Hemophagocytic Lymphohistiocytosis (HLH): Practical Approach to Diagnosis and Management

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Disclosures

- NIH Grant
- Clinical Trial Support from GlaxoSmithKline Company
- Own common stock in Johnson & Johnson Co.

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Goals

- Provide an understanding of what HLH is
- How do patients present?
- Diagnostic Criteria
- Associated signs and symptoms
- Biology and Genetics of HLH
- Treatment and management of HLH

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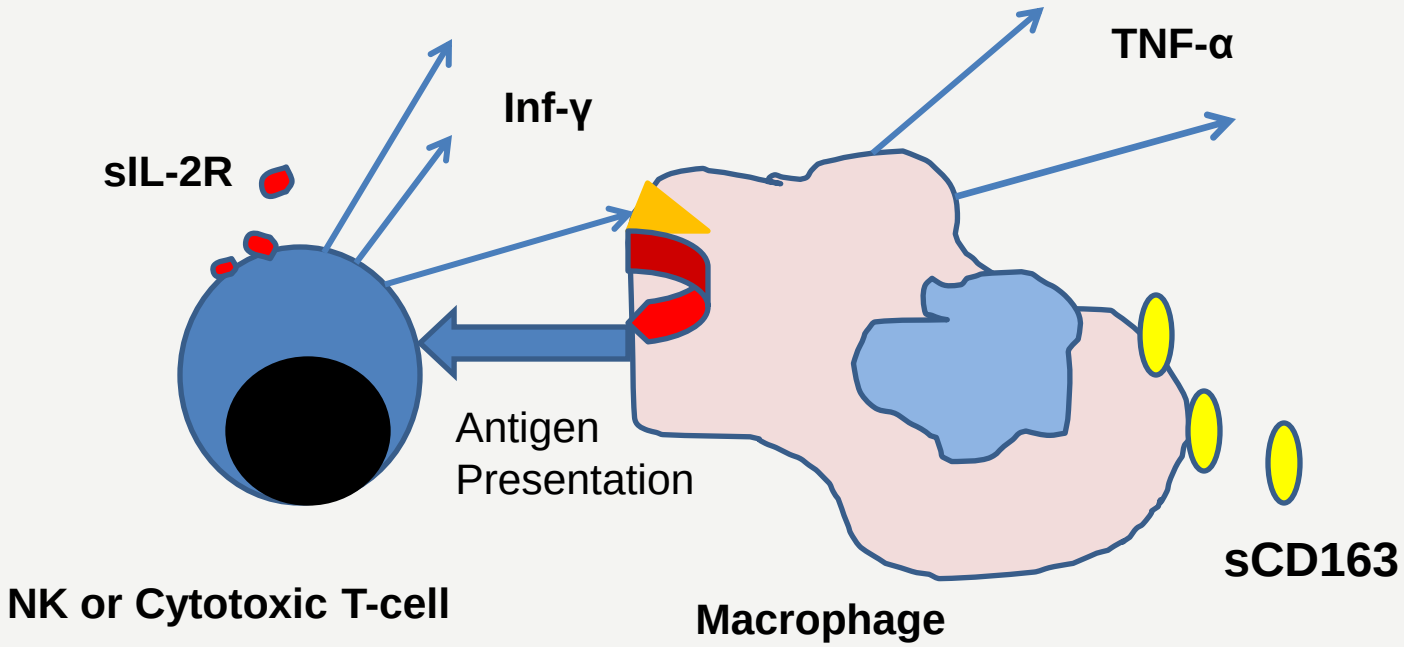
Epidemiology and Diagnosis of HLH

HLH Overview

- **Pathologic hyper-inflammation**
- **Familial HLH**
  - 1:50,000 live births – underestimate?
- **Usually associated with immune "trigger"**
  - EBV, CMV, HSV, VZV, sepsis, malignancy, autoimmune disease, immunizations
- **Inappropriate cytokine expression**
  - Highly elevated sIL-2R, TNFα, IL-6, Interferon-γ

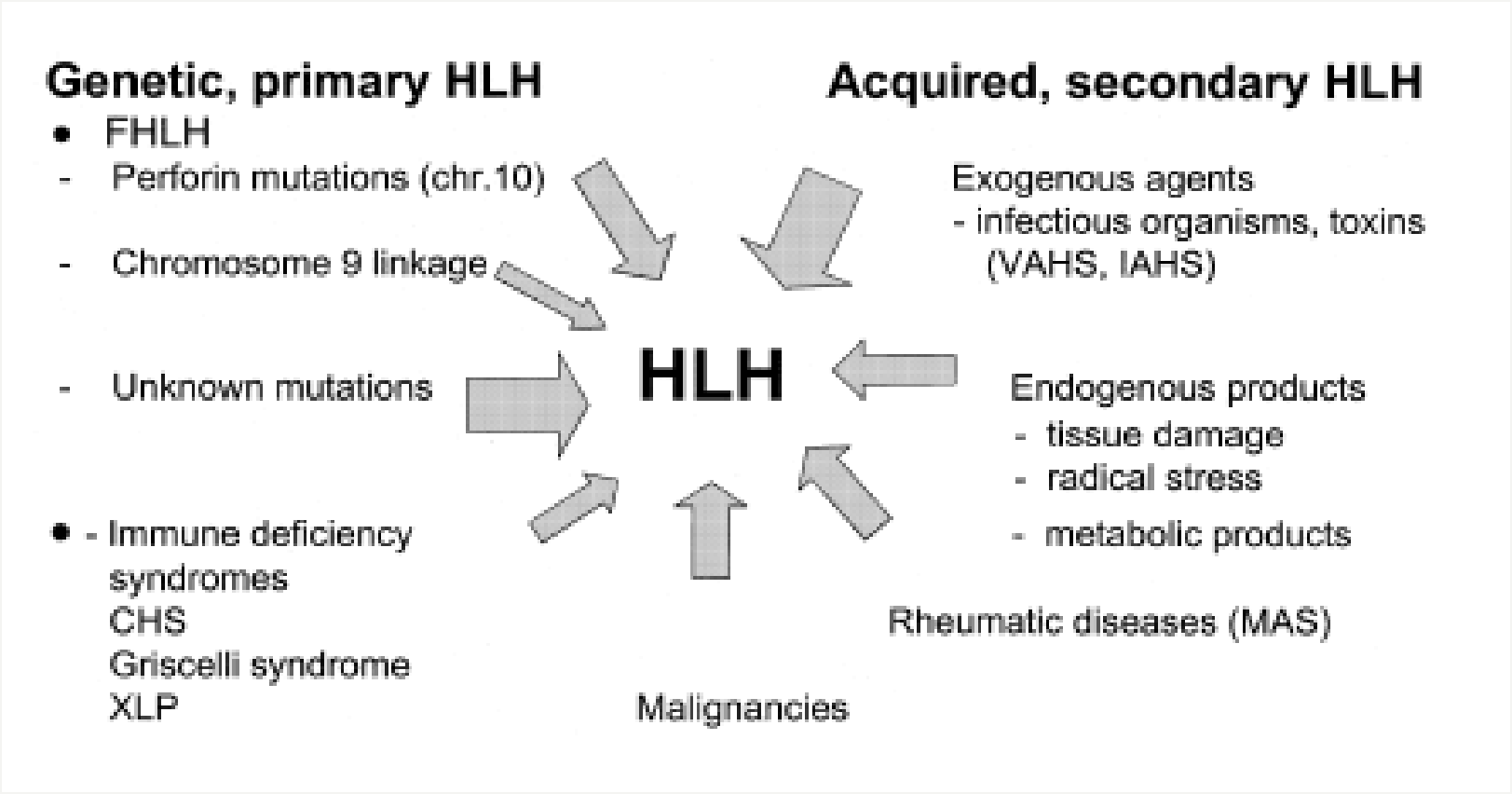
Dysregulated Cytokine Production in HLH



Familial HLH

- Average age of presentation 10 months
- Can occur in utero → hydrops fetalis
- 92% HLH < 12mo old = FHLH
- May present in 20 year olds
- Older siblings found with same genetic mutations

Who is at Risk for HLH?



