HỘI CHỨNG THỰC BÀO MÁU.

(HEMOPHAGOCYTIC LYMPHOHISTOCYTOSIS)

INTRODUTION.

- An aggressive and life-threatening syndrome of excessive immune activation.
- Was described in 1952.
- Most frequently affects infants

(from birth to 18 months of age)

but also observed in children and adults of all ages.

- Familial or sporadic disorder.

EPIDEMIOLOGY

- 1/50,000 live births.
- 1/3000 inpatient admissions to tertiary care hospitals.
- Infants are most commonly affected

the highest incidence in those <3 months

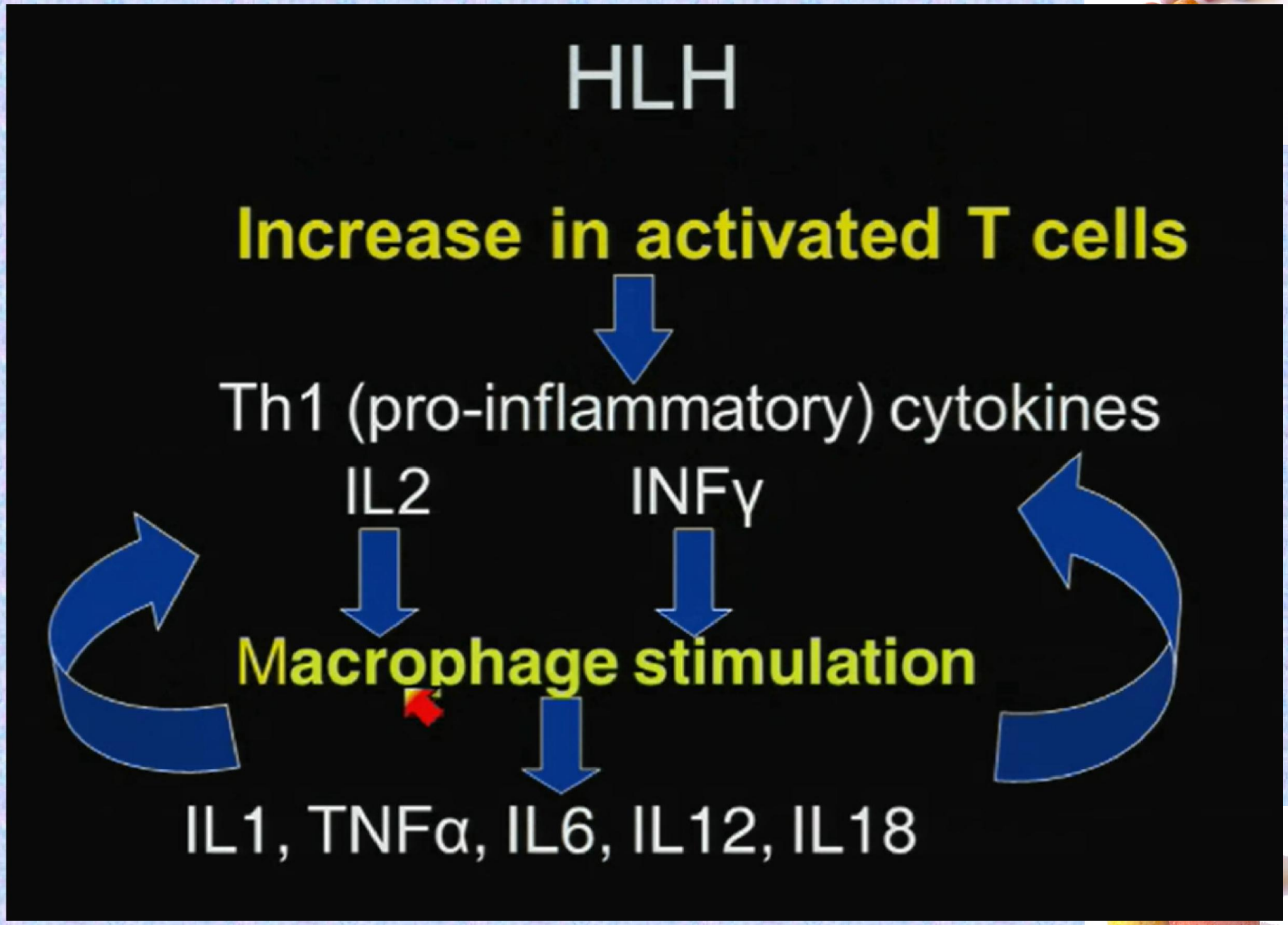
- Male:Female is 1:1 .
- 25 % of HLH cases are familial.
- Mutations in *STX11*, *PRF1*, and *UNC13D* were found in 20, 1.8, and 10 percent of affected individuals.

What is HLH?

A hyper-inflammatory condition

↓

Severe, life-threatening organ damage


HLH
Increase in activated T cells
Th1 (pro-inflammatory) cytokines
IL2
INFγ
Macrophage stimulation
IL1, TNFα, IL6, IL12, IL18

