Characterization of Children with Hemophagocytic Lymphohistiocytosis (HLH)

Anisha Gopu-Gilboy, MD, Bethany Verkamp, MD, Jennifer Jess, MD, Sarah McCormack-Spivey, MD, Adi Zoref-Lorenz, MD, Dafna Brik Simon, MD, Joanne Yacobovich, MD, Michael Jordan, MD

Background

Hemophagocytic lymphohistiocytosis (HLH) is a rare immune dysregulation disorder that presents with nonspecific features of severe inflammation[1–3]. This disorder is due to hypercytokinemia and an exaggerated and ineffective immune response usually in the setting of congenital or acquired defect in the natural killer (NK)/T-cell function in the cytotoxic pathway[1]. HLH can be divided into primary and secondary forms[1,2,4]. Primary HLH includes familial HLH (FHL) which is an autosomal recessively inherited condition that usually presents within the first year of life in 70-80% of cases[1], and other genetic forms. Common mutations associated with primary HLH are listed in **Table 1**. Secondary or acquired HLH can be associated with several hyperinflammatory states including but not limited to infection, malignancy, and rheumatologic diseases. HLH is often fatal and has up to a 95% mortality rate if not treated[1]. Treatment of HLH aims to suppress the exaggerated immune response through immunosuppression and chemotherapy agents[1,2,4]. As such, it is not benign and can involve hematopoietic stem-cell transplant, especially in genetic HLH given high risk of recurrence. Diagnosis is difficult as HLH can mimic other conditions with severe inflammation including but not limited to viral illness, rheumatologic diseases, and severe sepsis. Early and accurate diagnosis is crucial to provide optimal benefit and prevent harm. The diagnostic criteria used today are based on enrollment criteria for HLH trials in 1994 and 2004 despite their unknown sensitivity or specificity for HLH[5].

Disease	Gene	Protein	Function
fHLH-1	Unknown (9q21.3-22)		
fHLH-2	*PRF1*	Perforin	Cytotoxicity; forms pores in APCs
fHLH-3	*UNC13D*	Munc13-4	Cytotoxicity; vesicle priming
fHLH-4	*STX11*	Syntaxin 11	Cytotoxicity; vesicle fusion
fHLH5	*STXBP2*	Syntaxin binding protein 2	Cytotoxicity; vesicle fusion
Griscelli syndrome type II	*RAB27A*	Rab27A	Cytotoxicity; vesicle docking
Chediak–Higashi syndrome	*LYST*	LYST	Cytotoxicity; vesicle trafficking
Hermansky–Pudlak syndrome type II	*AP3B1*	AP-3 complex subunit beta-1	Cytotoxicity; vesicle trafficking
XLP1	*SH2D1A*	SAP	Signaling in cytotoxic NK and T cells
XLP2	*BIRC4*	XIAP	NF-kB signaling
ITK deficiency	*ITK*	IL-2 inducible T-cell kinase	IL-2 signaling in T cells
CD27 deficiency	*CD27*	CD27	Signal transduction in lymphocytes
XMEN syndrome	*MAGT1*	MAGT1	Magnesium transporter, induced by TCR stimulation

Table 1. **Genetic HLH Subtypes**. Adapted from *Janka and Lehmberg 2013*

Objective

This is a preliminary retrospective descriptive study to characterize patients with known HLH during their initial presentation to ultimately improve earlier provider recognition, limit misdiagnosis and therefore unnecessary exposure to toxic chemotherapy.

Methods

Patients with genetically validated HLH were identified from a maintained central database of patients at Cincinnati Children's Hospital Medical Center. Initial, peak and nadir values of diagnostic measures and other commonly deranged variables in HLH patients were obtained by retrospective chart review within 14 days of presentation. Central tendency markers for these measures, and time to peak/nadir values from initial presentation were measured as relevant.