Inheritance of HLH

- **Autosomal Recessive Genes**
  - *PRF1, UNC13D, STX11,STXBP2*
  - Griscelli Syndrome Type 2 *(RAB27A)*
  - Hermansky-Pudlak Syndrome Type 2 *(HPS2)*
  - Chediak-Higashi Syndrome *(LYST)*
  - Unknown gene chromosome *9*

- **X-Linked**
  - XLP1 *(SH3D1A)*
  - XLP2 *(BIRC 4)*

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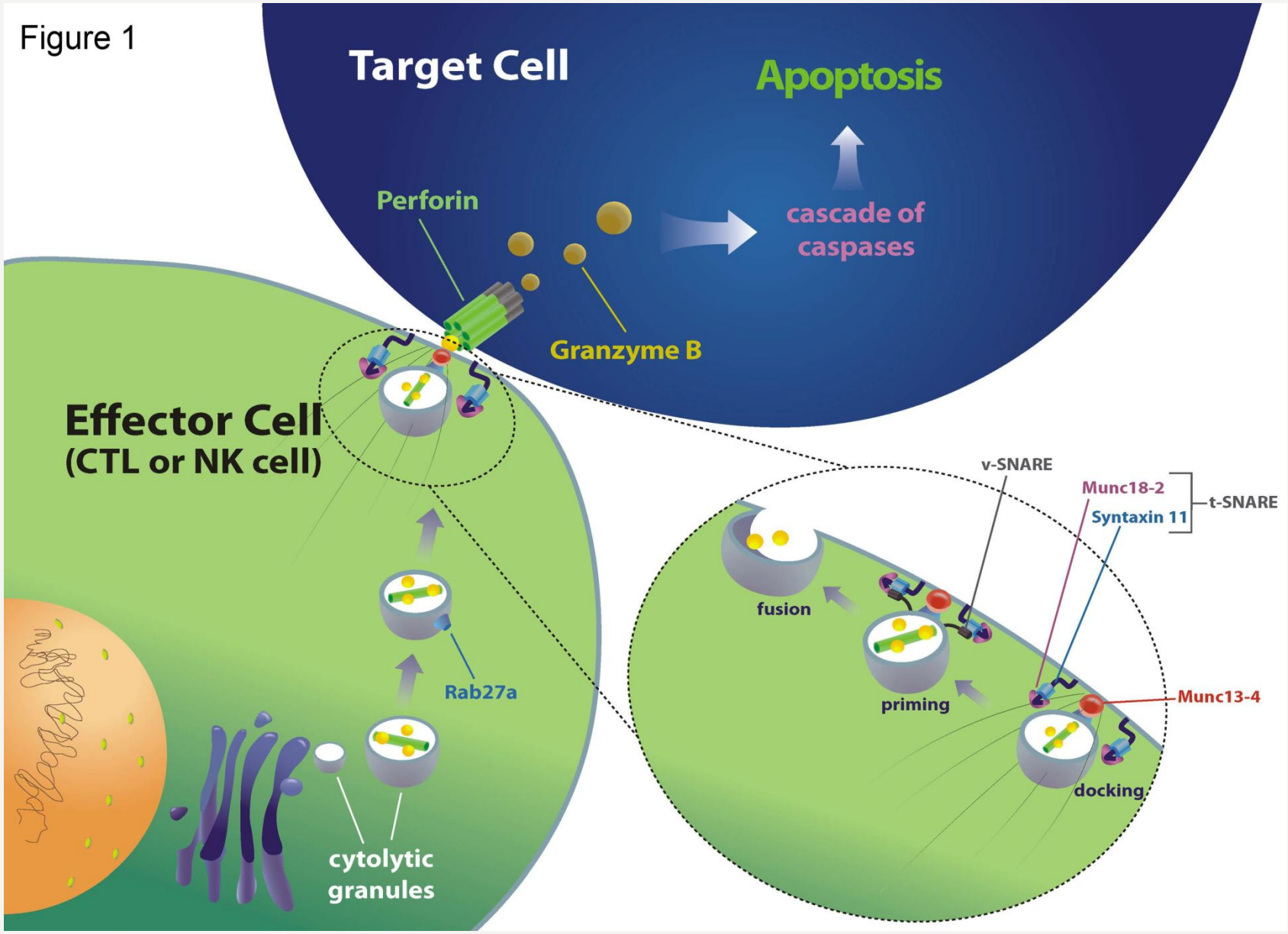
HLH-Associated Gene Mutations

Gene	Location	Disease
PRF1	10q21-22	FHL2
UNC13D	17q25	FHL3
STX11	6q24	FHL4
RAB27A	15q21	Griscelli syndrome
STXBP2	19p13	FHL5
Unknown	9q21.3-22	FHL1
SH2D1A	Xq24-26	XLP
XIAP (BIRC4)	Xq25	XLP2/X-linked HLH
Intron mutations UNC *LYST*	17q25 1Q42—Q43	FHL 6 & 7? Chediak-Higashi synd.

Blood 2011;118:4041-52

Figure 1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fusion
priming
Munc13-4
docking
Rab27a
cytolytic granules

Distribution of HLH-Associated Gene Mutations, by Ethnicity, in North American Patients with Identified Genetic Abnormalities (data provided by Judith Johnson, MS, CGC and Kejian Zhang, MD, MBA)

Gene	Caucasian	Hispanic	AA	Arabic	other/unknown
PRF1	**20 (27%)**	**41(71%)**	**44(98%)**	**8 (36%)**	**22 (88%)**
UNC13D	**35 (47%)**	**10 (17%)**	**0**	**6 (27%)**	**1 (4%)**
STX11	**1 (2%)**	**4 (7%)**	**0**	**2 (9%)**	**0 (0%)**
RAB27A	**2 (3%)**	**2 (3%)**	**0**	**2 (9%)**	**1 (4%)**
STXBP2	**16 (22%)**	**1 (2%)**	**1 (2%)**	**4 (18%)**	**1 (4%)**
Total	**74**	**58**	**45**	**22**	**25**