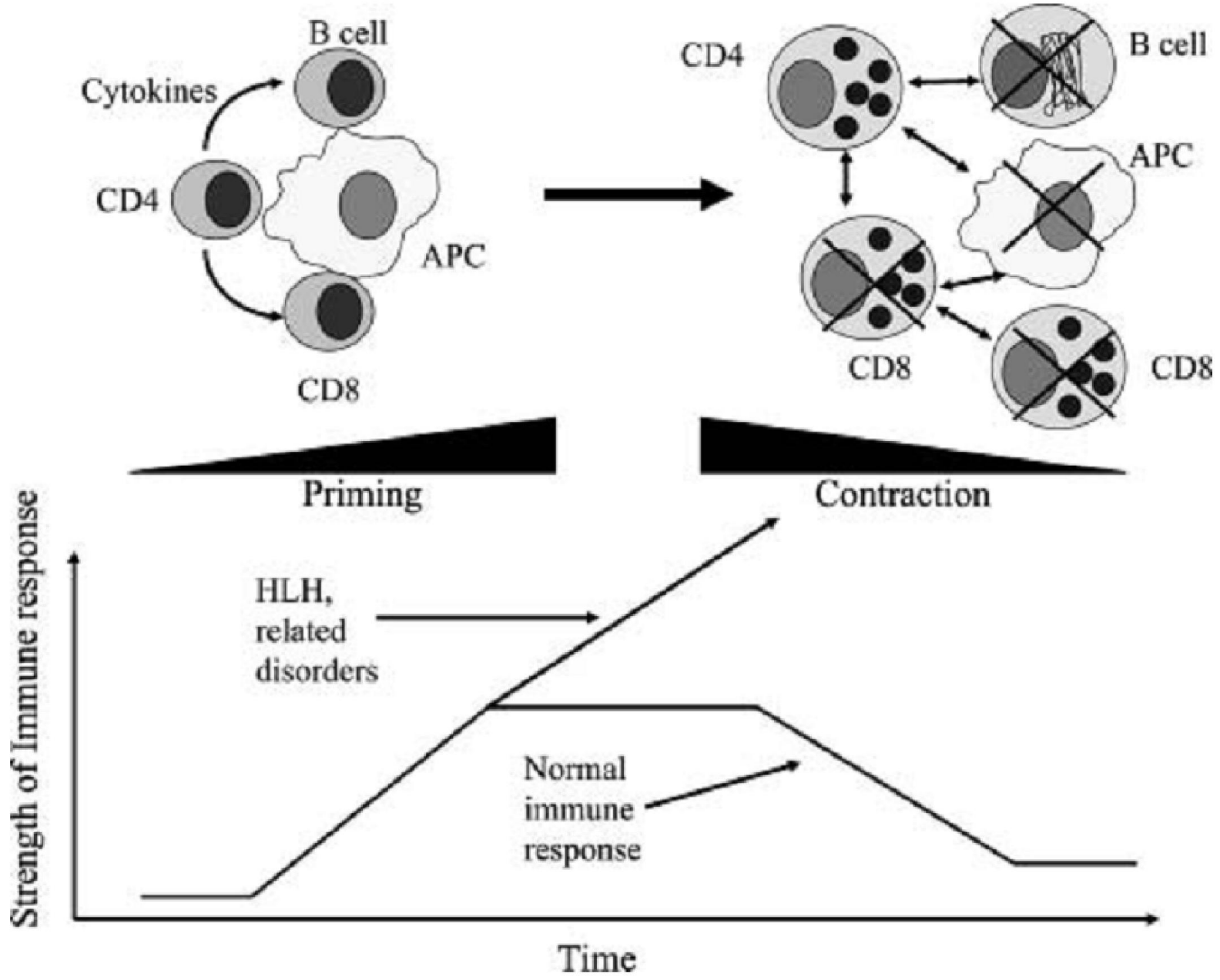
HLH -2 mechanisms suggested

- Failure of CTLs and NK cells to **clear** pathogen

↓

prolonged antigen presentation- excessive activation of lymphocytes and macrophages

- Failure to **terminate** the immune response


B cell
Cytokines
CD4
APC
CD8
CD4
B cell
APC
CD8
CD8
Priming
Contraction
Strength of Immune response
HLH,
related
disorders
Normal
immune
response
Time

