Pneumococcal Induced T-activation with Resultant Thrombotic Microangiopathy

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Abstract: Thrombotic microangiopathies are disorders resulting from platelet thromboses forming in the microvasculature with resultant schistocyte forms. Hemolytic uremic syndrome (HUS) is a microangiopathic hemolytic anemia often complicated by acute renal failure in children. HUS is typically caused by bacterial infection, most commonly enterohemorrhagic Escherichia coli. Neuraminidase-producing organisms, such as Streptococcus pneumoniae have also been reported as potential etiologies. The pathogenesis in these cases involves cleavage of sialic acid residues from the surfaces of erythrocytes, platelets, and glomerular capillary endothelial cells, exposing the Thomsen-Friedenreich antigen, a process known as T-activation. We describe a 2-year-old girl who presented with pneumococcal pneumonia and sepsis ultimately resulting in a thrombotic microangiopathy with acute renal failure, most consistent with HUS. The patient’s direct antiglobulin test was positive. Polyagglutination was observed with human adult serum, but not with umbilical cord serum. Her red blood cells (RBCs) were reactive against peanut and soybean lectins, but not Salvia sclarea or Salvia horminum lectins. These findings are consistent with T-activation. Clinicians should be cognizant of the possibility of T-activation with resultant HUS in patients infected with neuraminidase-producing bacteria. Such patients may be difficult to identify using monoclonal typing antisera, as these typically do not have anti-T antibodies. Whether such patients are at risk for transfusion-associated hemolysis is debatable.

Keywords: T-activation, hemolytic uremic syndrome, HUS, polyagglutination, Streptococcus pneumoniae, pneumococcus, thrombotic microangiopathies
Introduction

Hemolytic uremic syndrome (HUS), a common cause of acute renal failure in children, is one of a group of disorders referred to as “thrombotic microangiopathies.” HUS-related symptoms include fever, microangiopathic hemolytic anemia, thrombocytopenia and renal failure. Prior association of HUS resulting from preceding infection with veracytotoxin or shigella-like toxin-producing bacteria, in particular the enteric pathogen Enterohemorrhagic Escherichia coli (EHEC) O157:H7 and other E. coli serotypes have been documented.1–3 EHEC results in hemorrhagic bloody diarrhea. Additionally, infections involving neuraminidase-producing bacteria, especially Streptococcus pneumoniae, have also been documented as etiologies of atypical non-diarrheal HUS.5–7

The pathophysiology involves neuraminidase cleavage of the N-acetylneuraminic (sialic) acid from glycoproteins in the cell membranes of erythrocytes, platelets and glomerular endothelial cells with subsequent exposure of the Thomsen-Friedenreich antigen (T antigen).5 Anti-T IgM antibodies, present in plasma of humans greater than six months of age,8 subsequently bind the exposed antigen on involved RBCs, platelets, and endothelial cells (see Fig. 1) with resultant hemolysis, RBC polyagglutination, thrombocytopenia, and thrombotic renal microangiopathy.9–11

Case Report

A two year old Caucasian female presented with fever and respiratory difficulty including tachypnea, grunting, intercostal retractions, and a cough. A right-sided otitis media was also noted. Laboratory values are shown in Table 1. Initial X-ray results were significant for bilateral lobar pneumonia, which was consistent with the physical exam findings.

Her blood counts decreased over the first three days of hospitalization, as can be seen in Table 1. Also present were elevated fibrin degradation products and D-dimer. The patient typed as O positive. No reactivity was observed with the Anti-A and Anti-B typing reagents, which are of monoclonal origin and not human source material. Monoclonal antisera typically do not contain anti-T. Her direct antiglobulin test was 2+ positive, due to erythrocyte-bound complement (C3b, C3d) components. The antibody screen, using both low-ionic strength saline and polyethylene glycol-potentiated tests, was negative, as was a short cold panel. The Donath Landsteiner test for biphasic cold hemagglutinins was also negative.

When tested with several samples of human adult and cord serum, the patient’s red blood cells revealed strong reactivity with adult sera only. The sample was sent to a reference laboratory (ImmucorGamma, Houston, TX) for confirmation of polyagglutination and for classification of the particular polyagglutinable state. The patient’s red cells were found to be reactive (4+) with Arachis hypogaea (peanut lectin) and Glycine soja (soybean lectin) and non-reactive with Salvia sclarea and Salvia horminum lectins (see Table 2), most consistent with T-activation. She was transfused with washed RBCs and volume-reduced platelets.

