Successful Use of Etoposide for Transfusion-associated Hyperhemolysis Syndrome

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Discussant: Terry Gernsheimer, MD
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The Patient

30 yo female with congenital t(6;7), a history of Evans syndrome s/p splenectomy at age 8, and a strong cold red cell autoantibody who presents with dyspnea, lightheadedness, hematuria, and jaundice

Hct 19, MCV 99, LDH 836, retic 318, WBC 14.5, Plt 286, ferritin 13,000

- Coombs: new warm auto, strong cold antibody
- 3u pRBC c/b fever, flu and meningococcal vaccines given
- 1mg/kg prednisone daily and 1g/kg IVIG HD 3-5
- HD 2 CT CAP: R inguinal LAD
- HD 4 BMB: leukoerythroblastosis. normocellular. Erythroid hyperplasia. Increased histiocytes. Increased iron. Flow negative for myeloid, lymphoid and PNH clones
- HD 8 LNB: atypical clonal IgG-lambda restricted plasmacytoid cells. SPEP negative.
- HD 3-9 Hemolysis stabilized, transfusion independent, “adequate retic”
The Patient

- Hct 20, 2u pRBCs → hct 14. decreasing reticulocytes
- HD 10 Continued steroids, started rituximab 375mg/m2 weekly x3
- HD 16 Danazol 300mg BID x 4 days
- HD 19 1g/kg IVIG and 40K units epo.
- HD 21 1g/m2 cyclophosphamide
- Received B12 injections weekly, but never any folic acid during hospitalization
- HD 23 PET: post surgical changes R groin
- HD 25 excisional biopsy R groin: sheets of atypical plasmacytoid cells. EBER, HHV8, IL6, normal. 0.1g/dL IgG lambda
- HD 31 liver biopsy: severe hemosiderosis in Kupffer and hepatocytes. drug injury
- Infection: CMV, EBV, adenovirus, enterovirus, HSV, HBV, HCV, HIV, HHV6, HHV8, parvovirus all negative. Multiple blood and urine culture negative
- ANA weak +, normal C3, low C4, DSDNA+, SSA Ro+, Smooth muscle+
- HD 32 febrile transfusion reaction (39.4); 51st pRBCs

Due to difficulty treating hemolytic anemia, she was transferred to UWMC
**Physical Exam**

- T 36.9, P 118, RR 24, BP 104/68, SpO2 98%
- General: dysmorphic female, NAD
- HEENT: **sclera icteric**
- No cervical, supraclavicular, axillary, or inguinal LAD, seroma at biopsy site
- Lungs: CTAB no crackles
- Heart: RRR, no murmur, no edema
- Abd: mild tenderness in RUQ, **no HSM**, no ascites
- Extremities: **no joint swelling**, WWP
- Neuro: A&O, CN2-12 intact, normal strength/sensation

**Review of Systems**

- **Cyclic fevers, Cough, dark urine**
- Denies pain, SOB, n/v/d/c, and night sweats. Denies abnormal bleeding, good appetite
The Patient—UWMC Presentation

MCV 88
Retic 18 (0.9%)
Ferritin 23,230
% sat 118
LDH 1001
Plasma free Hgb 47
Haptoglobin <10
Epo 720
HFE wild type H63D & C282Y

ANA normal
IgG lambda 0.1 g/dL
UPEP oligoclonal bands
INR 1.2, PTT 31
Fibrinogen 538

Continued steroids, warmed antigen matched blood, folic acid, and limited marrow toxic meds
The Patient

- April 2009: PSBC strong cold autoantibody (likely anti PR)
- March 2014: OSH strong cold antibody and a weak warm autoantibody
- April 8, 2014: PSBC panagglutinin. cold (anti I) and warm autoantibodies no underlying allogeneic antibodies.
- April 12, 2014: Tx Rx eval-3+ poly, 2+ IgG, 3+ C3. cold panel negative. Back typing identified 1+antiA1. cold agglutinins neg.
- April 8-12, 2014: antigen matched cells-no alloantibodies
- April 8-13, 2014: blood warmer-no clinically relevant cold antibody
Hemolysis

Hematocrit

LDH

Reticulocytes
The Patient: Peripheral Smear
The Patient: Repeat Bone Marrow

CD163

EBER and CMV Neg
The Patient

- Increasing transfusions, recurrent fevers
- Unclear etiology of hemolysis
- HD 2: BMB erythroid hyperplasia, erythrophagocytosis
- HD 4: Repeat ferritin 11,000 (down >50%)
- HD 7: AMS, Ferritin 75K, repeat CMV PCR+
- High clinical suspicion for MAS
  - Fasting Triglyceride 260
  - Fibrinogen 538
  - NK activity: undetectable
  - sIL2 587pg/ml
Macrophage Activation Syndrome: AKA Hemophagocytic Lymphohistiocytosis

