Clinical Characteristics and Treatment of Familial Hemophagocytic Lymphohistiocytosis

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KEYWORDS

• Hemophagocytic lymphohistiocytosis • Hyperinflammation • Degranulation • Cytotoxicity • Cytokines • Therapy

KEY POINTS

- The clinical presentation of fHLH is variable but may involve multiple organ systems and progress to multiorgan failure, which can be fatal without treatment.
- fHLH is diagnosed through a combination of clinical, laboratory, and histologic criteria coupled with germline genetic testing and immunologic assays.
- Standard of care therapy for fHLH includes chemotherapy and/or immunosuppressive agents, followed by allogeneic hematopoietic cell transplantation.
- Emerging evidence in animal models and human clinical trials reveals that cytokine-directed therapies may dampen inflammation in fHLH.

INTRODUCTION

Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome driven by immune overactivation that results in end-organ damage and often mortality. The underlying immune dysregulation driving HLH is either inherited, as in familial HLH (fHLH), or acquired, as in secondary HLH (sHLH). fHLH affects about 1 in 50,000 to 100,000 children,[1,2] and historically refers to a group of autosomal recessive conditions resulting from biallelic variants in genes involved in cytotoxic T lymphocyte (CTL) and natural killer (NK) cell cytotoxicity (ie, *PRF1*, *UNC13D*, *STX11*, and *STXBP2*; **Table 1**). Specifically, the proteins encoded by these genes play a central role in the assembly and intracellular trafficking of perforin-containing and granzyme-containing

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Abbreviations	
CGs	cytotoxic granules
CHS	Chediak–Higashi syndrome
CMV	cytomegalovirus
CN	cranial nerve
CSF	cerebral spinal fluid
CTL	cytotoxic T lymphocyte
ECMO	extracorporeal membrane oxygenation
fHLH	familial hemophagocytic lymphohistiocytosis
GS	Griscelli syndrome
HCT	hematopoietic cell transplantation
HPS	Hermansky–Pudlak syndrome
ICU	intensive care unit
IL-18BP	IL-18 binding protein
IL-1βR	IL-1β receptor
IL-6	interleukin-6
IL-6R	IL-6 receptor
IS	immunologic synapse
IT	intrathecal
JAK	Janus kinase
MAS	macrophage activation syndrome
NK	natural killer
PALF	pediatric acute liver failure
sHLH	secondary HLH
sIL-2R	soluble interleukin-2 receptor
TNFα	tumor necrosis factor alpha
VUS	variants of uncertain significance
WES	whole exome sequencing
WGS	whole genome sequencing

cytotoxic granules (CGs) within CTLs and NK cells. These CGs are formed in the cytoplasm and localize to the site of target cell engagement at the immunologic synapse (IS). Perforin then forms pores in the target cell membrane through which granzymes enter to induce apoptosis.[3] Defects in this pathway result in an inability to effectively lyse target cells, including those that are infected or malignant, leading to persistent immune cell stimulation, proliferation, and exaggerated release of proinflammatory cytokines, which collectively mediate end-organ damage. This hyperinflammatory

Table 1
Subtypes of familial hemophagocytic lymphohistiocytosis[4]

fHLH Subtype	Genetic Mutation
fHLH Type 1	Unknown
fHLH Type 2	*PRF1*
fHLH Type 3	*UNC13D*
fHLH Type 4	*STX11*
fHLH Type 5	*STXBP2*

Each subtype of fHLH is associated with biallelic loss-of-function variants in a distinct gene that is involved in the lymphocyte cytotoxicity pathway. The earliest genetic locus linked to fHLH localized to a region on chromosome 9q21, but no causal gene has been identified.

Data from Ohadi M, Lalloz MR, Sham P, et al. Localization of a gene for familial hemophagocytic lymphohistiocytosis at chromosome 9q21.3-22 by homozygosity mapping. Am J Hum Genet 1999;64(1):165–71. https://doi.org/10.1086/302187.

phenotype is difficult to control even with immunosuppressive medications and ultimately requires hematopoietic cell transplantation (HCT).

DISCUSSION

Clinical Manifestations of Familial Hemophagocytic Lymphohistiocytosis

Patients with fHLH usually present at less than 1 year of age,[5,6] but HLH can present at birth or even prenatally.[7] Reported median age at diagnosis ranges from 3 months to 3.5 years[8–13] compared to 3 to 7 years[8,12,14] for pediatric patients with sHLH. The most common presenting symptoms of fHLH include fever, hepatosplenomegaly, bilineage or trilineage cytopenias, and less frequently lymphadenopathy, scleral icterus, rash, or edema.[13,15–17] In addition to cytopenias, there is typically laboratory evidence of inflammation (see "Diagnostic criteria" section). However, no single symptom or laboratory abnormality is specific to HLH. A list of reported symptoms and laboratory derangements is detailed in later discussion and summarized in Fig. 1.