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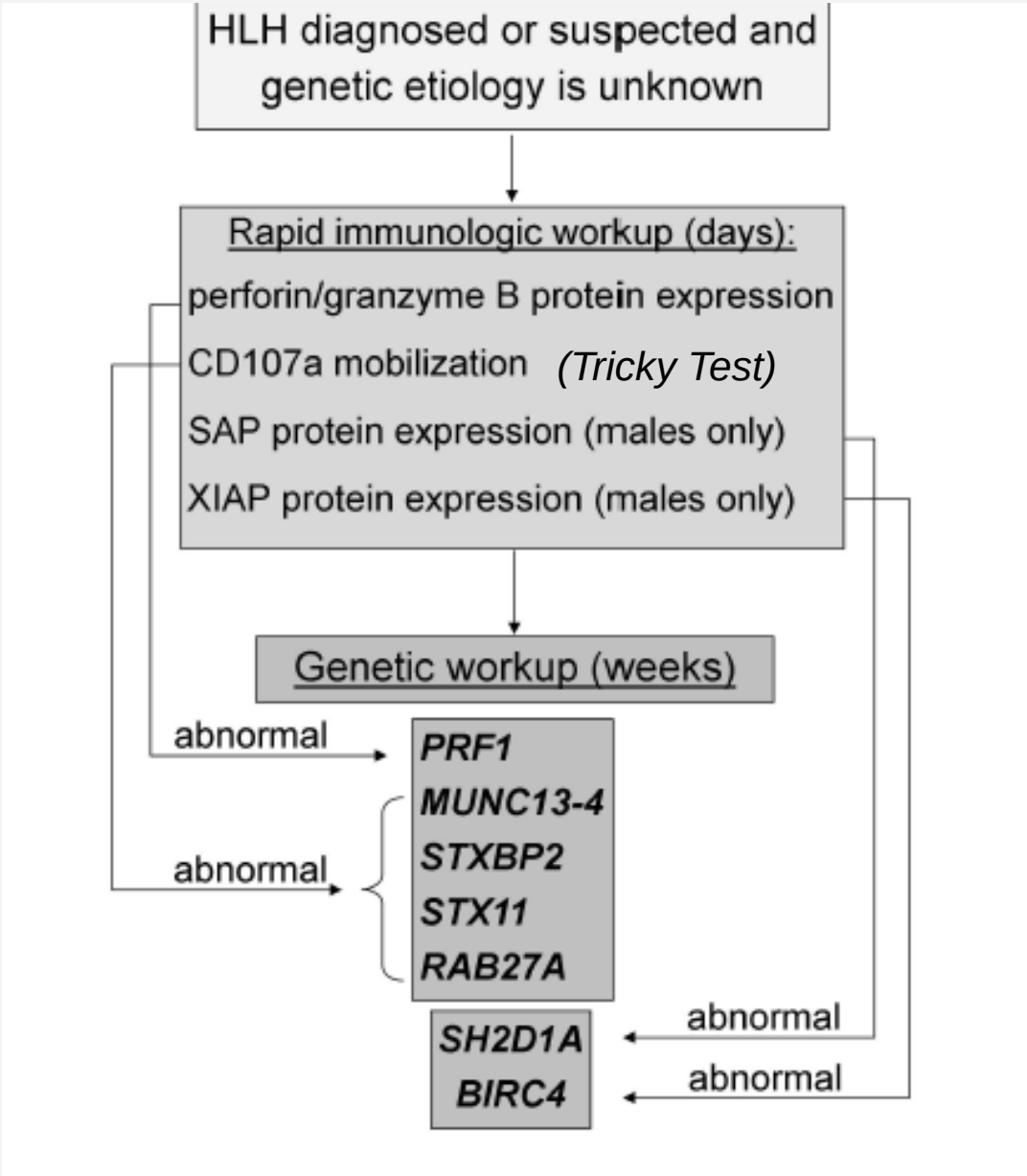
How Do I Evaluate for Inherited HLH?



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Diagnostic Criteria

A. Molecular diagnosis consistent with HLH: Pathologic mutations of *PRF1*, *UNC13D*, *Munc18-2*, *Rab27a*, *STX11*,*LYST, SH2D1A*, or BIRC4

B. At least 5/8 of the following:

- Fever
- Splenomegaly
- Cytopenias (at least 2 cell lines)
- Hypertriglyceridemia and/or hypofibrinogenemia
- Hemophagocytosis
- Ferritin >3000 mg/L
- Elevated sIL-2Ra >2400 units/ml
- Decreased NK cell activity

Pathophysiology of HLH

Re-Conceptualizing the Diagnostic Criteria and Other Common features of HLH

- Category 1: Predisposing Immunodeficiency
- Category 2: Significant Immune Activation
- Category 3: Abnormal Immunopathology

Predisposing Immunodeficiency

- Low or absent NK cell function
- Genetic defect of cytotoxicity
- Familial history of HLH
- Prior episode(s) of HLH or unexplained cytopenias
- Hypogammaglobulinemia/other immune deficiencies
- Lupus, rheumatoid arthritis, other rheum. dx

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Significant Immune Activation

- Fever
- Splenomegaly & hepatomegaly
- Elevated Ferritin (>3000 ng/ml)
- Elevated sCD25
- Elevated sCD163

Abnormal Immunopathology

- Cytopenias
- Decreased fibrinogen or increased triglycerides
  Elevated D-Dimers
- Hemophagocytosis
- Hepatitis: ↑ AST, ALT, & *GGT*
- CNS involvement

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How Often Are Clinical Signs Found?

	Early	At HLH Diagnosis
Fever	70%	100%
Rashes	43%	60%
Splenomegaly	70%	100%
Lymphadenopathy	42%	70%
Neurologic Sx	47%	70%
Resp. Distress	Variable	Up to 80%

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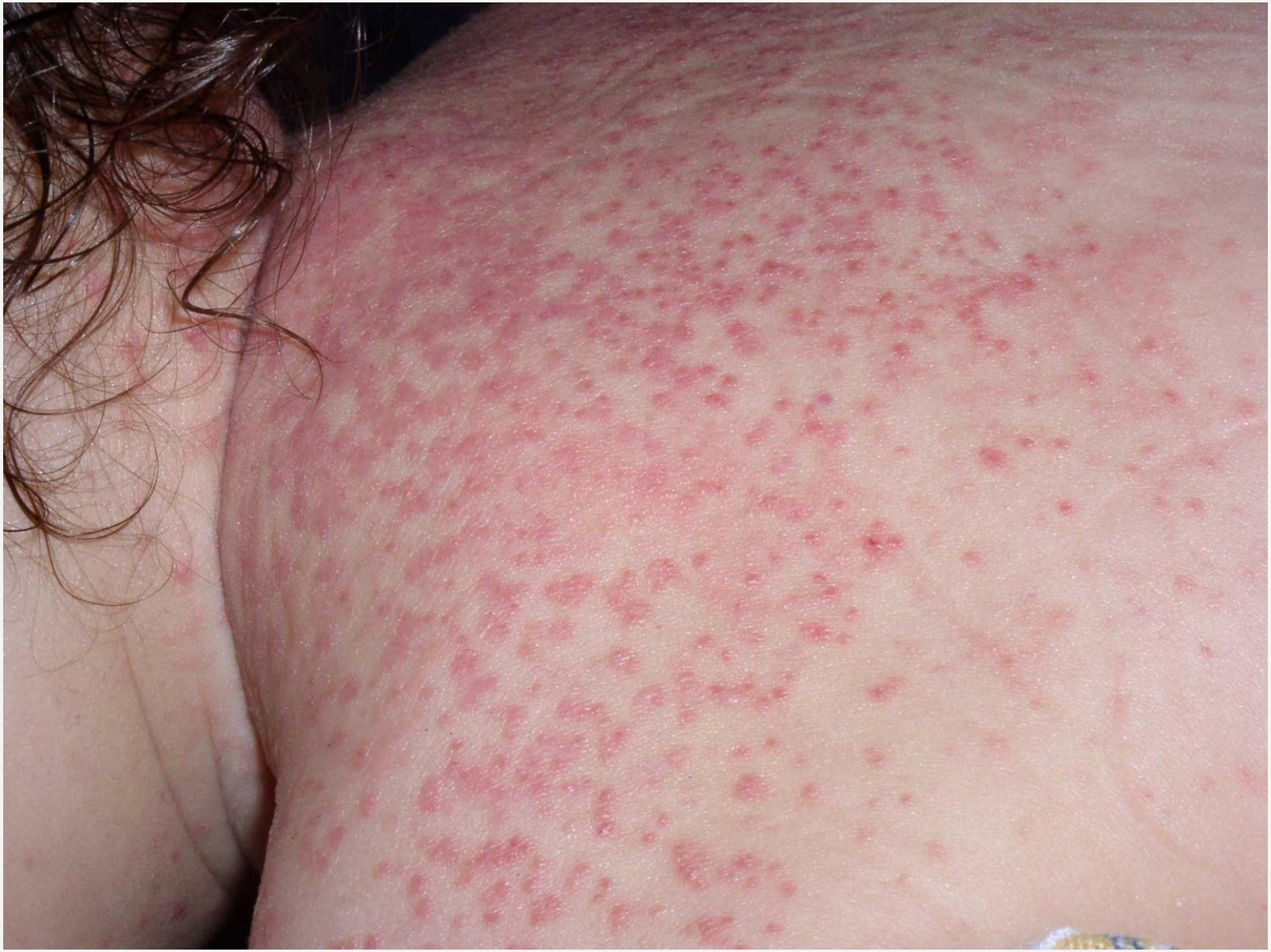
HLH-Associated Rash