- Life-threatening hyperinflammatory reactions
- Cytokine release from Macrophages
- Genetic vs acquired
  - Dysfunctional cytotoxic cells
  - Genetic mutations in 15% of adult presentations
- Autoimmune disease (MAS)
- Infections (EBV and Leishmania)
- Lymphoma (often Tcell origin)


## HLH/MAS Diagnosis

<table>
<thead>
<tr>
<th>Feature</th>
<th>Cutoff</th>
<th>Suggested mechanism</th>
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<tbody>
<tr>
<td>Fever</td>
<td></td>
<td>Elevated pyrogens</td>
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<tr>
<td>Splenomegaly</td>
<td></td>
<td>Infiltration by lymphocytes and histiocytes</td>
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<tr>
<td>Cytopenia</td>
<td></td>
<td>Multicausal: suppression by cytokines, ferritin, hemophagocytosis</td>
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<tr>
<td>- Hemoglobin</td>
<td>$&lt; 90 \text{ g/L}$ (neonates $&lt; 100 \text{ g/L}$)</td>
<td></td>
</tr>
<tr>
<td>- Platelets</td>
<td>$&lt; 100 \times 10^9/\text{L}$</td>
<td></td>
</tr>
<tr>
<td>- Neutrophils</td>
<td>$&lt; 1 \times 10^9/\text{L}$</td>
<td></td>
</tr>
<tr>
<td>Hyperferritinemia</td>
<td>$&gt; 500 \text{ µg/L}$</td>
<td>Macrophage activation GDF15</td>
</tr>
<tr>
<td>Hypofibrinogenemia or</td>
<td>$&lt; 1.5 \text{ g/L}$</td>
<td>Plasminogen-activator produced by macrophages</td>
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<tr>
<td>hypertriglyceridemia</td>
<td>$&gt; 3 \text{ mmol/L}$</td>
<td>Lipoprotein lipase suppression by cytokines</td>
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<tr>
<td>Elevated soluble CD25</td>
<td>$&gt; 2400 \text{ U/mL}$</td>
<td>T-cell activation</td>
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<tr>
<td>Hemophagocytosis</td>
<td>Bone marrow, other tissues</td>
<td>Macrophage activation</td>
</tr>
<tr>
<td>Reduced or absent NK cytotoxicity</td>
<td></td>
<td>Genetic defect, transient dysfunction</td>
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- Frequently diagnostic criteria are not met early in presentation
- Ferritin $> 3000$ or $> 10,000$ is likely more specific
- MAS fulfills HLH criteria at a much later stage, if at all

Macrophage Activation Syndrome

Figure 2: Treatment protocol overview for Hemophagocytic Lymphohistiocytosis (HLH-2004)

INITIAL THERAPY → SCT / CONTINUATION THERAPY

(dexamethasone daily)  (dexamethasone in pulses #)

Dexa (mg/m²)

| 10 mg |
| 5 mg |
| 2.5 mg |
| 1.25 mg |

VP-16

CSV

I.T. therapy

(↑ ↑ ↑ ↑)

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<tr>
<th>1 2 3* 4 5* 6 7 8 9*§ 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24</th>
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weeks

| 25 26 27* 28 29 30 31 32 33 34 35 36 37 38 39 40* |

The Patient

• HLH vs MAS
  • Undetectable NK activity: inherited HLH?
  • Infections: reactivation of CMV vs primary infection from transfusion?
  • Malignant lymphoma: LAD?
  • History of Evans and autoimmune hemolytic anemia? MAS?

• Erythroid hyperplasia and erythrophagocytosis-atypical for HLH
Excisional Lymph Node Biopsy
Marginal Zone Lymphoma

- Three unrelated subtypes: Nodal, extra-nodal, splenic
- Post-GC memory B cells under chronic immune stimulation
  - Autoimmune disease
  - Infection (Chlamydophilia, Lyme, Hpylori, Campylobacter)
- Stomach most common site (bowel, breast, head & neck, skin, lung)
- 30% associated with M-spike and plasmacytic differentiation
- Early stage disease usually treated locally (surgery or radiation)
- Stage III/IV disease is treated per FL algorithms (BR, RCHOP, RCVP; FR, lenolidomide + rituximab)
- Can transform to aggressive large cell lymphoma (DLBCL algorithms)
The Patient

- Hemophagocytic lymphohistiocytosis vs Macrophage Activation Syndrome
  - Undetectable NK activity: inherited HLH?
  - Infections: reactivation of CMV vs primary infection from transfusion?
  - Malignant lymphoma: LAD?
  - History of Evans and autoimmune hemolytic anemia? MAS?

