflammatory/hypercytokinemia syndrome clinicopathologically manifested by fever, hepatosplenomegaly, cytopenias, liver dysfunction, and hemophagocytosis.

Methods: We searched the medical literature for English-written articles and analyzed data regarding the diagnosis, pathoetiology, prognosis, and management of HLH.

Results: HLH can be subcategorized into primary/genetic (PHLH) or secondary/acquired (SHLH) according to etiology. PHLH, including familial HLH and inherited immune deficiency syndromes, typically occurs in children harboring underlying genetic defects, whereas SHLH frequently manifests in adults and is associated with infection, autoimmunity, immune suppression, or malignancy. The pathogenesis of HLH is still elusive. Its known mechanisms include somatic mutations in gene coding for proteins implicated in the cytotoxic pathways of cytotoxic T or natural killer cells. The impaired ability of these cells to kill target cells leads to an uncontrolled hypercytokinemia and hyperinflammatory process, triggering hemophagocytosis and multiorgan failure. Corticosteroids, chemotherapy, and immunotherapy are the mainstay therapeutic strategies. The consolidation with allogeneic hematopoietic stem cell transplantation is a potentially curative option for PHLH and refractory or relapsed SHLH.

Conclusions: Understanding of the pathophysiology of HLH has improved in the last decade. The establishment of diagnostic and treatment guidelines for PHLH and SHLH has resulted in earlier diagnoses and the rapid initiation of therapy, both of which are associated with favorable outcomes.

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Introduction

Hemophagocytic lymphohistiocytosis (HLH) is clinical syndrome characterized by a hyperinflammatory condition caused by increased levels of circulating inflammatory cytokines due to a highly stimulated but ineffective immune process, and it is uniformly manifested by an abnormal proliferation of histiocytes throughout the reticuloendothelial system with the engulfment of hematopoietic cells (hemophagocytosis).¹⁻³ The first case of HLH was described by Scott and Robb-Smith⁴ in 1939 as histiocytic medullary reticulosis in light of poorly controlled histiocytic proliferation; later, the term was changed to HLH and macrophage activation

syndrome.⁵ Familial HLH (FHLH) was first described in 1952 as an autosomal recessive immune dysregulation disorder of childhood.⁶

HLH can be subcategorized as primary/genetic (PHLH) or secondary/reactive (SHLH) forms. PHLH includes both FHLH and inherited immune deficiency syndromes, whereas SHLH is associated with

infection, autoimmunity, immune suppression, and malignancies (Table).^{1,7-10} Clinically, it is most often characterized by prolonged and persistent fevers, hepatomegaly, splenomegaly, hemophagocytosis, bilineage or trilineage cytopenias, hypertriglyceridemia, and/or hypofibrinogenemia. Neurological symptoms and multiorgan failures may be predominant in the beginning of the disease, or they may develop during the clinical course.^{7,11} Our understanding of the pathophysiology of HLH, particularly PHLH, has significantly improved in the last 10 years. Germline mutations in gene coding for proteins implicated in cytotoxic pathways have been described in patients with FHLH, hereditary immune deficiency syndromes, various viral infections, including Epstein-Barr virus (EBV), malignancies, and immunosuppression associated with SHLH.¹² In spite of these advances, HLH often poses a clinically diagnostic challenge and treatment dilemma.

This review summarizes the most important clinical, pathological, and molecular features of HLH and provides current treatment strategies for this rare, and sometimes, fatal disease.

Epidemiology
The incident rate of HLH is variable, occurring in 1 out of every 3,000 persons in North America,^{13,14} whereas the annual incidence of adult and pediatric cases of HLH in Japan was 1 per 800,000 persons.¹⁵ Approximately 25% of pediatric cases are PHLH, whereas nearly all adult cases are SHLH; the annual incidence rate of PHLH is 1.2 per 1 million children, whereas the incidence of SHLH among adults is uncertain.^{16,17} Approximately 80% of patients with FHLH are young children (< 1 year of age).¹⁶ One report showed that, for approximately every

Table. — Classification of Hemophagocytic Syndrome

Primary or Genetic Hemophagocytic Syndrome			
<i>Familial Hemophagocytic Lymphohistiocytosis</i>		<i>Immune Deficiency Syndrome</i>	
Type 1 <i>HPLH1</i> , 9q21.3-q22		Chédiak-Higashi syndrome (LYST) (1q42.1 – q42.2)	
Type 2 <i>PRF1</i> , 10q21-22		Griscelli syndrome (15q21)	
Type 3 <i>Munc13-4</i> , 17q25		X-linked lymphoproliferative syndrome	
Type 4 <i>STX11</i> , 6q24.1		Type 1: <i>SH2D1A (SAP)</i> (Xq25)	
Type 5 <i>STXB2</i> , 19p13.3-13.2		Type 2: <i>BIRC4 (XIAP)</i> (Xq25)	
		Wiskott-Aldrich syndrome (WAS, Xp11.4-p11.21)	
		Severe combined immunodeficiency (IL2RG, Xq13.1)	
		Lysinuric protein intolerance (SLC7A7, 14 (14q11.2)	
		Hermansky-Pudlak syndrome (HPS6, 10q24.32)	
Secondary or Reactive Hemophagocytic Syndrome			
<i>Infection-Associated Hemophagocytic Syndrome</i>			
Virus-associated hemophagocytic syndrome			
Herpesvirus			
HIV			
Other viruses			
Adenovirus	Mumps		
Hepatitis (A, B, C)	Enterovirus		
Parvovirus B19	Human T-lymphotropic virus		
Influenza	Flavivirus (dengue fever)		
Measles	H1N1		
Other infections associated with hemophagocytic syndrome			
Bacterial		Parasitic	Fungal
<i>Staphylococcus aureus</i>	<i>Salmonella typhi</i> sp	<i>Leishmania</i> sp	<i>Candida</i> sp
<i>Campylobacter</i> sp	<i>Rickettsia</i> sp	<i>Plasmodium</i> sp	<i>Cryptococcus</i> sp
<i>Fusobacterium</i> sp	<i>Brucella</i> sp	(<i>vivax, falciparum</i>)	<i>Pneumocystis</i> sp
<i>Mycoplasma</i> sp	<i>Ehrlichia</i> sp	<i>Toxoplasma</i> sp	<i>Histoplasma</i> sp
<i>Chlamydia</i> sp	<i>Borrelia burgdorferi</i>	<i>Strongyloides</i> sp	<i>Aspergillus</i> sp
<i>Legionella</i> sp	<i>Mycobacterium tuberculosis</i>	<i>Spirochetes</i> sp	<i>Fusarium</i> sp
Malignancy-associated hemophagocytic syndrome			
Hematopoietic malignancies		Solid tumors	
T-cell/NK-cell lymphoma/leukemia		Hepatocellular carcinoma	
Peripheral T-cell lymphoma (not otherwise specified)		Prostatic carcinoma	
Anaplastic T-cell lymphoma		Lung carcinoma	
Acute leukemia			
Classical Hodgkin lymphoma			
Non-Hodgkin B-cell lymphoma			
Macrophage activation syndrome (association with autoimmune disease)			
Systemic juvenile idiopathic arthritis			
Still disease			
Systemic lupus erythematosus			
Kawasaki disease			
Rheumatoid arthritis			