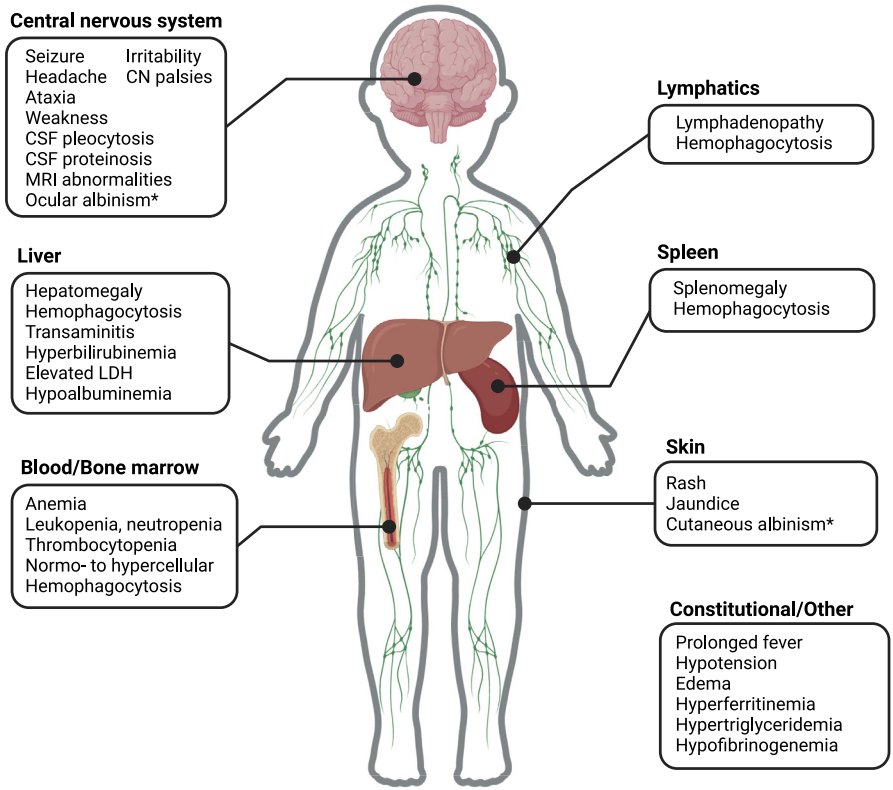


Fig. 1. Clinical manifestations of fHLH. Reported symptoms and laboratory derangements seen at presentation in pediatric patients diagnosed with fHLH. This list is not exhaustive but represents some of the most common clinical manifestations, including many of the features included in the diagnostic criteria. CN, cranial nerve; CSF, cerebral spinal fluid; LDH, lactate dehydrogenase. *Features of oculocutaneous albinism are exclusive to certain genetic disorders associated with HLH. (*Created in* BioRender. Keenan, C. (2025) https://BioRender.com/i24g203.)

Organ system involvement

Central nervous system. Central nervous system (CNS) involvement at diagnosis has been reported in 30% to 44% of pediatric patients with HLH.[7,8,18] Neurologic symptoms include ataxia, seizure, headache, irritability, weakness, vision changes, and/or cranial nerve palsies.[7,17,19] Patients with fHLH are more likely to have CNS involvement compared to patients with sHLH.[12,20] CNS involvement is typically diagnosed by cerebral spinal fluid (CSF) studies and/or brain MRI abnormalities rather than clinically apparent neurologic symptoms.[8,15] CSF abnormalities can include elevated white blood cell count, elevated protein, and hemophagocytosis.[7,19,21] Brain MRI findings vary widely, with no finding showing specificity for the disease. Reported findings include diffuse or multifocal white matter changes, periventricular changes, edema, and/or volume loss.[19,21–24] The cerebellum and/or brainstem are most often affected[19,24] and radiographic findings are usually bilateral.[22] In rare cases when brain biopsy has been performed, histologic findings included perivascular lymphocytic infiltration, scattered histiocytes with or without hemophagocytosis, and parenchymal and leptomeningeal inflammation.[25,26] CNS involvement can occur in isolation or in combination with systemic HLH-associated inflammation. When HLH presents with CNS findings alone, most patients do not progress to systemic HLH.[19] However, systemic HLH can sometimes develop years after onset of CNS-HLH.[19]

Liver. Although not part of current diagnostic criteria, many patients with HLH have evidence of hepatomegaly and significant acute liver injury.[15,18,27,28] The incidence of hepatic dysfunction at presentation of pediatric HLH is reported to be 50% to 80%.[8,18,28] One retrospective review found that total and direct bilirubin were significantly higher in patients with fHLH compared to those with sHLH.[29] Many patients with HLH meet criteria for pediatric acute liver failure (PALF) at presentation.[27] About 30% of children diagnosed with PALF and HLH who underwent genetic testing were found to harbor biallelic mutations in an fHLH gene.[30] Mortality rate was higher in patients with genetically confirmed fHLH. When comparing patients with HLH-associated liver failure versus other causes of PALF, patients with HLH had a significantly higher incidence of pleural effusions, cytopenias, fever, splenomegaly, and hypoalbuminemia.[31]

Kidney. Some patients have evidence of renal dysfunction at presentation or develop impairment during peak inflammation. Almost 20% of pediatric patients with HLH who are admitted to the intensive care unit (ICU) require renal replacement therapy.[32,33]

Skin. Some patients have a rash at symptom onset of HLH, although the appearance is not uniform.[13,15] Reported cutaneous descriptions include erythroderma, generalized purpuric macules and papules, and morbilliform eruptions.[34] Certain genetic disorders that cause oculocutaneous albinism are associated with HLH (discussed later).

Other clinical presentations

Multisystem organ failure. More than 60% of pediatric patients with HLH require ICU admission, often due to multiorgan failure.[35,36] Consensus guidelines recommend evaluating for HLH in all ICU patients with hyperinflammation that is unexplained or disproportionate to what is expected, especially in patients with rapidly worsening multiple organ dysfunction syndrome.[36]

Neonatal hemophagocytic lymphohistiocytosis. fHLH in the neonatal or prenatal stage can present with distinct clinical features relative to older children.[37,38] Specifically, fever and cytopenias are less common while neurologic and hepatic sequelae occur more frequently.[37,38] In one large meta-analysis of patients with HLH who were aged 30 days or younger, fever was absent in 19% of neonates and 39% of

preterm neonates, and neurologic manifestations were reported in 63% of cases.[38] Hepatic involvement is more commonly reported in neonatal HLH, with about 68% of patients having some degree of liver injury and 24% showing evidence of liver failure.[38] This is an important consideration since 2% to 11% of neonatal acute liver failure cases have been linked to genetically confirmed fHLH.[30,39,40] Often, no trigger is identified in patients with neonatal HLH.[41] Although neonatal HLH is rare, these patients generally have a high fatality rate.[42]