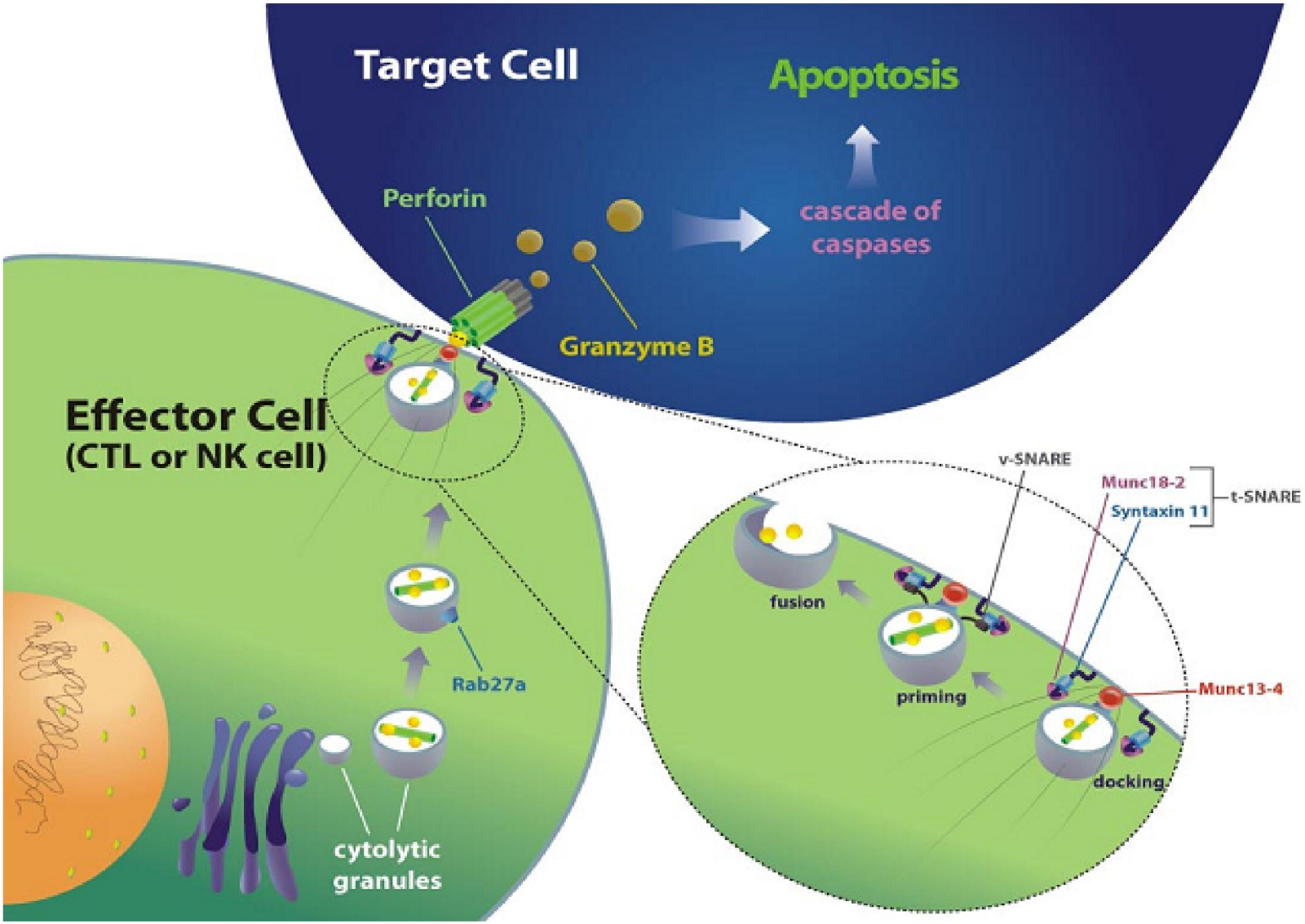


Figure 1. Mechanics of cytotoxic function revealed by HLH-associated gene mutations. HLH-associated genetic abnormalities (in the indicated genes) may affect granule-dependent lymphocyte cytotoxicity by impairing trafficking, docking, priming for exocytosis, or membrane fusion of cytolytic granules. The function of this pathway may also be severely impaired by loss of functional perforin, the key delivery molecule for proapoptotic granzymes. Diverse mutations in this pathway all give rise to similar clinical phenotypes (albeit of variable severity). *Lyst* (the gene affected in Chediak-Higashi syndrome) is not portrayed because its function is not entirely clear, although it appears to play an important role in the maintenance of normally sized (and functional) cytolytic granules.

TABLE 2. DIAGNOSTIC GUIDELINES FOR HLH-2004 (revision of ref 6)

The diagnosis HLH can be established if one of either 1 or 2 below is fulfilled.

1. A molecular diagnosis consistent with HLH.

2. Diagnostic criteria for HLH fulfilled (5 out of the 8 criteria below).

A) Initial diagnostic criteria (to be evaluated in all patients with HLH).

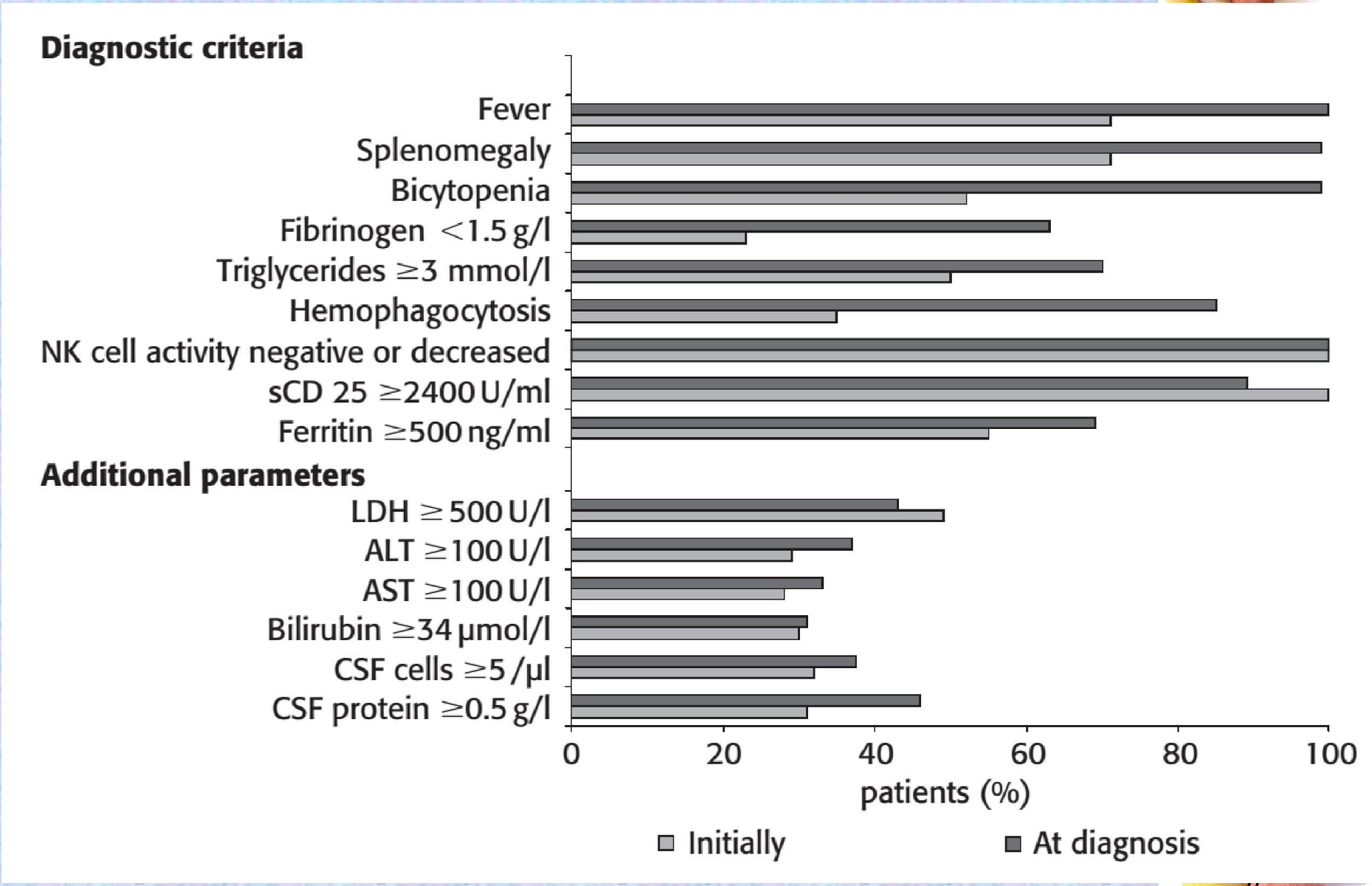
Clinical criteria
* Fever
* Splenomegaly