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Frequency of Laboratory Findings

	Early	At HLH Diagnosis
Bicytopenia	55%	99%
↓ Fibrinogen	20%	65%
↑ Triglycerides	50%	70%
↑ Ferritin (3000)	55%	>90%
↑ sCD25 (sIL-2R)	90%	100%
Hemophagocyt.	35%	20-100%

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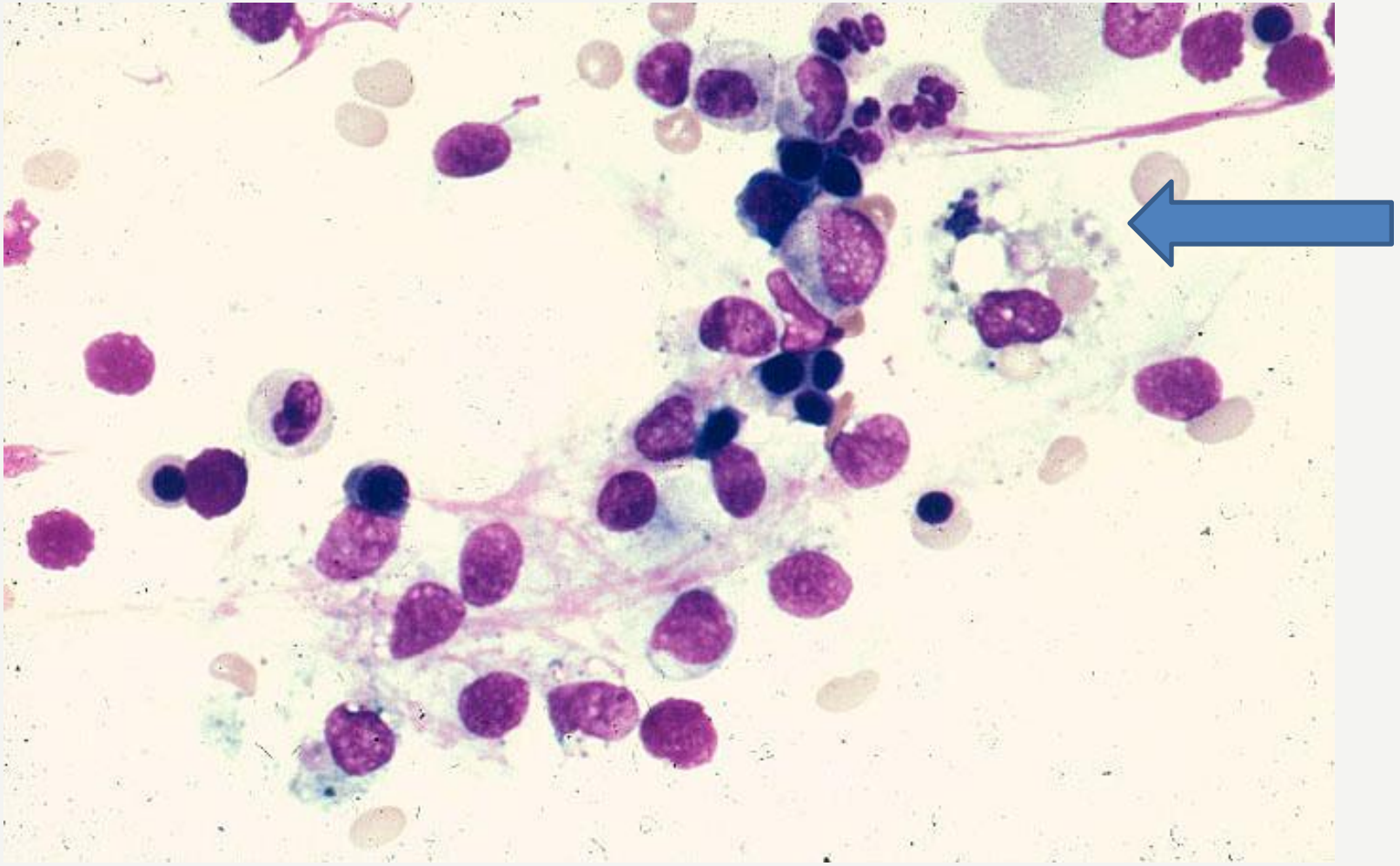
Hemophagocytosis in normal marrow