NK = natural killer.

Adapted from Rosado FG, Kim AS. Hemophagocytic lymphohistiocytosis: an update on diagnosis and pathogenesis. *AJCP* 2013;139:713-727. With permission by the American Society for Clinical Pathology.

2,000 inpatient admissions, there was approximately 1 diagnosis of HLH.¹⁷ HLH can occur in all age groups without predilection for race or sex.^{10,16} However, a higher incidence has been observed in Turkey, which is most likely due to increased consanguinity and a higher prevalence of genetic defects in the cytotoxic pathway.¹⁸

Pathoetiology

Natural killer (NK) cells comprise a subset of lymphocytes engaged in immune surveillance and host defense against cancer and primary or secondary viral infections. The steps for killing target cells via NK cells are complex, multistage processes (Fig 1A).¹⁹ When NK cells are activated, they secrete lytic or cytotoxic granules that contain perforin and granzymes at the immunological synapse to eliminate abnormal cells. As soon as these granules are delivered to a target cell, perforin permeabilizes the cell membranes of the

target cell so that granzymes can enter the cytoplasm and induce caspase-dependent and caspase-independent apoptosis.^{20,21} Thus, any defect of the normal NK cell cytolytic pathway will impair this function, resulting in the disruption of immune surveillance and host defense systems.

Cytotoxic T lymphocytes (CTLs) play a role similar to NK cells. CTLs express T-cell receptors that can recognize a specific antigen in the context of class I major histocompatibility complex molecules. When the immune response is triggered in a healthy individual, NK cells, CTLs, and histiocytes are activated to kill the infected or malignant cells. This process is followed by the elimination of the stimulating antigen and termination of the immune response via a feedback loop. All activated cells involved in this process interact with each other via normal receptors and secrete proinflammatory cytokines and chemokines (Fig 1).^{19,22,23}