Laboratory criteria
* Cytopenias (affecting ≥ 2 of 3 lineages in the peripheral blood:
  Hemoglobin (<90 g/L), Platelets (<100 x 10^9/L), Neutrophils (<1.0 x 10^9/L)
  (In infants <4 weeks: Hemoglobin <100 g/L)
* Hypertriglyceridemia and/or hypofibrinogenemia
  (fasting triglycerides ≥3.0 mmol/L (i e ≥265 mg/dL), fibrinogen ≤1.5 g/L)

Histopathologic criteria
* Hemophagocytosis in bone marrow or spleen or lymph nodes.
  No evidence of malignancy

B) New diagnostic criteria.

* Low or absent NK-cell activity (according to local laboratory reference)
* Ferritin ≥500 microgram/L
* Soluble CD25 (i.e. soluble IL-2 receptor) ≥2400 U/ml


Diagnostic criteria
Fever
Splenomegaly
Bicytopenia
Fibrinogen <1.5 g/l
Triglycerides ≥3 mmol/l
Hemophagocytosis
NK cell activity negative or decreased
sCD 25 ≥2400 U/ml
Ferritin ≥500 ng/ml
Additional parameters
LDH ≥500 U/l
ALT ≥100 U/l
AST ≥100 U/l
Bilirubin ≥34 µmol/l
CSF cells ≥5 /µl
CSF protein ≥0.5 g/l
0
20
40
60
80
100
patients (%)
Initially
At diagnosis

CLINICAL PRESENTATION

- Likely to be diagnosed more often in infants
- Clinical condition is not severely impaired initially
- Anemia/thrombocytopenia is usually present
- Patients may improve with non-specific therapies such as transfusions or antibiotics, but responses are usually

CLINICAL PRESENTATION

- Fever is uncommon in neonates
- HSV/EV
- Can be easily missed and mismanaged as sepsis

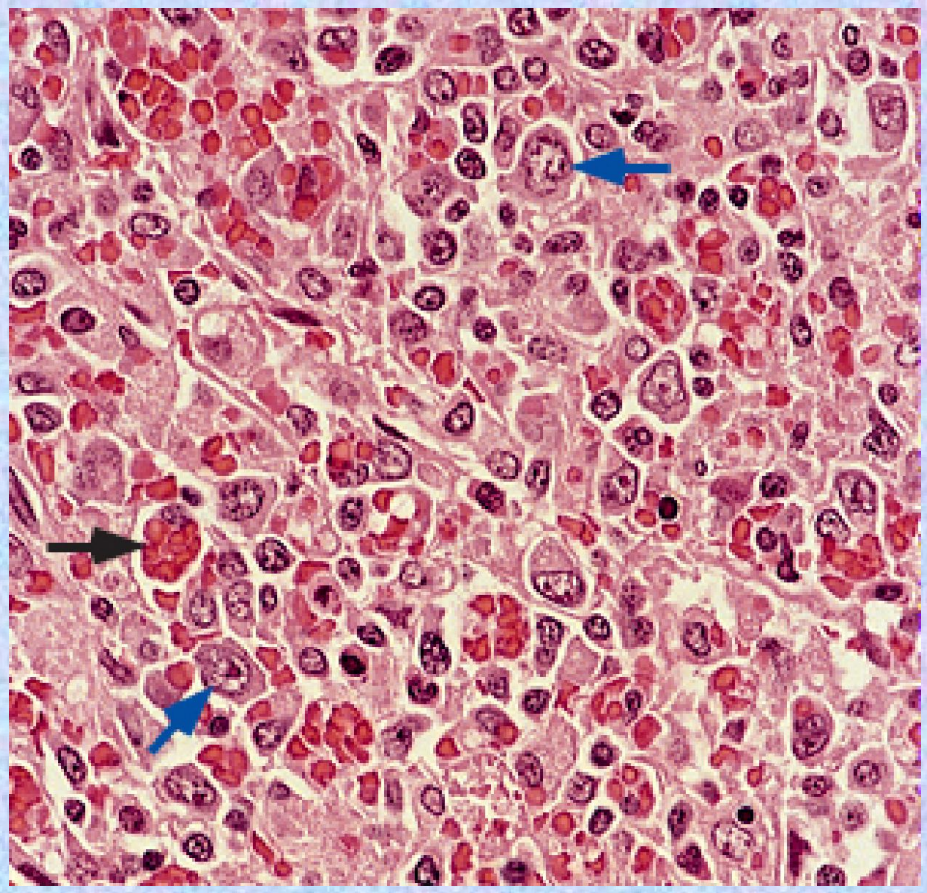
CLINICAL PRESENTATION

- Neurologic involvement is seen in 30-50%
- Seizures, altered mental status, brain stem symptoms, and ataxia
- Atypical presentation:
  - Colitis
  - Bleeding disorder
  - Hypogammaglobulinemia