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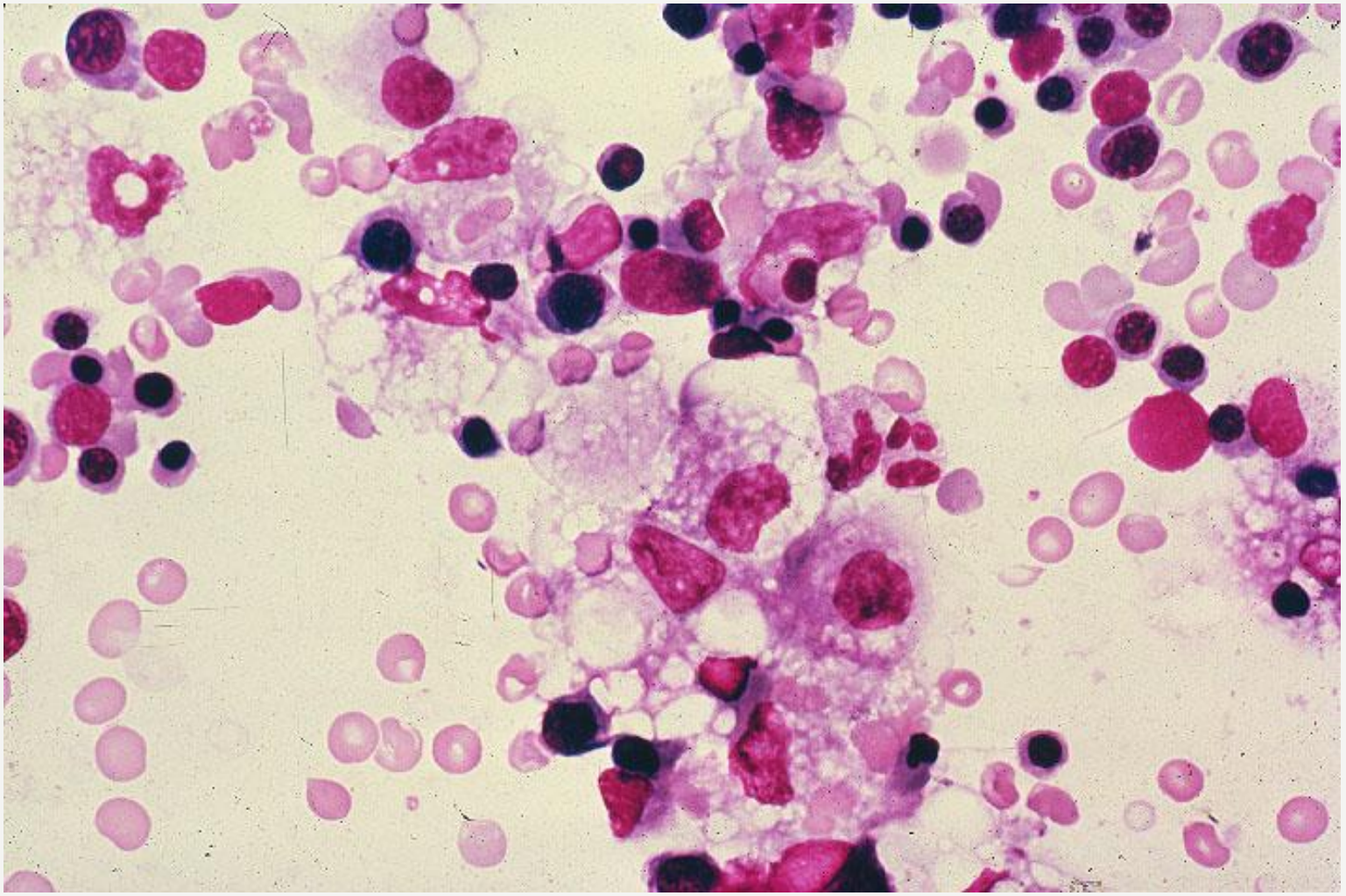
Hemophagocytosis in HLH



How Good is Hemophagocytosis as a Diagnostic Criterion?

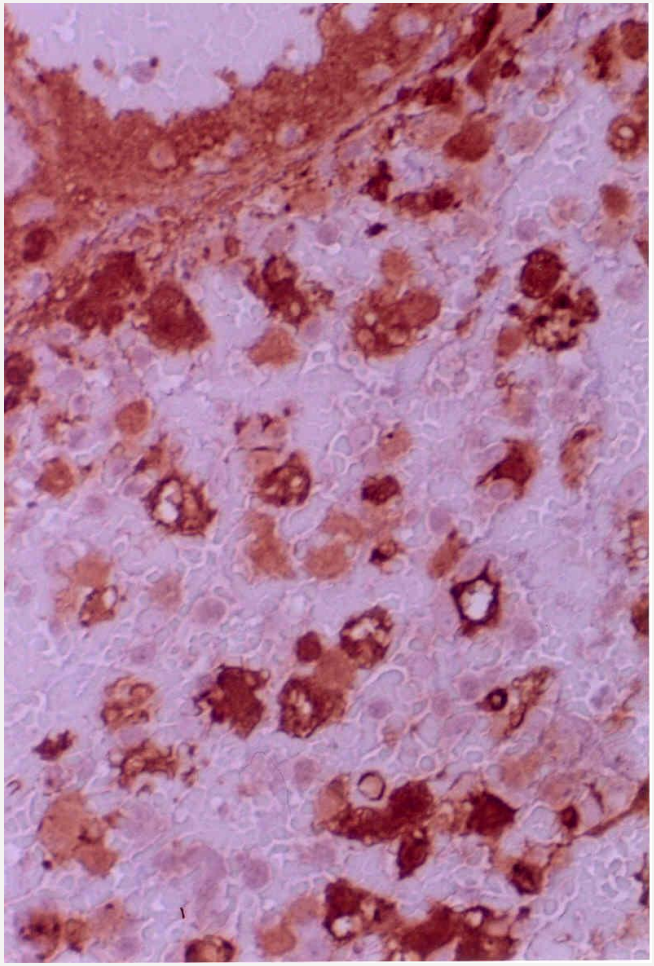
- **Neither sensitive nor specific for HLH!**
  - Found in 20-100% of patients in various series
  - *Staining marrow for macrophages (CD68) helps!*
- **Not seen in all patients particularly at diagnosis**
  - Spleen > nodes > BM > liver
- **Seen after transfusion reactions, surgery, IVIG administration, severe infections**
  - *Hemophagocytosis is an epiphenomenon*
  - Don't get hung up on finding this!
  - Look at the patient!