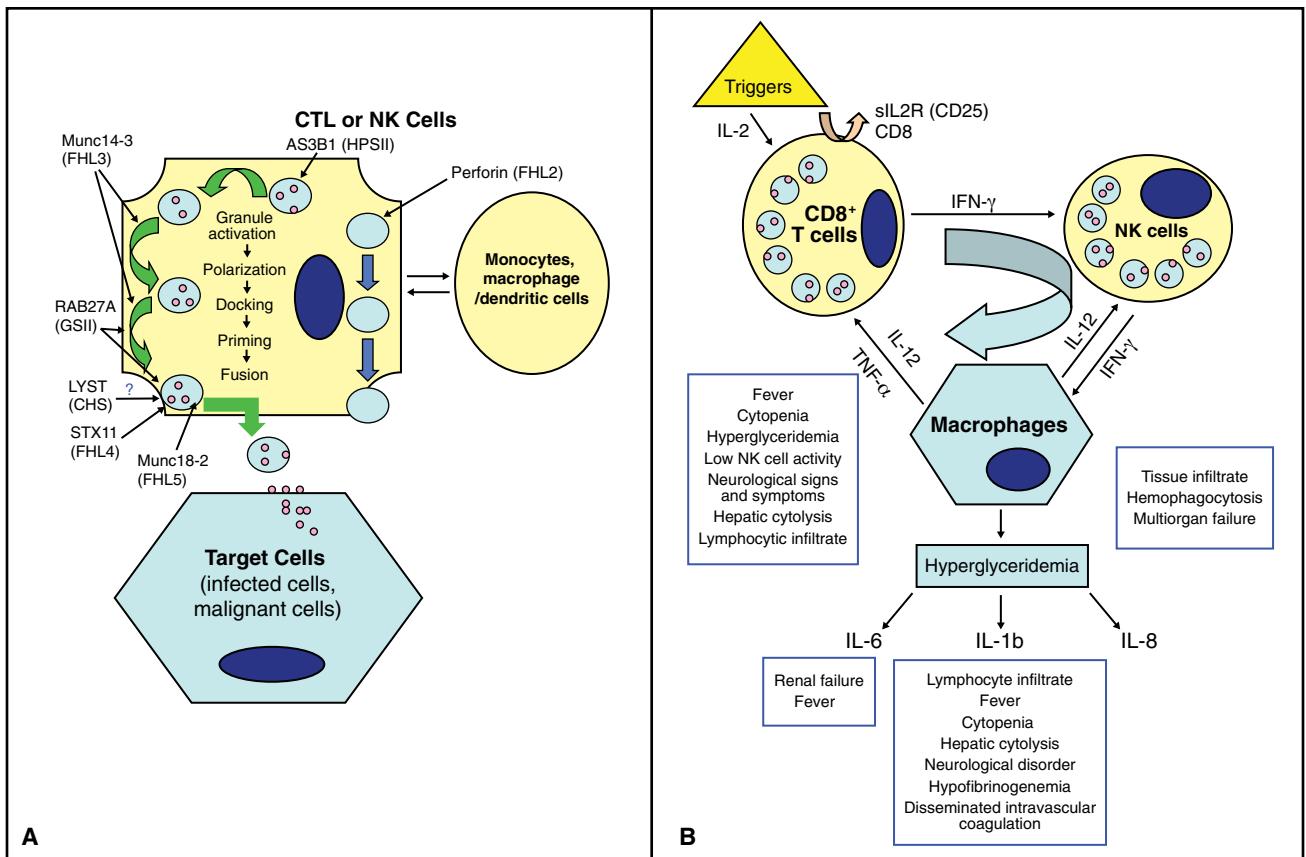


Fig 1A–B. — (A) The normal pathway goes through granule activation, polarization, docking, priming, and fusion. Cytotoxic granules are released into a synaptic gap, entering the target cells to kill them. The defects in FHL and immunodeficiency syndrome (GSII, CHS, and HPSII) impair the normal process of the cytotoxic pathway. Empty granules are seen in perforin deficiency. The question mark indicates that the function of *LYST*, which may be important for the correct size and function of lytic granules, is not entirely understood. (B) Activated CD8 T lymphocytes cause the activation and proliferation of NK cells with increased proinflammatory cytokines. Hypercytokinemia results in a hyperinflammatory reaction, which then leads to constitutional symptoms and systemic illness due to lymphocytic and histiocytic infiltrate. TNF- α and IFN- γ production contribute to macrophage activation with resulting hemophagocytosis. CHS = Chédiak–Higashi syndrome, CTL = CD8⁺ cytotoxic T lymphocyte, FHL = familial hemophagocytic lymphohistiocytosis, GSII = type 2 Griscelli syndrome, HPSII = type 2 Hermansky–Pudlak syndrome, IFN = interferon, IL = interleukin, NK = natural killer, TNF = tumor necrosis factor.

Panel A is adapted from Fig 1 in Bode SF, Lehmsberg K, Maul-Pavicic A, et al. Recent advances in the diagnosis and treatment of hemophagocytic lymphohistiocytosis. *Arthritis Res Ther*. 2012;14(3):213. © 2012 BioMed Central Ltd. Panel B is adapted from Fig 2 in Créput C, Galicier L, Buyse S, et al. Understanding organ dysfunction in hemophagocytic lymphohistiocytosis. *Intensive Care Med*. 2008;34(7):1177-1187. With kind permission from the originating authors and Springer Science and Business Media.

Although the precise pathogenesis of HLH is elusive, a strong link exists between the hyperinflammatory response and hemophagocytosis coupled with impaired CTL, NK activity (inherited or acquired), or both.^{21,24-27} The normal function of histiocytes in the innate immune reaction includes the presentation of antigen, phagocytosis, and the activation of the adaptive immune system through contact with infected or targeted cells and cytokine release.²¹ Antigen-presenting cells (eg, macrophages, histiocytes) are activated in HLH. The proinflammatory cytokines (ie, tumor necrosis factor [TNF] α), interferon [IFN] γ , interleukin [IL] 1b, IL-6, IL-8, IL-10, IL-12, IL-18, and soluble IL-2 receptor) are produced by the uncontrolled proliferation of histiocytes and T cells. The expansion of antigen-specific CTLs that produce a high level of cytokines further activates macrophages.^{21,28,29} The result of HLH at the tissue and cellular level is tissue necrosis and hemophagocytosis, leading to multiorgan failure. Hemophagocytosis, which is a hallmark of activated macrophages, is mediated via the CD163 heme-scavenging receptor.^{21,28} A brief schematic pathway of the pathophysiology of HLH is illustrated in Fig 1B.^{22,23}

Primary Hemophagocytic Lymphohistiocytosis

Familial Hemophagocytic Lymphohistiocytosis:

FHLH is inherited in an autosomal recessive fashion and has 5 subtypes. Most patients with FHLH present at younger than 1 year of age.^{16,30} FHLH has also been reported in adolescent and adult patients without a familial history.³¹ In addition to type 1 FHLH, other subtypes show defects in the perforin/cytotoxic pathway (see Table).^{9,10,32} The 5 hypomorphic *FHL* mutations might correlate with late-onset HLH.^{33,34} According to Zur Stadt et al,³⁴ types 2 to 4 FHLH account for 80% of the HLH cases of Turkish origin but only 30% of those of German descent.

Type 1. The mutation involved in type 1 FHLH is unknown.

Type 2. Approximately 20% to 40% of FHLH cases harbor a *PRF1* mutation.^{33,34} The *PRF1* gene was reported in 1999 and encodes a soluble pore-forming protein, perforin, synthesized and stored in cytotoxic lymphocytes, along with granzyme serine protease.³⁵ Perforin acts as an effector for NK cells and CD8⁺ CTLs. Mutations in *PRF2* impair the function of perforin to permeabilize the target cell membrane, allowing granzymes to enter the cells (see Fig 1A).^{19,21} The mutations are common in families of Middle Eastern descent.³⁶ When carrying nonsense perforin mutations, patients with these mutations were reported to have higher serum levels of ferritin and soluble IL-2 receptor when compared with other subgroups.³⁰

Type 3. Approximately 10% to 20% of cases of FHLH have a *UNC13D* gene mutation.³⁴ *UNC13D* encodes a protein unc-13 homolog D or Munc13-4. The

protein is required for cytolytic granule fusion with cytoplasmic membrane components to process degranulation or exocytosis.³⁷ *UNC13D* mutation results in defective degranulation.

Type 4. A total of 10% to 20% of FHLH cases have mutated *STX11*,³⁶ which belongs to a member of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (t-SNARE) family. *STX11* binds to *SNAP23* in NK cells. Similar to *UNC13D*, it is involved in accelerating the fusion in intracellular membrane trafficking processes.³⁸ Mutations in *STX11* result in decreased or absent *STX11* protein, leading to defects in the endocytotic and exocytotic pathway.³⁴

Type 5. Mutated *STXBP2*, also called *Munc18-2*, has been identified in type 5 FHLH.³⁹ The encoded protein plays a critical role in intracellular trafficking, the control of the SNARE complex assembly, and the release of cytotoxic granules by NK cells.⁴⁰ Study findings indicate that the *STXBP2* mutation could result in impaired granule mobilization of granules and loss of the ability to kill bacteria.⁴¹

Hereditary Immunodeficiencies

Type 2 Griscelli syndrome, Chédiak–Higashi syndrome, and type 2 Hermansky–Pudlak syndrome are all inherited in an autosomal recessive fashion that predispose patients to HLH.^{2,42} Common clinical and laboratory features for these diseases include oculocutaneous albinism, increased susceptibility to infections, and defects in CTL and NK cell activity resulting in immunodeficiencies.