Results

A total of 67 patients treated for confirmed HLH formed our patient cohort, 81% (54/67) of whom have genetic mutations known to cause HLH. The remaining 19% had indeterminate/ambiguous genetic mutations suspected to be associated with HLH. Average age at presentation was 3.9 years. The study population had a 25% (17/67) mortality rate overall. Ferritin, triglyceride, soluble IL2 receptor (sIL2R), and fibrinogen followed right-skewed F-distributions at initial presentation, with medians of 2924 mcg/L, 233 mg/dL, 14487 units/mL, and 159 mg/dL, as compared to peak/nadir values of 7458 mcg/L, 301 mg/dL, 14440 units/mL, and 108 mg/dL respectively. Patients reached peak/nadir values for absolute neutrophil count, hemoglobin, platelet count, ferritin, fibrinogen, triglyceride and sIL2R between 3.54 and 4.45 days. This data is further delineated in **Table 2** below. 83% (33/40) of the patients with measured natural killer (NK) cell function had decreased or absent function, and 68% (41/60) of the patients with bone marrow aspirations during the admission had identified hemophagocytosis on pathology. 85% (51/60) of patients evaluated had documented splenomegaly during the admission.

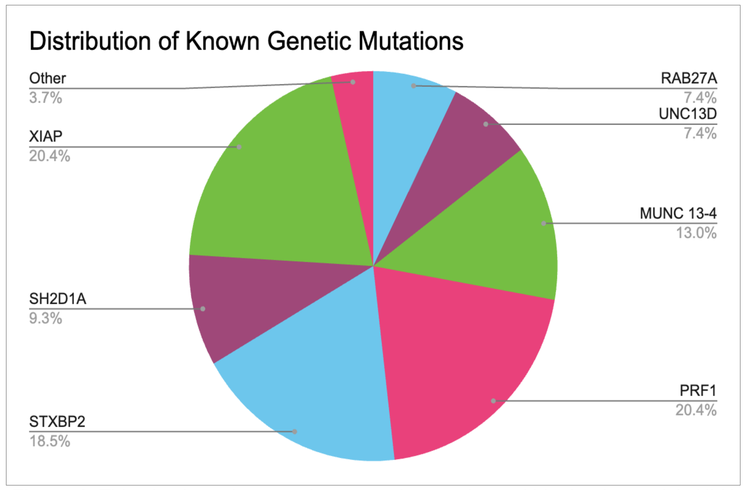

Figure 1. This pie chart shows various genetic mutations within the the study's HLH population. The category of "Other" is made up of 2 patients who have the following mutations in the following genes: MAGT1 and STX11.

Indeterminate/Ambiguous Mutations
VUS in PRF1 and LYST
VUS in PRF1 (heterozygous)
VUS in PRF1 (heterozygous)
VUS in PRF1 (heterozygous)
A91V mutation in PRF1 (heterozygous)
PRF1 indeterminant
MUNC 13-4 indeterminate
MUNC 13-4 (heterozygous)
MUNC 13-4 (heterozygous)
STXBP2 (heterozygous)
UNC13D (heterozygous)
Perforin (heterozygous)
VUS in SLC7A7
VUS in AP3B1

Table 2. Indeterminate/Ambiguous Mutations. Most mutations are within genes known to be associated with HLH.

	Initial Median (IQR)	Initial Mean	Peak/Nadir Median (IQR)	Peak/Nadir Mean	Days to Peak/Nadir	HLH-2004 Criteria[5]
ANC	1.2 (0.4-2.5) U/mcL	2.6 U/mcL	0.4 (0.1-0.8) U/mcL	0.7 U/mcL	3.54	<1 U/mcL
WBC	4.9 (2.9-8.6) U/mcL	8.1 U/mcL	2 (1.3-3.5) U/mcL	3.3 U/mcL	4.91	---
Hemoglobin	9 (7.3-10.3) g/dL	9.2 g/dL	6.9 (6.1-7.6) g/dL	7 g/dL	4.11	<9 g/dL
Platelets	70 (32-120) U/mcL	94.8 U/mcL	25.5 (16-59) U/mcL	41.1 U/mcL	4.28	<100 U/mcL
Ferritin	2924 (1198-10000) mcg/L	10616 mcg/L	7458 (2272-13124) mcg/L	14241 mcg/L	3.82	>500 mcg/L
Triglyceride	233 (148-352) mg/dL	261 mg/dL	301 (224-494) mg/dL	389 mg/dL	4.1	>265 mg/dL
Fibrinogen	159 (108-187) mg/dL	172 mg/dL	108 (67-136) mg/dL	120 mg/dL	4.45	<150 mg/dL
sIL2R	14487 (4777-30303) U/mL	19893 U/mL	14440 (4838-38647) U/mL	22806 U/mL	4.25	>2400 U/mL

