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

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

- NK or Cytotoxic T-cell
- Macrophage
- sCD163
- TNF-α
- sIL-2R
- Inf-γ
- Antigen Presentation
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?

Genetic, primary HLH
- FHLH
  - Perforin mutations (chr.10)
  - Chromosome 9 linkage
- Unknown mutations
- Immune deficiency syndromes
  - CHS
  - Griscelli syndrome
  - XLP

Acquired, secondary HLH
- Exogenous agents
  - Infectious organisms, toxins (VAHS, IAHS)
- Endogenous products
  - Tissue damage
  - Radical stress
  - Metabolic products
- Rheumatic diseases (MAS)
- Malignancies
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)
# HLH-Associated Gene Mutations

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

Target Cell

Apoptosis

cascade of caspases

Effector Cell (CTL or NK cell)

- Perforin
- Granzyme B

- Rab27a
- Cytolytic granules

- Priming
- Fusion
- Docking

v-SNARE
Munc18-2
Syntaxin 11
Munc13-4
t-SNARE
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)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Caucasian</th>
<th>Hispanic</th>
<th>AA</th>
<th>Arabic</th>
<th>other/unknown</th>
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</thead>
<tbody>
<tr>
<td>PRF1</td>
<td>20 (27%)</td>
<td>41 (71%)</td>
<td>44 (98%)</td>
<td>8 (36%)</td>
<td>22 (88%)</td>
</tr>
<tr>
<td>UNC13D</td>
<td>35 (47%)</td>
<td>10 (17%)</td>
<td>0</td>
<td>6 (27%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>STX11</td>
<td>1 (2%)</td>
<td>4 (7%)</td>
<td>0</td>
<td>2 (9%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>RAB27A</td>
<td>2 (3%)</td>
<td>2 (3%)</td>
<td>0</td>
<td>2 (9%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>STXBP2</td>
<td>16 (22%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>4 (18%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Total</td>
<td>74</td>
<td>58</td>
<td>45</td>
<td>22</td>
<td>25</td>
</tr>
</tbody>
</table>
How Do I Evaluate for Inherited HLH?

- HLH diagnosed or suspected and genetic etiology is unknown
  - Rapid immunologic workup (days):
    - perforin/granzyme B protein expression
    - CD107a mobilization
    - SAP protein expression (males only)
    - XIAP protein expression (males only)
  - Genetic workup (weeks)
    - abnormal
      - PRF1
      - MUNC13-4
      - STXBP2
      - STX11
      - RAB27A
    - abnormal
      - SH2D1A
      - BIRC4

Tricky Test
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
Significant Immune Activation

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

- Cytopenias
- Decreased fibrinogen or increased triglycerides
  \textit{Elevated D-Dimers}
- Hemophagocytosis
- Hepatitis: $\uparrow$ AST, ALT, & GGT
- CNS involvement
### How Often Are Clinical Signs Found?

<table>
<thead>
<tr>
<th>Clinical Signs</th>
<th>Early</th>
<th>At HLH Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>70%</td>
<td>100%</td>
</tr>
<tr>
<td>Rashes</td>
<td>43%</td>
<td>60%</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>70%</td>
<td>100%</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>42%</td>
<td>70%</td>
</tr>
<tr>
<td>Neurologic Sx</td>
<td>47%</td>
<td>70%</td>
</tr>
<tr>
<td>Resp. Distress</td>
<td>Variable</td>
<td>Up to 80%</td>
</tr>
</tbody>
</table>
HLH-Associated Rash
## Frequency of Laboratory Findings

<table>
<thead>
<tr>
<th></th>
<th>Early</th>
<th>At HLH Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bicytopenia</strong></td>
<td>55%</td>
<td>99%</td>
</tr>
<tr>
<td>↓ Fibrinogen</td>
<td>20%</td>
<td>65%</td>
</tr>
<tr>
<td>↑ Triglycerides</td>
<td>50%</td>
<td>70%</td>
</tr>
<tr>
<td>↑ Ferritin (3000)</td>
<td>55%</td>
<td>&gt;90%</td>
</tr>
<tr>
<td>↑ sCD25 (sIL-2R)</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>Hemophagocyt.</td>
<td>35%</td>
<td>20-100%</td>
</tr>
</tbody>
</table>
Hemophagocytosis in normal marrow
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
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

Target Cell

Apoptosis

cascade of caspases

Effector Cell (CTL or NK cell)

Perforin

Granzyme B

Rab27a

cytolytic granules

fusion

priming

docking

v-SNARE

Munc13-4

Syntaxin 11

Munc18-2

t-SNARE
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
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)

“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
How Good are Ferritin Levels Predicting HLH?

<table>
<thead>
<tr>
<th>Ferritin Level (µg/L)</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000</td>
<td>90%</td>
<td>77%</td>
</tr>
<tr>
<td>6000</td>
<td>90%</td>
<td>90%</td>
</tr>
<tr>
<td>10,000</td>
<td>90%</td>
<td>96%</td>
</tr>
</tbody>
</table>
# How High is Ferritin in Other Conditions?

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Median</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLH</td>
<td>15,830</td>
<td>189,721</td>
</tr>
<tr>
<td>Shock</td>
<td>5438</td>
<td>9,066</td>
</tr>
<tr>
<td>Liver Disease</td>
<td>1262</td>
<td>12,937</td>
</tr>
<tr>
<td>Chronic Transfusion</td>
<td>1775</td>
<td>6322</td>
</tr>
<tr>
<td>Autoimmune Dx</td>
<td>1356</td>
<td>37,407</td>
</tr>
<tr>
<td>Bacterial Infection</td>
<td>972</td>
<td>7508</td>
</tr>
</tbody>
</table>
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
Treatment of HLH
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 transplant

- DO NOT EXCEED 3 g/m² etoposide!!!
HLH Treatment Schema

Week
Dex.
Etoposide
IT MTX/HC
(CNS+ only)
2.5mg/m²
5mg/m²
10mg/m²
1.25 mg/m²

Blood 2011;118:4041-52
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
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
THANK YOU

QUESTIONS?

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