



Pediatric Cancer & Hematologic Disorder
PedHemOnc-PMK



Update in Pediatric hemophagocytic lymphohistiocytosis

Integrating Evidence-Based Clinical Practice Guidelines into Real Practice

16 March 2024

Chalinee Monsereenusorn, M.D.

Associate Professor

Pediatric Oncology Faculty

Division of Hematology-Oncology, Department of Pediatrics

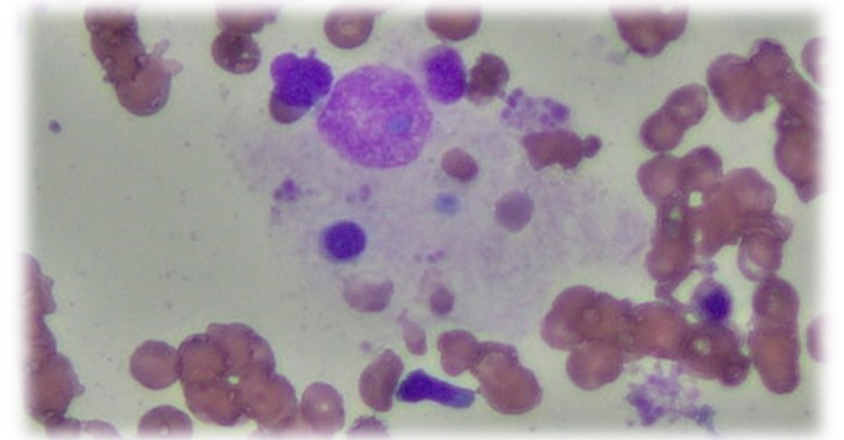
Phramongkutklao College of Medicine



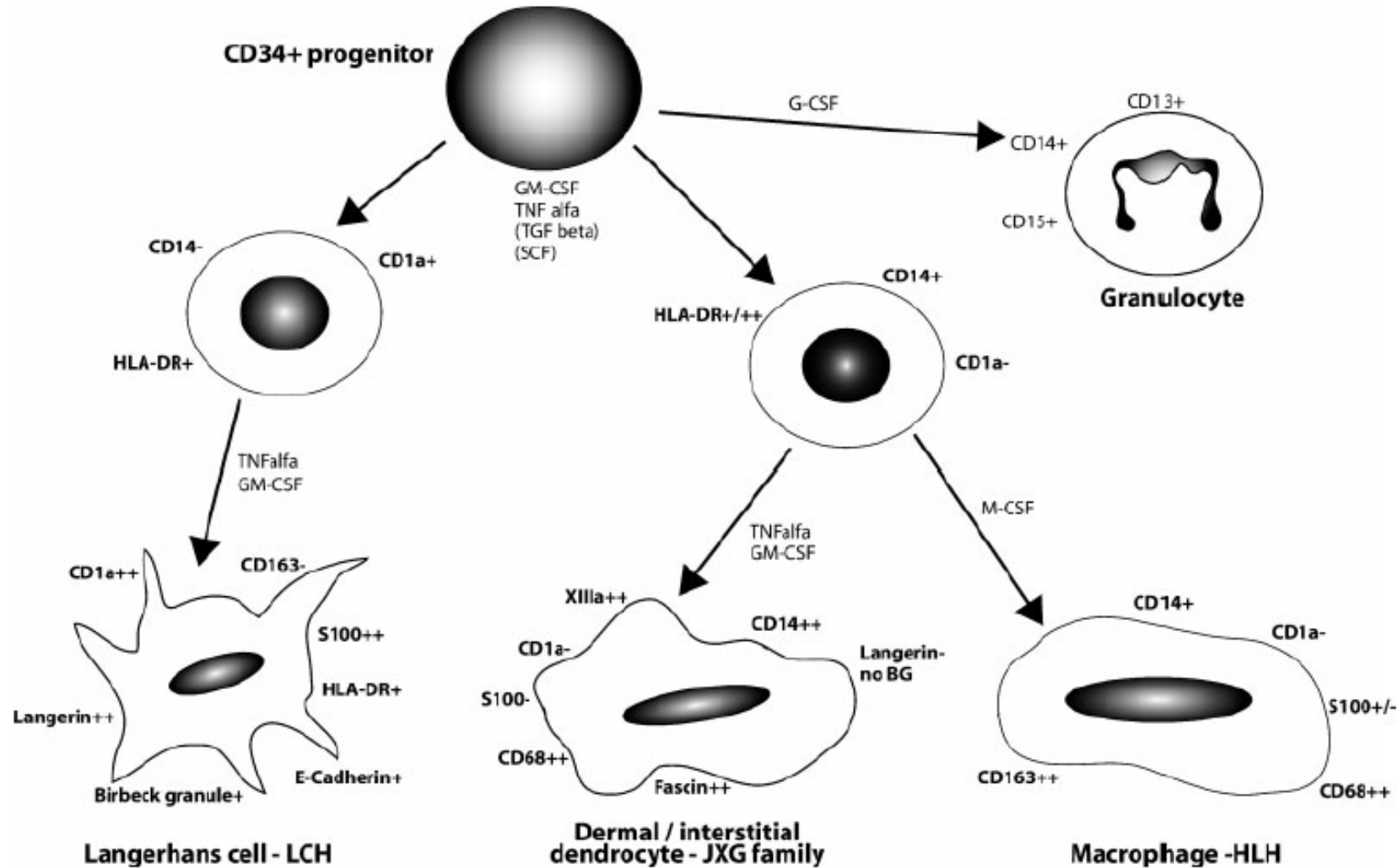
Outline



- Overview
- Classification of histiocytosis syndrome
- Pathophysiology and biology
- Diagnosis
- Primary vs. secondary HLH
- Clinical features
- Treatment
- Prognosis
- Outcomes
- Relapse and refractory disease



Histiocyte developmental pathway



Classification of histiocytosis syndrome in children

Class	Syndrome
I Dendritic/histiocytic disorder	• Langerhans cell histiocytosis (LCH) • Non-LCH ○ Erdheim-Chester Disease – primary in adult ○ Juvenile xanthogranuloma (JXG) – occur in children and adult
II Macrophage/monocytoid disorder	• Rosai-Dorfman Disease • Hemophagocytic lymphohistiocytosis (HLH) ○ Primary HLH – genetic disorder ○ Secondary HLH- infectious associated hemophagocytic syndrome (IAHS)
III Malignant disorder	• Malignant histiocytosis (histiocytic sarcoma) • Monocytic/myelomonocytic leukemias

Classification of histiocytosis (LCRMH)

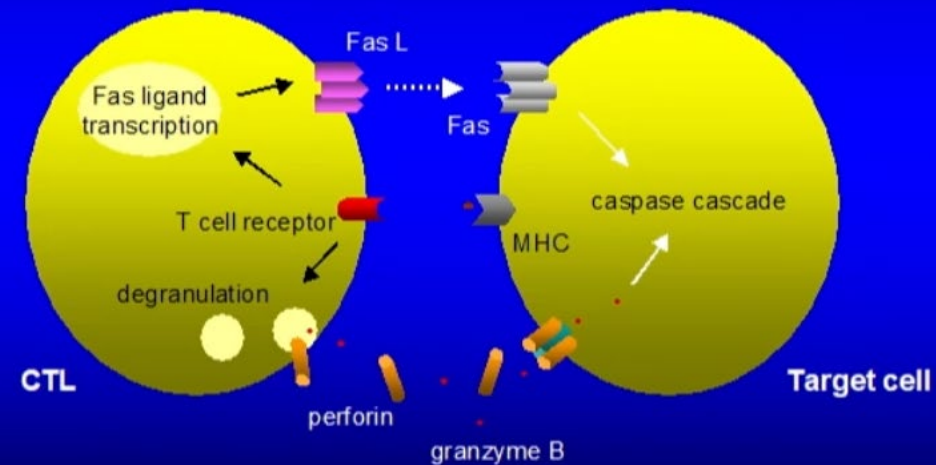
Group	Disease
L Group LCH	• LCH • Indeterminate-cell histiocytosis (ICH) • Erdheim-Chester Disease (ECD) • Mixed LCH/ECD
C Group Cutaneous non-LCH	• Cutaneous non-LCH • Xanthomatous granuloma (XG) family: JXG • Non-XG family: cutaneous RDD, NXG, other • Cutaneous non-LCH with a major systemic component • XG family: XD • Non-XG family: MRH
R Group RDD	• Familial RDD • Sporadic RDD
M Group Malignant histiocytoses	• Primary Malignant Histiocytoses • Secondary Malignant Histiocytoses
H Group HLH	• Primary HLH • Secondary HLH (non-Mendelian HLH) • HLH of unknown/uncertain origin

Classification of histiocytosis (LCRMH)

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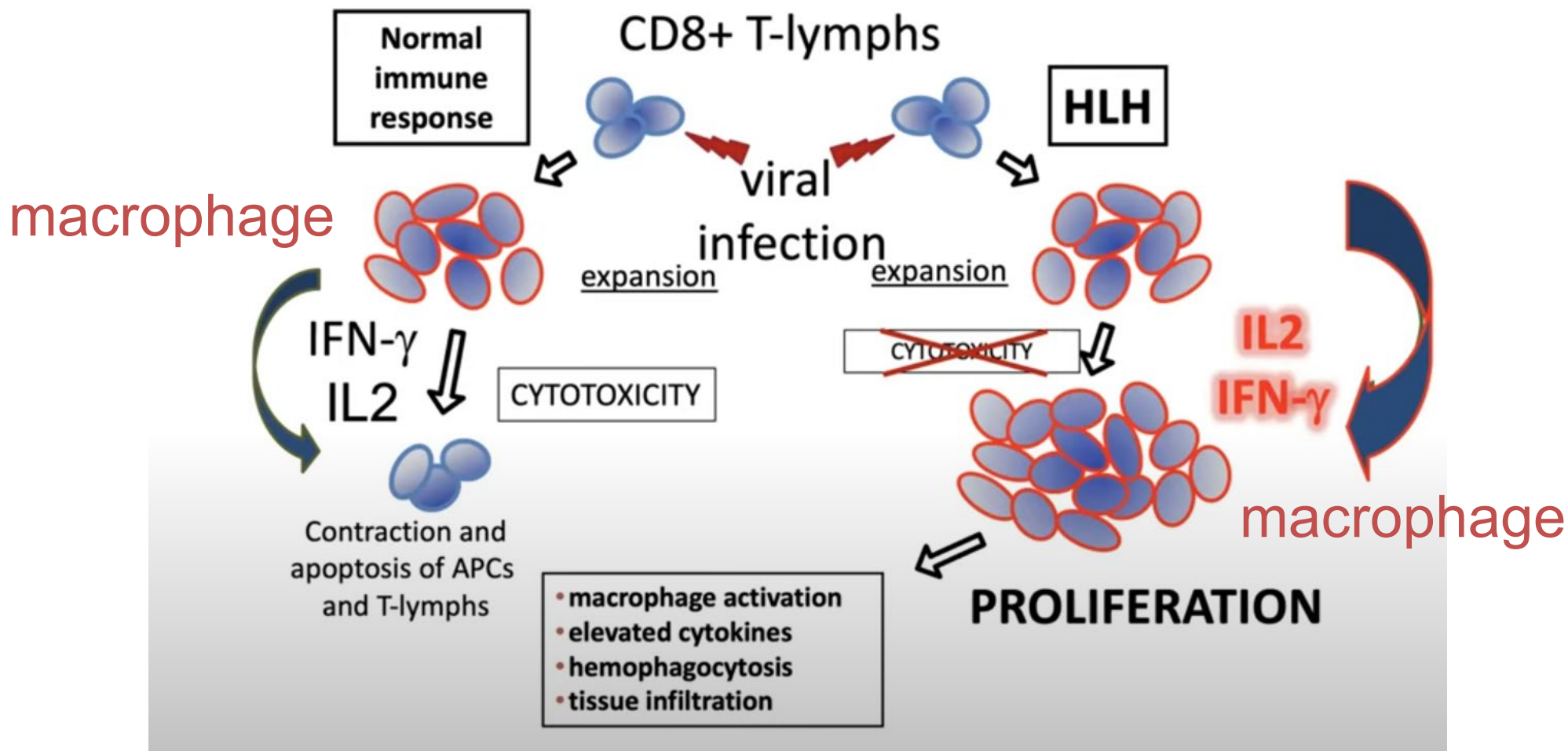
Pathophysiology and biology