Table 3. Descriptive Statistics for HLH Criteria. The median, interquartile range, and mean are listed for each laboratory parameter at initial presentation and at peak/nadir. The HLH-2004 criteria are listed to the side for comparison.

Discussion

The diagnosis of HLH is currently either based on identification of a known associated genetic mutation or if at least 5 of the following 8 criteria are identified (thresholds are listed in **Table 3**): 1. fever, 2. splenomegaly, 3. cytopenias 4. hypertriglyceridemia and/or hypofibrinogenemia, 5. hemophagocytosis in bone marrow, spleen, liver, or other tissues., 6. low or absent NK cell activity, 7. hyperferritinemia, 8. elevated sIL2r. These guidelines were established based on inclusion criteria for the HLH-2004 clinical trial[5] without knowing sensitivity or specificity. Many of the above criteria are observed in this study's population of genetic HLH. In fact, the values are often more extreme for certain criteria including sIL2R and ferritin, such that other studies have suggested cutoff values for ferritin >10,000 mcg/L are 90% sensitive and 96% specific for HLH[6,7]. Patients on average reached peak/nadir values within 3-5 days of presentation for all measured variables suggesting there may be a critical period for laboratory studies if suspicious for HLH. Unfortunately, the study population had a mortality rate of 25% overall that could potentially have been improved with earlier recognition and initiation of treatment.

Conclusion

HLH diagnosis is often delayed due to variability in clinical presentation and similarity to other hyperinflammatory states. We hope to improve HLH prognosis with earlier provider recognition and limit unnecessary exposure to toxic therapy from misdiagnosis of HLH through future studies that will compare this cohort to a large control group to better define specificity and sensitivity of current and novel criteria using receiver operating curves to optimize diagnostic thresholds.

References

1. Rosado FGN, Kim AS. Hemophagocytic Lymphohistiocytosis. *Am J Clin Pathol*. 2013;139(6):713-727. doi:10.1309/AJCP4ZDKJ4ICOUAT
2. Allen CE, McClain KL. Pathophysiology and epidemiology of hemophagocytic lymphohistiocytosis. *Hematology*. 2015;2015(1):177-182. doi:10.1182/asheducation-2015.1.177
3. Zhou YH, Han XR, Xia FQ, Poonit ND, Liu L. Clinical Features and Prognostic Factors of Early Outcome in Pediatric Hemophagocytic Lymphohistiocytosis: A Retrospective Analysis of 227 Cases. *J Pediatr Hematol Oncol*. 2022;44(1):e217-e222. doi:10.1097/MPH.0000000000002283
4. Jordan MB, Allen CE, Weitzman S, Filipovich AH, McClain KL. How I treat hemophagocytic lymphohistiocytosis. *Blood*. 2011;118(15):4041-4052. doi:10.1182/blood-2011-03-278127
5. Henter J, Horne A, Aricó M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48(2):124-131. doi:10.1002/pbc.21039
6. Cleves D, Lotero V, Medina D, et al. Pediatric hemophagocytic lymphohistiocytosis: A rarely diagnosed entity in a developing country. *BMC Pediatr*. 2021;21(1):411. doi:10.1186/s12887-021-02879-7
7. Allen CE, Yu X, Kozinetz CA, McClain KL. Highly elevated ferritin levels and the diagnosis of hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2008;50(6):1227-1235. doi:10.1002/pbc.21423
8. Gritta E. Janka, Kai Lehmberg; Hemophagocytic lymphohistiocytosis: pathogenesis and treatment. Hematology Am Soc Hematol Educ Program 2013; 2013 (1): 605–611. doi: https://doi.org/10.1182/asheducation-2013.1.605