Case Study: T-activation in the newborn

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Clinical presentation

- The patient is a 3 week old female who was born on 6/15/14 at 27\(\frac{6}{7}\) weeks via C-section

- She was intubated after birth for respiratory distress and had a complicated hospital course
  - anemia of prematurity, indirect hyperbilirubinemia requiring bili lights, possible sepsis near birth (completed 7 days of ampicillin and gentamicin)

- Because of the anemia, she was transfused 1 aliquot of pRBCs on 6/26 and again on 6/27
27.1%

Normal high 60.0
Normal low 39.0
Critical low 21.1%
On 7/6 her hematocrit was 29.4% and by 7/10 it had fallen to 26.4%. She was transfused one aliquot on 7/10 with pRBCs.

During the evening on 7/10, she was noted to have abdominal distention.

Distention continued throughout the night. Repeat x-rays showed pneumoperitoneum and pneumointestinalis consistent with perforated bowel, likely necrotizing enterocolitis (NEC).
Surgical management

- She was intubated and underwent laparotomy. She was found to have gangrene of the small bowel.

- 30 cm of bowel was resected and abdomen was packed and left open (for a possible second look procedure in the next couple days)
Intra-operatively

- Surgeon noted a brownish-red fluid in the peritoneal cavity with a “sickly” odor

- Sent a STAT Gram stain to microbiology
  - Gram positive rods (also some Gram negative rods)
  - Presumptive dx of Clostridium perfringens

- Sent peritoneal fluid for anaerobic and aerobic culture
Friday 7/11/14

- Blood bank resident was called at 4:30 pm because there was a concern for intravascular hemolysis.
  - The patient had blood drawn for coags that was grossly hemolyzed straight from her peripheral IV. There was gross blood in her urine. Her potassium sample was critically high at 7.5 mmol/L (normal 3.5-5.1)

- The clinical team referred us to an 1987 article from Arch Dis Child “T-activation haemolysis and death after blood transfusion” and asked about this as a possible cause for her hemolysis

- They were also concurrently transfusing a neonatal unit of pRBCs to bump her Hgb before imminent surgery and wanted to know if this would be a problem
  - Her Hgb had been 10.3 at 12:15 pm and fell to 6.6 by 3:25 pm
She is critically ill with active hemolysis.

What is T-activation and how do we test for it?
Laboratory Evaluation of Polyagglutination
Initial IRL Testing Results

- Sample collected 07/14/2014
- Initial testing demonstrated group O RBCs
- Rh type positive, mixed-field noted due to recent transfusion of O Negative red cells
- DAT Negative with Polyspecific AHG
- Antibody screen negative at IS, LISS-37°C, and LISS-AHG

<table>
<thead>
<tr>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-A,B</th>
<th>Anti-D</th>
<th>Rh Cont.</th>
<th>Anti-A (human)</th>
<th>Anti-B (human)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>O</td>
<td>O</td>
<td>2⁺⁶⁺⁺⁺MF</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
</tbody>
</table>

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Polyagglutination Investigation

- Patient’s Red Cells were reactive with all examples of fresh normal adult serum
- A lectin panel revealed the following results:

<table>
<thead>
<tr>
<th></th>
<th>Arachis hypogea</th>
<th>Salvia sclarea</th>
<th>Dolichos biflorus</th>
<th>Glycine soja</th>
</tr>
</thead>
<tbody>
<tr>
<td>07/10/2014 Sample</td>
<td>○</td>
<td>○</td>
<td>○</td>
<td>○</td>
</tr>
<tr>
<td>07/15/2014 Sample</td>
<td>3+MF</td>
<td>○</td>
<td>○</td>
<td>○</td>
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</tbody>
</table>

- While normal red blood cells are nonreactive when tested with these lectins, positive reactions are noted when polyagglutinable cells are tested. The pattern of reactivity can help identify the type of polyagglutination.

