Polyagglutination in a Child with Pneumococcal Hemolytic Uremic Syndrome

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Case Presentation to LCH Blood Bank

• Resident phone call:
  • Infectious Disease recommended RBCs should be washed for a patient with “T-cell activation” [?]

• Patient had been admitted 4 days previously
  – Had received 3 regular RBC aliquots, 4 platelet aliquots
Lurie Children’s Hospital Patient

• 13-month-old girl - previously healthy, vaccinated
  - 7d: fever, cough
  - 4d: dx pneumonia, antibiotic
  - 2d: switch antibiotic
  - 0d: Outside ED, hypoxia -> LCH

• T 103°F, decreased L breath sounds, CXR pneumonia, intubated

• Low urine output LCH day 2, became anuric day 3
Key Laboratory Results

- Hgb 10.6 -> nadir 7.1, Plt nadir 25K/uL
- LDH peak 1289 U/L (nl 209-463)
  - RBCs: acanthocytes, burr cells [no schistocytes]
- PT 10.5 sec, aPTT 53 sec, fibrinogen 242 mg/dL (nl 180-410)
  - D-dimer 1.04 ug/ml FEU (nl <0.65) [no clear DIC]
- Complement C3 44 mg/dl (nl 87-181), C4 8 mg/dl (nl 16-52)
- Group A, D+; negative antibody screen; DAT 1+ IgG, wk+ C3

- Day 1: Outside ED blood culture/PCR: *S. pneumoniae*
  - No serotype sent; ?not covered in PCV13 vaccine at 2, 4, 6 and 12-15 months?
**Dx: Pneumococcal Hemolytic Uremic Syndrome**

- Invasive pneumococcal disease
- Anemia, thrombocytopenia, hemolysis, elevated LDH
- Acute renal injury
- Positive direct antiglobulin test [not seen in other HUS types]
- DIC not present

**Dx: PHUS**

- Two plasma exchanges, albumin replacement, days 2 & 4
- Continuous hemofiltration, days 2-8

**Infectious Disease consult, day 4: recommended washed RBCs**

- Concern for potential hemolysis from transfused plasma
RBC T-Activation: Polyagglutination Testing

- LCH Blood Bank investigated patient’s RBCs for polyagglutination
- *Judd’s Methods*, 2008, procedure slightly modified

- Group $A_1$ reagent RBCs
- Group A patient’s RBCs
- Each individually mixed with 4 plasmas, group A patients >1 yr old
- Room temp incubation 15 min, centrifuge for agglutination

1) All plasmas nonreactive with reagent RBCs [rule out cold agglutinins]
2) All plasmas 2-4+ reactive with patient’s RBCs
- Presumptive evidence of RBC polyagglutination
Polyagglutination: Reference Laboratories

• Polyagglutination confirmation?
• Discussed with two reference labs

• Neither had a procedure for testing RBCs with normal plasma
• Both had only outdated lectin reagents for investigational testing
  – Not performed in this patient
Transfusion Management

• 3 RBC aliquots and 4 platelet aliquots had been given before blood bank was called on day 4
  – Day 2 plasma exchange circuit primed with RBCs
  – No evidence of extra hemolysis associated with transfusions
    • No transfusion reactions; Hgb levels responded initially
    • Total bilirubin normal days 1, 3, 4
    • Haptoglobin normal day 3

• 4 washed RBC aliquots days 4-12, similar Hgb responses
• Platelets were to be hyperconcentrated—none needed
Patient’s Outcome

- Hemofiltration discontinued day 8
- Extubated day 18
- Home day 31
- Pulmonary clinic, ongoing followup
Pneumococcal Hemolytic Uremic Syndrome

- PHUS in 0.5% of invasive pneumococcal infections
- 2-12% mortality, 10-16% of survivors with end-stage renal disease
- T-activation of RBCs and possibly other cell membranes
- Hemolytic anemia, thrombocytopenia, renal injury
- Natural anti-T in patient’s plasma
  - Thought to cause positive DAT, contribute to hemolysis
  - Might injure T-activated renal glomeruli?
- Complement pathway mutations in some patients, like atypical HUS
  - Szilagyi A, Nephrol Dial Transplant 28:2237, 2013 (3 of 5 patients)
- Plasmapheresis considered in selected cases (ASFA Class III indication)
- Washed RBCs, plasma avoidance often recommended
  - Although transfusion hemolysis rarely if ever reported in PHUS