The kiss of death: cytotoxic T and NK cells kill target cells by apoptosis



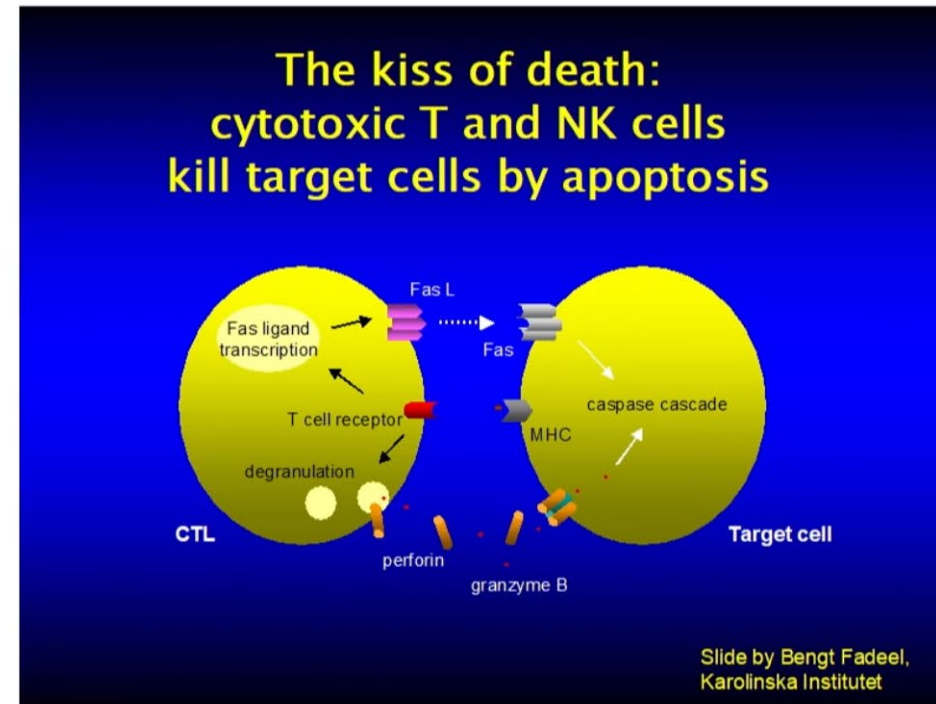
Slide by Bengt Fadeel,
Karolinska Institutet

Pathophysiology and biology



FHL biology: Defect in the cytotoxic lymphocytes (CTL + NK cells)

- Familial HLH = Appropriate initiation of the immune response - **inability to terminate it**
- **Defect in the cytotoxic lymphocytes, within the perforin system** which is central in human immune regulation
Stepp et al. *Science* 1999; 286:1957-59
- Cytotoxic lymphocyte functions:
 - Eliminate virus infected cells
 - Eliminate cancer transformed cells
 - **Downregulate the immune system**
 - **If defect: Hyperinflammation**



Criteria diagnosis



HLH-94

Criteria

Clinical

Fever

Splenomegaly

Laboratory

Cytopenias (affecting at least 2 of 3 lineages in the peripheral blood)

Hemoglobin (< 90 g/L), platelets ($< 100 \times 10^9$ /L), neutrophils ($< 1.0 \times 10^9$ /L)

Hypertriglyceridemia and/or hypofibrinogenemia

Fasting triglycerides ≥ 2 mM or ≥ 3 SDs of the normal value for age

Fibrinogen ≤ 1.5 g/L or ≤ 3 SDs of the normal value for age

Histopathologic

Hemophagocytosis in the BM or spleen or lymph node

No evidence of malignancy

HLH-2004

Diagnosis will be established if one of either (1) or (2) is fulfilled

- (1) Molecular diagnosis consistent with HLH
- (2) Diagnostic criteria for HLH fulfilled (5 out of the 8 criteria shown below)
 - ① Fever $\geq 38.5^\circ\text{C}$ for ≥ 7 days
 - ② Splenomegaly ≥ 3 finger breadth below the left subcostal margin
 - ③ Cytopenias affecting ≥ 2 of 3 lineages in peripheral blood
 - Hemoglobin < 9 g/L
 - Platelets $< 100 \times 10^9$ /L
 - Absolute neutrophil count $< 1.0 \times 10^9$ /L
 - ④ Hypertriglyceridemia and/or hypofibrinogenemia
 - Fasting triglycerides ≥ 265 mg/dL, Fibrinogen ≤ 1.5 g/L
 - ⑤ Hemophagocytosis in the bone marrow or spleen or lymph node
 - ⑥ Low or absent NK cell activity (according to the local laboratory reference)
 - ⑦ Ferritin ≥ 500 $\mu\text{g/L}$
 - ⑧ Soluble CD25 (sIL-2 receptor) $\geq 2,400$ U/mL

Abbreviations: HLH, hemophagocytic lymphohistiocytosis; NK, natural killer; sIL-2, soluble interleukin-2.

***Add 3 more criteria (Hyperferritinemia, Low or absent NK cell activity and elevated soluble CD25)**

Revised HLH-2004 diagnostic guidelines

- **Three options for the Clinical Criteria**
- Number of diagnostic criteria fulfilled (i.e. “5 of 8 criteria”)
 - Easy to use in the clinic
 - How precise is it?
- Numerical score using cutoffs for continuous lab values (as in HScore)
 - Ferritin >2000-5000 = 10 points; Ferritin >5000 = 25 points. Add the scores !
 - Somewhat tricky to use in the clinic (need a calculator)
 - How precise is it?
- Numerical score using a continuous scale for continuous lab values
 - Tricky to use in the clinic (need a webpage or an app)
 - How precise is it?
- The three options were compared, and compared with current criteria

Hscore for diagnosis secondary HLH

Parameter	No. of points (criteria for scoring)
Known underlying immunosuppression*	0 (no) or 18 (yes)
Temperature (°C)	0 (<38.4), 33 (38.4–39.4), or 49 (>39.4)
Organomegaly	0 (no), 23 (hepatomegaly or splenomegaly), or 38 (hepatomegaly and splenomegaly)
No. of cytopenias†	0 (1 lineage), 24 (2 lineages), or 34 (3 lineages)
Ferritin (ng/ml)	0 (<2,000), 35 (2,000–6,000), or 50 (>6,000)
Triglyceride (mmoles/liter)	0 (<1.5), 44 (1.5–4), or 64 (>4)
Fibrinogen (gm/liter)	0 (>2.5) or 30 (≤2.5)
Serum glutamic oxaloacetic transaminase (IU/liter)	0 (<30) or 19 (≥30)
Hemophagocytosis features on bone marrow aspirate	0 (no) or 35 (yes)

* Human immunodeficiency virus positive or receiving long-term immunosuppressive therapy (i.e., glucocorticoids, cyclosporine, azathioprine).

† Defined as a hemoglobin level of ≤9.2 gm/dl and/or a leukocyte count of ≤5,000/mm³ and/or a platelet count of ≤110,000/mm³.

The probability of having HLH

- <1% with an HScore <90
- >99% with an HScore ≥250

- Both HLH-2004 criteria (using 4 criteria) and HScore proved to be of good diagnostic accuracy (in ICU)

- Knaak C, et al. Crit Care 2020; 24: 244

- The important part is **to early identify patients at risk** of mortality and severe morbidity

→ **Do not delay HLH-directed therapy**, particularly if organ dysfunction is imminent or there is CNS affection

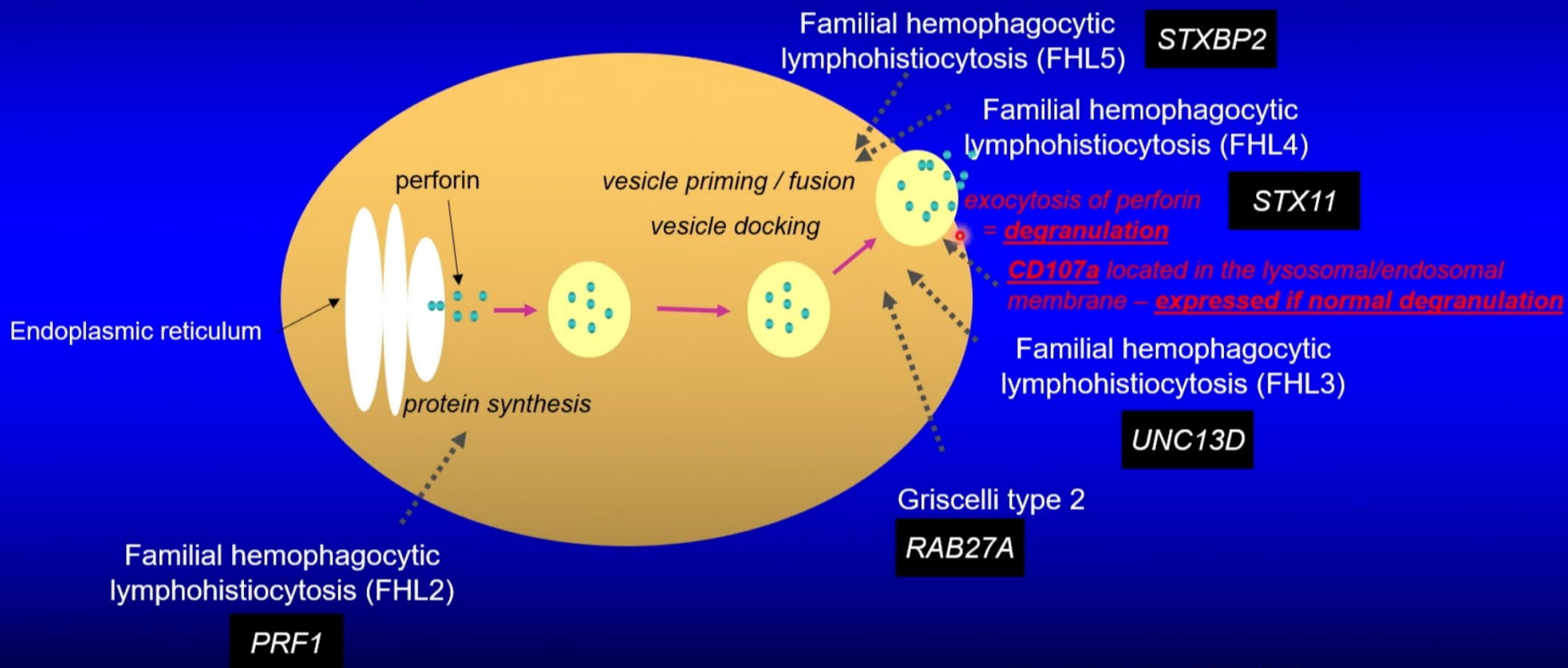
- Hines M, et al. Crit Care Med 2022; 50: 860-72

HLH-2004 diagnostic guidelines for FHLH



- HLH-2004 criteria were based on expert opinion
- Pathways
 - Genetic data
 - Cytotoxic data
 - Clinical and laboratory data – diagnostic criteria