Clinical features of related genetic syndromes

Griscelli syndrome type 2. Griscelli syndrome (GS) is an autosomal recessive disorder characterized by partial albinism and immunodeficiency caused by mutations in *MYO5A* (type 1), *RAB27A* (type 2), or *MLPH* (type 3).[43,44] RAB27A is a GTPase that is essential for CG trafficking, and as such, patients with GS type 2 commonly develop HLH, typically within the first year of life.[43–45] Like classic fHLH, outcomes are poor without curative HCT. One study reported 50% mortality in patients with GS type 2 who developed HLH and did not undergo HCT.[44]

Chediak–Higashi syndrome. Chediak–Higashi syndrome (CHS) is another autosomal recessive disorder associated with partial albinism and immune dysfunction secondary to mutations in the *LYST* gene, impacting regulation and trafficking of lysosomes, including CGs.[43,46] Patients also exhibit a bleeding diathesis and progressive neurologic deterioration.[43,47,48] Up to 85% of patients with CHS are reported to develop HLH within their first decade of life.[43,47–50]

Hermansky–Pudlak syndrome type 2. Patients with Hermansky–Pudlak syndrome (HPS) type 2 similarly have oculocutaneous albinism and immune deficiencies related to impaired lysosomal protein trafficking, resulting from mutations in *AP3B1*. However, there has only been one reported case of HLH in a patient with HPS type 2, and that patient also had a heterozygous *RAB27A* mutation.[51]

In addition to these conditions, patients with other inherited immunodeficiency disorders associated with genes involved in the lymphocyte cytotoxicity pathway are also at an increased risk for HLH. Other HLH predisposition syndromes involving genes outside of this pathway are beyond the scope of this review, but are discussed elsewhere.[17]

Diagnosis of familial hemophagocytic lymphohistiocytosis

Diagnostic criteria

The most widely utilized diagnostic schema for fHLH are the HLH-2004 criteria,[52] which were developed as inclusion criteria for HLH-2004, an international clinical trial for pediatric HLH. These criteria consist of the following: (1) fever; (2) splenomegaly; (3) bilineage or trilineage cytopenias; (4) hyperferritinemia; (5) hypertriglyceridemia and/or hypofibrinogenemia; (6) elevated soluble interleukin-2 receptor (sIL-2R); (7) low or absent NK cell activity; and (8) hemophagocytosis in bone marrow, spleen, or liver. Under these guidelines, patients are diagnosed with HLH either by fulfilling at least 5 of 8 criteria or by the presence of molecular findings consistent with fHLH, defined by biallelic loss-of-function mutations in an fHLH gene (**Box 1**).[53]

To further evaluate the validity of these criteria, Henter and colleagues[54] performed a case–control study including 366 children with fHLH and 703 controls with either systemic juvenile idiopathic arthritis or infection. They demonstrated that the HLH-2004 criteria showed an accuracy of 97.4%. A slightly higher accuracy of 99% was obtained when NK cell functional studies were omitted. As a result, the authors proposed a modification to existing diagnostic criteria. Specifically, they proposed that

Box 1
HLH-2004 criteria

1. A molecular diagnosis consistent with fHLH
2. At least 5 of 8 of the following criteria:
   - Fever
   - Splenomegaly
   - Cytopenias affecting at least 2 lineages:
     - Hemoglobin <90 g/L
     - Platelets <100 × 10^9/L
     - Neutrophils <1.0 × 10^9/L
   - Hypertriglyceridemia and/or hypofibrinogenemia
     - Fasting triglycerides ≥265 mg/dL
     - Fibrinogen ≤1.5 g/L
   - Ferritin ≥500 μg/L
   - sIL-2R ≥2400 U/mL
   - Hemophagocytosis in bone marrow, spleen, or lymph nodes
   - Low or no NK cell activity[a]

According to the HLH-2004 criteria, HLH is diagnosed in patients with a molecular diagnosis consistent with fHLH or in patients who meet at least 5 of 8 clinical, laboratory, and histologic features consistent with HLH. [a]A recent study by Henter and colleagues has modified these criteria to remove NK cell functional studies from the main diagnostic criteria (see discussion in text).

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):124–31. https://doi.org/10.1002/pbc.21039; Henter JI, Sieni E, Eriksson J, et al. Diagnostic Guidelines for Familial Hemophagocytic Lymphohistiocytosis Revisited. Blood 2024:blood.2024025077. https://doi.org/10.1182/blood.2024025077.

the HLH syndrome can be diagnosed in patients meeting at least 5 of the 7 remaining criteria, excluding NK cell functional studies. Additional diagnostic testing, in the form of genetic studies and/or immunologic assays (see later discussion), can subsequently be used to identify patients with fHLH. Another widely used diagnostic tool for HLH is the HScore. This tool has not been validated in a pediatric population or in patients with fHLH.[55] As such, the HLH-2004 criteria remain the gold standard for diagnosis of fHLH.[52]

Of these HLH-2004 criteria, elevated ferritin and sIL-2R are particularly specific for the diagnosis of HLH, including fHLH. While the HLH-2004 criteria use a ferritin cutoff of 500 μg/L,[53] other studies have demonstrated that a higher cutoff may show improved sensitivity and specificity for diagnosing HLH. In an analysis of 330 children with a peak ferritin value greater than 500 μg/L, Allen and colleagues[56] demonstrated that a ferritin level of 10,000 μg/L or greater showed a 90% sensitivity and 96% specificity for the diagnosis of HLH, though the study did not differentiate between fHLH and sHLH. Lehmberg and colleagues[57] subsequently showed that 2000 μg/L represents the optimal cutoff value for the diagnosis of HLH, with a sensitivity and specificity of 68% in patients with fHLH.

In terms of sIL-2R, the HLH-2004 criteria use a cutoff of 2400 U/mL for differentiating HLH from non-HLH.[53] sIL-2R is a truncated protein that is cleaved from the IL-2R on activated T cells, thereby serving as a surrogate marker of Tcell activation.[58] In one study of patients with HLH, sIL-2R of 7900 U/mL or greater optimally differentiated patients with fHLH from those with sHLH.[59] Thus, while Henter and colleagues[54] evaluated multiple cutoff values for these two parameters and did not find justification to alter the values put forth in the HLH-2004 criteria, these studies suggest that in

patients with elevated levels of these markers, HLH should be included in the differential diagnosis.