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The initial results indicate that the red cells are possibly Tk-activated:

<table>
<thead>
<tr>
<th>Lectin</th>
<th>Normal Cells</th>
<th>Polyagglutinable Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T</td>
</tr>
<tr>
<td><em>Arachis hypogea</em></td>
<td>O</td>
<td>+</td>
</tr>
<tr>
<td><em>Salvia sclarea</em></td>
<td>O</td>
<td></td>
</tr>
<tr>
<td><em>Dolichos biflorus</em></td>
<td>O</td>
<td></td>
</tr>
<tr>
<td><em>Glycine soja</em></td>
<td>O</td>
<td>+</td>
</tr>
</tbody>
</table>

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What is Polyagglutination?

- Initial testing with the lectin panel indicates that the patient’s red cells are Tk transformed, however there are other types of polyagglutination that would give similar patterns of reactivity.
- To determine the true type of polyagglutination, we have to think about the different types and causes of polyagglutination.
- A polyagglutinatable cell is agglutinated by most human ABO-matched adult sera, but not by cord sera. The abnormality is a property of the red cells and not the sera.
- Polyagglutination can cause:
  - Erroneous blood typing results
  - Incompatible crossmatches when the donor’s cells are polyagglutinable
  - Delay in transfusion
  - Hemolytic transfusion reaction
  - Hemolytic anemia and myelodysplasia
  - Leukopenia and thrombocytopenia
What is Polyagglutination?

Polyagglutination results from the exposure of cryptic antigens on the red cell membrane.

The different types of polyagglutination represent the exposure of different cryptic antigens on the red cell.

These antigens are naturally occurring on the surface of all RBCs, but are normally concealed.

The cryptic antigens are exposed via inheritance, mutation, or by the action of bacteria or viruses.

All normal adult serum contains antibody to these cryptic antigens.

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Classification of Polyagglutination

- **Transient forms**
  - Include T, Th, Tk, Tx, VA, Acquired B
  - T-activation is associated with or occurs with bacterial or viral infections, most often in children

- **Persistent forms**
  - Include Tn, H.E.M.P.A.S.
  - These types of polyagglutination result from a somatic mutation of pluripotent hematopoietic stem cells and are permanently acquired

- **Inherited forms**
  - Cad, NOR, Tr

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T-activation

- The T antigen is normally concealed by sialic acid (N-acetylneuraminic acid)
- Bacterial neuraminidase removes the N-acetylneuraminic acid residues from normal disialylated tetrasaccharides of MN, Ss glycoproteins and exposes the T receptor
- The T-receptor is recognized by a specific anti-T polyagglutinin present in most normal adult human sera.

![Diagram of Normal tetrasaccharide]

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Tk-activation

- The Tk antigen is part of the paragloboside antigen
- Bacterial β-galactosamine removes β-galactose from the membrane exposing the Tk receptor

Paragloboside

Gal\_β(1-4) GluNAc\_β(1-3) Gal\_β(1-3) Glu-Ceramide

Tk Receptor
Polyagglutination Characterization

- Additional testing was performed to further characterize the type of polyagglutination noted.
- The red cells were treated with papain which eliminated the reactivity with *Arachis hypogea*.

<table>
<thead>
<tr>
<th>Patient Sample</th>
<th>Arachis hypogea</th>
</tr>
</thead>
<tbody>
<tr>
<td>07/15/2014</td>
<td></td>
</tr>
<tr>
<td>Neat cells</td>
<td>3+MF</td>
</tr>
<tr>
<td>Papain-treated cells</td>
<td>0</td>
</tr>
</tbody>
</table>

- The Tk receptor is enhanced by enzyme treatment while the T and Th receptors are damaged.
- This testing puts doubt on the preliminary conclusion of Tk activation.

