

Invasive *Streptococcus pneumoniae* infection causing hemolytic uremic syndrome in children: Two recent cases

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INTRODUCTION: *Streptococcus pneumoniae* is an uncommon cause of hemolytic uremic syndrome (HUS) with a unique pathophysiology that differs from Shiga toxin-related HUS.

METHODS: Case descriptions for each patient are provided. Each strain of *S pneumoniae* was subjected to a pulsed-field gel electrophoresis (PFGE) analysis, Shiga toxin assay and polymerase chain reaction to detect Shiga toxin genes. A review of the current literature was conducted.

CASE PRESENTATIONS: Two patients with *S pneumoniae*-related HUS that presented to the Alberta Children's Hospital, Calgary, Alberta, within four weeks of each other in 2001 are described. Both presented with pneumonia and empyema with associated HUS. Both patients required dialysis, one patient for 10 days and the other for 18 days. Neither patient demonstrated evidence of Shiga toxin-related disease. *S pneumoniae* isolated from blood or pleural fluid was penicillin susceptible. One isolate was serotype 3 and the other was serotype 14. The two strains had different PFGE patterns. Both patients recovered well with no persistent renal dysfunction.

CONCLUSIONS: *S pneumoniae* continues to be an uncommon but important cause of HUS. Most cases can be confirmed or at least considered probable without performing a renal biopsy.

Key Words: Hemolytic uremic syndrome; *Streptococcus pneumoniae*

Streptococcus pneumoniae has long been reported to be an uncommon cause of hemolytic uremic syndrome (HUS) (1,2). The pathogenesis of *S pneumoniae*-associated HUS is different from Shiga toxin-associated HUS. The primary factor appears to be the production of neuraminidase by *S pneumoniae* (1). Patients treated with washed blood products tend to have a better outcome, likely related to decreased binding of naturally occurring antibodies to activated T antigen (3-7). The lack of a consistent case definition has resulted in debate about the distinction between cases of *S pneumoniae*-associated HUS and disseminated intravascular coagulation associated with invasive *S pneumoniae* infections, especially when diagnostic renal biopsy is not performed (8).

HUS is the most common cause of acute renal failure in children (3). Most cases are caused by Shiga-like toxins (9,10), and the etiology for Shiga-like toxin-associated HUS is usually

Deux cas récents d'infection envahissante à *Streptococcus pneumoniae*, qui ont dégénéré en syndrome hémolytique et urémique chez des enfants

INTRODUCTION : Les infections à *Streptococcus pneumoniae* dégénèrent rarement en syndrome hémolytique et urémique (SHU) qui, par sa physiopathologie unique, diffère du SHU causé par la toxine de Shiga.

MÉTHODE : Les deux cas ont fait l'objet de description. Chaque souche de *S. pneumoniae* a été soumise à une électrophorèse sur gel en champ pulsé, à un dosage de la toxine de Shiga et à une réaction en chaîne de la polymérase pour détecter la présence des gènes de la toxine de Shiga. Nous avons passé en revue la documentation sur le sujet.

EXPOSÉ DE CAS : Deux patients atteints d'un SHU lié à *S. pneumoniae* se sont présentés à notre établissement en l'espace de quatre semaines en 2001. Les deux présentaient à la fois une pneumonie et un empyème associés au SHU. Les deux ont dû être traités par dialyse : l'un pendant 10 jours, l'autre pendant 18 jours. Aucune trace de la présence de la toxine de Shiga n'a été décelée chez les deux enfants. La souche de *S. pneumoniae* isolée dans les prélèvements de sang et de liquide pleural s'est montrée sensible à la pénicilline. Un isolat était de sérotype 3; l'autre, de sérotype 14. Les deux souches ont réagi différemment à l'électrophorèse. Les deux patients se sont bien rétablis, sans subir de séquelles rénales.

CONCLUSION : *S. pneumoniae* continue d'être une cause rare mais importante de SHU. Sa présence peut être confirmée ou tout au moins fortement présumée dans la plupart des cas, sans biopsie rénale.

enterohemorrhagic *Escherichia coli* 0157:H7 (3,9,11). However, many other *E coli* serotypes, as well as *Shigella dysenteriae*, *Aeromonas* species and the human immunodeficiency virus can cause HUS (9). In addition, other neuraminidase-producing organisms, such as influenza A virus, have been reported to cause HUS (12,13). Hereditary, drug-induced, disease-associated and idiopathic HUS have been reported (9,11). The disease is heralded by the sudden onset of microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure, often preceded by an acute gastroenteritis with bloody diarrhea (9,10).