Gene sequencing

Definitive diagnosis of fHLH requires identifying biallelic loss-of-function variants in an fHLH gene. Gene sequencing can take the form of targeted sequencing panels, whole exome sequencing (WES), or whole genome sequencing (WGS). Targeted sequencing is most useful when a specific diagnosis is suspected, as this strategy requires the clinician to select a gene panel that correlates with the clinical phenotype. In contrast, WES and WGS enable identification of potential disease-associated variants in all genes.[17] To date, targeted sequencing panels have been the most widely utilized for diagnosing fHLH.[60–64] However, as next generation sequencing (NGS) technologies continue to advance, they may provide deeper insights into mechanisms of genetic predisposition and disease pathophysiology. For example, Chinn and colleagues[65] performed clinical genetic sequencing of children with HLH and identified biallelic fHLH gene variants in 19% of patients. Subsequent research-based WES on 47 patients without fHLH gene variants revealed likely molecular explanations for the associated HLH phenotype in over 50% of patients, including the identification of gene variants associated with other primary immunodeficiencies and immune dysregulation syndromes. While expanded use of this technology is likely to inform an understanding of HLH biology, WES and WGS commonly identify protein-altering variants in multiple genes even in healthy individuals, many of which are variants of uncertain significance (VUS) that are challenging to interpret. Immunologic assays are, therefore, essential for evaluating the functional impact of such VUS and potential associations with a clinical phenotype.[66]

Immunologic assays

In conjunction with gene sequencing, immunologic assays aid in differentiating fHLH from sHLH and in interpreting the functional significance of gene variants. Given that they can often be performed more rapidly than gene sequencing, some institutions use immunologic assays to screen patients with suspected fHLH, prioritizing those who have an abnormal immunologic screen for genetic testing.[67–69] Immunologic assays can be subdivided into phenotyping and functional assays.

Phenotyping assays. The most widely used phenotyping assay for diagnosing fHLH measures intracellular perforin protein expression by flow cytometry in CTLs or NK cells (**Fig. 2**).[70] Abdalgani and colleagues[71] performed a large study of 750 samples from children with HLH in which they compared perforin flow cytometry to genetic testing and demonstrated that loss of perforin protein expression has a sensitivity of 94% and specificity of 97% for the identification of patients with biallelic loss-of-function *PRF1* mutations.

Functional assays. Functional immunologic assays can be subdivided into degranulation and cytotoxicity assays. Degranulation assays involve flow cytometric measurement of CD107a protein expression on the surface of NK cells. CD107a is a protein on the inner membrane of CTL and NK cell CGs. When CGs fuse with the plasma membrane at the IS, the CD107a protein is translocated to the cell surface, where it is detected by flow cytometry. CD107a expression on NK cells strongly correlates with cytotoxic function. Historically, this assay has been performed by coculturing patient NK cells with K562 cells, an immortalized human erythroleukemic cell line that does not express major histocompatibility complex (MHC) class I, thereby stimulating NK cell degranulation.[72] Chiang and colleagues[73] reported the accuracy

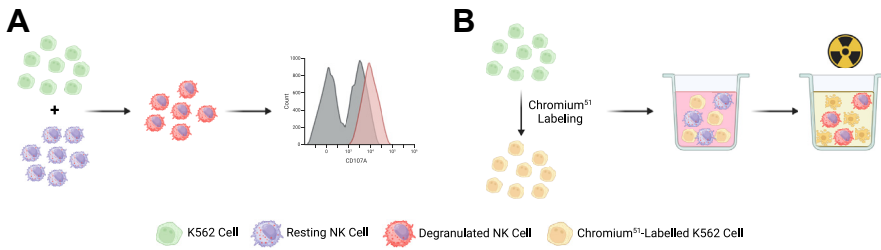


Fig. 2. Schematic of immunologic assays. (*A*) NK cell degranulation assays are traditionally performed by coculturing K562 target cells with NK cells, which contain cytotoxic granules with CD107a protein on their inner surface (red intracellular granules). Following NK cell activation, CD107a protein is translocated to the NK cell surface, where it can be detected by flow cytometry. (*B*) NK cell cytotoxicity assays are traditionally performed by prelabeling K562 target cells with radioactive chromium.[52] These labeled cells are then cocultured with NK cells in a culture dish. Upon lysis of K562 cells, chromium[52] (shown in *yellow*) is released into the media, where the radioactivity can be detected as a readout of cytotoxic activity. (*Created in* BioRender. Meyer, L. (2025) https://BioRender.com/y77i215; and *Data from* Kogawa K, Lee SM, Villanueva J, et al. Perforin expression in cytotoxic lymphocytes from patients with hemophagocytic lymphohistiocytosis and their family members. Blood 2002;99(1):61–6. https://doi.org/10.1182/blood.v99.1.61.)

of a degranulation assay that utilizes the P815 murine mastocytoma cell line rather than K562 cells. P815 cells express cell surface fragment crystallizable gamma receptor II, which binds murine immunoglobulin G antibodies. The authors incubated P815 cells with anti-CD3 or anti-CD16 antibodies prior to coculture with CTLs or NK cells, respectively, after which degranulation could be assessed by CD107a flow cytometry. They found that combining the results of the CTL and NK cell P815 degranulation assays resulted in the highest accuracy at 99.3% for identification of patients with fHLH types 3 to 5. Other degranulation assays have been developed that do not rely on a cell line, including stimulating CTLs and NK cells with chemical or biologic agents, with many of these assays demonstrating excellent reliability.[74–76]

The CD107a degranulation assay performs well in diagnosing fHLH. Bryceson and colleagues[77] analyzed CD107a expression in cells from 494 patients with suspected HLH, and demonstrated a 96% sensitivity and 88% specificity for diagnosing fHLH types 3 to 5. Importantly, while loss of CD107a expression identifies patients with these fHLH subtypes, its expression is normal in patients with *PRF1* loss-of-function, as CGs still form properly and fuse with the plasma membrane despite their ineffective cytotoxic capacity. Rubin and colleagues[78] retrospectively reviewed the performance of immunologic assays in 1,614 patients with HLH and showed that CD107a degranulation combined with perforin flow cytometry demonstrates optimal sensitivity for diagnosing patients with all subtypes of fHLH.