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Polyagglutination Characterization

- The patient’s cells were incubated with a 1% solution of Polybrene and no aggregation was noted.
- Polybrene is a polycation potentiator. It is a positively charged polymer which acts by neutralizing the negative charge on normal RBCs which causes spontaneous aggregation of red cells possessing normal sialic acid levels. Since the negative charge is almost entirely due to sialic acid groups, cells that lack sialic acid (T, Th, and TN) will not aggregate.
- The cells were tested with Glycine soja lectin and were nonreactive and with Ulex europaeus and were 3+.
- This pattern of reactivity is consistent with Th-activation.

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## Testing Summary

<table>
<thead>
<tr>
<th>Other Testing</th>
<th>Lectin</th>
<th>T</th>
<th>Tk</th>
<th>Tn</th>
<th>Cad</th>
<th>Th</th>
<th>Patient Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Arachis hypogea</strong></td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>O</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td><strong>Salvia sclarea</strong></td>
<td>O</td>
<td>O</td>
<td>+</td>
<td>O</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td><strong>Dolichos biflorus</strong></td>
<td>O</td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td></td>
<td><strong>Glycine soja</strong></td>
<td>+</td>
<td>O</td>
<td>+</td>
<td>+/-</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Enzyme</td>
<td><strong>Arachis hypogea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td><strong>Polybrene</strong></td>
<td>O</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>O</td>
<td>O</td>
</tr>
<tr>
<td>Ulex europaeus</td>
<td><strong>(H lectin)</strong></td>
<td>Normal</td>
<td></td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>3+</td>
</tr>
</tbody>
</table>
Th-activation

- Th-activation is considered to be a weakened form of T-activation with fewer sialic acid residues removed.
- This type of polyagglutination is seen in patients with infections and newborns with neonatal necrotizing enterocolitis or hemolytic uremic syndrome.
- Th-activation is most often associated with *E. coli*, *Bacteriodes*, and *Clostridia* infections.
How does polyagglutination affect the patient clinically?

- Polyagglutination can be suspected in patients with infection, intravascular hemolysis, hemoglobinuria, and hemoglobinemia after transfusion or those who don’t get post-txn hemoglobin increase.
- The condition is usually transient lasting days or weeks but may persist for months.
- The amount of neuraminidase in the circulation influences the degree of T/Th-activation and the severity of hemolysis if present.
- Once the infection is resolved, there is no more bacterial neuraminidase. Newly produced RBCs are not exposed to the enzyme and will remain normal.
Our patient has T activation, but is it causing hemolysis?

- Largest case series in 1989 of 1672 infants who were all screened for T activation. Only 10 had T activation and only 4 of those had hemolysis (only 1 of the 4 with hemolysis didn’t have a transfusion. The other 3 got FFP with low titer anti-T). Because hemolysis didn’t occur in patients without T activation, authors advocated hemolysis was related to plasma-blood products and advocated for widespread T activation screening.

- Osborn et al in 1990 studied 201 infants with NEC and found that infants with T activation had higher mortality (35% vs 7%) and hemolysis rate (71% vs 21%), use of low titer anti-T blood products didn’t reduce mortality.

- Boralessa et al in 2002 studied 375 neonates and found 48 (12.8%) developed T activation during stay in NICU. NO infants (including the 5.6% with NEC) developed hemolysis in association with transfusion of blood products.

- Hall et al in 2002 studied 104 infants with NEC. 23 infants (22%) tested positive for T-activation. There was no difference in requiring operative treatment for infants with T activation vs not. They reported 39% mortality for those with T activation and 28% mortality without (not significant). Used washed products.
  - “Although activation of the T antigen per se does not pose any threat to the infant with NEC, it does alert us to the risk of hemolysis and advanced disease.”

- Wang et al in 2011 examined 43 infants with NEC. 4 (9%) had weak T activation and RBC transfusion did not result in hemolysis regardless of washed/unwashed products.
Let’s say you believe in T activation. How does it work?