Type 2 Griscelli syndrome caused by the *RAB27A* mutation is characterized by hypomelanosis with immunological abnormalities (defective CTL and NK cell cytotoxic activity) with or without neurological impairment.^{39,43} The *RAB27A*-encoded protein interacts with *Munc13-4* during the docking of cytotoxic granules to the cell membrane.⁴⁴ Chédiak–Higashi syndrome is associated with granulated cells and enlarged lysosomes because of biallelic mutations in *LYST*, resulting in the ineffective release of cytotoxic granules.^{39,43,44} The *AP3B1* mutation leads to type 2 Hermansky–Pudlak syndrome, which is characterized by platelet storage disease, prolonged bleeding, congenital neutropenia, pulmonary fibrosis, granulomatous colitis, and albinism.⁴⁵

X-Linked Lymphoproliferative Syndromes

Types 1 and 2 X-linked lymphoproliferative (XLP) syndrome are due to the hemizygous mutation of *SH2D1A* and the mutation of *XIAP*, respectively, and both are associated with a high risk of developing HLH.^{12,28,34,46} *SH2D1A* and *XIAP* are responsible for XLP syndrome due to signaling lymphocytic activation molecule-associated protein and deficiencies of the X-linked inhibitor of apoptosis protein, respectively.

XLP is characterized by extreme vulnerability to EBV infection,²⁸ and the signaling lymphocytic activation molecule-associated protein is a key regulator of normal immune function in T cells, NK cells, and B cells. *XIAP* encodes a 497-amino-acid antiapoptotic molecule. Although the pathophysiology of HLH in patients deficient in the X-linked inhibitor of apoptosis protein is not fully understood, it may be due to defects in CTLs or the NK cell cytotoxic pathway (see Fig 1A).¹⁹ *SH2D1A* and *XIAP* are proximally located on the same chromosome and may interact with each other.²⁸

Secondary Hemophagocytic Lymphohistiocytosis

Causes of SHLH may include viral, fungal, bacterial, or parasitic infections, as well as hematological malignancies, autoimmune disorders, or immunosuppression, and particularly post-solid organ transplantation (see Table).^{9,10} Similar to PHLH (see Fig 1),^{19,22,23} the acquired defects in CTL or NK cell cytotoxic pathways have been observed, but the exact molecular mechanisms resulting in SHLH are unclear.⁴⁷ It has been speculated that hypercytokinemia may impair the normal functions of CTLs, NK cells, or both, and individual genetic polymorphisms on leukocyte common antigen might increase susceptibility for HLH in such patients.^{48,49}

Infections: EBV is a ubiquitous γ -herpesvirus and is the most common pathogen associated with HLH.^{50,51} It causes a clonal proliferation and the hyperactivation of EBV-infected T cells in patients with SHLH.⁸ Of interest, most cases of EBV infection with concurrent HLH have been reported in children and adolescents, with the highest incidence occurring in East Asia.⁵²

According to a study of adult patients with HLH, in addition to EBV infection, histoplasmosis and cytomegalovirus (CMV) were the other 2 common infectious agents, comprising 19% (4 patients) and 14% (3 patients) of cases, respectively.¹⁷ In a large study of 96 patients with HLH, 30 were associated with infection.⁵³ The most common types of infection were viral (41%), mycobacterial (23%), bacterial (23%), and fungal (13%).⁵³ In addition to EBV, CMV, and histoplasmosis, other viral agents implicated in HLH include HIV, human herpesvirus 8, parvovirus B19, the hepatitis viruses, enterovirus, flavivirus (dengue fever), and H1N1, among others.^{8,54-62} Other infectious agents associated with SHLH appear in the Table.^{9,10}

Autoimmune Processes: HLH may be associated with rheumatology or autoimmune diseases.⁶³ A comprehensive literature review of patients with associated HLH and rheumatological diseases identified 117 papers describing 421 patients, with the most common rheumatological diseases being systemic juvenile arthritis (50.2%), systemic lupus erythematosus (22.3%), Still disease (8.8%), and Kawasaki disease (5.9%).⁶³ In addition, connective tissue diseases such

as dermatomyositis, systemic sclerosis, and mixed connective disorder were also observed in patients with HLH.^{63,64}

Associated Malignancies

Lymphoma is the most common hematological malignancy associated with HLH.⁶⁵ Among lymphomas, T-cell lymphoproliferative disorders, such as anaplastic large cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, and NK cell lymphoma, were the most frequently observed.^{64,66-70} HLH has also been reported in patients with classical Hodgkin lymphoma^{71,72} and other B-cell lymphoproliferative disorders.⁷²⁻⁸⁴ Less frequently, myeloid malignancies such as acute myeloid leukemia have been reported in association with HLH.^{73,74}

Clinical Findings

In general, the early signs and symptoms of HLH are nonspecific. No specific laboratory tests are available for diagnosing HLH.⁷⁵ The most common clinical symptoms and laboratory abnormalities include unexplained fevers, cytopenia, and hepatosplenomegaly. Neurological symptoms such as altered mental status, seizures, and nerve palsies can be observed.⁷⁵ Cerebrospinal fluid cytology can reveal hemophagocytic cells, but the absence of these cells does not exclude HLH.^{21,76,77} In the late phase of HLH, most patients develop multiorgan failure (frequently accompanied with coagulopathy) and acute respiratory distress syndrome.²¹

Typical clinical scenarios in which PHLH should be considered in the differential diagnosis include infectious mononucleosis in an infant or young child, aseptic meningitis associated with cytopenias, or a viral-like syndrome or illness with fever, cytopenias, and organomegaly.⁷⁸ Of note, in cases of systemic juvenile arthritis, 30% to 40% of such patients had a subclinical manifestation of the disease, with 10% to 20% of them presenting with overt clinical symptomatology.^{79,80}

Laboratory Findings

Characteristic laboratory findings include elevated serum levels of ferritin, fasting hypertriglyceridemia (≥ 265 mg/dL), transaminitis, hyperbilirubinemia, and elevated levels of lactate dehydrogenase, along with decreased levels of fibrinogen (< 1.5 g/L).⁸⁵ Elevated blood levels of proinflammatory cytokines, including IL-6, IL-8, IL-10, IL-12, IL-18, macrophage colony-stimulating factor, IFN- γ , and TNF- α , as well as elevated plasma levels of soluble IL-2 receptor (CD25), sCD95 ligand, and sCD163, have also been reported.^{1,81-84} The decreased or loss of NK cell activity is another laboratory abnormality that supports the diagnosis of HLH.⁸⁵