Unlike degranulation assays, cytotoxicity assays can identify patients with all fHLH subtypes. Historically, cytotoxicity was assessed by prelabeling K562 cells with radioactive chromium-51 prior to NK cell coculture. When lysed by lymphocytes, the target cells release chromium into the supernatant, where radioactivity can be detected.[79] Due to safety concerns related to radioactivity, other methods have been developed that instead use fluorescent dyes.[80,81]

Overall, while phenotyping and functional assays have a role in diagnosing fHLH, both are subject to important caveats. For example, both assays require technical expertise that is not available in many clinical laboratories, and as such, samples

must be sent to specific laboratories where these assays are routinely performed. This can result in logistical challenges and diagnostic delays. Phenotyping assays are subject to limitations in antibody technology, including the lack of commercially available antibodies against proteins of interest. Furthermore, some pathogenic gene variants may produce defective proteins with preserved antibody binding sites, leading to false negative results, whereby it would appear that the protein of interest is present; however, the protein might not function properly. Functional assays require a large number of viable cells, which can be challenging to obtain from critically ill patients and can be compromised in the process of sending samples to centralized laboratories.[82] As a result, it remains essential to interpret immunologic assays in conjunction with gene sequencing in patients with suspected fHLH.

Additional diagnostic studies

Multiple inflammatory cytokines are elevated in HLH,[83–85] and their levels can be measured in the serum, most commonly through an immunoassay platform.[86,87] While these assays are becoming more widely available, their clinical interpretation remains complicated by several factors. Most importantly, it is often unclear how serum cytokine levels correlate with those in the tissues upon which cytokines exert their activity. Additionally, it is challenging to interpret cytokine levels obtained from a single timepoint, with trends over time often more useful in a clinical context. For this purpose, it is essential that the same assay platform be used at all timepoints, because studies have demonstrated significant interassay differences in the levels of reported cytokines.[85] Therefore, while cytokine panels may corroborate a diagnosis of HLH, additional studies are needed to better understand how to integrate them into clinical management.

Evaluation for familial hemophagocytic lymphohistiocytosis triggers

There are multiple clinical mimics of fHLH, and definitive diagnosis requires the gene sequencing and immunologic assays discussed earlier. Even when the diagnosis is confirmed, testing to identify potential underlying disease triggers, including infections, malignancies, and autoimmune disorders, is essential. Infectious triggers are most common.[17,28,41,52,88,89] Autoimmune disorders and malignancy are less likely in young patients with fHLH, although careful investigations for these disorders should be carried out in parallel to the fHLH diagnostic workup.

Infection/sepsis. Viral, bacterial, fungal, and parasitic infections have been implicated as infectious triggers for HLH.[7,17,52,89] Viruses are the most common, specifically herpesviruses including Epstein-Barr virus (EBV), cytomegalovirus (CMV), and herpes simplex virus (HSV).[17,38] Bacterial blood cultures and serum polymerase chain reaction (PCR) viral testing is recommended.[52] Evaluation for less common infectious triggers such as fungal or insect-borne diseases should be performed, in coordination with infectious disease consultation, on a case-by-case basis depending on a patient's risk.

Malignancy. The likelihood of an underlying malignancy increases with age and is, therefore, relatively uncommon at the typically young age of fHLH presentation. However, one meta-analysis reported a 2% incidence of malignancy in patients aged 30 days or younger diagnosed with HLH.[38] Evaluation with bone marrow and/or tissue biopsy and cross-sectional imaging with a computed tomography scan of the neck, chest, abdomen, and pelvis is recommended for evaluation of possible malignancy in coordination with oncology consultation.[52] Preferably, these tests are obtained prior to initiation of corticosteroids when clinical status allows. Additionally,

a PET scan should be considered to evaluate for possible lymphoma, one of the most common malignant triggers of HLH.[17,52]

Autoimmune/rheumatologic diseases. Although more common in older children and adults, assessment for possible autoimmune/rheumatologic disorders is an important consideration. Nearly 3% of neonatal patients diagnosed with HLH were found to have an underlying rheumatologic diagnosis.[38] Systemic juvenile idiopathic arthritis is the most commonly reported rheumatologic disorder in pediatric patients with HLH.[17] Therefore, it is recommended to send appropriate autoimmune/rheumatologic laboratory workup in coordination with rheumatology consultation.

Treatment of familial hemophagocytic lymphohistiocytosis

Initial therapy for fHLH conventionally involves a combination of corticosteroids and chemotherapy.[52,90,91] Without treatment, fHLH is typically fatal.[10,18,92–94] After initial inflammatory control, definitive cure requires allogenic HCT to replace the dysfunctional immune system.