Blood culture was positive for S. pneumoniae and was identified as Serotype 19A by the Texas Department of Health. Nasopharyngeal cultures were negative for adenoviruses, influenza virus types A and B, and parainfluenza types 1, 2, and 3. Fecal cultures were negative for E. coli O157:H7 and Campylobacter spp. as well as other enteric pathogens. She was hyperkalemic and hyperbilirubinemic on hospital day #4, consistent with hemolysis. Peripheral blood smear examination revealed schistocytes and thrombocytopenia consistent with microangiopathic hemolytic anemia (Fig. 2) in addition to neutrophilic toxic granulation as expected in septicemia. This was considered most likely HUS because of acute renal failure (as evidenced by increased serum creatinine and

Figure 1. Left, Schistocytes in peripheral blood, indicative of microangiopathic hemolytic anemia (Wright-Giemsa, x600). Right, Closer view of schistocyte and neutrophilic toxic granulation (Wright-Giemsa, x1000).
blood urea nitrogen) and known association with pneumococcal infections and T-activation, although confirmation with ADAMTS13 was not performed. The patient ultimately required mechanical ventilation to treat metabolic acidosis prior to transfer to an institution capable of pediatric dialysis.

The patient’s diagnoses upon transfer included thrombotic microangiopathy most likely HUS with acute renal failure secondary to pneumococcal pneumonia and sepsis, acute respiratory distress syndrome, pneumonia thoraces, and pleural effusions ultimately requiring bilateral thoracostomy tube placement after transfer. Upon transfer, the patient underwent 3 weeks of continuous ambulatory peritoneal dialysis (CAPD) followed by nighttime intermittent peritoneal dialysis (NIPD). The patient’s urine output slowly improved. A renal biopsy performed some time later at the outside institution revealed acute tubular necrosis (ATN) without evidence of cortical necrosis. Fibrosis involved approximately 25% of the interstitium. Surprisingly, no microthrombi were seen. Considered was the possibility that ATN secondary to sepsis was the primary disease process; ATN, however, is not typically associated with microangiopathic hemolytic anemia or T-activation. The patient was ultimately discharged on hospital day #36. At the one week follow-up appointment, her physical exam was normal as was her blood pressure. Significant laboratory values are listed in Table 1.

The patient was instructed to avoid crowded places for 2–3 months and to receive the influenza vaccine in order to minimize the chances of acquiring any respiratory illness. The long-term renal prognosis for this patient appears to be good in light of her normal physical exam, normal blood pressure, and normal renal function, although careful follow-up is ongoing because of her prolonged ATN and the presence of interstitial fibrosis on biopsy.

### Table 1. Pertinent laboratory data.

| Test                        | Admission | Day #3 | Day #4 | Day #10 | Follow-up | Reference range |
|-----------------------------|-----------|--------|--------|---------|-----------|-----------------|
| WBC count                   | 24.7      | 34.2   | 14.3   | 6.5     | 6–17 k/µL |                 |
| Hemoglobin                  | 11.6      | 5.0    | 7.3    | 8.8     | 10.5–13.5 mg/dL | |
| Hematocrit                  | 35.5      | 14.4   |        | 27.0    | 33%–39%   |                 |
| Platelet count              | 471       | 44     | 85     | 366     | 140–400 k/µL|                 |
| Sodium                      | 142       | 145    | 137–145 mmol/L |         |           |                 |
| Potassium                   | 6.6       | 3.9    | 3.6–5.0 mmol/L |         |           |                 |
| Chloride                    | 116       | 111    | 98–107 mmol/L |         |           |                 |
| Bicarbonate                 | 17        | 24     | 22–30 mmol/L |         |           |                 |
| Blood urea nitrogen (BUN)   | 20        | 71     | 82     | 8       | 9–20 mg/dL |                 |
| Creatinine                  | 3.5       | 0.4    | 0.8–1.5 mg/dL |         |           |                 |
| Glucose                     | 91        | 92     | 65–110 mg/dL |         |           |                 |
| AST                         | 194       | 21–72 IU/L |         |           |                 |
| ALT                         | 33        | 0.2–1.3 mg/dL |         |           |                 |
| Total bilirubin             | 5.1       | 2.3    | 13.8   | 41.1    | 24.3–32.8 sec |                 |
| PT                          |           | 0.4–1.3 mg/dL |         |           |                 |
| PTT                         |           | 0.4–1.3 mg/dL |         |           |                 |