A laboratory search for infectious agents is necessary in patients with suspected HLH. Serological assays specific for EBV may be nondiagnostic in some

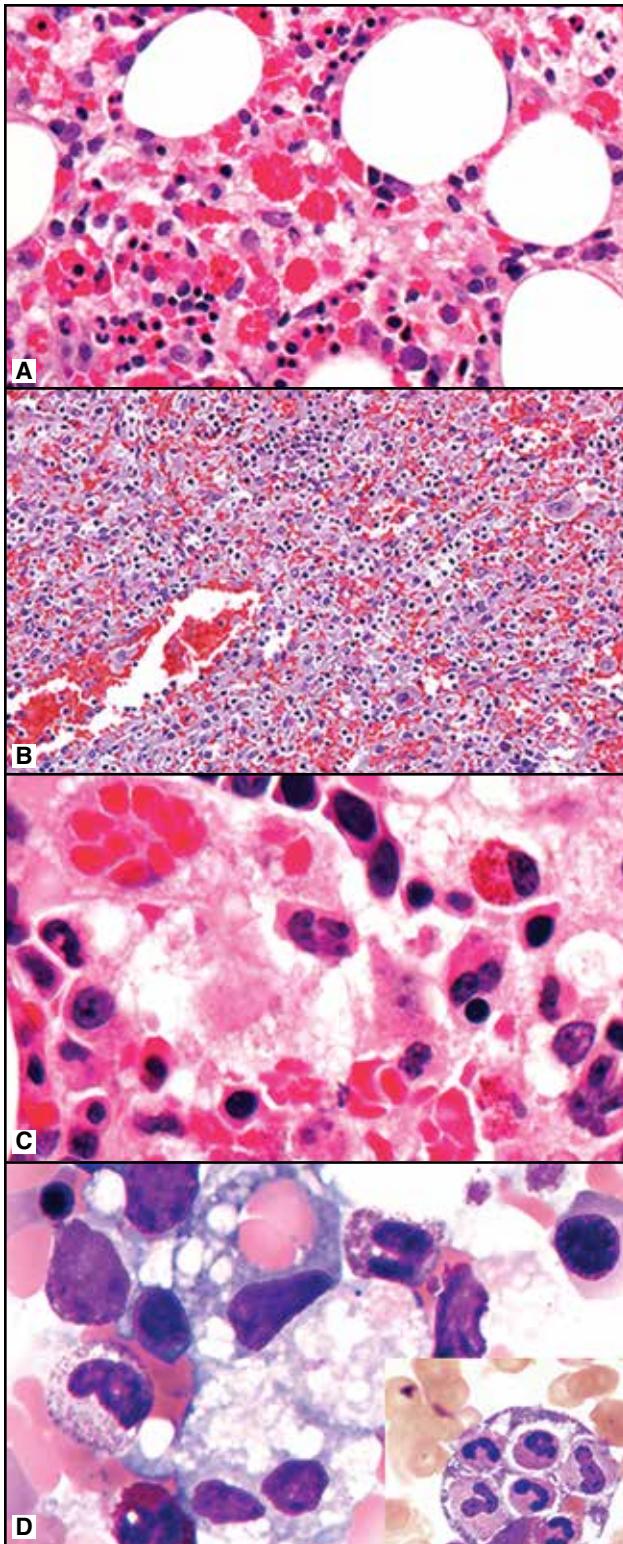


Fig 2A–D. — (A) Bone marrow core biopsy showing an increased number of histiocytes engulfing erythrocytes, granulocytes, or platelets associated with focal granulomatous change (H & E, $\times 200$). (B) Spleen with abnormal proliferation of medium- to large-sized atypical histiocytes associated with increased hemophagocytosis (H & E, $\times 200$). (C) Bone marrow core biopsy showing enlarged histiocytes with phagocytosis predominantly in dilated sinusoidal areas. Normal trilineage hematopoiesis is decreased in number (H & E, $\times 1000$). (D) Bone marrow aspirate exhibiting enlarged histiocytes, some containing erythrocytes, or various kinds of hematopoietic elements (inset; Wright-Giemsa stain, $\times 1000$). H & E = hematoxylin & eosin.

patients; however, the presence of a high EBV DNA load in plasma supports the diagnosis of EBV-associated HLH.^{50,52} Thus, direct molecular virological assays may allow better detection of this potentially underdiagnosed disease.^{50,52}

Histological Findings

Biopsies of bone marrow and other tissues (eg, lymph nodes) are useful for identifying hemophagocytosis. In general, bone marrow typically shows reactive lymphocytosis, slightly to markedly increased histiocytes, and a marked left-shift myeloid maturation regardless of etiology. In the bone marrow aspirate smear, enlarged histiocytes, engulfing red blood cells, granulocytes, lymphocytes, and occasional plasma cells can be seen; in addition, the spleen with red-pulp expansion and increased hemophagocytosis can be seen on autopsy (see Fig 2). Immunohistochemical studies using histiocyte-specific antibody, such as CD68, CD163, and CD14, are useful for highlighting phagocytic cells as well as engulfed, negative-stained hematopoietic cells.

Diagnostic Criteria

The diagnostic criteria for HLH were established in 1991⁵ and then subsequently revised in 1997⁸⁶ and then updated again in 2004.⁸⁵ These diagnostic criteria have been widely adopted clinically and represent the current guidelines for HLH. A diagnosis of HLH requires either a documented molecular confirmation or the presence of at least 5 of the following 8 clinical or laboratory parameters⁸⁵:

- Fever
- Splenomegaly
- Cytopenia affecting ≥ 2 lineages in the peripheral blood:
 - Hemoglobin < 90 g/L (< 100 g/L for infants < 4 weeks of age)
 - Platelets $< 100 \times 10^9$ /L
 - Neutrophils $< 1.0 \times 10^9$ /L
- Hypertriglyceridemia and/or hypofibrinogenemia, fasting triglycerides ≥ 265 mg/dL, fibrinogen ≤ 1.5 g/L
- Hemophagocytosis found in the biopsy specimen of bone marrow, spleen, or lymph nodes
- Decreased or absent NK cell activity
- Ferritin ≥ 500 mg/L
- sCD25 $\geq 2,400$ U/mL