Mechanisms of defective cellular cytotoxicity in FHL



Courtesy Bengt Fadeel,
Karolinska Institutet

Biology in the diagnostics of primary HLH

Cytotoxicity assays

- **NK/CTL degranulation** (CD107a) (pHLH)
 - Degranulation defects – accuracy >99%
(*UNC13D*, *STXBP2*, *STX11*, *RAB27A*, *LYST*)
 - **Flow cytometry** (pHLH)
 - Perforin, SAP and XIAP protein deficiencies
 - **Low NK cell activity** (Lytic units) (pHLH)
-
- **NK and Cytotoxic T cell numbers** (sHLH)

Genetic testing

- Familial HLH (FHL)
 - *PRF1*, *UNC13D*, *STXBP2*, and *STX11*
- Griscelli syndrome type 2
 - *RAB27A*
- XLP (X-linked lymphoproliferative syndrome)
 - *XLP1*: *SAP* (= *SH2D1A*)
 - *XLP2*: *XIAP* (= *BIRC4*)
- Chediak-Higashi syndrome
 - *LYST*

Results for various options of the clinical diagnostic pathway and various combinations of variables

Option of clinical pathway	Variables included	No. of criteria**	AUC***	Accuracy	Sensitivity	Specificity	PPV	NPV
Number of diagnostic criteria fulfilled	Exact values based on current optimization [#]	4/7	0.9976	97.7%	98.1%	97.6%	89.1%	99.6%
	- as above + fever + sCD25 ^{##}	6/9	0.9991	99.1%	97.1%	99.5%	97.6%	99.4%
	Rounded values (ferritin ≥ 500 $\mu\text{g/L}$) [§]	5/9	0.9961	99.1%	97.1%	99.5%	97.6%	99.4%
	Rounded values (ferritin $\geq 1,000$ $\mu\text{g/L}$) [§]	5/9	0.9967	99.1%	97.1%	99.5%	97.6%	99.4%
HLH-2004 criteria	Current criteria [■]	5/8	0.9920	97.4%	99.0%	97.1%	87.3%	99.8%
	Current criteria except natural killer cell activity [■]	5/7	0.9924	99.0%	96.2%	99.5%	97.6%	99.2%
	- ferritin ≥ 500 $\mu\text{g/L}$, sCD25 $\geq 2,400$ U/mL	4/7	0.9924	93.8%	99.0%	92.8%	73.3%	99.8%
	- ferritin $\geq 1,000$ $\mu\text{g/L}$, sCD25 $\geq 2,400$ U/mL	5/7	0.9932	99.4%	96.2%	100%	100%	99.2%
	- ferritin $\geq 1,000$ $\mu\text{g/L}$, sCD25 $\geq 2,400$ U/mL	4/7	0.9932	94.6%	99.0%	93.8%	76.0%	99.8%
	- ferritin ≥ 500 $\mu\text{g/L}$, sCD25 $\geq 5,000$ U/mL	5/7	0.9951	96.2%	96.2%	100%	100%	99.2%
	- ferritin ≥ 500 $\mu\text{g/L}$, sCD25 $\geq 5,000$ U/mL	4/7	0.9951	98.2%	99.0%	98.1%	91.2%	99.8%
	- ferritin $\geq 1,000$ $\mu\text{g/L}$, sCD25 $\geq 5,000$ U/mL	5/7	0.9954	99.4%	96.2%	100%	100%	99.2%
	- ferritin $\geq 1,000$ $\mu\text{g/L}$, sCD25 $\geq 5,000$ U/mL	4/7	0.9954	99.1%	99.0%	99.0%	95.4%	99.8%
Numerical score using cut-offs for continuous laboratory values	At score 100, FHL probability was 59.9%. Variables: Splenomegaly, neutrophils, platelets, triglycerides, fibrinogen, ferritin, bilirubin		0.9980	98.1%	98.1%	98.1%	91.1%	99.6%
Numerical score using a continuous scale for lab values	Variables: splenomegaly, hepatomegaly, hemoglobin, neutrophils, platelets, triglycerides, fibrinogen, ferritin, ALT, LDH, bilirubin		0.9976	NA	NA	NA	NA	NA

Diagnostic Guidelines for Familial Hemophagocytic Lymphohistiocytosis Revisited

Tracking no: BLD-2024-025077R1

Table 5: Revised diagnostic criteria for the diagnosis of FHL

The diagnosis of FHL can be established if at least one of either 1, 2 or 3 below is fulfilled*:

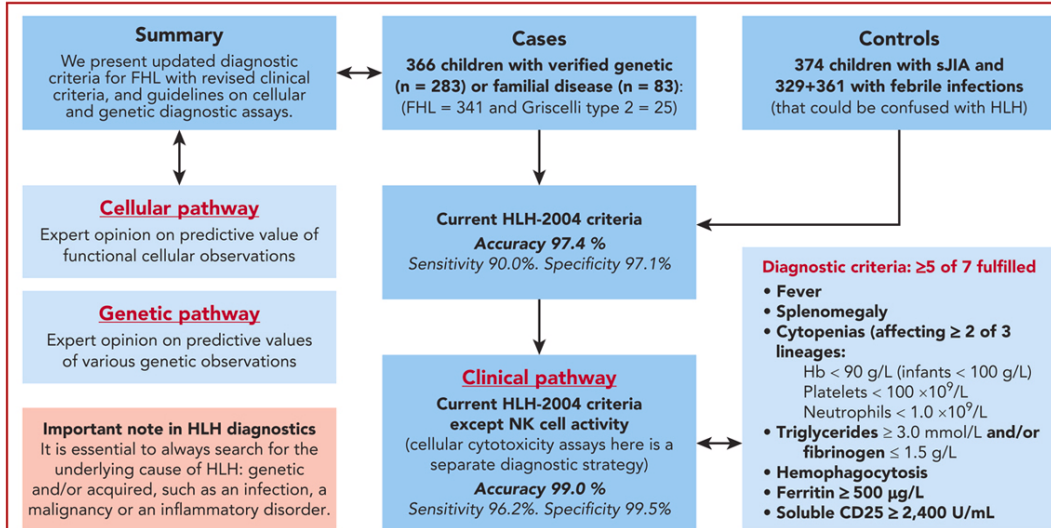
1. A **molecular** diagnosis consistent with FHL in a patient with signs / symptoms suggestive of HLH
2. Functional **cellular findings** consistent with FHL in a patient with signs / symptoms suggestive of HLH

3. Clinical diagnostic criteria for FHL with at least **5 out of the 7** criteria below fulfilled

- * Fever $\geq 38.5^{\circ}\text{C}$
- * Splenomegaly (≥ 2 cm below the costal margin)
- * Cytopenias (affecting ≥ 2 of 3 lineages in the peripheral blood:
Hemoglobin (< 90 g/L); Platelets ($< 100 \times 10^9/\text{L}$); Neutrophils ($< 1.0 \times 10^9/\text{L}$)
(In infants < 4 weeks: Hemoglobin < 100 g/L)
- * Hypertriglyceridemia and/or hypofibrinogenemia
Fasting triglycerides ≥ 3.0 mmol/L; Fibrinogen ≤ 1.5 g/L
- * Hemophagocytosis
- * Ferritin ≥ 500 $\mu\text{g/L}$
- * Soluble CD25 (*i.e.*, soluble interleukin-2 receptor) $\geq 2,400$ U/mL

* It is always of vital importance to search for the underlying cause(s) for the HLH syndrome, which may be either genetic, acquired, such as an infection, a malignancy or an autoimmune or autoinflammatory disorder.

Diagnostic Guidelines for Familial Hemophagocytic Lymphohistiocytosis Revisited

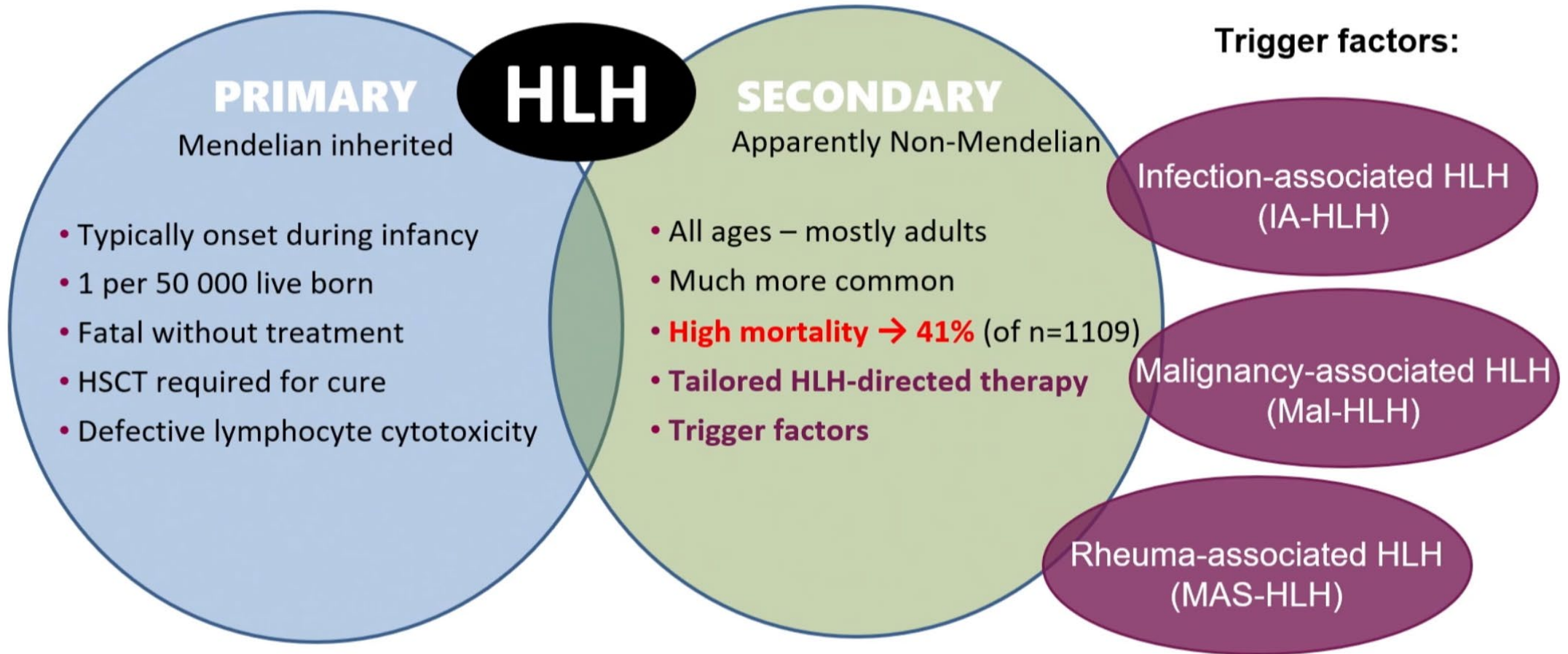


Conclusions: The HLH-2004 criteria, without NK-cell function, have significant validity (accuracy 99%) when tested against infections and systemic juvenile idiopathic arthritis. The complementary cellular and genetic diagnostic guidelines presented can facilitate the necessary confirmation of clinical diagnosis.