- Erythroid hyperplasia and only erythrophagocytosis-atypical for HLH
Hyperhemolysis Syndrome

- Sickle cell disease*, myelofibrosis, thalassemia, etc
- Rare complication of transfusion (50% mortality with 1L pRBCs)
  - Post transfusion hematocrit lower than pre-transfusion
  - Fever = a possible hemolytic transfusion reaction (rigors, chills, “impending doom”, hematuria, SCD pain crisis).
- Reticulocytopenia
  - Bystander hemolysis (non-specific antibodies against general red cell antigen)
  - Suppression of erythropoiesis
  - RBC destruction by activated macrophages (liver, spleen, marrow)
- Hypertransfusion: more blood in the setting of a HTR

Hyperhemolysis Syndrome

- Acute vs Delayed
  - Acute: <7 days, DAT -, no alloantibodies
  - Delayed: >7 days, DAT+, new alloantibodies

- Monitor retics, plasma free hgb, and hematuria for early diagnosis of acute HS

- KEY TREATMENT: avoid additional transfusions!!
  - steroids (1mg/kg)
  - IVIG (400mg/kg x 5d)
  - Erythropoietin
  - Rituximab
  - Eculizumab

- Antigen-matched, compatible blood does NOT prevent HS

Hyperhemolytic Transfusion Reaction

Hematocrit

LDH

Ferritin
Etoposide Decreases Use of pRBCs

Units of pRBC transfused weekly

Absolute Neutrophil Count
Macrophage Activation Syndrome

Figure 2: Treatment protocol overview for Hemophagocytic Lymfohistiocytosis (HLH-2004)

- INITIAL THERAPY
  - Dexamethasone daily
  - Dexa (mg/m²)
    - 10 mg
    - 5 mg
    - 2.5 mg
    - 1.25 mg

- SCT / CONTINUATION THERAPY
  - Dexamethasone in pulses

- VP-16
  - Week 1-4

- CSA
  - Week 9-10

- I.T. therapy
  - Week 1-4
  - Week 9-10

Etoposide Leads to MAS Resolution

- Hematocrit
- Ferritin
- LDH
- Absolute Reticulocyte Count
Take Home Points

- “Why give 2 when 1 (or none) will do”
- Consider IVIG when interpreting antibody screen
- Hyperhemolysis: a life threatening transfusion complication
  - Hct lower after transfusion
  - Fever
  - reticulocytopenia
- HLH/MAS: clinical diagnosis
Acknowledgements

- Terry Gernsheimer
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- Vivian Oehler
- Mike Linenberger
- David Garcia
References

- Vamvakas and Blajchman. 2009. Blood. 113: 3406
The Patient

- HD 9 Hct dropped from 20 to 14 after 2u pRBC: started rituximab 375mg/m2 weekly x3
- HD 16 danazol 300mg BID x 4 days
- HD 17 hct 25, retic 66 (1.4%), LDH 687
- HD 19 1g/kg IVIG and 40K units epo. dramatic rise of LFTs (5xULN); improved after danazol stopped
- HD 20 Hct 21.5, retic 41 (0.9%). LDH 702
- HD 21 Hct 14.8, retic 34 (0.8%), LDH 542. 1g/m2 cyclophosphamide
- HD 23 PET: mildly hypermetabolic R internal and external iliac, pelvic sidewall LAD and post surgical changes (no convincing evidence of lymphoma)
- HD 25 R externalinguinal LAD (exisional biopsy): effacement with atypical plasmacytoid cells (clock face chromatin). No clonal aberrations. Negative flow. EBER, HHV8, IL6, SPEP/UPEP normal
- HD 31 liver biopsy: severe hemosiderosis in both Kupffer and hepatocytes. Evidence of drug injury
- Infection: CMV, EBV, adenovirus, enterovirus, HSV, HBV, HCV, HIV, HHV6, HHV8, parvovirus all negative. Multiple blood and urine culture negative
- ANA weak +, normal C3, low C4, DSDNA+, SSA Ro+, Smooth muscle+
- Polyclonal hypergammaglobulinemia
- Spiked a fever to 39.4 after her 51th unit of pRBCs (2u pRBCs daily from HD 9-32)
- Received B12 injections weekly, but never any folic acid during hospitalization

Due to difficulty treating hemolytic anemia, she was transferred to UWMC
- She has had very aggressive marginal zone lymphoma treatment since March 2014 including high dose steroids, 3 doses of rituximab, 1g/m2 cyclophosphamide, and etoposide. We have no evidence to think that MZL is driving macrophage activation syndrome (MAS) currently as MAS has stabilized and we are holding further etoposide based therapy. She is also having no symptoms from lymphoma. Specifically she denies fevers, chills, night sweats, or fatigue. She has had a persistent swelling that may have been increasing in her R groin on exam at the site of her prior lymph node resection.
- We had obtained an ANA on admission in early April and repeated an ANA again last week. Both labs were normal.
- hemolytic anemia (haptoglobin >100, plasma free hemoglobin undetectable, decreased LDH, transfusion independent)
- pure red cell aplasia (retic >250)
- MAS (ferritin <10K from >75K)
- marrow suppression with rising neutrophils, platelets and hematocrit, and
- CMV reactivation (CMV viral load remains undetectable off valgancyclovir).

http://www.uni-ulm.de/expane/docs/HLH%202004%20Study%20Protocol.pdf