Bone marrow hemophagocytosis has a high sensitivity rate because rare hemophagocytic histiocytes can be detected prior to patients exhibiting overt clinical symptoms of HLH; however, the specificity of this test is too low to incorporate it into the panel of screening tests for diagnosing HLH.⁸⁷ Therefore, the 2004 HLH diagnostic guidelines set forth by Henter et al⁸⁵ suggest obtaining materials

from the other organs if the bone marrow specimen is inconclusive. In addition, the presence of any of the following findings may also provide strong supportive evidence for the diagnosis: (a) spinal fluid pleocytosis (mononuclear cells), elevated spinal fluid protein, or both, and (b) histological results from liver biopsy resembling chronic persistent hepatitis.⁸⁵ Other abnormal clinical and laboratory findings can include cerebromeningeal symptoms, lymph node enlargement, jaundice, edema, skin rash, hepatic enzyme abnormalities, hypoproteinemia, hyponatremia, increased very-low density lipoprotein, and decreased high density lipoprotein.^{85,88}

A search for novel markers for an HLH diagnosis has revealed that serum S-SMase/ceramide activity is elevated in cases of HLH; however, these patients eventually died despite appropriate treatment.⁸⁹

Using flow cytometry to diagnose HLH is not specific. However, qualitative abnormalities of atypical cytotoxic T cells have been reported in the majority of EBV-associated HLH cases.⁹⁰ According to a large cohort study of 494 patients with suspected HLH, the performance of degranulation assays based on surface up regulation of CD107a on NK cells and CTLs may provide a diagnostic value in FHLH.⁹¹ A resting NK cell degranulation level below 5% was associated with sensitivity and specificity rates of 96% and 88%, respectively, for diagnosing genetic degranulation disorders.⁹¹

Molecular studies of gene mutations have involved *PRF129*, *UNC13D* (*Munc13-4*), *STX11*, and *STXBP2* (*Munc18-2*).^{37,38,92} *RAB27A*, *LYST*, and *AP3B12* have been useful in aiding the diagnosis of inherited immunodeficiency syndromes.⁴²

Differential Diagnosis

HLH can be a diagnostic challenge when distinguishing between HLH and the reactive or malignant histiocytic proliferations (eg, infection-related histiocytosis).^{10,93-95} An autoimmune lymphoproliferative syndrome might mimic HLH.⁹⁶ In neonates, HLH might be difficult to differentiate from neonatal hemochromatosis when patients present with acute liver failure⁹⁷ or storage disease with hyperglycerinemia.¹⁶ A newly proposed and validated scoring system for reactive HLH called HScore may be a practical way to exclude non-HLH cases.⁹⁸

Treatment

HLH has an aggressive clinical course with a high mortality rate in all age groups. Prognosis depends on early diagnosis and timely treatment.⁸⁵ The HLH 2004 protocol is the most commonly used frontline treatment regimen, with the immediate goals of extinguishing the hyperinflammatory and hypercytokine status, controlling underlining disorders (eg, infection, tumor, autoimmune), and eliminating overactive

macrophages in the reticuloendothelial system.^{65,85} In PHLH, the ultimate goal of treatment is to replace the mutated gene or defective immune system.⁸⁵

General Management

The HLH protocol is widely accepted as the standard therapeutic approach for HLH (Fig 3).⁸⁵ The main difference distinguishing the 2004 protocol from the 1997 protocol is the administration of cyclosporine A at the onset of therapy instead of at week 9.^{85,86} Both protocols include dexamethasone, etoposide, and cyclosporine A for 8 weeks in patients with SHLH who do not have an identifiable genetic defect.^{85,86} In cases of PHLH, therapy is administered for more than 8 weeks until hematopoietic stem cell transplantation (HSCT) is initiated. Intrathecal therapy with methotrexate and corticosteroids is recommended for patients with central nervous system manifestations.⁸⁵

Supportive care with prophylactic antibiotics, blood and platelet transfusions, and treatment with fluids and electrolytes are all important steps in the treatment of HLH.⁹⁹

Familial Hemophagocytic Lymphohistiocytosis

Given the high mortality rate of FHLH, the initial steps of treatment are to suppress the hyperinflammatory process and eliminate abnormal T and NK cells, antigen-presenting cells, and phagocytes. A response to treatment typically takes up to 8 weeks. In a patient with low-risk HLH, corticosteroids and/or intravenous immunoglobulin or cyclosporine A may be sufficient to control the dysregulated biological processes.^{51,85} Moreover, etoposide is recommended to reverse lymphohistiocytic dysregulation in patients at high risk.^{8,85}

HSCT is recommended for patients with FHLH and genetic defects and is considered the only known curative approach.⁴⁷ A matched related donor is preferred over an unrelated, partially, or umbilical cord blood-matched donor to achieve maximal hematopoietic stem cell engraftment and reduce the risk of severe graft-host and transplant-related mortality.⁴⁷ Only a subset of patients (approximately 20%) have matched sibling or parental donors.⁴⁷ The long-term experience with HSCT in patients with FHLH is limited due to the rarity of the disease.^{100,101} The current use of reduced intensity conditioning protocols for HSCT decreases chemotherapy-associated toxicity, including veno-occlusive disease.^{100,101} Patients who do not respond to treatment (based on the 2004 protocol⁸⁵) within 4 to 8 weeks may benefit from second-line therapy (eg, antithyroglobulin, alemtuzumab).⁴⁷

Secondary Hemophagocytic Lymphohistiocytosis

Typically, patients with idiopathic SHLH are treated per the 2004 protocol, which includes a 2-week in-

duction phase followed by a 6-week tapering phase.⁸⁵ In patients with organ dysfunction or failure, immediate treatment should be started to reduce mortality.⁸⁵ Treatment may be also beneficial for patients who have relapsed SHLH but are without genetic defects.⁴⁷

Underlying Disease

Treatment is largely dependent on clinical status.⁸⁵ When HLH is triggered by an acute infection or another condition (eg, rheumatoid disease), treatment of the trigger is necessary to eliminate the hyperinflammatory stimulus or hyperimmunological activation. Stable patients who are less acutely ill may be able to tolerate initial treatment without HLH-specific therapy.¹⁰²

Malignancies

The treatment of HLH secondary to lymphoma frequently includes combined chemotherapy regimens