NATURAL HISTORY

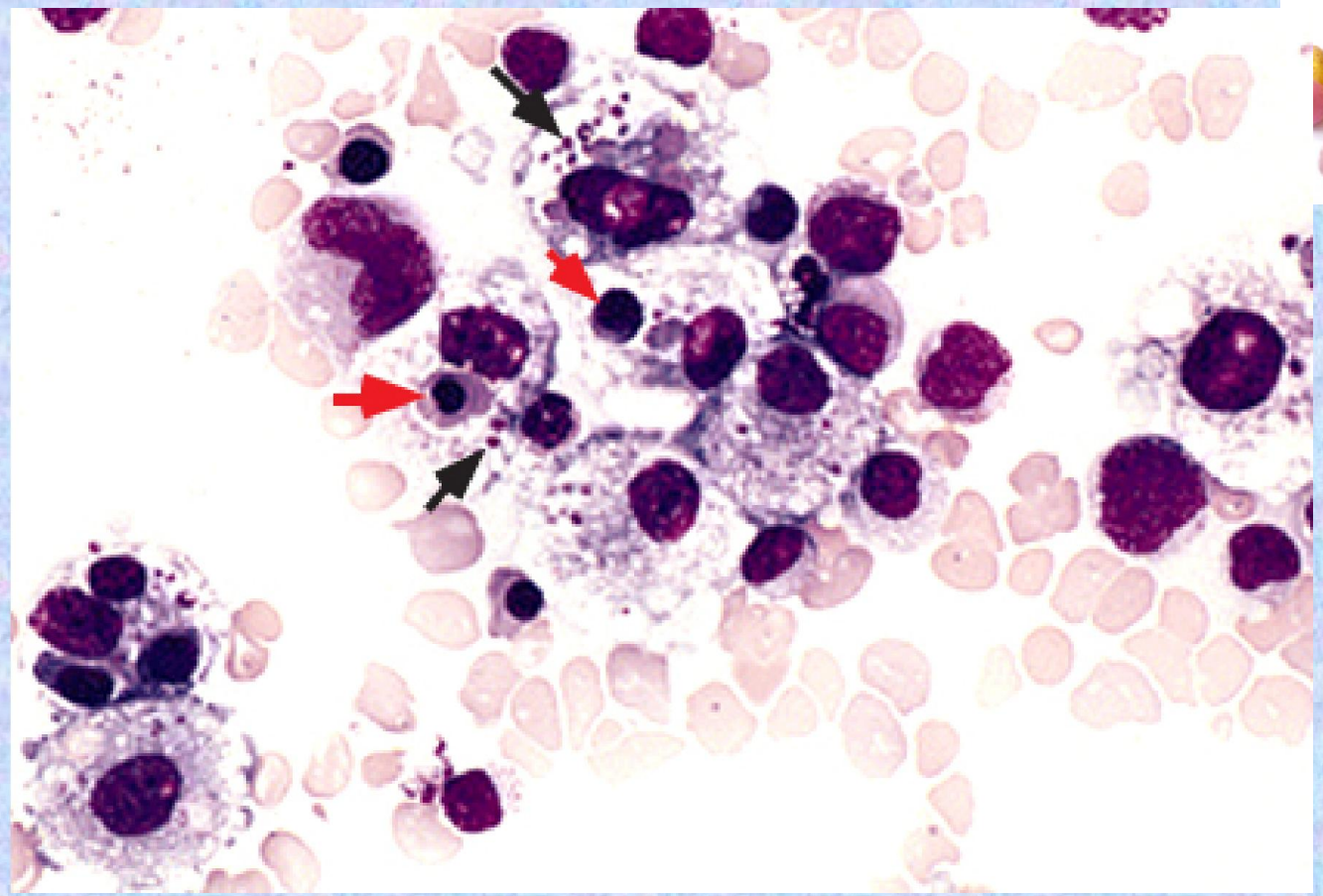
	Year reported	# patients	Survival
Janka review	1983	121	5% (1 yr)
Arico et al	1996	122	22% (5 yr)
HLH-94	2002	113	55% (5 yr)
HLH-94 (familial)	2002	25	51% (5 yr)

Henter et al (2002) Blood



Large T cell lymphoma with reactive hemophagocytosis

The red pulp of the spleen is diffusely permeated by large lymphoma cells (T lineage, blue arrows) and histiocytes showing erythrophagocytosis (black arrow). The histiocytes have smaller, bland-looking nuclei with delicate chromatin.



Infection-associated hemophagocytic syndrome

Bone marrow from a child with hemophagocytic syndrome, secondary to Epstein-Barr virus infection. Reactive histiocytes show phagocytosis of nucleated red blood cells (red arrows) and platelets (black arrows). Wright-Giemsa stain.

#

EBV - HLH

Original article

Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis: a retrospective study of 78 pediatric cases in mainland of China

JIN Ying-kang, XIE Zheng-de, YANG Shuang, LU Gen and SHEN Kun-ling

Keywords: *Epstein-Barr virus; hemophagocytic lymphohistiocytosis; risk factors*

Background The clinical characteristics of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) are largely unreported in the pediatric patients in mainland of China. The main aim of this study was to recognize the clinical features of EBV-HLH in children and to explore its prognosis and risk factors.

Methods A retrospective study was performed on 78 pediatric patients with EBV-HLH who were admitted to Beijing Children's Hospital between 2003 and 2008. All patients' medical records were reviewed and analyzed. For each patient, demographic, clinical, laboratory and outcome information was collected. Statistical analysis was conducted via multivariate and univariate analysis.

EBV - HLH

- 78 pts
- No genetic testing was done
- Group 1: 33 received (gancyclovir or/and interferon) and immunotherapy (steroid and IVIG)
- Group 2: 45 received HLH 94 protocol
- Treatment group was decided by physician