### Table 2. Differentiation of polyagglutinable states. Characteristic agglutination patterns to various lectins.

| Lectin                    | Acquired | B | T | Tk | Tn | Cad | Patient results |
|---------------------------|----------|---|---|----|----|-----|-----------------|
| Arachis hypogaea          | –        | + | + | –  | –  | –   | 4+              |
| Dolichos biflorus         | +/-      | – | – | –  | –  | –   |                 |
| Glycine soja              | –        | + | – | +  | +  | +   | 4+              |
| Salvia horminum           | –        | – | – | +  | +  | –   |                 |
| Salvia sclarea            | –        | – | – | +  | –  | –   |                 |
Discussion
This case is not meant to provide new information on the topic of T-activation, but is meant to be a review of a relatively uncommon clinical phenomenon.

Polyagglutination
Polyagglutinable RBCs are red blood cells agglutinated by a large proportion of human adult serum, regardless of blood group, and are usually non-reactive with their autologous serum or serum from cord blood samples. Principle causes of polyagglutination are modification of RBC membrane structure by microbial enzymes, incomplete biosynthesis of RBC membrane-associated carbohydrates, and inheritance of uncommon haplotypes. Several forms of polyagglutination have been described. Those associated with microbial infection are T, Tk, Th, Tx, and acquired B. Tn syndrome is a typical cause of polyagglutination by incomplete biosynthesis, while those due to inheritance are Cad, NOR, HEMPAS, VA, and Hemoglobin M-Hyde Park. Differentiation can be made by use of seed extracts, known as lectins (except for NOR). Reaction patterns of some of the polyagglutinable states with a lectin panel are depicted in Table 2. T-activation caused by bacterial infections may occur quickly in vivo, but is usually transient, resolving within weeks or months. Additionally, previous research has revealed approximately 0.5% of healthy individuals may also be affected with this condition.

Anti-T, a naturally occurring antibody, reacts best at room temperature or colder, rather than at 37 °C, and does not appear to activate complement. The antibody is predominately IgM, which likely forms as a result of exposure to intestinal flora with bacterial structures that have antigenic similarity to red blood cell cryptantigens. T antibodies, found in all human adult sera, begin to develop between 3 and 6 months of age and are present at adult levels by two years of age.

Transfusion of patients with T-polyagglutination
The concern of transfusing patients with T-activated RBCs is related to the possibility of anti-T antibodies present in plasma-containing blood products causing hemolysis. Hemolysis in patients with T-activation has been reported, whether or not the patient has been transfused. Some reports show that no hemolysis was observed after transfusion of anti-T containing products, while very severe hemolysis and death have been reported in other patients. Post-transfusion hemolysis may be related or dependent on the cause of T-activation. In addition to hemolysis, patients with S. pneumoniae associated-HUS with T-activation may be subject to renal damage from transfusion of anti-T containing blood products. However, transfusion may be required for the hemostatic factors provided in these components and may outweigh the risk of hemolysis associated with passive transfusion of anti-T.

Therefore, the prudent course of action would be to limit transfusions to products containing low titers demonstrated on red cells, platelets, white blood cells, and tissue cells. In HUS patients specifically, the T-antigen has been demonstrated to be present on the glomerular capillary loops and renal tubular epithelium. T antigens, normally hidden, are exposed when neuraminidases are released from bacterial organisms which then cleave the sialic acid residues covering the T-antigens (Fig. 2). T-activation can be identified by the red cells’ reactions with the lectins derived from peanuts, Arachis hypogaea and their reactions with soybean lectin, Glycine soja. (Table 2). T-activation caused by bacterial infections may occur quickly in vivo, but is usually transient, resolving within weeks or months. Additionally, previous research has revealed approximately 0.5% of healthy individuals may also be affected with this condition.
of anti-T antibody. Blood products and derivatives containing small amounts of IgM include cryoprecipitate, purified albumin concentrates, and IVIG. If RBC transfusion is indicated, washed RBCs or red cells suspended in 10% albumin or plasma protein fraction may decrease hemolytic complications. For selection of donor plasma, a minor crossmatch may be beneficial in identifying those units with the lowest anti-T titers. However, other authors have demonstrated that there is minimal to no risk of transfusion-associated hemolysis in patients with T-activation, making this a very controversial issue.

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Disclosures
This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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