We present two patients with *S pneumoniae*-associated HUS who presented to the Alberta Children's Hospital, Calgary, Alberta within one month of each other in 2001, and who were not epidemiologically linked. We also review the published literature concerning HUS associated with *S pneumoniae*.

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METHODS

A chart review was performed for each case. The organisms were identified from the clinical specimens using standard techniques, and antibiotic susceptibility testing was performed. Serotyping of the *S pneumoniae* isolates was performed by the Quellung method at the National Streptococcus Centre in Edmonton, Alberta.

Pulsed-field gel electrophoresis (PFGE) analysis was performed on both isolates. The *Sma* I restriction endonuclease was used in 1% agarose gel using 0.5 × tris-borate-ethylene diamine tetraacetic acid buffer at 10°C. The Chef Mapper (BioRad, USA) was used, and switch and run times were 0.20 to 35 s over 21 h, with a voltage gradient of 6.0 V/cm.

S pneumoniae exotoxin was determined by both toxicity assay and polymerase chain reaction (PCR) to identify the genes responsible for exotoxin production. The toxicity assay was performed using Vero cells. Controls used were *S pneumoniae* ATCC 27336, *S pneumoniae* ATCC 49619 and *E coli* pJB 128 (harbouring the VT1 operon on a multicopy plasmid, VT1 positive) (14). After overnight growth of the organism in brain heart infusion broth, 1.5 mL was removed and filtered using a Millex-GV syringe filter (Millipore, USA). This was then used for the exotoxin production assay. The plate was incubated in a humid chamber at 37°C and observed every 24 h for cytopathic effect.

PCR to identify Verotoxin genes was also performed. Control strains included *S pneumoniae* ATCC 27336 (negative control), an *E coli* 0157:H7 strain known to be VT1 positive (laboratory clinical isolate) and an *E coli* 0157:H⁻ strain known to be VT2 positive (laboratory clinical isolate) (positive controls). A suspension containing one of the above organisms was pelleted, resuspended in 200 µL 10% Triton X-100 (Sigma, USA) and boiled for 20 min. The specimens and controls were then centrifuged for 20 min at 13,200 rpm, and the supernatant was used for the PCR. The following PCR conditions were used: hold for 15 min at 95°C, 25 cycles (denature for 40 s at 94°C, anneal for 40 s at 55°C, and elongate for 40 s at 72°C), hold for 6 min at 72°C and finally hold forever at 4°C.

The literature review consisted of an electronic search of the National Library of Medicine MEDLINE index using the search terms "*Streptococcus pneumoniae*", "pneumococcus" and "hemolytic uremic syndrome". In addition, the reference lists of articles found with this search were examined to identify more articles. We identified 27 articles that reported on 70 cases of HUS associated with *S pneumoniae* (1-7,10,11,15-32).

CASE PRESENTATIONS

Case report 1

A two-year old, previously well, white boy presented with a seven-day history of upper respiratory symptoms with gradual worsening. He presented with fever, cough, vomiting, lethargy and decreasing urine output. There was no history of diarrhea, urinary tract infections, easy bruising or bleeding, travel, poorly cooked meats or unpasteurized dairy products. He attended daycare and had a 10-month-old sibling with an upper respiratory tract infection. Family history was noncontributory.

On admission, he was afebrile (however, fever had been noted by family), with a pulse of 150 beats/min and a blood pressure of 120/80 mmHg. His respiratory rate was 50 breaths/min with significantly increased effort. He was moderately dehydrated. His oxygen saturation remained normal in room air. Chest examination revealed dullness to percussion and decreased breath sounds on the right. Jaundice and pallor were

also present. A chest x-ray showed right middle and lower lobe consolidation and a right-sided effusion.