Table 3. Univariate analysis of factors related with EBV-HLH fatality

Factors †	χ^2 value	*P* values
Age (years) <2 (24 of 43) vs. ≥2 (14 of 24)	0.40	0.842
Time prior to diagnosis (weeks) ≥4 (6 of 19) vs. <4 (22 of 48)*	8.166	0.004
Chemotherapy, no (21of 25) vs yes (17 of 42)*	12.093	0.001
Neutrophil (10^9/L) <0.5(13 of 21) vs. ≥0.5 (25 of 46)	0.335	0.562
Hemoglobin (g/L) <60 (12 of 12) vs. ≥60 (26 of 55)*	4.219	0.040
Platelet (10^9/L) <20 (15 of 20) vs. ≥20 (23 of 47)*	3.882	0.049
ALT (IU/L) ≥100 (21 of 39) vs. <100 (17 of 28)	0.313	0.576
LDH (IU/L) ≥ 2000 (16 of 23) vs. <2000 (22 of 44)	2.355	0.125
TB (mmol/L) ≥34 (17 of 28) vs. <34 (21 of 39)	0.004	0.469
TG (mmol/L) ≥3 (28 of 44) vs. <3 (10 of 23)	2.500	0.114
FIB (g/L) <0.5 (20 of 28) vs. ≥0.5 (18 of 39)*	4.241	0.039
Albumin (g/L) <20 (17 of 23) vs. ≥20 (21 of 44)*	4.219	0.040
Ferritin (μg/L) ≥3000 (8 of 15) vs. <3000 (18 of 33)	0.006	0.938

*Factor is considered statistically significant (*P* <0.05). †Numbers in parenthesis represent the deceased of total patients.

EBV - HLH

- Conclusion
  - EBV-specific therapy using aciclovir or ganciclovir did not improve the survival rate and neither did conventional immunomodulatory therapy (corticosteroids and immunoglobin infusion)
  - Chemotherapy (HLH-94 or HLH-04) yielded better results than non-chemotherapy treatments (40% vs 84%)
  - The high fatality of our patients in the chemotherapy group can be attributed to the lack of early etoposide-based chemotherapy, as well as delayed diagnosis

EBV - HLH

Hemophagocytic Syndromes and Infection

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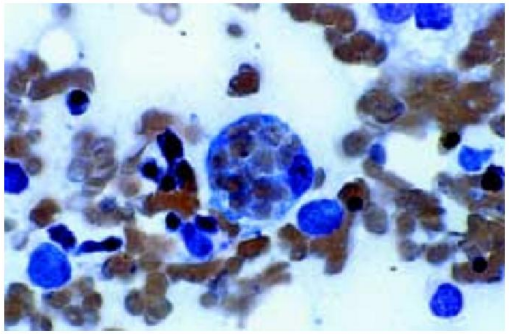
Hemophagocytic lymphohistiocytosis (HLH) is an unusual syndrome characterized by fever, splenomegaly, jaundice, and the pathologic finding of hemophagocytosis (phagocytosis by macrophages of erythrocytes, leukocytes, platelets, and their precursors) in bone marrow and other tissues. HLH may be diagnosed in association with malignant, genetic, or autoimmune diseases but is also prominently linked with Epstein-Barr (EBV) virus infection. Hyperproduction of cytokines, including interferon-γ and tumor necrosis factor-α, by EBV-infected T lymphocytes may play a role in the pathogenesis of HLH. EBV-associated HLH may mimic T-cell lymphoma and is treated with cytotoxic chemotherapy, while hemophagocytic syndromes associated with nonviral pathogens often respond to treatment of the underlying infection.

The term hemophagocytosis describes the pathologic finding of activated macrophages, engulfing erythrocytes, leukocytes, platelets, and their precursor cells (Figure 1) (1). This phenomenon is an important finding in patients with hemophagocytic syndrome, more properly referred to as hemophagocytic lymphohistiocytosis (HLH) (2). HLH is a distinct clinical entity characterized by fever, pancytopenia, splenomegaly, and hemophagocytosis in bone marrow, liver, or lymph nodes. The syndrome, which has also been referred to as histiocytic medullary reticulosis, was first described in 1939 (3). HLH



EBV - HLH

- Acyclovir does not appear to be useful in the treatment of EBV-associated HLH*
- Adenovirus – vidarabine
- HHV-8 -- foscarnet

* CDC website – Hemophagocytic syndromes and infection

Requirement for Etoposide in the Treatment of Epstein-Barr Virus–Associated Hemophagocytic Lymphohistiocytosis

By Shinsaku Imashuku, Kikuko Kuriyama, Tomoko Teramura, Eiichi Ishii, Naoko Kinugawa, Masahiko Kato, Masahiro Sako, and Shigeyoshi Hibi

Purpose: **We sought to identify the clinical variables most critical to successful treatment of Epstein-Barr virus (EBV)–associated hemophagocytic lymphohistiocytosis (HLH).**

Patients and Methods: **Among the factors tested were age at diagnosis (< 2 years or ≥ 2 years), time from diagnosis to initiation of treatment with or without etoposide-containing regimens, timing of cyclosporin A (CSA) administration during induction therapy, and the presence or absence of etoposide.**

Results: **By Kaplan-Meier analysis, the overall survival rate for the entire cohort of 47 patients, most of whom had moderately severe to severe disease, was 78.3% ± 6.7% (SE) at 4 years. The probability of long-term survival was significantly higher when etoposide treatment was begun less than 4 weeks from diagnosis (90.2% ± 6.9% *v* 56.5% ± 12.6% for patients receiving this agent later or not at all; $P < .01$, log-rank test). Multivariate analysis with the Cox proportional hazards model demonstrated the independent prognostic significance of a short interval from EBV-HLH diagnosis to etoposide administration (relative risk of death for patients lacking this feature, 14.1; 95% confidence interval, 1.16 to 166.7; $P = .04$). None of the competing variables analyzed had significant predictive strength in the Cox model. However, concomitant use of CSA with etoposide in a subset of patients appears to have prevented serious complications from neutropenia during the first year of treatment.**

Conclusion: **We conclude that early administration of etoposide, preferably with CSA, is the treatment of choice for patients with EBV-HLH.**

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- 47 Patients with EBV-HLH
- **Group 1**: 21 pts were treated first with steroid alone/IVIG alone/CSA alone/combination
  - →17 pts were switched to etoposide containing regimens
  - →4 did not receive etoposide (one recovered, 3 died)
- **Group 2**: 26 pts were treated with etoposide containing regimens