Other Clinical and Immunologic Features

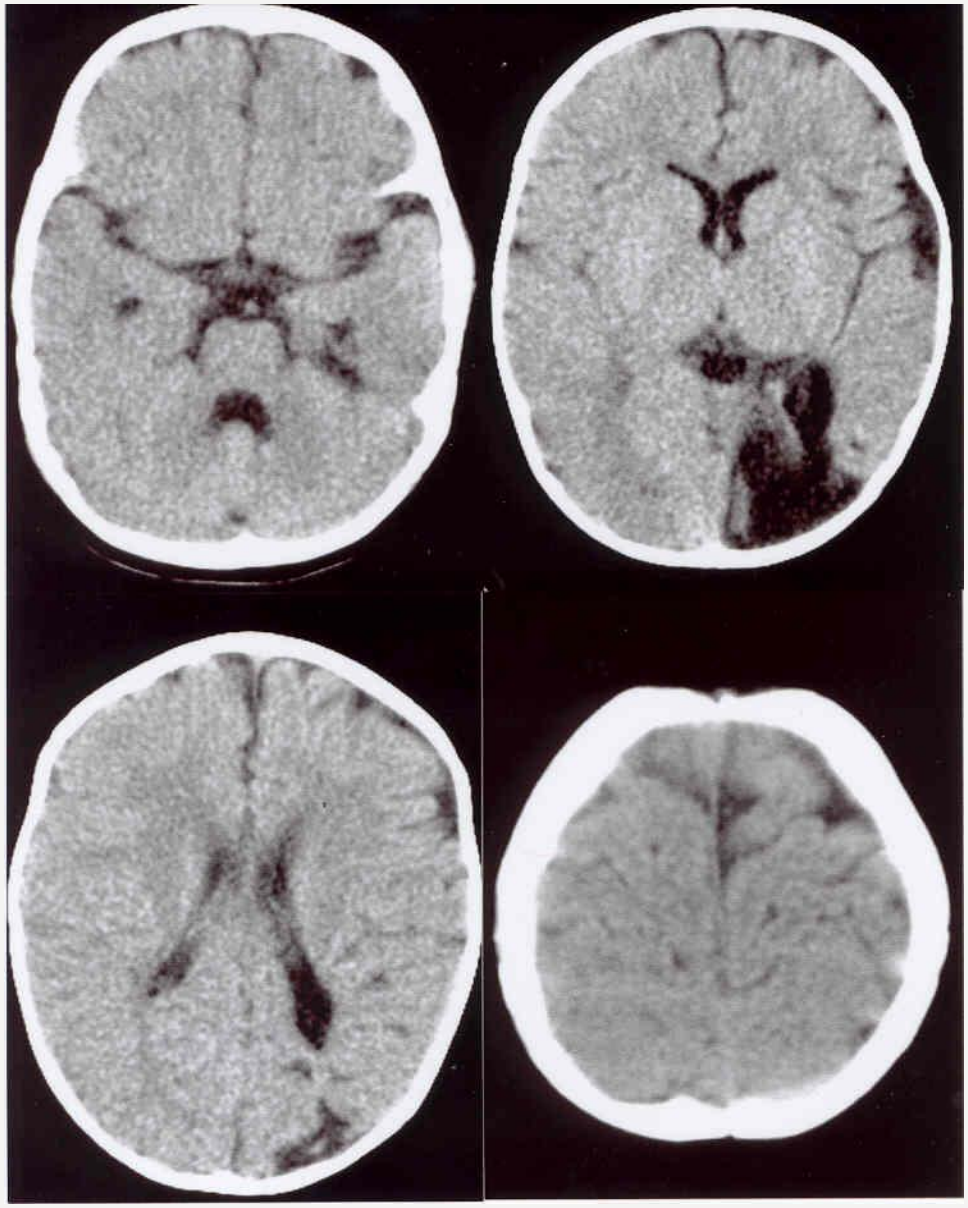
CNS Problems in HLH

- Cranial nerve signs
- Confusion, seizures, increased intracranial pressure
- Brain stem symptoms, ataxia
- Subdural effusions & bleeds, retinal hemorrhage
- CSF: mononuclear pleocytosis (lymphs & monos), rarely see hemophagocytosis, RBC
- MRI: parameningeal infiltrations, masses or necrosis – hypodense areas

Parameningeal Macrophages



Brain Necrosis from HLH



HLH: Multi-organ dysfunction

- Lung infiltration – activation of alveolar macrophages → respiratory distress (ARDS)
- Liver – synthetic function often diminished → hyperbilirubinemia, coagulopathy, increased transaminases, hypoalbuminemia →

  liver failure
- Renal failure, hyponatremia

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HLH: Helpful CBC Trends

- **Bicytopenia**
  - ↓Hemoglobin
  - ↓Platelets
- **WBC**
  - 1/3 low
  - 1/3 normal
  - 1/3 high
- ***Don't respond to transfusions***

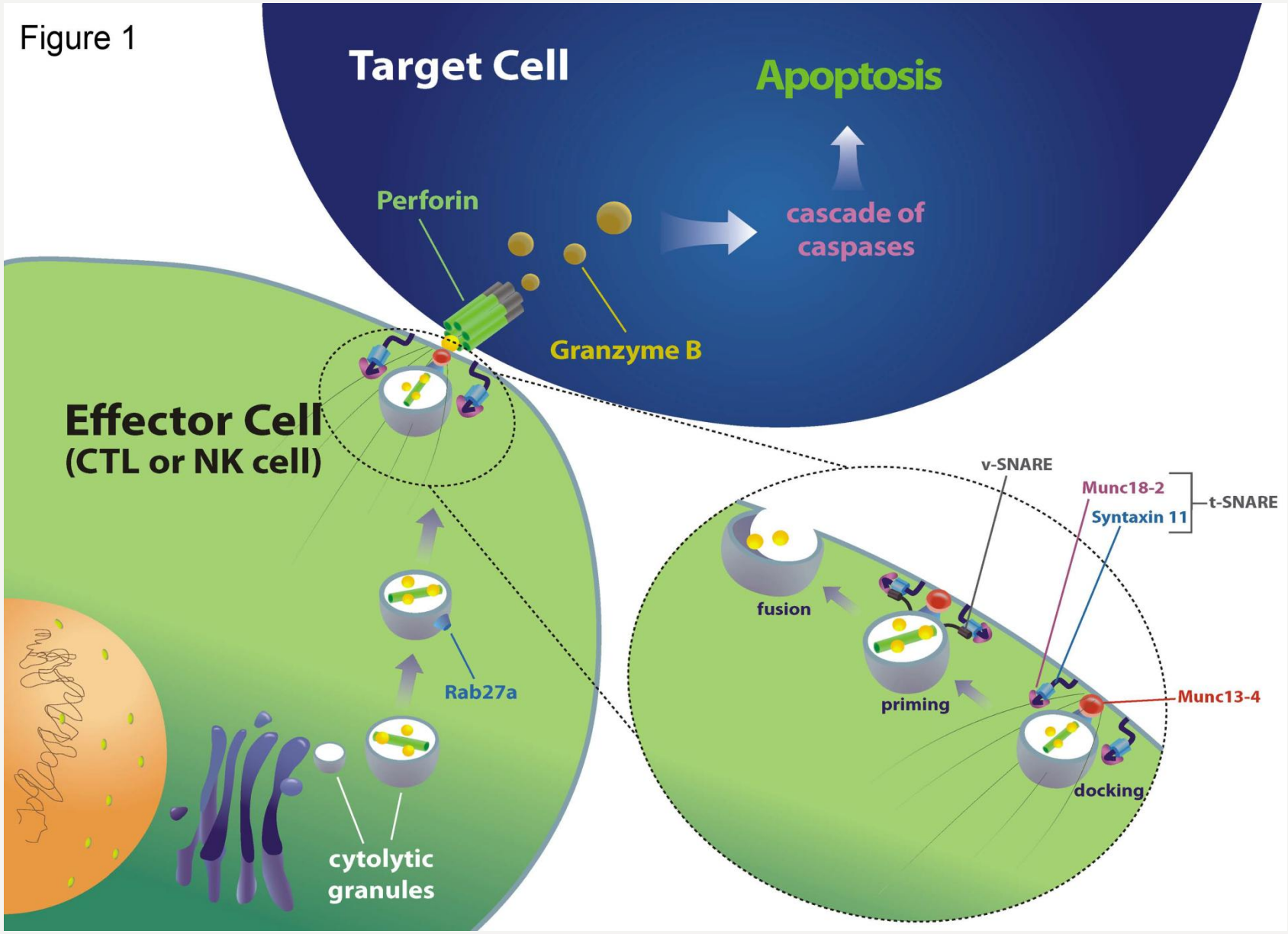
HLH: Other Supportive Laboratory Data

- High LDH
- High transaminases
- High conjugated bilirubin
- Histiocytes/lymphocytes home to biliary tract in liver biopsies

Immune Dysfunction in HLH

- **Defective NK cell function (number variable)**
  - Decreased killing of target cells
  - Decreased perforin (usually)
- **Defective Cytotoxic T cells**
  - May differ from NK cell findings
- **Effects of above: unregulated cytokine production**
  - No apoptosis of lymphs and monos

Figure 1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Rab27a
cytolytic granules

Perforin Defects in HLH

- **Perforin: cytolytic effector protein regulates NK and cytotoxic T cell function**
- **Levels depend mutation types**
  - May be normal in patients with MUNC-13 or other mutations
- **> 50 mutations in the *PRF1* gene known: cause absence of functional protein or truncated proteins**
  - No gross deletions or insertions

HLH "Cytokine Storm"

- **Increased sCD25 (sIL2R) = activated T-cells**
  - Age-specific norms
- **Increased sCD163 = activated macrophages**
  - Sepsis: 1.8 mg/L but in HLH: > 39 mg/L (p <0.001)
- **Combination may be very useful in diagnosis and follow-up to assess activity**

Secondary HLH

"Secondary" HLH – *Really, HLH is HLH*

- **EBV, CMV, HSV, Parvovirus, HHV6, etc**
- **Bacteria and Fungi**
- **Leishmaniasis, Brucella, etc.**
- **Malignancies:**
  - Usually T-cell, NK cell, or Anaplastic Large Cell Lymphomas
  - ALL patients at various treatment stages
- **Transplant patients**

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Tricky Situations

- Kawasaki Disease
- Rheumatologic Syndromes
- Sepsis/Multi-organ failure

What to do for “Secondary” HLH?