- Mechanism is unclear
- Most arguments that support anti-T as the cause of hemolysis are based on temporal association between hemolysis and plasma-containing blood product (our patient)
- Animal studies (rabbit, rat, mouse) suggest hemolysis of T-activated RBCs is due to faster clearance because of decreased sialic acid residues NOT immune mediated with interactions between anti-T and T. Increased clearance may be related to net charge
  - Remember anti-T is IgM and active at low thermal ranges and doesn’t fix complement….pretty difficult to make this a culprit
  - Except for a few early (1950s-ish) reports, the DAT is negative in patients with T activated RBCs. Early reports were likely false positives from anti-T in polyclonal antiglobulin reagents
Supporting T activation... Implications for transfusion of products

- **RBCs** – have approximately 30-50 mL of plasma
  - Washing RBCs (preserved with CPDA) shortens shelf life from 35 days to 24 hours with 20% loss of the unit
  - May increase risk of bacterial contamination
  - Delay in availability (time of washing and transport)
  - Increases donor exposures. Instead of using one 7 day old unit in aliquots, must use new one daily
  - Increased cost because of waste- washing an entire unit to give neonatal aliquot

- **Platelets** – contains 100-500 mL of plasma
  - Washing decreases platelets in a unit by as much as 25% and may effect hemostatic function

- **FFP** –
  - Delay in time/availability
The other side of the aisle

- T-activation is not uncommon, 10-30% of infants with NEC, but severe hemolysis is rarely only attributable to plasma-containing products. Especially significant because these patients can require lots of product.
- Many infants are not screened for T-activation so likely patients with T-activation are frequently getting plasma with high titers of anti-T without problems.
- There are other reasons for hemolysis...
Other possibilities for hemolysis

1. **Necrotizing enterocolitis (NEC)** is characterized by abdominal distention, bloody stools, shock, metabolic acidosis, and DIC. Usually affects premature neonates
   - Fatality rate of 9-28% from septicemia or coagulopathy

2. **Intravascular hemolysis from Clostridium perfringens**
   - Clostridium perfringens is part of GI flora in 70% of newborns
   - Is an anaerobic Gram positive rod that produces at least 12 exotoxins, including a hemolysin
   - **Hemolysin** is an alpha-toxin (lecithinase), which hydrolyzes phospholipids in RBC membranes and causes **spherocytosis**. Spherocytes are sensitive to osmotic lysis (hemolysis).
   - DAT will be negative in cases of Clostridium induced hemolysis
Our patient’s peripheral smear
The rest of the story...

- On the evening of 7/11, the patient had an additional 50 cm of bowel resected including the ileocecal valve.

- On 7/15/14, had a 3rd look exploratory-laparotomy with abdominal wall and ostomy debridement. Sampled fluid for additional cultures.

- Original peritoneal fluid cultures (from 7/10) confirmed the gram positive rods were Clostridium perfringens.
7/18 - 4th look abdominal washout and wound-vac change. Discovered additional bowel perforations. Additional bowel removal was incompatible with life.
- Also had positive wound cultures with heavy Candida tropicalis

7/20 patient noted to have pneumothorax requiring thoracentesis. Overnight on 7/20 patient had worsening bradycardia and acidosis

On 7/21 it was decided with withdraw ventilator support and patient died at 7:03 am
Product usage after 7/11 hemolytic episode

- 7/12 – 2 aliquots washed pRBCs
- 7/13 - 1 unwashed aliquots pRBCs
- 7/14 – 3 aliquots platelets, 2 aliquots washed pRBCs
- 7/15 – 2 aliquots platelets, 1 aliquots washed pRBCs
- 7/16 – 1 aliquots platelets
- 7/17 - 1 aliquots washed pRBCs
- 7/19 – 1 aliquots platelets
- 7/20 – 1 aliquots washed pRBCs
1 unwashed pRBC
2 washed pRBCs
1 washed pRBC

HCT

%
References


Thank you!