Table 1. Characteristics of the Patient Population and Treatment Results*

Feature	No. of Patients		P†
	Group 1 (N = 21)	Group 2 (N = 26)	
Age, years			.325
Median	5.2	4.7	
Range	0.8-31	0.6-17	
Sex			.738
Male	10	10	
Female	11	16	
Underlying disease			.99
None	20	21	
XLP/FHL	1	1	
Neutropenia			.166
< 500 ANC/μL	12	8	
500-1,000 ANC/μL	5	12	
> 1,000 ANC/μL	4	6	
LDH			.387
< 2,000 IU/L	6	6	
2,000-5,000 IU/L	8	7	
> 5,000 IU/L	6	13	
Ferritin			.333
< 3,000 ng/mL	3	4	
3,000-10,000 ng/mL	9	6	
> 10,000 ng/mL	9	16	
Total bilirubin			.303
< 2.0 mg/dL	12	9	
2.0-10.0 mg/dL	8	15	
> 10.0 mg/dL	1	2	
C-reactive protein			.002
< 1.0 mg/dL	10	0	
1.0-10.0 mg/dL	7	12	
> 10.0 mg/dL	4	7	
Karyotype of bone marrow cells			.859
Abnormal	3	2	
Normal	10	13	
Weeks from diagnosis to initial therapy			.113
< 1	5	14	
1-4	12	9	
> 4	4	3	
Treatment with an etoposide-containing regimen	17	26	.072
Weeks from diagnosis to etoposide therapy			.002
< 4	7	23	
≥ 4	10	3	
Weeks from diagnosis to CSA therapy‡			.364
< 4	6(2)	8(8)	
≥ 4	4	1	
Treatment with ET, PE, PP, or HD	5	10	.449
Off treatment within 2 months	4	9	.330
Maintenance with CSA required	12	13	.846
Hemopoietic stem cell transplantation required	2	4	.99
Kaplan-Meier survival estimates (mean ± SD) at 4 years after diagnosis, %	68.5 ± 10.9	85.7 ± 8.0	.112
Deaths from acute complications	4	3	.459

Table 2. Initial Treatment of Patients (group 1) Subsequently Switched to an Etoposide-Based Regimen

Case No.*	Treatment	Start Time After Diagnosis (weeks)	Duration (weeks)	Reason for Switch to Etoposide	Time to Etoposide After Diagnosis (weeks)
1	PE/PSL/IVIG	1	3	PD	4
4	mPSL	2	2	PD	4
5	PSL/IVIG	1	2	PD	3
7	PSL, mPSL pulse	3	4	PF	7
8	PSL/IVIG	1	2	RF	3
9	CSA	1	2	PF	3
10	Dex/CSA	2	1	PF	3
11	CSA	1.4	0.7	NR	1.9
12	ET/PE/IVIG/Dex	4	0.4	PD	4.4
13	PP/IVIG/PSL	4	1	PD	5
14	PSL/IVIG	3	1	NR	4
15	IVIG/PSL	2	2	RF	4
16	Dex	3	1	NR	4
17	PSL/IVIG	4	2	PF	6
18	PSL/IVIG, Dex/CSA	2	3	PD	5
19	IVIG/PSL, mPSL pulse	2	1.5	NR	3.5
20	PE/PSL	3	1	PD	4

Abbreviations: PE, plasma exchange; PSL, prednisolone; IVIG, intravenous immunoglobulin; Dex, dexamethasone; mPSL, methylprednisolone; CSA, cyclosporin A; ET, exchange transfusion; PP, plasmapheresis; PD, progression of disease; PF, persistent fever; RF, reemergence of fever; NR, no response.

*Four cases in group 1 were excluded because of early death (three cases) or complete response (one case).

Table 3. Univariate and Multivariate Analyses of Overall Survival by Risk Factor

Variable*	Univariate Analysis† *P*	Multivariate Analysis‡ RR	95% CI	*P*
Age, years				
< 2 (13 of 14) *v* ≥ 2 (24 of 33)†	.15	3.93	0.41-37.0	.23
Time from diagnosis to initial therapy				
Early (33 of 39) *v* late (4 of 8)	.01	0.98	0.21-4.70	.98
Time from diagnosis to etoposide				
Early (28 of 30) *v* late/no (9 of 17)	< .01	14.1	1.16-166.7	.04
Timing of CSA during induction therapy				
Early (12 of 14) *v* late/no (28 of 33)§	.32	2.74	0.62-12.1	.18
Timing of CSA in early etoposide group				
Early (10 of 10) *v* late/no (16 of 20)‖	.91	—	—	—
Timing of CSA for neutropenic patients				
Early (7 of 11) *v* late/no (17 of 22)	.49	—	—	—
Group 1 (15 of 21) *v* group 2 (22 of 26)	.11	2.00	0.25-15.9	.51

Abbreviations: CSA, cyclosporin A; RR, relative risk; CI, confidence interval.

*Numbers in parenthesis represent surviving/total patients.

†Log-rank analysis of Kaplan-Meier plots.

‡Cox proportional hazards model. RR refers to the risk of death associated with the first versus the second variable.

§Survival at 3 months.

‖Survival at 12 months.

Table 4. Comparison of Clinical Data Between the Two Groups Treated Early or Late/No With Etoposide (< 4 weeks or > 4 weeks/no from diagnosis)

Feature	No. of Patients: Early-Etoposide Group (N = 30)	Late/No-Etoposide Group* (N = 17)	P†
Age, years			.129
Median	4.7	4.5	
Range	0.8-16	0.6-31	
Sex			.448
Male	14	6	
Female	16	11	
Neutropenia			.364
< 500 ANC/μL	13	7	
500-1,000 ANC/μL	9	8	
> 1,000 ANC/μL	8	2	
LDH			.671
< 2,000 IU/L	8	6	
2,000-5,000 IU/L	9	5	
> 5,000 IU/L	13	5	
Ferritin			.194
< 3,000 ng/mL	7	6	
3,000-10,000 ng/mL	6	6	
> 10,000 ng/mL	17	5	
C-reactive protein			.425
< 1.0 mg/dL	4	6	
1.0-10.0 mg/dL	12	7	
> 10.0 mg/dL	5	6	
Weeks from diagnosis to CSA therapy			.056
< 4	10	4	
≥ 4	1	4	
Treatment with ET, PE, PP, or HD	7	8	.176
Off treatment within 2 months	8	5	.99
Maintenance with CSA required	19	6	.121
Hemopoietic stem cell transplantation required	4	2	.99
Kaplan-Meier survival estimates (mean ± SD) at 4-years after diagnosis, %	90.2 ± 6.9	56.5 ± 12.6	< .01
Deaths from acute complications	1	6	.011

Abbreviations: LDH, lactate dehydrogenase; ET, exchange transfusion; PE, plasma exchange; PP, plasmapheresis; HD, hemodialysis; ANC, absolute neutrophil count; CSA, cyclosporin A.