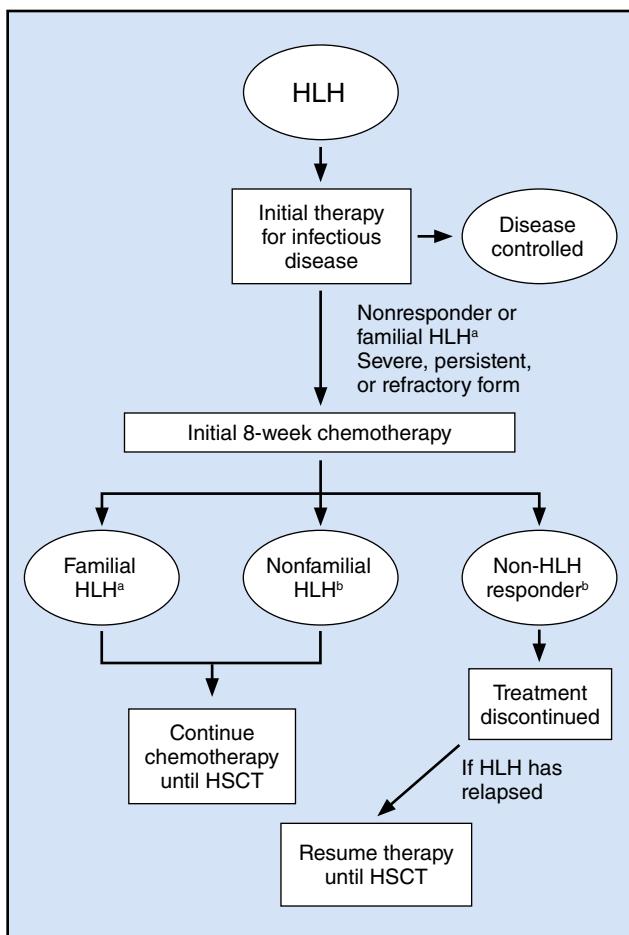


Fig 3. — Schematic algorithm for the treatment of children according to the HLH 2004 protocol. Refer to the text and Fig 1 for information on whether to start continuation therapy and perform HSCT. Data from Henter JI, Horne A, Aricó M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48(2):124-131.

^aFamilial HLH with or without genetic mutation verified. ^bNonfamilial HLH and nongenetic mutations identified.

HLH = hemophagocytic lymphohistiocytosis, HSCT = hematopoietic stem cell transplantation.

according to lymphoma subtype. The use of HSCT in patients during their first remission may be of benefit for those with partial remission or refractory HLH. Selected patients with recurrent HLH in complete remission should be considered for HSCT.^{85,99,103} However, the proportion of adult patients with acquired HLH who are eligible for HSCT is low due to poor performance status, malnutrition, active infections, and complications from lymphoma therapy. Similar approaches using disease-specific therapy first should be employed for other malignancies associated with HLH.¹⁰⁴

Infections

Although they are rare, cases of EBV infection associated with HLH can occur in apparently EBV-immunocompetent individuals, particularly in adults living in western countries. The high mortality rate among patients with EBV-associated HLH is usually due to a delay in diagnosis or ineffective therapy.⁵⁰ However, the 2004 protocol has improved survival rates of patients with HLH (see Fig 3).^{85,88} Per Kleyenberg and Schiller,⁷ studies have emphasized the importance of etoposide for the treatment of EBV infection associated with HLH, describing the drug as the most effective single agent against activating histiocytes. Although intravenous immunoglobulin has been recommended for the treatment of reactivated EBV infection, patients with EBV-associated B-lymphoproliferative disorders taking rituximab have also been found to have improved outcomes.¹⁰⁵ The efficacy of a rituximab-containing regimen was investigated in 42 patients with HLH who received, on average, 3 rituximab infusions (range, 1–10) at a median dose of 375 mg/m², along with steroids, etoposide, and/or cyclosporine.¹⁰⁵ The therapy was well tolerated and resulted in clinical improvements among 43% of patients. A significantly reduced EBV viral load was also observed.¹⁰⁵ Because EBV can also infect T and NK cells, recurrence of EBV infection can occur in patients despite rituximab therapy; in such patients, alemtuzumab has been shown to be beneficial.¹⁰⁶

Salvage Therapy

The removal of cytokines with plasma or transfusion exchange in very young infants has been shown to stabilize patients until other therapies have enough time to work.¹⁰⁶⁻¹⁰⁸ Other salvage therapies include high-dose pulse corticosteroids and alemtuzumab, which suppresses CD52-expressing T or B cells and histiocytes.¹⁰⁶⁻¹⁰⁸ CMV and adenovirus viremia were common complications of this therapy. Monitoring CMV DNA viral load by weekly polymerase chain reaction is standard in patients treated with this agent.¹⁰⁶ Other reported salvage therapies include an anti-TNF antibody, infliximab,¹⁰⁹ and the anti-CD25 antibody

daclizumab.^{110,111} In some patients with macrophage activation syndrome, inhibiting IL-1 and IL-6 was successful.¹¹²

In recent clinical trials with blinatumomab, drug-induced HLH was observed.¹¹³ Therapy with tocilizumab resulted in a rapid clinical improvement;¹¹³ Tocilizumab is a drug that could be potentially effective in other types of HLH and is currently undergoing testing in a clinical trial in children and young adults (NCT02007239). Failure of salvage regimens is an indicator for considering allogeneic HSCT.^{72,114}

Allogeneic Hematopoietic Stem Cell Transplantation

Allogeneic bone marrow transplantation from a related, human leukocyte antigen identical donor is the treatment of choice for patients with FHLH. However, few patients have a disease-free sibling who is an identical human leukocyte antigen match. Presently, HSCT is the only available treatment to cure FHLH; thus, it represents the definitive therapy of choice for many patients.^{101,114} However, it is not uncommon for patients to develop recurrence of HLH before a suitable donor is identified. Thus, preparation for allogeneic HSCT should be initiated at the time of diagnosis, and it should include human leukocyte antigen typing and a search for a suitable donor for HSCT.^{101,114}

It is worth noting that, in 1 study, a median disease-free survival rate was achieved in 9 patients with FHLH during a follow-up period of 33 months (range, 8–69 months).¹¹⁴ In a meta-analysis, 11 studies comprising data from 342 patients with EBV-associated HLH were collected and analyzed.¹¹⁵ A total of 54 of the 342 patients underwent HSCT. The transplantation-related mortality rate was 20% (11 of 54 patients), which was lower than that seen in the control arm (32%; 93 of 288 patients); however, no statistically significant difference was seen in mortality rates found between those treated with HSCT and those treated with conventional immunochemotherapy.¹¹⁵ Therefore, HSCT may not be suitable for patients with EBV-associated SHLH compared with those who have FHLH.