Henter et al. DOI: 10.1182/*blood*.2024025077

 **Visual Abstract**

Primary vs. Secondary HLH



Mortality rate from Ramos-Casals M, et al. Lancet 2014; 383:1503-16

Familial HLH

- 70% of cases, age onset is less than 1 year old
- Sporadic/Autosomal recessive
- Rapid fatal without treatment (median survival of about 2 months)

Defective immune function	Gene* (locus)	Syndrome	Clinical features	Laboratory findings
Cytotoxic granule content	<i>PRF1</i> (10q21-22)	FHL2		Decreased/absent perforin expression (FC)
Cytotoxic exocytosis pathway	<i>UNC13D</i> (17q2)	FHL3		Low CD107a expression (degranulation assay, FC)
	<i>STX11</i> (6q24)	FHL4		Low CD107a expression (degranulation assay, FC)
	<i>STXBP2</i> (19p13)	FHL5	Colitis, sensorineural hearing loss	Low CD107a expression (degranulation assay, FC)
	<i>RAB27A</i> (15q21)	Griscelli type 2	Hypopigmentation	Abnormal granule pattern in CBC and hair shafts, low CD107a expression
	<i>LYST</i> (1q42-43)	Chediak-Higashi	Hypopigmentation	Abnormal granule pattern in CBC and hair shafts, low CD107a expression
Cytotoxic T-cell signaling	<i>SH2D1A</i> (Xq24-25)	XLP1 Duncan disease	EBV lymphoproliferation	Decreased/absent SAP expression, reduced NK T cells (FC), hypogammaglobulinemia
Inflammasome regulation, excess apoptosis, NOD signal	<i>BIRC4</i> (Xq25)		Refractory colitis, EBV lymphoproliferation	Decreased/absent BIRC4 protein expression, reduced NK T cells
Inflammasome constitutive activation	<i>NLR4</i> (2p22.3)	XLP2	Recurrent autoinflammation, enterocolitis	High circulating IL-18 levels

Table 4. Frequency of HLH-associated gene mutations by age in North American patients

Age at referral	No. of HLH patients	<i>PRF1</i>	<i>UNC13D</i>	<i>STXBP2</i>	<i>STX11</i>	<i>RAB27A</i>	Mutation identified, %
< 1 mo	58	16	5	0	0	0	45
2 mo to 1 y	100	23	15	1	0	0	39
1-2 y	55	7	4	0	0	0	20
> 2 y	263	7	3	2	2	1	6

Table 5. Distribution of HLH-associated gene mutations, by ethnicity, in North American patients with identified genetic abnormalities

Gene	White	Hispanic	Black	Arabic	Other/unknown
<i>PRF1</i>	20 (27)	41 (71)	44 (98)	8 (36)	22 (88)
<i>UNC13D</i>	35 (47)	10 (17)	0 (0)	6 (27)	1 (4)
<i>STX11</i>	1 (2)	4 (7)	0 (0)	2 (9)	0 (0)
<i>RAB27A</i>	2 (3)	2 (3)	0 (0)	2 (9)	1 (4)
<i>STXBP2</i>	16 (22)	1 (2)	1 (2)	4 (18)	1 (4)
Total	74	58	45	22	25

La Rosée P, Horne A, Hines M, et al. *Blood*. 2019;133(23):2465-2477.

Jordan MB, Allen CE, Weitzman S, Filipovich AH, McClain KL. *Blood*. 2011;118(15):4041-4052.



Thinking of Primary HLH

Acute liver failure

78 children aged <24 mo with **acute liver failure**
(UK pediatric hepatology center)

- 30/78 (**38%**) had **HLH**
- 19/78 (**24%**) had **genetic HLH variations**
 - 9 (**12%**) **biallelic primary HLH mutations (→ HSCT)**
 - 10 heterozygous mutations and/or polymorphisms

Hadzic N, et al, J Pediatr 2022; 250: 67-74

Unknown encephalitis

CNS affection is common in HLH: 122/193 children (**63%**) had neurological symptoms and/or pathological CSF at HLH diagnosis

Horne et al. Br J Haematol 2008; 140: 327-35

HLH may present as severe, progressive encephalitis

Henter JI, et al. Lancet 1992; 339: 104-7

Feldmann J, et al. Blood 2005; 105: 2658-63

Blincoe A, et al. J Clin Immunol. 2020 Aug;40(6):901-916.

Zhao C, et al, Ann Hematol 2024; 103: 17-28

- Essential to be aware of the seriousness of CNS disease in HLH
 - The most important cause of late effects !!!
- **Prompt treatment** of active HLH at onset or relapse and **early HSCT** may reduce neurological sequelae

Critically ill patients

Secondary HLH in adult

Panel 1: List of triggers and associated diseases and processes detailed in 2197 adult haemophagocytic lymphohistiocytosis cases identified through search strategy

Infection (1108)

a) Viruses (762)

- Epstein-Barr virus (330)
- HIV (173)
- Herpes viruses (74)
- Cytomegalovirus (69)
- Viral hepatitis (20)
- Influenza (14)
- Human parvovirus B19 (14)
- Other viruses or not specified (68)

b) Bacteria (206)

- *Mycobacterium tuberculosis* (78)
- *Rickettsia* spp (17)
- *Staphylococcus* spp (15)
- *Escherichia coli* (11)
- Other bacteria or not specified (85)

c) Parasites (53)

- *Leishmania* spp (17)
- *Plasmodium* spp (14)
- *Toxoplasma* spp (10)
- Other parasites (12)

d) Fungi (37)

- *Histoplasma* spp (18)
- Other fungi (19)

e) Infection not specified (50)

Neoplasms (1047)

a) Haematological (981)

- T-cell or natural-killer lymphoma (369)
- B-cell lymphoma (333)
- Leukaemia (67)
- Hodgkin's lymphoma (61)

- Not specified lymphoma (35)
- Castleman's disease (22)
- Other haematological neoplasms or not specified (94)

b) Solid (32)

c) Not specified neoplasm (34)

Autoimmune diseases (276)

a) Systemic (244)

- Systemic lupus erythematosus (133)
- Adult-onset Still's disease (54)
- Rheumatoid arthritis (18)
- Vasculitis (11)
- Other or not specified (28)

b) Organ-specific (32)

- Inflammatory bowel disease (11)
- Other diseases (21)

Other circumstances or diseases (184)

a) Transplantation (95)

- Kidney (53)
- Haematological (29)
- Other (13)

b) Other circumstances (76)

- Drugs (20)
- Surgery or biopsies (11)
- Vaccination or acute injuries (10)
- Diabetes or chronic liver disease (14)
- Pregnancy (11)
- Haemodialysis (10)

c) Other or not specified (13)

Idiopathic or unknown (81)

Number of reported cases in parentheses.

EBV = 43%

- predominant virus in Asia and North America. Both adults and children.

Malignancies = common trigger in adults, much less in children.

Secondary Hemophagocytic Lymphohistiocytosis in Children : An Analysis of Etiology and Outcome

GAVIVANN VEERAKUL, M.D.*,
VORAVARN S. TANPHAICHITR, M.D., M.S.*,
NONGNAPA JIRARATTANASOPA, M.D.*

KLEESABAI SANPAKIT, M.D.*,
CHULARATANA MAHASANDANA, M.D.*

Organisms	N
Bacteria	
<i>Salmonella</i> spp	3
Enterobactor	2
<i>Staphylococcus</i>	2
<i>Serratia marcescens</i>	1
Virus	
Dengue	3
Ebstein-Barr virus	1
Parasites	
Malaria (<i>P vivax</i> 1, <i>P falciparum</i> 1)	2
<i>Penicillium marneffe</i>	1

TABLE 3 Classification and causes of children with HLH (n = 78)

	N (%)
IAHS*	59 (75.6)
Viruses	49 (62.8)
EBV	37 (47.4)
Dengue virus	3 (3.8)
CMV	2 (2.6)
Enterovirus	2 (2.6)
Influenza B virus	2 (2.6)
Adenovirus	2 (2.6)
Parvovirus B19	1 (1.3)
Bacteria	10 (12.8)
Mycobacteria	2 (2.6)
<i>Mycobacterium tuberculosis</i>	2 (2.6)
Gram negative bacterias	6 (7.7)
<i>Burkholderia pseudomallei</i> (Melioidosis)	2 (2.6)
<i>Salmonella</i> spp.	2 (2.6)
<i>Rickettsia</i> (Scrub typhus)	1 (1.3)
<i>Acinetobacter baumannii</i>	1 (1.3)
Gram positive bacterias	2 (2.6)
<i>Staphylococcus aureus</i>	1 (1.3)
<i>Streptococcus viridans</i>	1 (1.3)
Fungi	2 (2.6)
<i>Histoplasma capsulatum</i>	1 (1.3)
<i>Aspergillus</i> spp. (Invasive pulmonary aspergillosis)	1 (1.3)
Rheumatologic disease-associated HLH	4 (5.1)
MAS	4 (5.1)
MA-HLH	2 (2.6)
B cell ALL	1 (1.3)
T cell ALL	1 (1.3)
Unspecific	13 (16.7)



Whole-Exome Sequencing Reveals Mutations in Genes Linked to Hemophagocytic Lymphohistiocytosis and Macrophage Activation Syndrome in Fatal Cases of H1N1 Influenza

Grant S. Schulert,¹ Mingce Zhang,³ Ndate Fall,¹ Ammar Husami,² Diane Kissell,² Andrew Hanosh,⁴ Kejian Zhang,² Kristina Davis,⁴ Jeffrey M. Jentzen,⁴ Lena Napolitano,⁵ Javed Siddiqui,^{4,7} Lauren B. Smith,⁴ Paul W. Harms,^{4,6,7} Alexei A. Grom,¹ and Randy Q. Cron³

¹Division of Pediatric Rheumatology, and ²Division of Human Genetics, Cincinnati Children's Hospital Medical Center, Ohio; ³Division of Pediatric Rheumatology, Children's Hospital of Alabama/University of Alabama at Birmingham; ⁴Department of Pathology, ⁵Department of Surgery, ⁶Department of Dermatology, and ⁷Michigan Center for Translational Pathology, University of Michigan Medical School, Ann Arbor

- The fatal influenza A (H1N1) infections correlate with HLH.
- HLH gene mutations increase risk of mortality among individuals with influenza A (H1N1) infection

Malignancy-associated hemophagocytic syndrome (MAHLH)



1. Development of a hemophagocytic syndrome before and/or during the treatment for malignancy, such as:
 - Acute lymphoblastic leukemia
 - Acute myeloid leukemia
 - Multiple myeloma
 - Germ cell tumor
 - Thymoma
 - Carcinoma
2. Development of a hemophagocytic syndrome with a masked hematolymphoid malignancy in the background, such as:
 - a. T/NK-cell leukemia
 - b. Lymphomas
 - c. Large cell anaplastic lymphoma
 - d. Adult B-cell lymphoma

From Janka, G., Imashuku, S., Elinder, G., Schneider, M., Hente, J.J., 1998. Infection and malignancy associated hemophagocytic syndromes. *Hematol. Oncol. Clin. N. Am.* 12, 435–443.

Macrophage activation syndrome (MAS) in SoJIA and other chronic conditions



Table 2. Genes of interest with variations in patients with systemic JIA and MAS*

Family ID no./ patient ID no.	Heterozygous variants of known genes implicated in clinically similar syndromes	New genes		
		Recessive pairs		De novo variants
		Homozygotes	Compound heterozygotes	
21/53	<i>STXBP2</i> †	–	6 (<i>XIRP2</i>)	<i>C7orf70, ACIN1</i>
60/155	<i>LYST, STXBP2</i>	<i>CCDC141</i>	7	–
88/241	–	–	3	–
119/327	<i>STXBP2</i> †	–	3	<i>NIPBL, ZIK1</i>
342/900	<i>MUNC13-4</i>	<i>FAM160A2</i>	4 (<i>ARHGAP21</i>)	–
3784/7431	<i>LYST</i>	–	6	<i>WDR66</i>
3790/7437	–	–	7	<i>KIF24, GPR132</i>
3807/7472	–	–	4	–
3932/7894	–	–	12 (<i>XIRP2</i>)	–
4218/8463	–	–	3	–
4272/8610	–	–	5	–
4388/8872	–	–	10 (<i>SLAC2B</i>)	–
12778/70087	–	–	3	–
12937/72575	–	<i>LRGUK</i>	3	<i>CAMK1, OGG1</i>

* See Table 1 for definitions.

† Variant reported in late-onset familial HLH (12).