- **Consistent follow-up of critical labs:**
  - CBC, Ferritin, D-Dimer, GGT
- **Treat possible underlying conditions:**
  - Infections, malignancy
- **Some (minority) improve spontaneously or with treatment of trigger**
- **Need to treat HLH early!**

EBV HLH

- 20 cases young adults (15-34)
  - 11 newly acquired
  - 6 reactivation
  - 3 non-specific
- Early etoposide
  - 85.7% ±13.2% Survival
- No/late etoposide
  - 10.3% ±9.4% Survival (p=0.014)

(Imashuku S, Med Pediatr Oncol,2003)

“Atypical” Kawasaki Syndrome= HLH?

- Patient seems to fit criteria, but not quite
- Doesn’t respond to IVIG or relapses quickly
- Lab values – especially ferritin and D-dimers uncharacteristically high
- Think HLH and treat it!

Serum Ferritin and HLH: Why?

- Esumi *et al* Cancer 1988:
  - Malignant histiocytosis (3) HLH (5)
  - Ferritin ranged from 12,000 to 68,000 ng/ml

- Ferritin from monocyes as inflammatory marker
  - Ferritin transcription enhanced by TNF & Interferon-α

Texas Children's Ferritin Study

- **Hypothesis**
  - Highly elevated ferritin levels are specific to HLH
- **Retrospective review of all patients with ferritin >500 at TCH** (Chosen because of HLH-04 Criteria)
  - 10/1/03-10/1/05
  - 1093 ferritin levels
  - 320 patients
  - Median: 1454 mg/L
  - Range: 503-189, 721 mg/L

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How Good are Ferritin Levels Predicting HLH?

Ferritin Level (µg/L)	Sensitivity	Specificity
3000	90%	77%
6000	90%	90%
10,000	90%	96%

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How High is Ferritin in Other Conditions?

Diagnosis	Median	Maximum
HLH	15,830	189,721
Shock	5438	9,066
Liver Disease	1262	12,937
Chronic Transfusion	1775	6322
Autoimmune Dx	1356	37,407
Bacterial Infection	972	7508

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Helpful Hints for Ferritin and other labs

- Ferritin levels change dramatically in HLH but not in other conditions
- Rate of ferritin decline is a prognostic marker:
  - *>96% drop in 2 wks → 30% die*
  - *<50% drop → 68% die*
- Follow Ferritin, D-dimer, GGT often to track response to therapy.
- sCD25 (sIL-2R) more sensitive than ferritin

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Treatment of HLH

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When Should I Start Treatment for Suspected HLH?

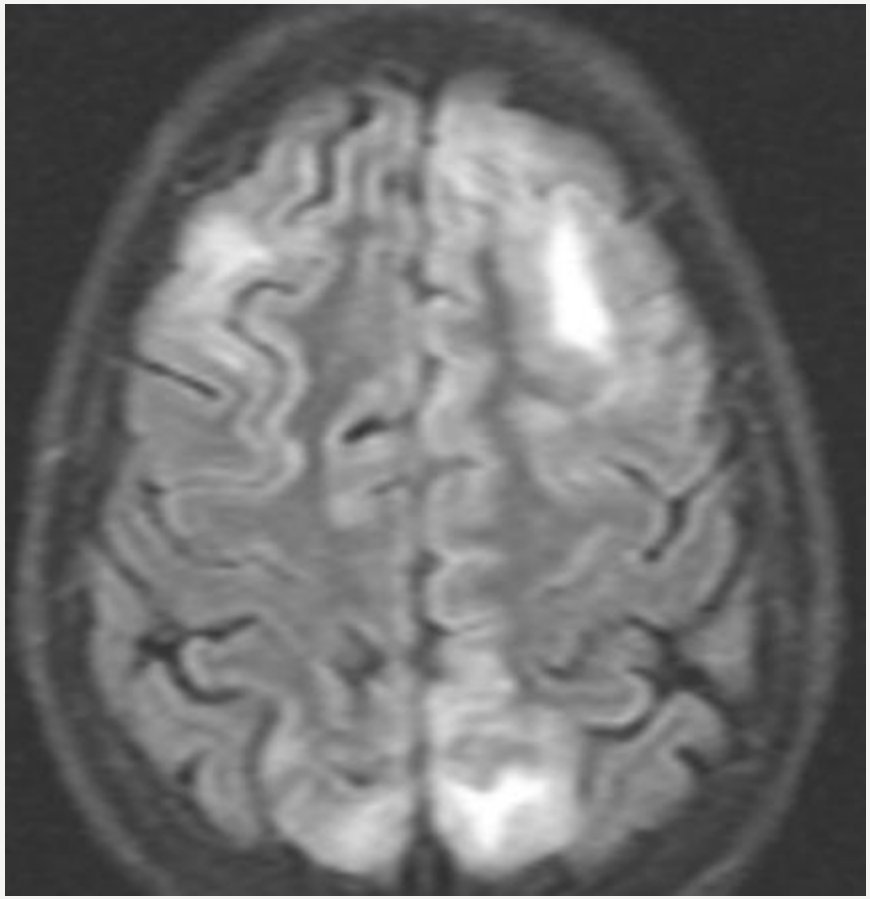
- Easy answers: Familial, >5 criteria, or 4 criteria *and respiratory, renal, blood pressure unstable*
- Not so easy:
  4 Criteria, no hemophagocytosis: Treat When-
  *Ferritin, D-dimers, GGT rising
  *Renal, respiratory, BP status deteriorating
  *Not responding to antibiotics
- Can I start with decadron alone?
  Yes, but follow ferritin, D-Dimers,GGT daily!
- *McClain's advice: No Guts, No Glory*