Spinale JM, Curr Opin Pediatr 25:203, 2013
Polyagglutination

• RBCs with modified carbohydrate antigens, agglutinated by:
  – Normal plasmas: naturally occurring anti-carbohydrate antibodies
  – Lectins: specific carbohydrate-binding proteins from plants
• Microbial infections: enzymes modifying RBCs
  – T polyagglutination: pneumococcus, clostridium, cholera, influenza
    • Invasive pneumococcal disease, necrotizing enterocolitis
  – Other types: Tk, Th, Tx, acquired-B
• Genetic carbohydrate transferase mutations:
  – Congenital: Sd(a++) (“super-Sid”) (aka CAD)
  – Congenital dyserythropoietic anemia type II (aka HEMPAS)
  – Acquired: Tn clonal mutation, usually in hematologic diseases

Functions of >10 Pneumococcal Glycosidases in Airways

Various carbohydrate cleavage sites

King SJ, Molec Oral Microbiol 25:15, 2010
**Pr and M Structures: Enzyme Effects**

- **M/N at N-terminal**
- **Ficin** removes M/N and Pr sites
- **Neuraminidase** removes NAc-neuraminic acids
- Uncovers T antigen

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**Figure 15-2** To illustrate the amino acid sequence at the NH₂ terminal end of Glycophorin A when produced under control of an M (left) and N (right) gene.

**Amino acids**
- S = Serine
- L = Leucine
- T = Threonine
- G = Glycine
- E = Glutamic acid

**Carbohydrates**
- GN = N-acetyl-galactosamine
- G = Galactose
- N = N-acetyl-neuraminic acid (NeuAc)

*Leo A, Vox Sang 86:141, 2004*
*Issitt, Applied Blood Group Serology, 1998*
### Selected Polyagglutination Lectins

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Common name</th>
<th>T: acquired--infection</th>
<th>Tn: acquired--mutation</th>
<th>Sd(a++) (CAD): congenital</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine max (soja)</td>
<td>Soybean</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Arachis hypogaea</td>
<td>Peanut</td>
<td>+*</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dolichus biflorus (group O and B RBCs**)</td>
<td>Horse gram</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salvia horminum</td>
<td>Annual clary</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Salvia sclarea</td>
<td>Clary sage</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

- *Peanut lectin also reacts in infection-acquired Th, Tk, and Tx types
- **Dolichus biflorus is the ABO A₁ typing lectin

Adapted from AABB Technical Manual
T-Activation for Diagnosis of PHUS

- RBC T-activation detectable after ≥ 25-55% removal of sialic acids
  - Springer GF, J Biol Chem 257:2744, 1982

- PHUS tests, case series & review (Loupiac A, Pediatr Infect Dis J 32:1045, 2013)
  - T-activation 97% (90/93)
  - Polyagglutination 88% (7/8)
  - Positive DAT 62% (31/50)

- In HUS cases of unknown cause:
  - T lectin test “might be most appropriate test for direct diagnosis”

- Positive T lectin test can suggest PHUS before microbiology confirms
  - Strobel E, Blood Transfus 12:425, 2014
Blood Bank Lectin Kits (Not) in US

• T-lectin in FDA In-Vitro Diagnostics database of approved tests:
  – Only Gamma Biologicals
  – But no longer made by Gamma/Immucor

• Hemo Bioscience (Morrisville, NC) had made blood bank lectin kits
  – But production discontinued in 2016

• Current US testing options -- investigational testing:
  – Use dwindling stocks of outdated lectins
  – Import blood bank lectin kits, e.g., Source BioScience, UK
  – Make “homemade” reagent from raw materials or purified lectins
    • Recipes: Judd’s Methods in Immunohematology, 2008
Case Summary

• 13-mo-old, pneumococcal pneumonia, hemolytic uremic syndrome
  – Serious condition, substantial mortality and morbidity

• Patient’s RBCs were polyagglutinable in simple plasma testing

• T-lectin testing is advocated for assisting diagnosis of PHUS

• But blood bank lectin kits are no longer made in US
  – Reference labs would need to adopt non-approved tests or develop “in-house” reagents
The Blood Banker’s “In-House” T-Lectin Production

[or a Super Bowl party recipe]