WES shows
overlap between
SoJIA MAS-HLH
and FHLH
mutations (5/14)

sHLH/MAS TRIGGERS

Medication

Infliximab
Sulfasalazine
Methotrexate
Etoposide
Etanercept
NSAIDs
Adalimumab

Malignancy

Lymphoid/solid cancers
Hodgkin lymphomas
Leukemias
Multicentric Castleman Disease

Cell therapy

CAR-T CAR-NK CAR-macrophage

Autoimmune diseases

High frequencies

Adult onset Still disease (10-40%)
Systemic juvenile idiopathic arthritis (sJIA) (10-30%)
Systemic lupus erythematosus (SLE) (0.9~4.6%)
Kawasaki disease (~2%)

Others

Systemic sclerosis Vasculitic syndrome
Sjogren syndrome Dermato-myositis
Inflammatory bowel disease
Enthesitis arthritis Rheumatoid arthritis

Infection

Viruses

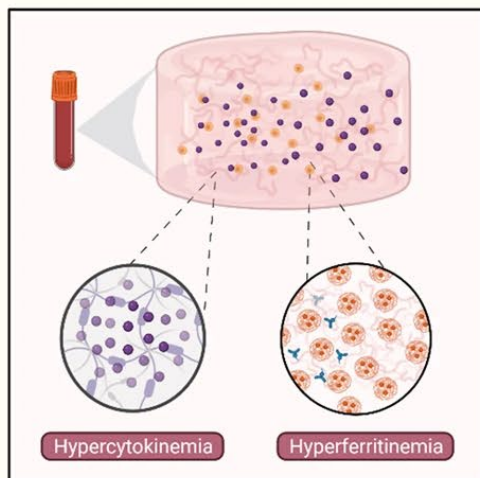
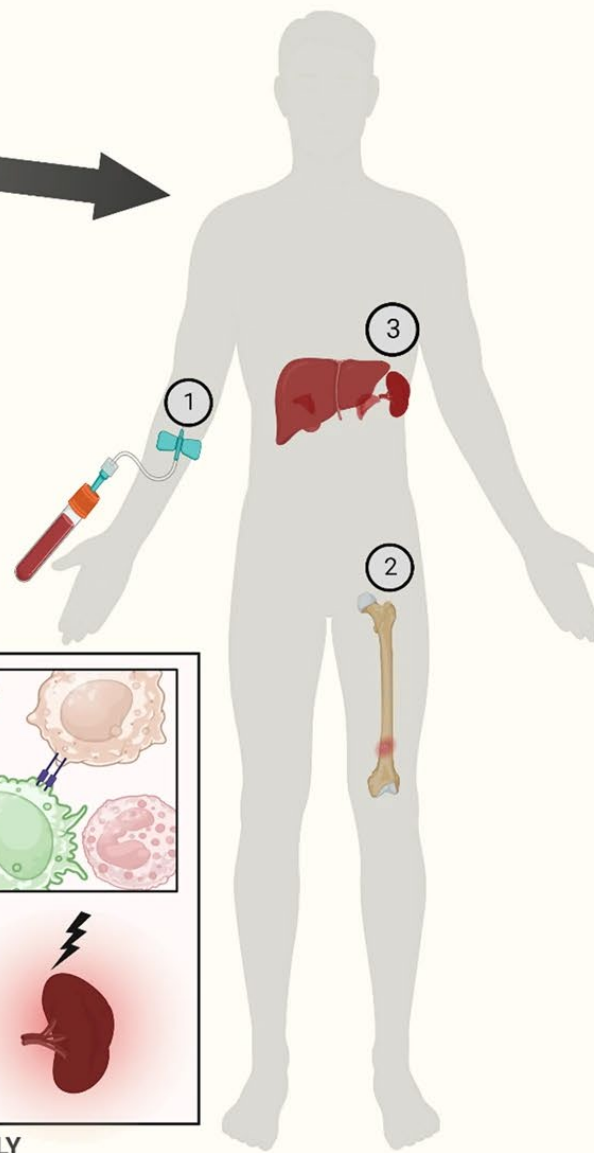
Epstein-Barr virus (25%) Herpes HIV
Cytomegalovirus Varicella Adenovirus
COVID-19 Influenza Enterovirus Coxsaki

Bacteria

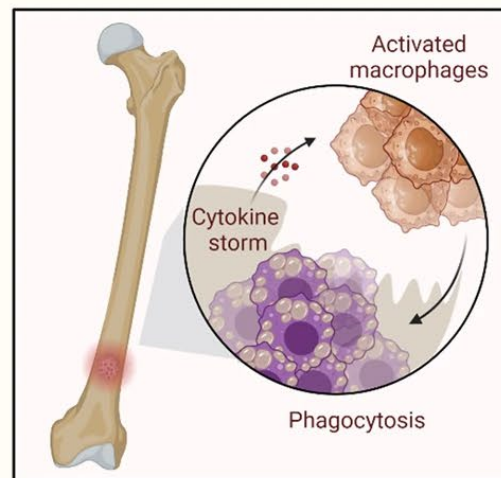
Mycobacterium tuberculosis *Rickettsia*
Staphylococcus aureus *Pneumococcus*
Brucellosis *Enterobacteria* *Leptospirosis*

Parasite/Fungi

Leishmania *Malaria* *Candida sp.* *Babesia*
Toxoplasma *Aspergillus sp.* *Fusarium sp.*

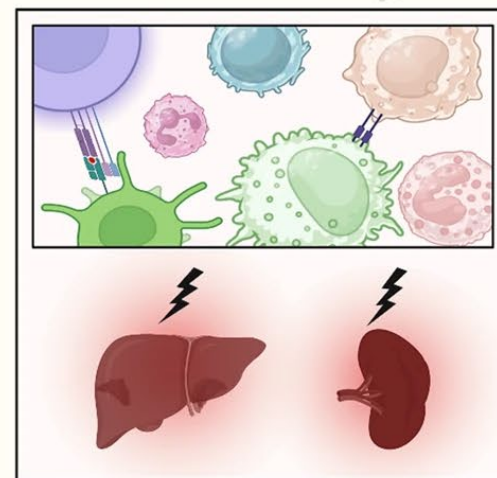


- 1) Ferritin > 500 ng/ml
- 2) Cytokine storms: $\uparrow$ IFN- γ , TNF- α , IL-1, IL-6, IL-18, sCD25 (IL-2R α)



2) HEMOPHAGOCYTOSIS

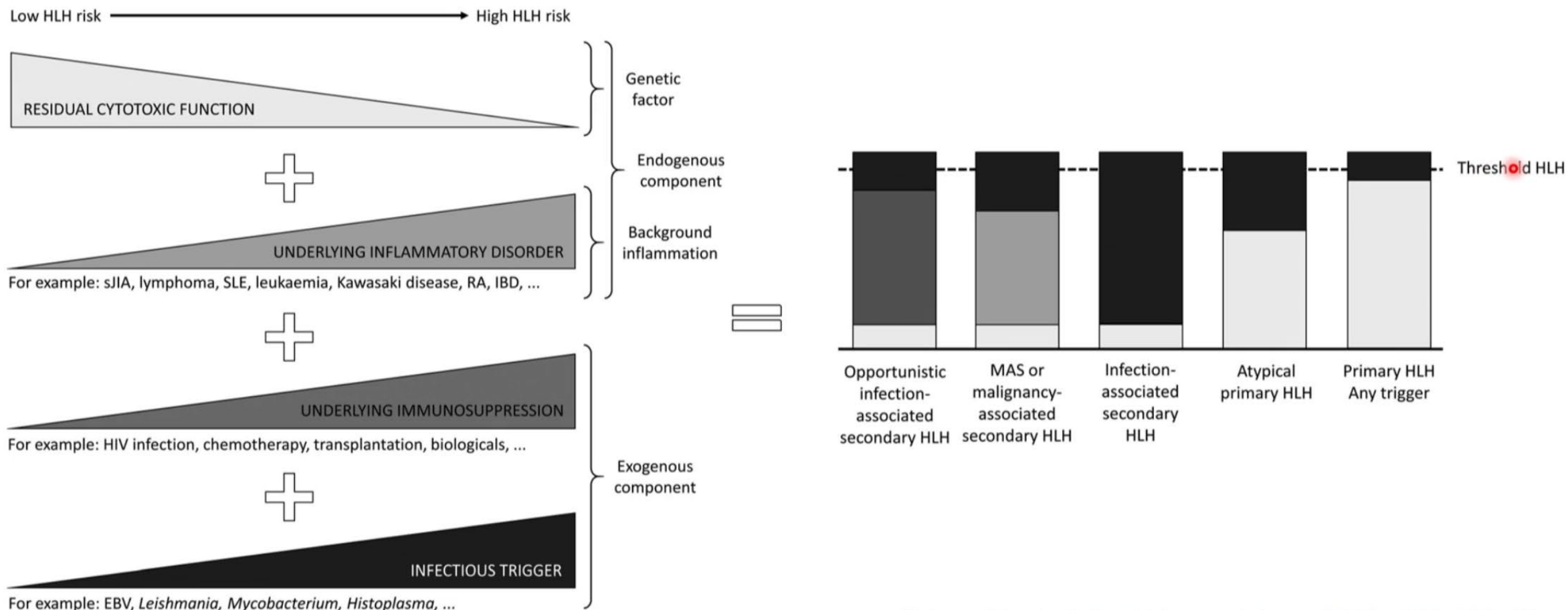
Bone marrow biopsy showing the phagocytosis of erythrocytes, lymphocytes or other hematopoietic precursors by activated macrophages as a result of cytokine storms



3) HEPATOSPLENOMEGALY

Cytokine storms caused by overaction and interactions of immune cells cause organ failure; enlargement of the liver and spleen is signature feature of MAS, correlation with $\uparrow$ ALT, AST, TG, $\downarrow$ albumin and fibrinogen

Threshold model of the HLH spectrum



Clinical features and laboratory findings

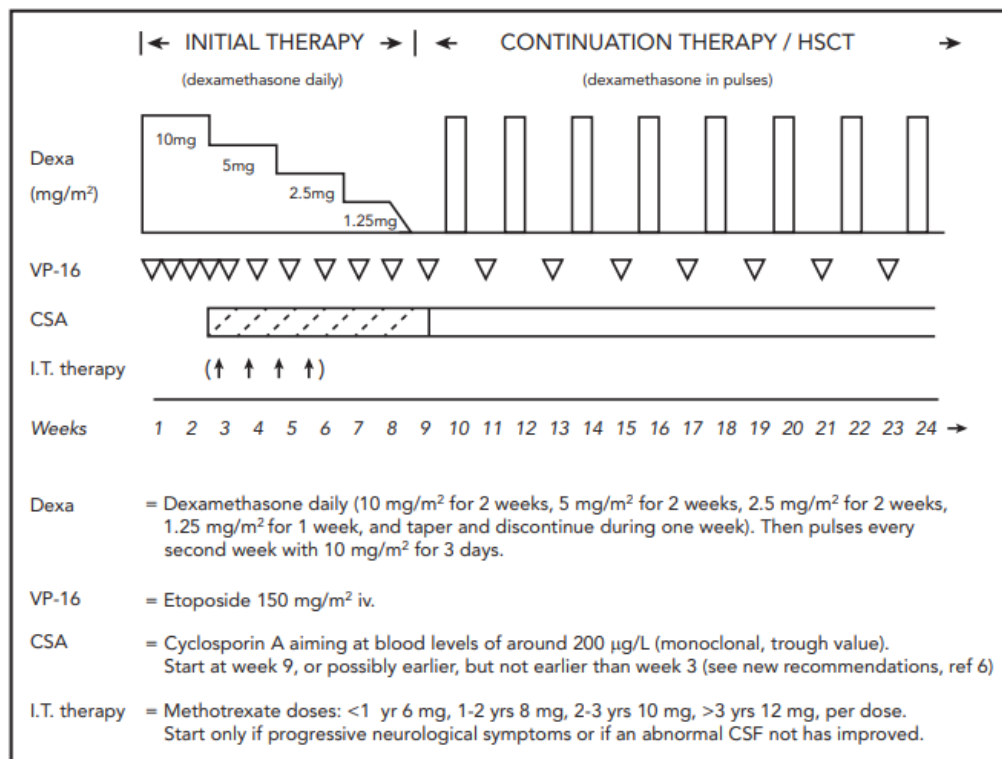
Fever	90-100 %
Maculopapular and/or petechial rash	10-60 %
Hepatosplenomegaly	70-100 %
Lymphadenopathy	20-50 %
Respiratory distress	40-90 %
Jaundice	~50 %
CNS involvement	~50 %