*Consists of patients with late etoposide (n = 13) and no etoposide (n = 4).

†All *P* values refer to comparisons between the two groups.

- Conclusion
  - 4 year survival 78% ± 6.7
  - The probability of long term survival was significantly higher when etoposide treatment was begun less than 4 weeks from diagnosis for patients receiving this agent later or not at all (90.2% ± 6.9% vs 56.5% ± 12.6%)
  - Multivariate analysis
    - Short interval from diagnosis to etoposide
    - RR 14

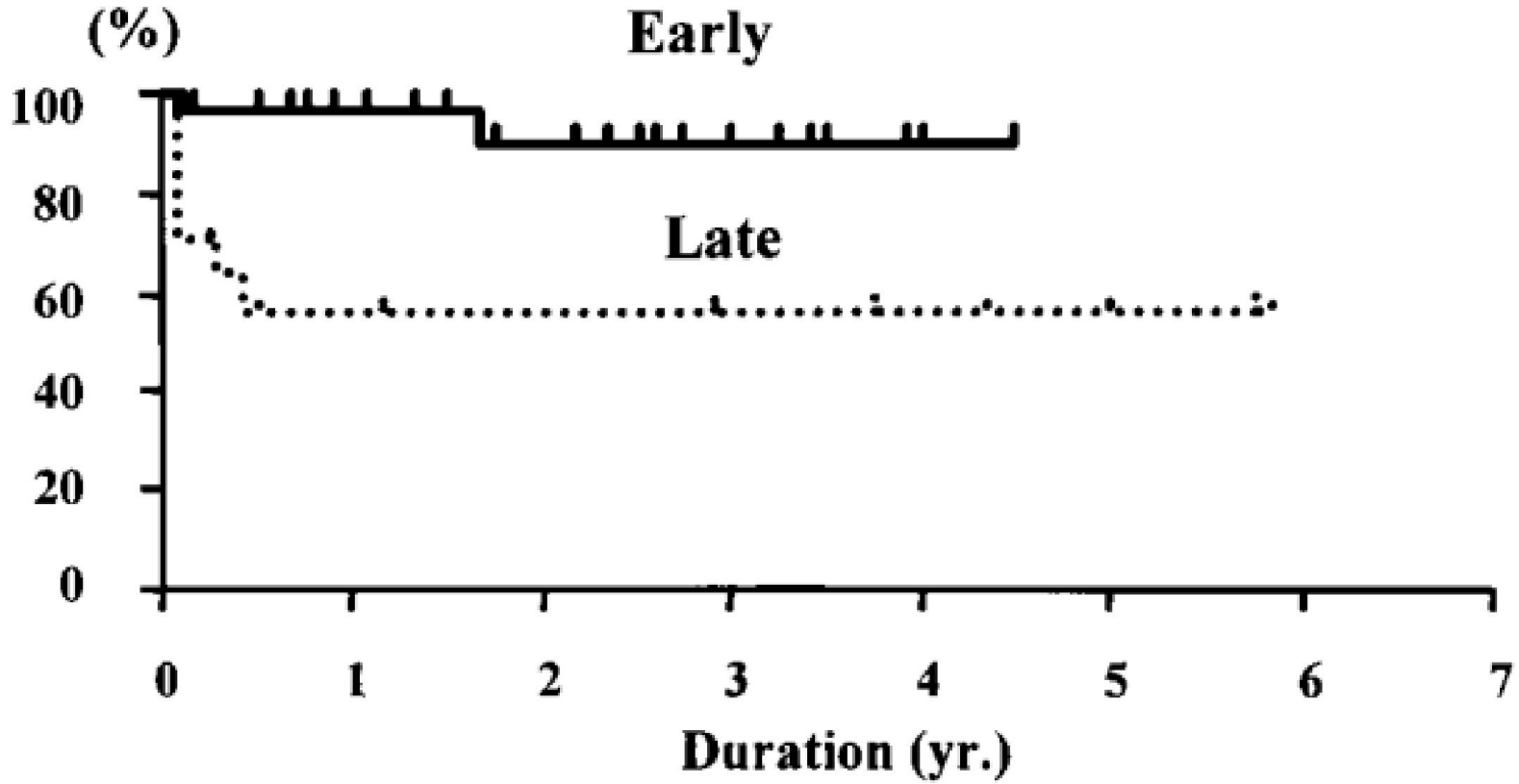

Fig 1. Kaplan-Meier analysis of survival times for patients receiving etoposide early (n = 30) versus late or not at all (n = 19). The difference between the curves is significant at the $P < .01$ level.

CONCLUSION.

- Hemophagocytic lymphohistiocytosis (HLH) is an aggressive and life-threatening syndrome of excessive immune activation. It is most common in infants and young children but can affect patients of any age, with or without a predisposing familial condition.
- The diagnosis of HLH is made by identifying a mutation in an HLH gene, or by fulfilling five of eight diagnostic criteria.
- Patients may improve with non-specific therapies such as transfusions or antibiotics, but responses are usually short-lived.
- Etoposide is important HLH drug.

THANK FOR YOUR ATTENTION.