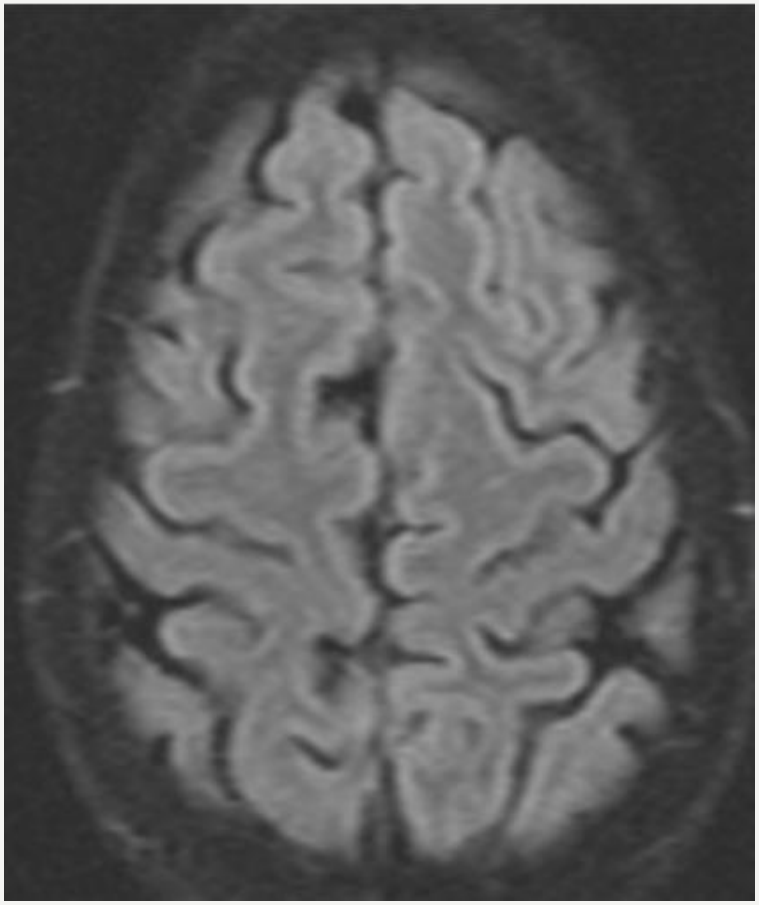
HLH Treatment

- "Gold Standard": etoposide/decadron
- Cyclosporine causes CNS & renal problems (*I no longer recommend using it)*
- IT methotrexate/hydrocortisone for CNS+ (pleocytosis, ↑protein, MRI changes)
- IVIG may help a little at first, but not sufficient
- Plasmapheresis also temporizing

MRI image of PRES and Resolution

After cyclosporine discontinued



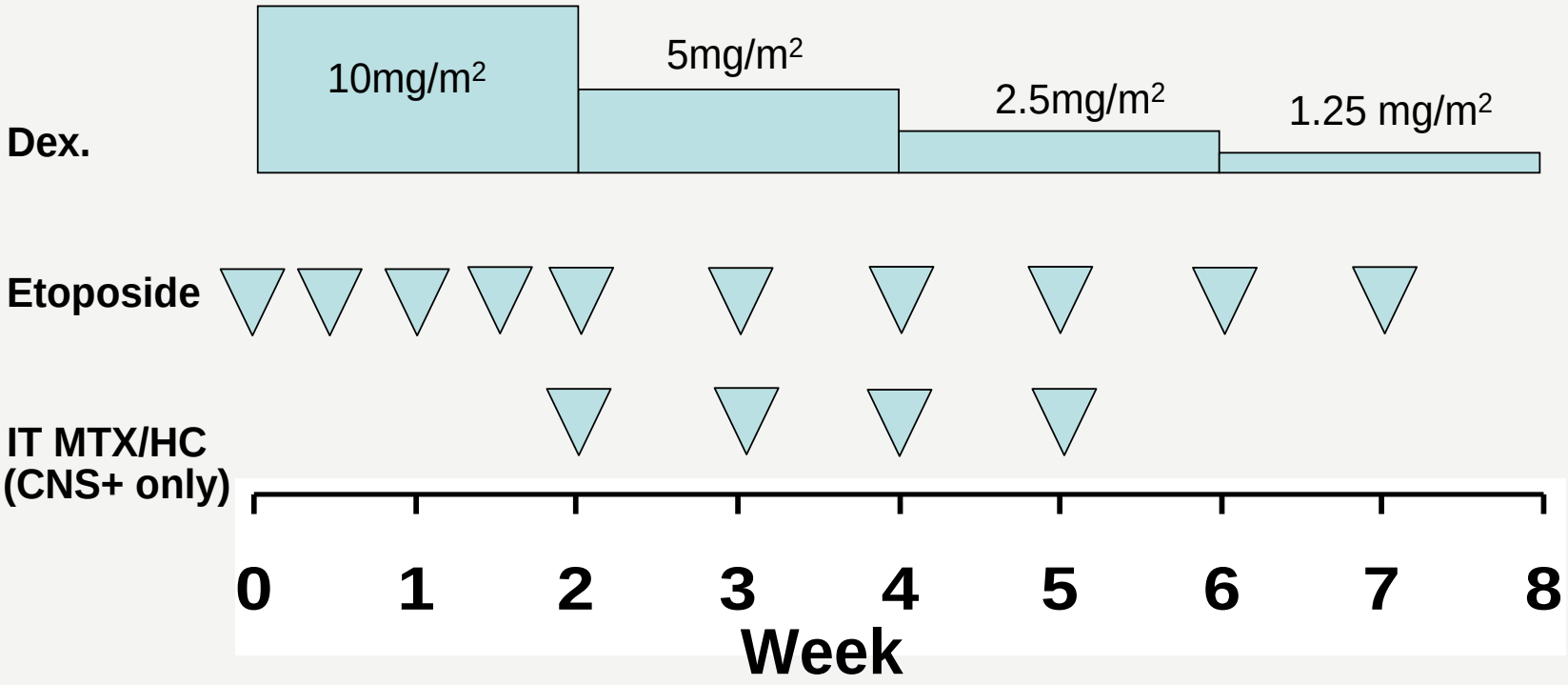


MRI images illustrating the rapid resolution of the imaging abnormalities associated with PRES. **A:** FLAIR sequence of an MRI one day after Patient 4 developed seizures. **B:** Follow-up images obtained 12 days later showing complete resolution of the abnormalities.

How Long Do I Treat?

- **8 Weeks if all goes well**
  - *Etoposide 2x/wk for 2 wks, then weekly*
  - *Decadron drop dose by 50% every 2 wks*
- **Patients often "flare" with changes**
  - *Be prepared to increase decadron or etoposide*
- **Stop at 8 wks IF: no flare, no CNS, no mutations**
- **"Continuation": Alternating weeks of etoposide & Decadron for control before translplant**
- ***DO NOT EXCEED 3 g/m^2 etoposide!!!***

HLH Treatment Schema



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The Ways HLH Patients Will Trick You

- **Fungal infections**
  - Fluconazole prophylaxis from day 1
  - High index of suspicion with new fevers - *Need to follow fungal serologies and scan early!!!!*
- **CNS bleeds and HLH damage**
- **New rashes and pain: Can be HLH or infections**

What Do I Do When the HLH Comes Back, or Doesn't Respond?

- Increase frequency of etoposide or increase decadron
  - *Beware, this → fungal infections*
- Alemtuzumab (Campath): overall the best back-up
- Rituximab if high EBV DNA levels
- Anti-TNF agents: so, so

Who Needs a Stem Cell Transplant?

- Essentially all patients <3 yrs: if you don't find a mutation, they probably have a new one
- Family history +
- Any of the 8 mutations
- Patients who relapse
- CNS +
- *Advice: draw HLA typing on day 1*

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Treatment Results

- **Pre-cytotoxic therapy**
  - *All "HLH" <10% Survival*
- **HLH-94 study survival: 55% overall, 51% familial**
- **Current results: 65+% survival with BMT**
  Standard conditioning: Busulfan/Cytoxan/
  +/- etoposide

BMT Issues for HLH Patients

- Reduced intensity conditioning is better:
  75% OS with unrelated and haploidentical donors
  Less veno-occlusive disease
- Mixed chimerism still protects against reactivation
- Alemtuzimab (D-14) in conditioning regimen helps
  Fludarabine/melphalan (Days -3 to 0)

Take Home Points

- **HLH is clearly the most dangerous disease we treat- my humble opinion**
- **HLH patients are tricky to diagnose**
  - Follow ferritin, D-dimers, GGT especially
  - Look for underlying causes (2 diagnoses are possible)
- **HLH patients are hard to treat**
  - Don’t always respond quickly
  - Frequently get fungal infections

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THANK YOU

QUESTIONS?

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