*Occasionally, ocular, heart, skeletal muscle, and kidney involvement have been noted

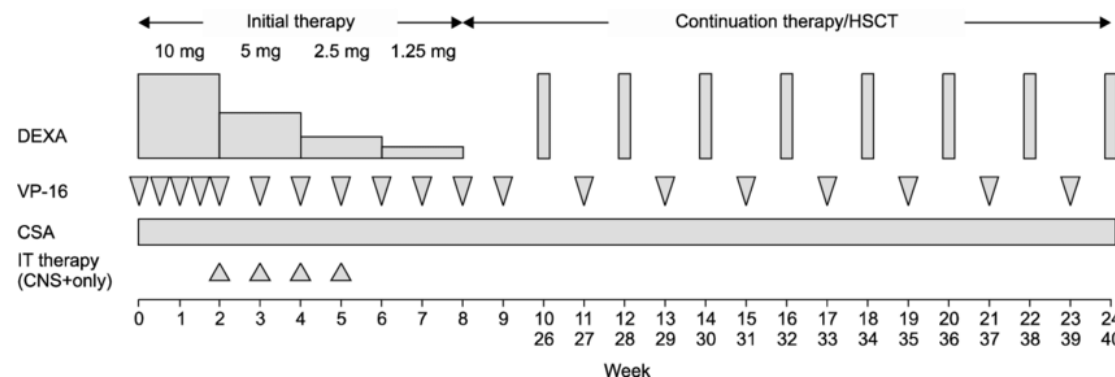
Characteristics	N (%)
Diagnostic criteria findings	
Fever	78 (100)
Splenomegaly	65 (83.3)
Hyperferritinemia	76 (97.4)
Hemophagocytosis	75 (96.2)
Cytopenia	63 (80.8)
Hypertriglyceridemia	60 (76.9)
Hypofibrinogenemia	37 (47.4)
Nondiagnostic criteria findings	
Liver transaminitis	67 (85.9)
Hypoalbuminemia	63 (80.8)
High LDH	58 (74.4)
Direct hyperbilirubinemia	49 (62.8)

Treatment

HLH-94



HLH-2004



- **Etoposide:** potent selective **deletion of activated T-cell**, efficient suppression of inflammatory cytokine production
- **CSA:** **inhibit gamma-IFN**
- **Dexa:** Long half-life, via BBB, **anti-inflammation**
- **IVIg:** for IAHS only

Treatment-ThaiPOG 2023



Phase I Initial therapy (Week 1-8)

Date start ____/____/____

Week	1	2	3	4	5	6	7	8
Day	1	8	15	22	29	36	43	50
Etoposide _____mg	☐	☐	☐	☐	☐	☐	☐	☐
Dexa _____ mg	10 mg/m ²		5 mg/m ²		2.5 mg/m ²		1.25mg/m ²	taper off
IVIG _____ mg	◆				◆			
CSA _____ml q12hr	____ ml	____ ml	____ ml	____ ml	____ ml	____ ml	____ ml	____ ml
IT*			T*	T*	T*	T*		

*Give IT chemotherapy if progressive neurological symptom or abnormal cell persist in CSF only

Phase II Continuation therapy (Week 9-40)

Date start ____/____/____

Given dose	Drug	Dosage	Day
_____ mg	Etoposide	150 mg/m ² IV every 2 weeks	1
_____ mg	Dexamethasone	10 mg/m ² /day PO x 3 days every 2 weeks	8-10
_____ mg	Cyclosporin A	8-10 mg/kg/day PO q 12hr	daily
_____ mg	Cotrimoxazole	5 mg of TMZ/kg/day PO bid 3 day/wk (Fri-Sat-Sun)	

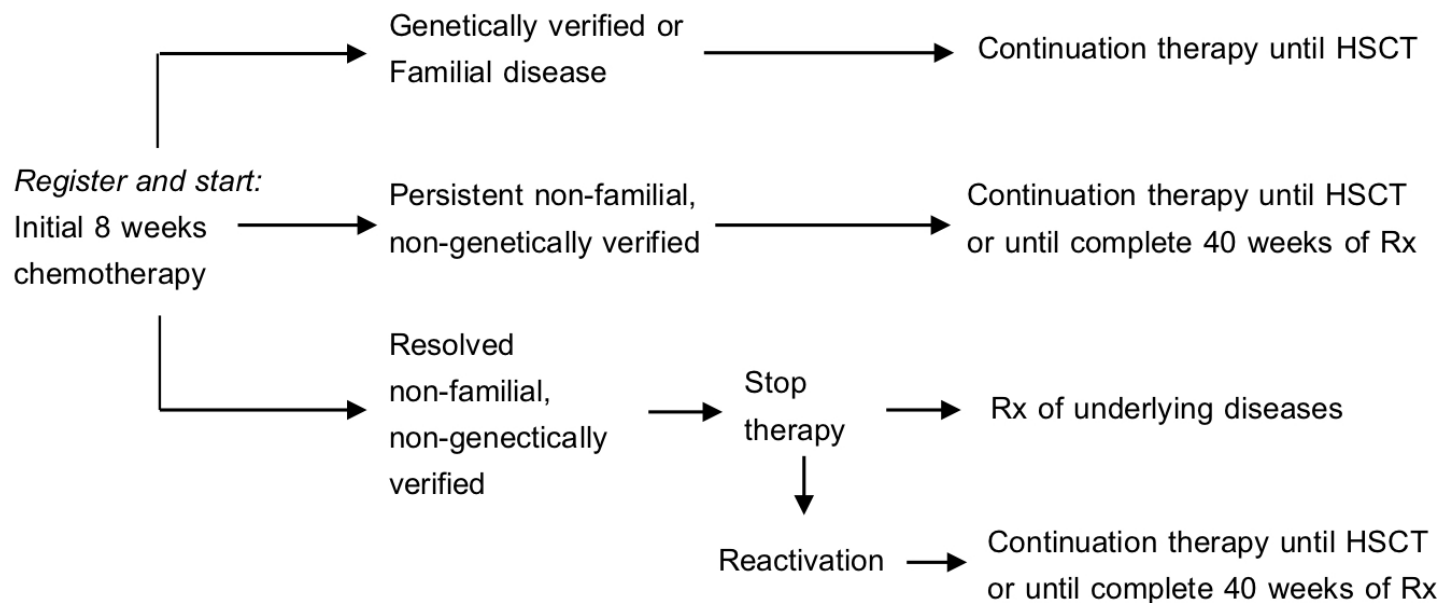


Thai Pediatric Oncology Group
ชมรมโรคมะเร็งเด็กแห่งประเทศไทย



Goal of treatment

- Suppressed life-threatening inflammation by destroying immune cells
- Identify and treat underlying triggers of HLH
- Supportive treatment: Anemia, thrombocytopenia, or bleeding



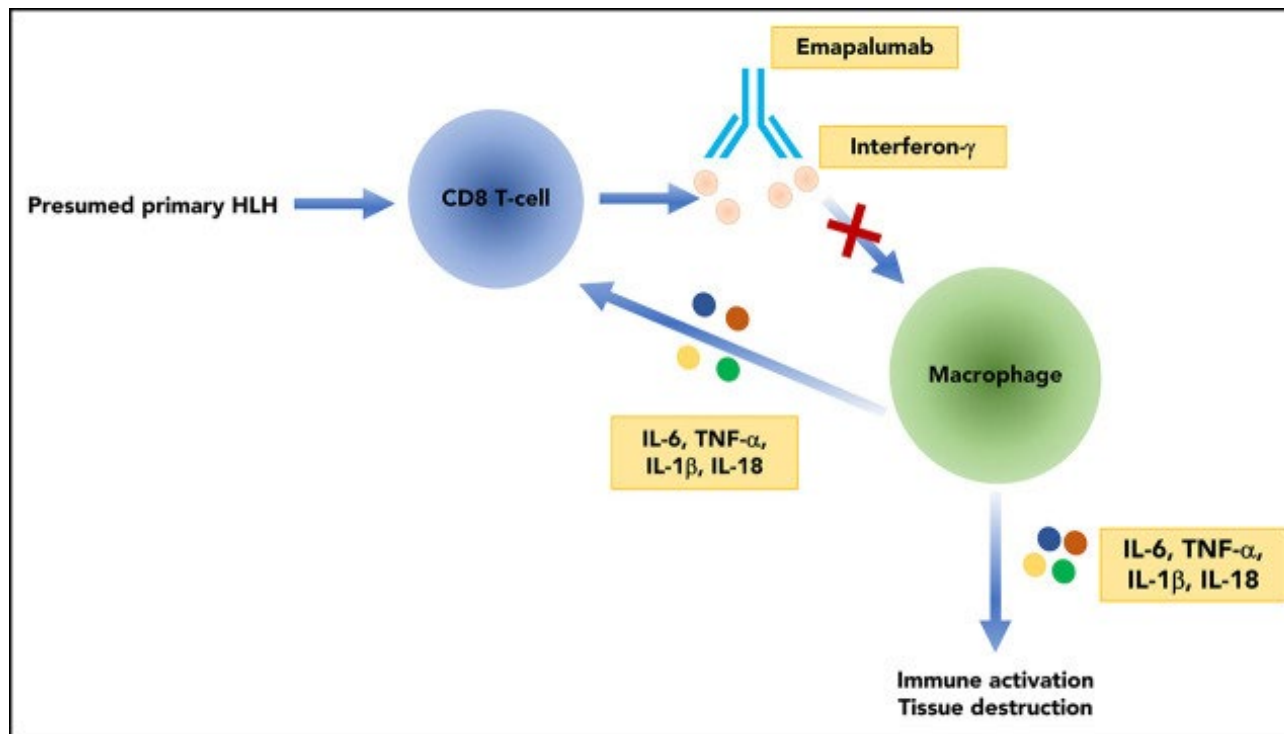
Emapalumab in Children with Primary Hemophagocytic Lymphohistiocytosis

F. Locatelli, M.B. Jordan, C. Allen, S. Cesaro, C. Rizzari, A. Rao, B. Degar, T.P. Garrington, J. Sevilla, M.-C. Putti, F. Fagioli, M. Ahlmann, J.-L. Dapena Diaz, M. Henry, F. De Benedetti, A. Grom, G. Lapeyre, P. Jacqmin, M. Ballabio, and C. de Min

► *Blood*. 2019 Nov 21;134(21):1783–1786. doi: [10.1182/blood.2019002289](https://doi.org/10.1182/blood.2019002289)

Emapalumab for the treatment of relapsed/refractory hemophagocytic lymphohistiocytosis

Mounica Vallurupalli¹, Nancy Berliner¹,[✉]



Locatelli F, Jordan MB, Allen C, et al. *N Engl J Med*. 2020;382(19):1811-1822.

Vallurupalli M, Berliner N. *Blood*. 2019 Nov 21;134(21):1783-1786.