Conventional therapy

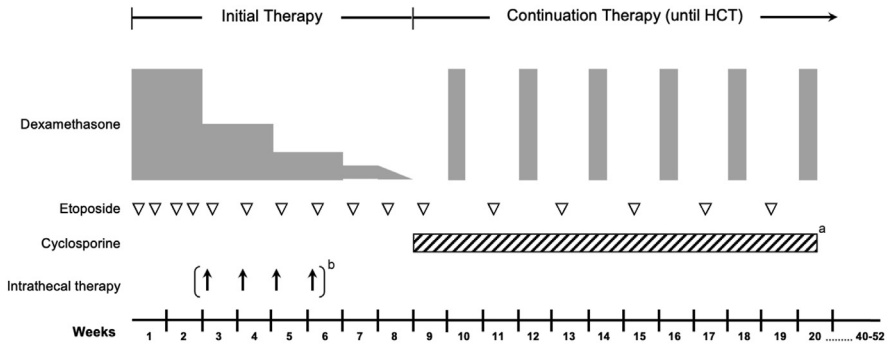
Conventional upfront treatment of fHLH is based on the HLH-94 clinical trial, which combined etoposide and dexamethasone, as well as intrathecal (IT) methotrexate if evidence of CNS involvement, into an initial eight weeks of intensive induction therapy (**Fig. 3**).[90] This is followed by bridging continuation therapy with dexamethasone, etoposide, and cyclosporine (CSA) to provide ongoing disease control until HCT.[90] Additionally, IT corticosteroids are often given in combination with methotrexate for patients with CNS involvement, as per the HLH-2004 clinical trial.[52,53] Similar to the "continuation" therapy in the HLH-94 and HLH-2004 protocols, providers may choose to administer dexamethasone pulses and occasionally CSA after initial therapy as bridging therapy to HCT; however, the benefit of bridging therapy has never been examined.[95] Many patients will experience refractory or relapsed disease despite this conventional approach, especially during induction when dexamethasone is tapered.[11,18] If relapse occurs during this time, re-escalation of therapy can be helpful in some patients.[95] Beyond this approach, salvage therapy options for patients with relapsed/refractory fHLH vary widely. Alternative therapy approaches often involve anticytokine therapy, discussed later.

Hematopoietic cell transplantation

No patients with fHLH on the HLH-94 trial survived without receiving HCT. The 5 year overall survival for patients who successfully underwent HCT was 66%; however, it was slightly higher at 72% among patients without evidence of active disease at time of transplant.[90] This is consistent with other reports showing significantly higher rates of survival in patients able to achieve HLH remission prior to HCT.[96] Using modified HLH-94 or HLH-2004 regimens followed by HCT, outcomes for patients with fHLH remain in the 65% to 77% range.[11,96–100] It is generally recommended that asymptomatic family members carrying pathogenic biallelic mutations undergo pre-emptive HCT.[95,101] One study reported a higher probability of survival if patients received HCT prior to becoming symptomatic (95%) versus those in whom HLH developed prior to HCT (45%).[101]

Targeted therapies

There have been significant efforts to develop therapeutic strategies that target inflammatory cytokines and their downstream signal transduction mediators as a means of attenuating hyperinflammation in HLH (**Fig. 4**).[85]



Dexamethasone	PO or IV	Given twice daily during initial therapy (5 mg/m2 BID weeks 1-2, 2.5 mg/m2 BID weeks 3-4, 1.25 mg/m2 BID weeks 5-6, 0.625 mg/m2 BID week 7, then taper and discontinue by end of week 8). Given as 3-day pulses of 5mg/m2 BID during continuation therapy.
Etoposide	IV	150 mg/m2 given twice weekly on weeks 1-2, once weekly on weeks 3-8, then every other week starting on week 9.
Cyclosporine (CSA)	PO	[a]CSA use during continuation is optional and can be started earlier if desired but not recommended sooner than week 3. If utilized, recommended starting dose 3mg/kg BID with goal trough level 200ug/L.
Intrathecal (IT) therapy	IT	[b]Weekly IT only recommended if progressive neurologic symptoms during weeks 1-2 or if any CSF or brain MRI evidence of CNS-HLH at diagnosis. Administer IT therapy until neurologic improvement or CSF/MRI normalization, up to 4 total doses. IT methotrexate only was used in HLH-94 trial; IT prednisolone was added in HLH-2004 trial. IT methotrexate doses by age (<1 year = 6 mg, 1-2 years = 8 mg, 2-3 years = 10mg, ≥3 years = 12 mg) IT prednisolone doses by age (<1 year = 4 mg, 1-2 years = 6 mg, 2-3 years = 8mg, ≥3 years = 10 mg)

Fig. 3. HLH-94 treatment regimen for fHLH. Schematic depicting recommended fHLH treatment regimen adapted from HLH-94 clinical trial. Initial therapy (weeks 1–8) consists of dexamethasone and etoposide. Intrathecal therapy is included during initial therapy if there is evidence of CNS involvement. Continuation therapy (weeks 9 and beyond) consists of dexamethasone pulses alternating with etoposide doses, with optional cyclosporine therapy. Duration of therapy varies dependent on availability and timing of eventual curative HCT. BID, bis in die (twice per day); CNS, central nervous system; CSF, cerebrospinal fluid; HCT, hematopoietic stem cell transplant; IV; intravenous; PO, per os (by mouth). [b]Of note, the HLH-94 trial includes IT prednisolone, though this can be substituted with IT hydrocortisone per institutional or provider preference. (*Data from* Trottestam H, Horne A, Aricò M, et al. Chemoimmunotherapy for hemophagocytic lymphohistiocytosis: long-term results of the HLH-94 treatment protocol. Blood. 2011;118(17):4577–84. https://doi.org/10.1182/blood-2011-06-356261.)

Interferon gamma. Interferon gamma (IFNγ) is produced primarily by hyperactivated CD8 T cells[102] and signals downstream to activate a large number of interferon-stimulated genes.[103] CD8 T cell depletion or IFNγ neutralization in murine models of fHLH resolves the HLH phenotype.[104,105] These preclinical data led to the development of emapalumab, a fully humanized monoclonal antibody that neutralizes IFNγ. Locatelli and colleagues[106] evaluated the safety and efficacy of emapalumab, given in combination with dexamethasone and/or other HLH-directed therapies, for patients with newly diagnosed or relapsed/refractory disease, the majority of whom were reported to have fHLH. Of the relapsed/refractory patients, 63% showed some degree of response with this combination, while 65% of the total patient cohort responded and 65% were able to proceed to HCT. Based on these results, emapalumab was approved by the FDA in 2018 for pediatric and adult patients with refractory, recurrent, or progressive fHLH or those who demonstrated intolerance to conventional therapy. Subsequent real-world data were collected through the REAL-HLH study, where Chandrakasan and colleagues[107] evaluated treatment patterns and outcomes of 46