A nationwide retrospective analysis indicated that reduced intensity conditioning followed by cord blood transplantation is an alternative and feasible treatment for PHLH or FHLH.¹¹⁶ The overall survival rate reached in that study was 65.4% ± 6.6% in 13 patients, a rate comparable with other therapeutic strategies.¹¹⁶ The treatment dilemma was with patients with engraftment failure; however, HLH could be managed in these patients through the use of secondary cord blood transplantation.¹¹⁶

Data indicate that HSCT should be used in HLH refractory to conventional therapy. A single institutional study focusing on therapy for HLH associated with T- or B-cell lymphomas reported that the median

overall survival rates of patients who had HLH and T- or B-cell lymphoma were 96 days and 330 days, respectively.¹¹⁷ Rituximab might have partially contributed to longer survival rates in patients with B-cell lymphoma-associated HLH; by contrast, allogeneic HSCT should be recommended for patients with T-cell lymphoma-associated HLH.¹⁰⁵

Outcome and Prognosis

Despite advances in therapy and supportive care, the cure rate for HLH, particularly in patients with multiorgan failure, is low.^{19,23} Clinical outcomes for children with HLH have been previously determined in 2 important clinical trials. Prior to the 1997 protocol,⁸⁶ patients with FHLH were not likely to survive beyond 1 year.⁷⁶ Moreover, a study conducted in 2002 suggested that overall survival rates increased once the 1997 protocol was put into practice.⁹⁹ Patients with all types of HLH treated per the 1997 protocol had a 3-year overall survival rate of 55%, and a subgroup of patients who underwent HSCT had a 3-year overall survival rate of 62%.^{99,118} In a single, institutional, retrospective study of pediatric patients, the 3-year overall survival rate was 92% in patients treated with allogeneic HSCT after reduced intensity conditioning and 43% in patients who underwent myeloablative allogeneic HSCT.¹⁰¹ A review of antithymocyte globulin-based therapy in 38 patients with FHLH demonstrated a complete response rate of 73%.¹¹⁹ Sixteen of the 19 patients (84%) who underwent consolidation with HSCT were considered to be cured, and overall survival for all study participants was 55%.¹¹⁹ Japanese patients with EBV-associated HLH were shown to have a survival rate of 86%.¹²⁰ The results of another important HLH study have not yet been published (NCT00426101).

To date, most studies concentrate on the management of PHLH in children. Limited trials study adult patients with SHLH,^{17,67,121} and data demonstrate inferior median overall survival rates, ranging from 35 days¹²¹ to approximately 2 months.¹⁷ Among trials relating to SHLH, patients with HLH due to malignancy had the poorest clinical outcome (median overall survival, 1–12 months).^{122–126} Parikh et al¹²⁷ reported that patients with HLH associated with malignant tumors had a much shorter median overall survival rate of 1.4 months compared with 22.8 months among patients who had HLH without infection, autoimmune disease, or idiopathic entity. A report of EBV-associated HLH outcomes among children revealed a 90% overall response rate to multiagent therapy, including corticosteroids, etoposide, and cyclosporin,¹²⁸ whereas many other patients with infection-associated HLH died within days or months.⁶¹ Dhote et al¹²⁹ reported an overall mortality rate of 38.5% among patients with autoimmune disease-associated HLH.

However, in a different study, a subset of patients with autoimmune disease-associated HLH treated with immunosuppressive agents, such as cyclosporine, cyclophosphamide, or tacrolimus, achieved a remission rate of 80%.¹³⁰

Long-term complications of HLH encompass therapy-related morbidity — particularly following HSCT — and neurological deficits. The latter can manifest months to years following HLH; however, most patients return to their normal lives.¹³¹

Validated prognostic factors are lacking among prospective studies in order to guide treatment decisions in patients with HLH. Most of the currently available prognostic factors have been derived from literature reviews or from single institutional studies.

Earlier studies revealed that liver function abnormalities and cytopenias, along with increasing in serum levels of ferritin, soluble CD25, and soluble CD163, may be indicators of relapse.¹⁰⁶ Kaito et al¹³² suggest that age older than 30 years, a fibrinogen degradation product level above 10 mcg/mL, and a ferritin level above 500 ng/mL are risk factors associated with death. Another study revealed that an elevated level of soluble CD25 (> 10,000 U/mL) has a negative impact on prognosis, with a 5-year survival rate of 36% compared with 78% in the control group.¹³³

The severity of hyperbilirubinemia, thrombocytopenia, hyperferritinemia, and cerebrospinal fluid pleocytosis may also be key risk factors for early death among patients with HLH, as are lack of improvement in hemoglobin or fibrinogen levels, persisting thrombocytopenia, and persistent fever following the initiation of therapy.¹³⁴ In EBV-associated HLH, a high viral DNA load is associated with poor outcomes.¹³⁵ Active HLH at the time of HSCT and central nervous system involvement has been associated with worse outcomes.^{103,118} A single institutional, retrospective study of 62 adult patients with HLH showed that a low serum albumin level and tumor-associated HLH were 2 independent factors.¹⁷ In a univariate analysis, old age, a high lactate dehydrogenase level, a low serum albumin level, a high ferritin level, and tumor-associated HLH were all associated with a worse prognosis.¹⁷ In a study focused on infection-associated HLH, age older than 50 years, fever not subsiding within 3 days of diagnosis of HLH, and the development of disseminated intravascular coagulation were considered to be strong indicators of mortality.⁵³

Conclusions

Comprehensive clinical, immunological, and genetic workups are required to diagnose hemophagocytic lymphohistiocytosis. Despite recent advances in the diagnostics and therapy for hemophagocytic lymphohistiocytosis, the disease is incurable for the majority of adults with secondary hemophagocytic lympho-

histiocytosis. More research into the molecular biology, immunology, and genetics of hemophagocytic lymphohistiocytosis is needed to discover effective treatment options for patients with this rare disorder.

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