CLINICAL TRIALS AND OBSERVATIONS

Survival in primary hemophagocytic lymphohistiocytosis, 2016 to 2021: etoposide is better than its reputation

- **Material:** All primary HLH patients in the HLH registry 2016 - 2021 with 1-yr follow-up
 - 88 patients
 - 63 had FHL2-5, 13 had GS2/CHS, 9 had XLP1/2 and 3 had undefined pHLH
 - 76 had symptoms: 71 (93%) fulfilled HLH-2004-criteria; 3 = CNS-HLH; 2 = CNS-HLH + <5 HLH-criteria
- **Treatments:**
 - 66 major HLH-directed drugs, 57 (85%) first-line etoposide; alemtuzumab (n=7); emapalumab (n=2)
- **Outcome:**
 - Asymptomatic:** 12/12 (100%) of patients alive and well (10/10 post-HSCT)
 - Symptomatic: Alive until HSCT; 52/57 (91%) of etoposide first-line patients.** 69/76 (91%) of all
 - 65 underwent HSCT: 44/52 (85%) of etoposide first-line patients survived HSCT; 58/65 (89%) of all
 - 79% of pat receiving first-line etoposide were alive after 1 year – 77% after 3 years

Treatment outcomes in Primary HLH

1st line drugs

1 st line drugs	<u>Emapalumab</u>	<u>Alemtuzumab</u>	<u>Etoposide</u>		
	n=27	n=7	n=25	n=167	n=57
<u>Characteristics at study start</u>	<u>Previously treated</u>	<u>Untreated</u>	<u>Prospective</u>	<u>HLH-2004 (ver FHL)</u>	<u>HLH Registry (pHLH)</u>
▪ Median age at HLH onset	0.9 years	0.85 yrs	3-4 mo	3 mo	5 mo
▪ Genetically confirmed	22/27 (81%)	5/7 (71%)	25/25=100%	157/167=94 (-100)%	54/57 (95%)
▪ Pre-SCT survival	22/27 (81%)	4/7 (57%)	23/25 (92%)	= 81% (135/167)	52/57 (91%)
▪ Post-SCT survival	20/22 (91%)	3/4 (75%)	20/23 (87%)	= 70% (93/133)	44/52 (85%)
▪ Alive at last follow-up	20/27 (74%)	3/7 (43%)	20/25 (80%)	= 59% 5-yr	44/57 (77%, 3-yr)

Locatelli F, et al. N Engl J Med 2020; 382: 1811-22 (incl Suppl Tables 4, 5, 8)
Moshous D, et al. Blood 2019; 134 (Suppl 1): 80 + Pers communication
Bergsten E, et al. Blood 2017; 130: 2728-2738
Böhm S, et al. Blood 2024; 143: 872-881

Current standard-of-care for pre-SCT therapy in familial HLH

- Two very successful treatment protocols
 - International studies: Etoposide-based (HLH-94 / HLH-2004)
 - Readily available
 - Very affordable
 - France: Alemtuzumab-based
 - Very early SCT (often <7 weeks)
 - Access limited
- **My conclusion: Standard of care in familial HLH = HLH-94 or Modified HLH-2004**
 - Modified HLH-2004: Avoid CSA first 2 weeks
 - Consider less steroid tapering

EBV-associated HLH

1. EBV-HLH has a high mortality - untreated

- **Etoposide + steroids = dramatic effects**

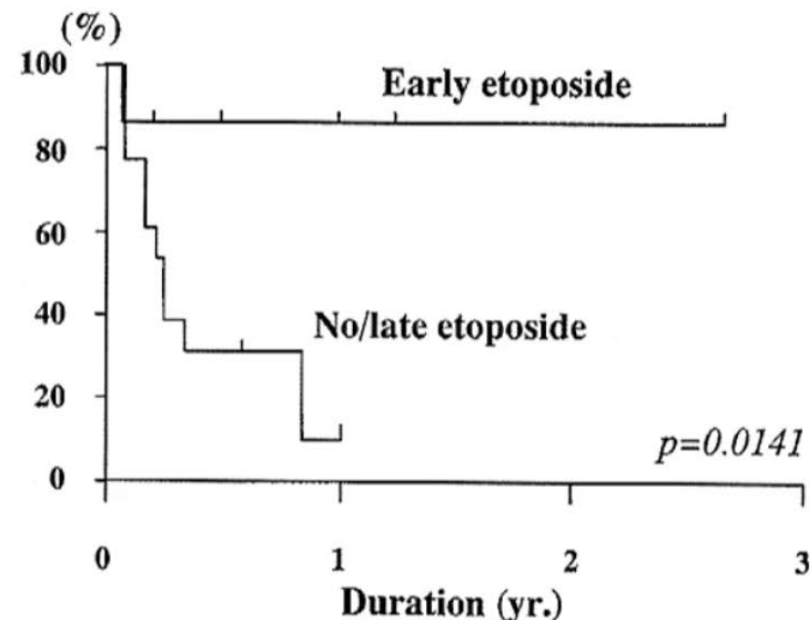
Imashuku S, et al. Blood 1999; 93: 1869-74

Imashuku S, et al. Med Pediatr Oncol 2003; 41: 103-9

2. Later findings: All do not need etoposide

- Revised Japanese approach:

- **Low risk**
→ Dexamethasone (Dexa) only
- **Intermediate risk (37.5°C or higher at 48 hours)**
→ Dexa + Etoposide + Cyclosporine A (CSA)
- **High risk (2 years old or less, and/or EBV-DNA load >1,000 copies/mL):**
→ Dexa + Etoposide + CSA + Rituximab



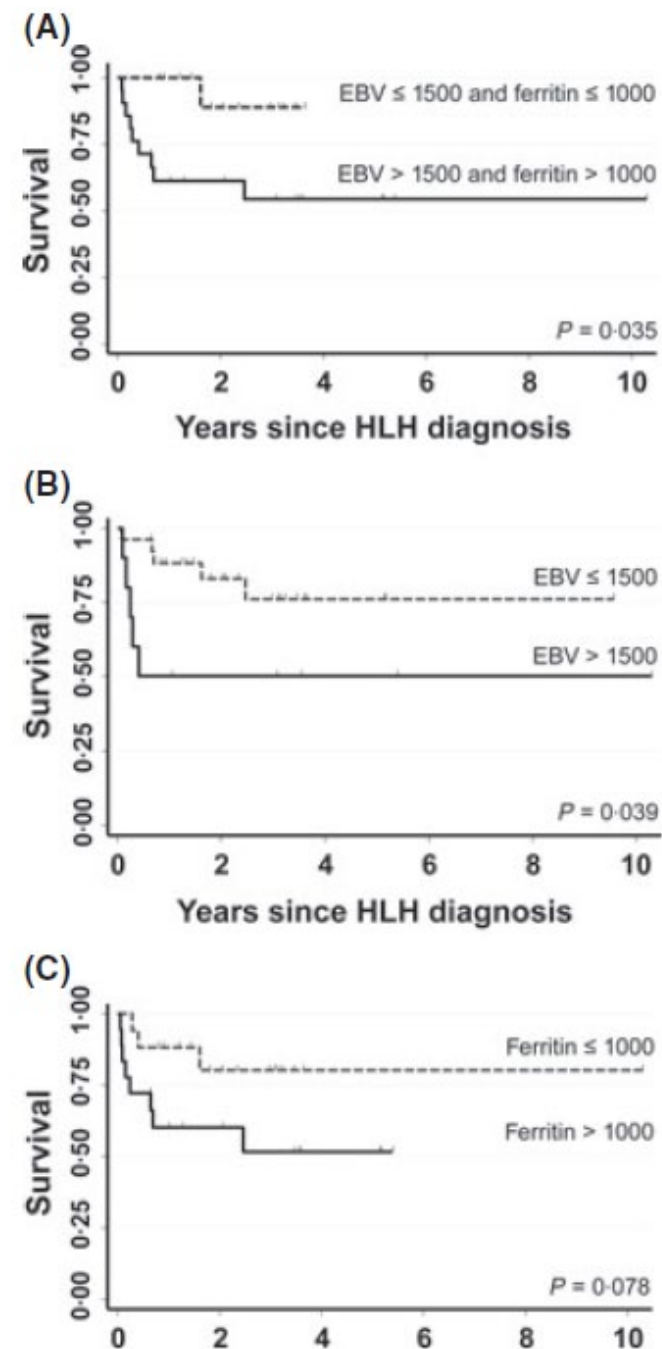
Courtesy Dr Kenichi Sakamoto, Principal Investigator

Treatment of Epstein Barr virus-induced haemophagocytic lymphohistiocytosis with rituximab-containing chemo-immunotherapeutic regimens

Table II. Dosing and schedule of rituximab administration.

	Patients (n)	Median, range
Dosage information		
Dose (mg/m ²)	42	375 (175–412)
Days between diagnosis and first rituximab	42	13 (1–210)
<30 d	28	5.5 (1–28)
≥ 30 d	14	73.5 (30–210)
Number of doses*	42	3 (1–10)
	Patients (n)	% of total cohort
Schedule		
Weekly	26	62
Monthly	1	2
Other†	15	36
Other medications		
Chemotherapy	38	90
Steroids	41	98
IVIG	33	79
Ciclosporin	32	76
SCT	20	48
Antiviral therapies		
Acyclovir	13	31
Ganciclovir	14	33
Other‡	7	17

Patients received 3 rituximab infusions (range 1–10) at a median dose of 375 mg/m²



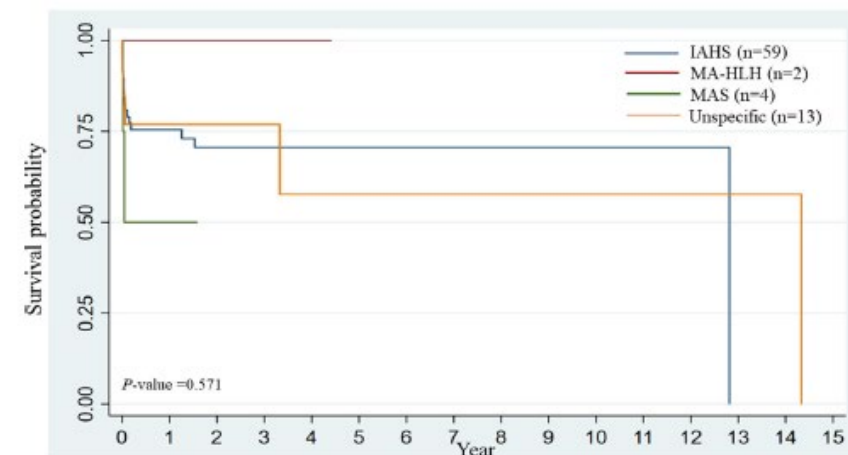
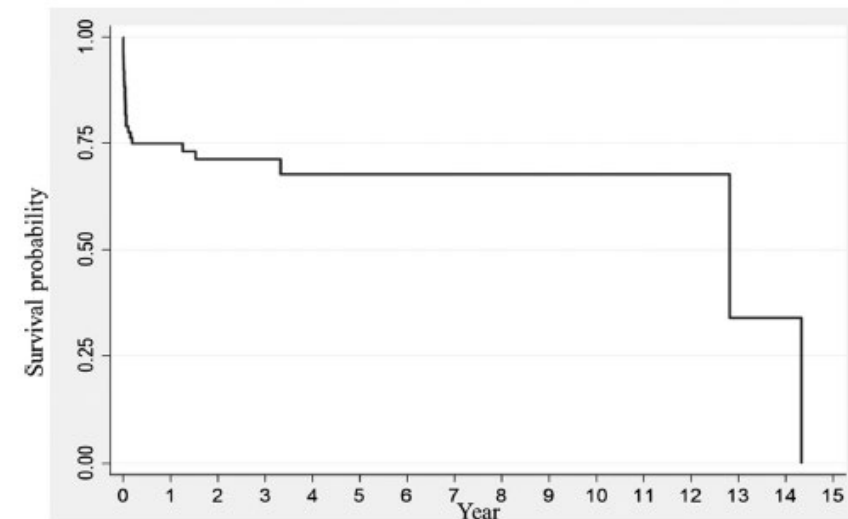