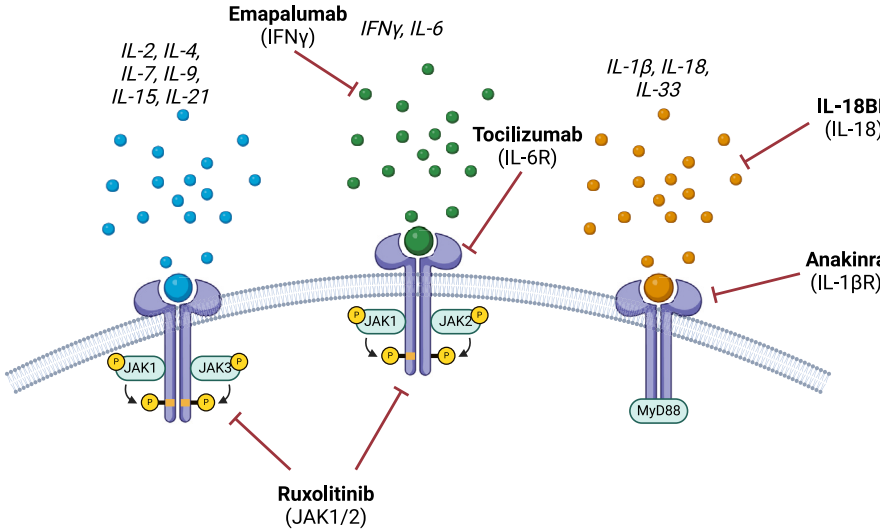


Fig. 4. Cytokine receptor signaling and targeted inhibitors. Common γ-chain cytokines signal via JAK1 and JAK3, while IFNγ and IL-6 signal via JAK1 and JAK2. The JAK1/2 inhibitor ruxolitinib inhibits signaling downstream of the receptors corresponding to each of these cytokines. Emapalumab is a monoclonal antibody that inhibits the activity of circulating IFNγ. Tocilizumab is a monoclonal antibody that binds to soluble and membrane-bound IL-6 receptor (IL-6R), inhibiting its downstream signaling. Anakinra is a small molecule inhibitor of the IL-1β receptor (IL-1βR). Finally, IL-18 binding protein (IL-18BP) binds to IL-18, thereby inhibiting its ability to bind its receptor and induce downstream signaling. (*Created in* BioRender. Meyer, L. (2025) https://BioRender.com/k92e877; and *Data from* Behrens EM. Cytokines in Cytokine Storm Syndrome. Adv Exp Med Biol 2024;1448:173–83. https://doi.org/10.1007/978-3-031-59815-9_13.)

patients receiving emapalumab for the management of fHLH, 35 of whom had received prior therapy. They demonstrated excellent pretransplant survival of 90%, with 75% of patients who received a transplant alive at the end of the follow-up period.

Janus kinase (JAK)/Signal Transducer and Activator of Transcription (STAT) pathway. Another therapeutic strategy increasingly used in the treatment of fHLH involves inhibiting signal transduction downstream of inflammatory cytokine receptors. Many cytokines that are implicated in HLH pathogenesis, including IFNγ as well as interleukin-6 (IL-6), IL-12, and common γ-chain cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21) signal via the Janus kinases (JAKs), and specifically via JAK1 and/or JAK2.[108] As such, the JAK1/2 inhibitor ruxolitinib has gained increasing attention in the treatment of HLH. Multiple preclinical studies have demonstrated the efficacy of ruxolitinib in murine models of fHLH.[109–114] Preclinical data have also demonstrated that ruxolitinib may be particularly effective when combined with dexamethasone, a component of existing HLH standard of care.[115] Consistent with these data, ruxolitinib has shown efficacy in patients with both newly diagnosed and relapsed/refractory fHLH. In the first prospective trial of ruxolitinib in pediatric patients with newly diagnosed HLH, which included several patients with fHLH, there was a 69% overall response rate to 28 days of ruxolitinib monotherapy, with 42% of patients achieving a complete remission.[116] In a retrospective study of children with newly diagnosed and relapsed/refractory fHLH who received ruxolitinib treatment, all 21 patients had an objective disease response within eight weeks of starting therapy and 90% were

alive at one year. Seventeen patients subsequently underwent HCT, with 15 patients alive posttransplant.[117] Case reports have also demonstrated successful HCT in pediatric patients with relapsed/refractory fHLH who received ruxolitinib as a component of salvage therapy. These data together suggest that ruxolitinib may be safe and effective as a bridge to transplant in children with fHLH.

Interleukin-1 family. The IL-1 family of cytokines includes IL-1β, IL-18, and IL-33. IL-1β and IL-18 exist and circulate in preprotein forms until they are activated by inflammasomes.[85,118,119] As such, these cytokines tend to be produced at higher levels in patients with inherited inflammasomopathies and various forms of sHLH and are thought to be less abundant in patients with fHLH.[120] However, there have been some initial studies to evaluate therapeutic blockade of these pathways in patients with fHLH. The IL-1β receptor antagonist anakinra, which has shown efficacy in patients with sHLH and macrophage activation syndrome (MAS),[121] has been used successfully in at least two published reports of pediatric patients with fHLH.[122,123] These initial reports suggest that further studies may be warranted to evaluate the utility of anakinra in patients with fHLH.

IL-18 has similarly been shown to play a central role in the pathophysiology of MAS and other forms of sHLH, where it functions, in part, to enhance the production of IFNγ, and is thought to be less relevant in patients with fHLH.[124] The activity of IL-18 is inhibited by another circulating protein called IL-18 binding protein (IL-18BP), which has high affinity for binding free IL-18 and inhibiting its activity. As such, recombinant IL-18BP has been explored as a therapeutic strategy in patients with MAS,[125] and limited preclinical data suggest that this may be effective in fHLH. Chiossone and colleagues[126] treated CMV-infected *Prf1*$^{-/-}$ mice with recombinant IL-18BP and observed decreased IFNγ production, a reduction in the burden of hemophagocytosis, and reversal of liver and spleen pathology. It remains to be seen whether inhibition of IL-18 is a viable therapeutic strategy in patients with fHLH.