Pediatric hemophagocytic lymphohistiocytosis in a tropical country: Results of a multicenter study in Thailand

Nattapol Rungrojjananon^{1,2} | Samart Pakakasama³ | Angkana Winaichatsak⁴ |
Rapee Siriwanawong⁴ | Piya Rujkijyanont⁵ | Chanchai Traivaree⁵ |
Apichat Photia⁵ | Chalinee Monsereenusorn⁵

TABLE 4 Influential factors associated with outcomes in pediatric HLH patients (n = 78)

	Alive (n = 54)	Expired (n = 24)	Crude			Adjusted		
	N (%)	N (%)	HR	95% CI	p-value	HR	95% CI	p-value
Duration from onset of disease to diagnosis (days)	3 (0–30)	3 (0–42)	1.037	.999–1.076	.055	.999	.939–1.063	.980
Cytopenia								
Pancytopenia	13 (72.2)	5 (27.8)	1			1		
Bicytopenia	30 (66.7)	15 (33.3)	1.107	.394–3.106	.847	2.036	.486–8.52	.330
Hemoglobin (g/dl)	8.55 (6.3–11.5)	8.3 (5.8–11)	.770	.542–1.093	.144	.626	.368–1.061	.082
ANC (cells/mm ³)	1,082 (0–28,116)	1192 (0–12,985)	1.000	1.000–1.000	.467	1.000	.999–1.000	.350
Platelets (cells/mm ³)								
<50,000	18 (54.5)	15 (45.5)	2.975	1.258–7.038	.013	3.593	1.091–11.839	.035
≥50,000	36 (80)	9 (20)	1			1		
DB (mg/dl)	.5 (0–8.6)	2.1 (.1–23.5)	1.089	1.015–1.168	.018	1.059	.940–1.192	.344



Outcome and Prognostic Factors of Hemophagocytic Lymphohistiocytosis in Children: Experience From a Low- and Middle-Income Country

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Benish Hira ¹, Abdul Wahab Siddique ¹, Shakeel Ahmed ¹, Ayesha Latif ¹, Rabiha Manzoor ¹, Tariq Ghafoor ², Awaish Arshed ¹

¹. Department of Paediatric Oncology, Combined Military Hospital, Rawalpindi, PAK ². Armed Forces Bone Marrow Transplant Centre, Department of Paediatrics, Combined Military Hospital, Rawalpindi, PAK

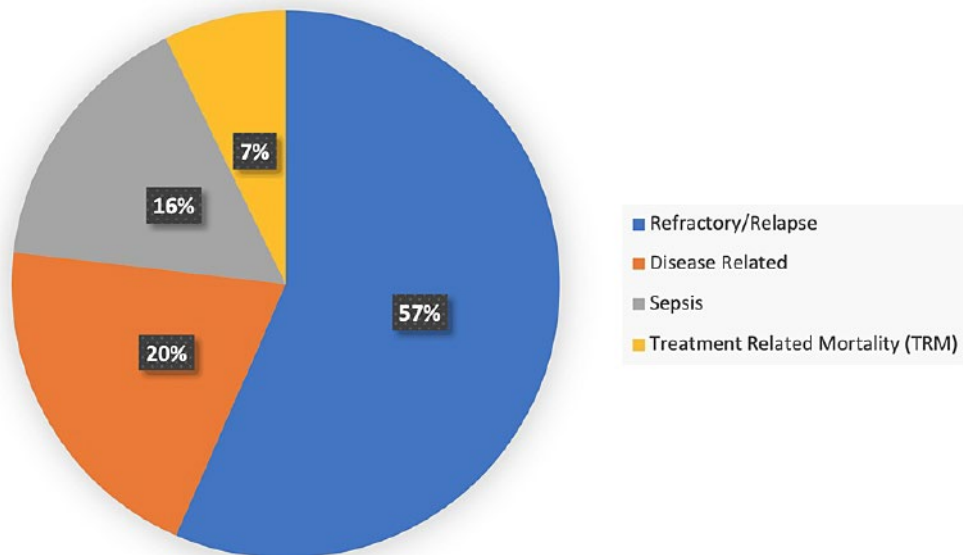
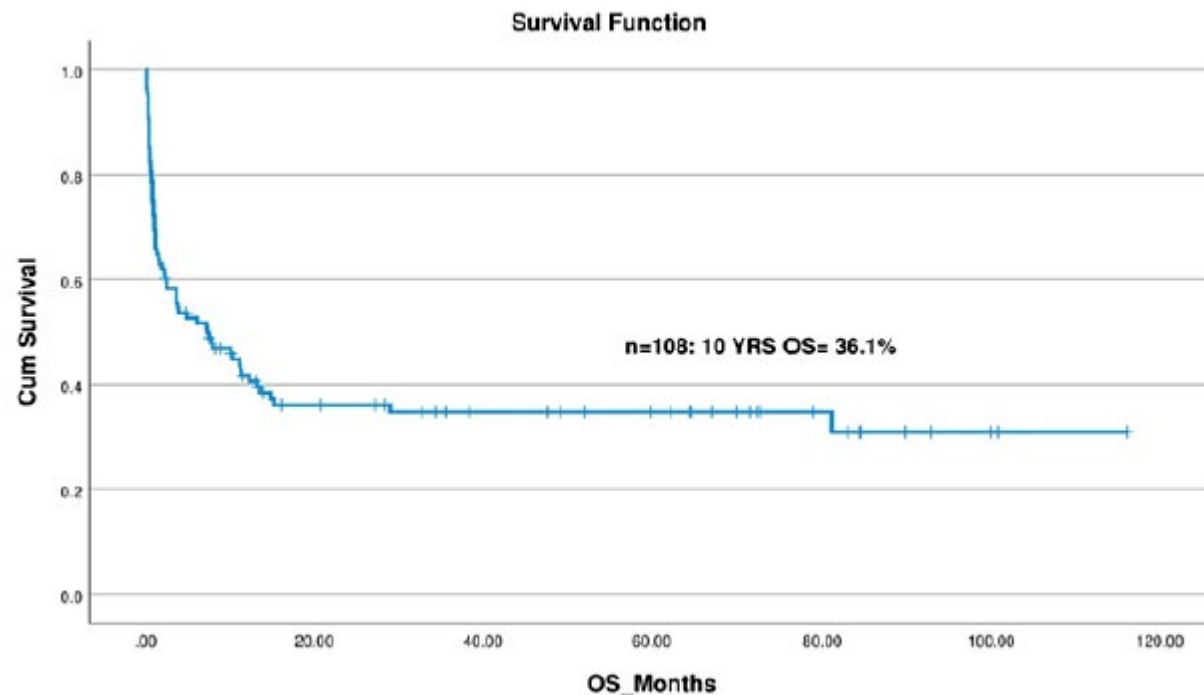


FIGURE 1: Mortality distribution of cases (n = 69)





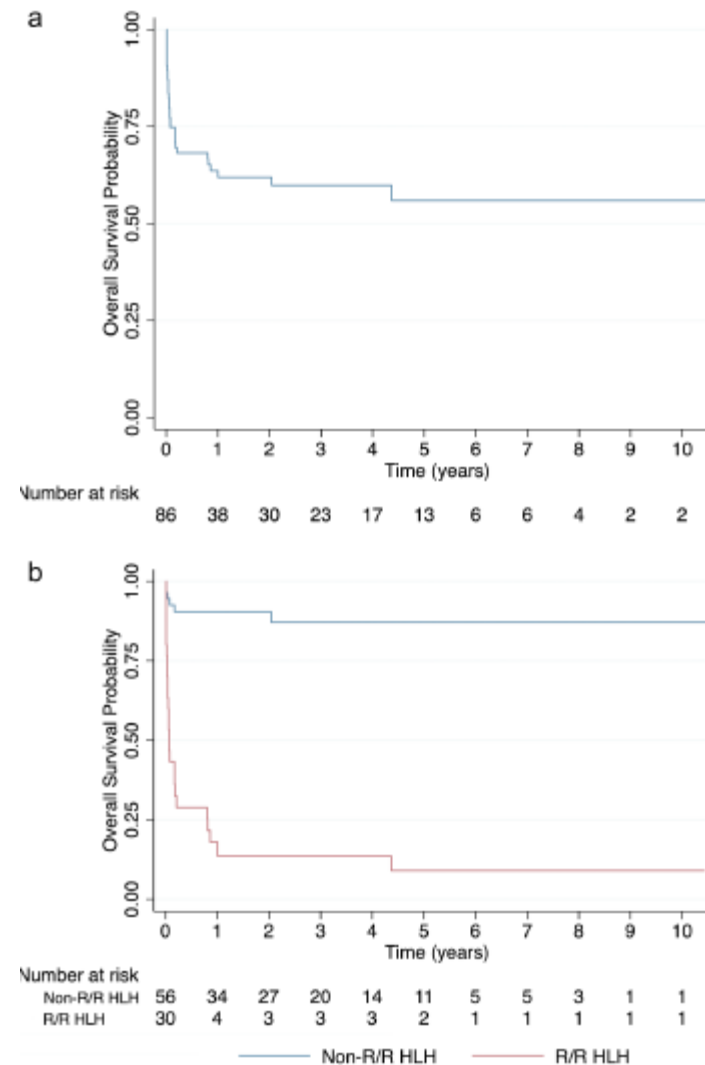
Predicting relapsed/refractory disease in childhood hemophagocytic lymphohistiocytosis based on clinical features at diagnosis: A 13-year single-institute retrospective study in Thailand

Pattranan Kusontammarat¹ · Chane Choed-Amphai² · Lalita Sathitsamitphong² · Watchareewan Sontichai³ · Rungrote Natesirinilkul² · Pimlak Charoenkwan^{2,4}

Table 3 Univariable and multivariable logistic regression analysis of predictors for R/R HLH

Variables	Univariable			Multivariable		
	OR	95%CI	p-value	Adjusted OR	95%CI	p-value
Age < 3 years	4.95	1.86–13.14	0.001	3.62	1.04–12.63	0.044
Severe disease	6.4	2.33–17.58	< 0.001	1.32	0.23–7.51	0.753
HLH-2004 criteria score ≥ 6	2.73	1.08–6.92	0.034	0.84	0.21–3.37	0.811
H-score ≥ 251	3.75	1.48–9.53	0.005	2.5	0.64–9.72	0.188
pSOFA score ≥ 8	12	4.13–34.85	< 0.001	6.35	1.18–34.19	0.032
Overt DIC	3.43	1.34–8.76	0.01	1.98	0.56–6.97	0.285
Na < 135 mmol/L	1.61	0.65–3.99	0.306	1.12	0.32–3.96	0.858
AST ≥ 175 U/L	3.11	1.24–7.82	0.016	0.84	0.23–3.07	0.795
TB ≥ 1.2 mg/dL	4.5	1.74–11.62	0.002	3.12	0.48–20.34	0.234
DB ≥ 0.8 mg/dL	3.75	1.48–9.53	0.005	0.41	0.06–2.77	0.363

- 30/86 (34.9%) relapse/refractory HLH
- Children with HLH who have a pSOFA score of ≥ 8 , or are <3 years, had a higher risk of relapsed or refractory disease



Summary

- HLH is a syndrome of pathologic immune activation, caused by familial or a sporadic condition, in association with a variety of triggers, with a high mortality rate
- MCM cause of HLH is IAHS of which EBV-HLH is the MCM
- HLH-2004 criteria (without NK-cell function) have significant validity in their current form when tested against severe infections or SoJIA
- Prompt initiation of immunochemotherapy is essential for survival
- Relapse and refractory diseases remain challenging



for
children with cancer