Other cytokines. Targeted therapies exist for several other cytokines that are elevated in both fHLH and sHLH. These have been evaluated for safety and efficacy in sHLH, though minimal to no data exist on their utility in fHLH. One such cytokine is tumor necrosis factor (TNF). Inhibition of TNF with etanercept has shown efficacy in patients with MAS.[127,128] Etanercept has not been evaluated in patients with fHLH, but TNF neutralization in *Prf1*$^{-/-}$ mice infected with lymphocytic choriomeningitis virus did not enhance survival,[104] suggesting that this may not be an effective therapeutic strategy in patients with fHLH. Similar findings were seen with neutralization of IL-6 in this experiment.[104] IL-6 is a cytokine produced by multiple immune cell subtypes and is effectively inhibited by the monoclonal antibody tocilizumab, which has been used primarily in the context of rheumatologic disease. Conflicting data exist regarding its efficacy in MAS and other forms of sHLH,[129] and it has not yet been evaluated in the context of fHLH.

Gene therapy

With the advent of gene therapy technologies, exciting preclinical studies have begun to show the promise of gene therapy for the treatment of patients with fHLH, either as a curative therapy or as a bridge to HCT. For example, studies in murine models of fHLH have shown that introduction of wild-type gene sequences into deficient CTLs or NK cells restores cytotoxic function and ameliorates inflammation.[130–134] HCT studies have demonstrated that donor chimerism as low as 20% can protect against disease sequelae in a patient with fHLH.[135] Thus, correction of the gene defect in a similar

percentage of effector cells may serve to control hyperinflammation in patients with HLH, possibly as a bridge to HCT. Other studies have corrected gene defects in hematopoietic stem cells,[136,137] creating the potential for development of an autologous HCT gene therapy strategy involving transplantation of a patient's own edited stem cells. Ongoing and future studies will see the transition of these gene-editing strategies into humans with fHLH, further altering the landscape of treatment options available to patients.

Prognosis of familial hemophagocytic lymphohistiocytosis

Lack of clinical response after two weeks of initial therapy is a poor prognostic feature for patients with fHLH.[92,95] One study found that improvement in laboratory criteria on day seven of therapy was most predictive of outcomes. Most notably, a decrease in sIL-2R to less than 25% of the pretreatment value by day seven was associated with improved survival.[138] Laboratory abnormalities that portend a poor prognosis vary among studies but have included hyperferritinemia, thrombocytopenia, anemia, and hyperbilirubinemia.[11,92,139,140] Clinical features reported to be associated specifically with early mortality risk include CNS involvement, heart failure, respiratory failure, severe thrombocytopenia, and hypoproteinemia.[33,92,140,141] In one study of pediatric patients with HLH requiring ICU admission, factors related to longer ICU admission included higher lactate dehydrogenase or total bilirubin, mechanical ventilation, vasopressor support, and secondary infection.[32,33] Patients with HLH who require extracorporeal membrane oxygenation (ECMO) have higher reported mortality rates compared to the overall pediatric ECMO population at 30% versus 59%, respectively.[142]

SUMMARY

Our understanding of the genetics, clinical features, and treatment options for patients with fHLH has advanced tremendously over the last two decades. For example, it is now well-recognized that fHLH represents a group of autosomal recessive diseases characterized by defective lymphocyte cytotoxicity leading to excessive immune cell activation, proliferation, and cytokine secretion. Together, these factors contribute to often life-threatening organ damage. Fortunately, advances in immunologic assays can suggest a diagnosis in as little as one week, with molecular confirmation of the underlying genetic defect occurring in approximately four weeks. Initial treatment of fHLH is aimed at controlling hyperinflammation, which can include administration of chemoimmunosuppressive therapies and/or targeted inhibitors of inflammatory cytokines, followed by curative allogeneic HCT.

Despite these significant advances, many biological and clinical questions remain to be answered. For example, while the genetic causes of classic fHLH are well-established, the full spectrum of genetic predisposition to pathologic hyperinflammation remains incompletely understood. Even in those patients with identified variants in fHLH genes, technologies required to interpret the functional significance of VUS have not yet advanced from the research stage into clinical use.[143] Although numerous anti-cytokine therapies have been developed and have shown tolerable safety profiles in both pediatric and adult populations, it is currently unclear how to optimally integrate these agents into the treatment of patients with fHLH. Further, more needs to be done to identify the clinical or serum biomarkers that can be used to best predict responsiveness or resistance to HLH-directed therapies. Additionally, while gene therapy strategies have shown promise in preclinical settings, it remains to be seen whether these approaches will be safe and efficacious in humans. Finally, long-term outcome

data exist for patients treated with conventional chemoimmunosuppressive therapy,[100] but such data do not exist for patients receiving targeted anticytokine therapies. Collection of these data through prospective clinical trials will be essential to establish the long-term safety profiles of these agents and to evaluate the durability of treatment responses.

CLINICS CARE POINTS

- fHLH has a variable clinical presentation, and clinicians should maintain a high index of suspicion for HLH in any patient with unexplained hyperinflammation, including in patients with acute liver failure, neurologic symptoms, and multisystem organ failure.
- The HLH-2004 diagnostic criteria remain the gold standard for the diagnosis of fHLH.
- Gene sequencing studies and immunologic assays, consisting of phenotyping assays and functional assays, are essential for the accurate diagnosis of fHLH.
- The HLH-94 treatment regimen is the most widely utilized regimen for initial treatment of fHLH, though targeted inhibitors of inflammatory cytokines and their receptors have shown safety and efficacy, both in newly diagnosed patients and relapsed/refractory disease.
- HCT is required as definitive therapy in most patients with fHLH.

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