His hemoglobin level was 95 g/L, which decreased to 67 g/L after rehydration. Platelets were 15×10⁹/L, with a normal white blood cell count of 4.8×10⁹/L. The erythrocyte sedimentation rate was 148 mm/h. His creatinine level was 77 µmol/L and urea level was 21 mmol/L. The bilirubin and lactate dehydrogenase concentrations were 145 µmol/L and 2805 U/L, respectively. Serum cold agglutinins were strongly positive. There was gross hematuria, and the urinalysis demonstrated proteinuria. A smear of the peripheral blood demonstrated fragmented red blood cells and schistocytes consistent with microangiopathic hemolytic anemia. The partial thromboplastin time was 74 s (normal 25 to 35 s), international normalized ratio (INR) was 1.1, D-dimers were 0.2 to 0.4 mg/L (normal less than 0.2 mg/L) and fibrinogen was 9.3 g/L (normal 2 to 4 g/L). Cefotaxime and vancomycin were started for empiric therapy. Blood cultures and subsequent pleural fluid obtained by thoracentesis grew *S pneumoniae*, serotype 3 – susceptible to penicillin, cefotaxime and vancomycin. Pleural fluid chemistry revealed a concentration of glucose less than 0.5 mmol/L, a concentration of lactate dehydrogenase greater than 4500 U/L, a pH of 7.73 and a protein level of 35 g/L. Urine culture was negative. Acute and convalescent serology for *Mycoplasma pneumoniae* was also negative. Stool cultures were negative for *E coli* 0157 and *Shigella* species, but serology was positive (titre greater than 1:128) for antibody to Shiga toxin 2 by neutralization assay by the National Laboratory for Public Health, Winnipeg, Manitoba. This may have indicated past exposure. After antibiotic susceptibilities were known, the antibiotic treatment was changed to intravenous penicillin G.

His course in hospital included six washed, packed red blood cell transfusions, five washed platelet transfusions, nine days of hemodialysis and an additional nine days of peritoneal dialysis. His course was complicated by the empyema, treated conservatively without chest drain, pancreatitis (lipase level 3041 U/L) and hepatitis (aspartate aminotransferase level 800 U/L and alanine aminotransferase level 269 U/L). At 11 months following admission, his renal function was normal, with a creatinine concentration of 42 µmol/L and a calculated creatinine clearance of 95 mL/min/1.73 m².

Case report 2

A four-year-old, previously well, white boy presented with intractable epistaxis and a seven-day history of upper respiratory symptoms, including sore throat. During the five days preceding admission, symptoms of abdominal pain, vomiting, increasing fevers with progressive tachypnea and dyspnea were present. He had no history of diarrhea, fast food or poorly cooked meat products. There was no history of travel. His mother and sister also had upper respiratory infections. His immunizations were incomplete. Family history was significant for urinary tract infections but not renal disease.

On admission, he was afebrile, with a pulse of 159 beats/min and a blood pressure of 105/67 mmHg. His respiratory rate was 55 breaths/min with obvious increased effort. He was moderately dehydrated. His oxygen saturation was normal in room air. Chest examination revealed dullness to percussion with decreased breath sounds to the left hemithorax. Jaundice and pallor were also present. A chest x-ray confirmed left lower lobe consolidation with effusion.

On admission, his hemoglobin level was 69 g/L. His platelet count was $4 \times 10^9/L$ and his white blood cell count was $13.7 \times 10^9/L$, with 52% neutrophils and 29% band forms. The erythrocyte sedimentation rate was 148 mm/h. His creatinine level was 110 $\mu\text{mol/L}$ and his urea level was 20 mmol/L. The total bilirubin and lactate dehydrogenase concentrations were 75 $\mu\text{mol/L}$ (20 $\mu\text{mol/L}$ conjugated) and greater than 4500 U/L, respectively. The aspartate aminotransferase level was 400 U/L, and the alanine aminotransferase level was normal. The urine demonstrated gross hematuria and proteinuria. A smear of the peripheral blood demonstrated fragmented red blood cells and schistocytes consistent with microangiopathic hemolytic anemia. The patient's partial thromboplastin time was 47 s, INR was 1.4, D-dimers were 0.8 to 1.6 mg/L and fibrinogen was 5.1 g/L. Cefotaxime and vancomycin were empirically started.

Blood cultures and subsequent pleural fluid cultures grew *S pneumoniae*, serotype 14 – susceptible to penicillin, cefotaxime and vancomycin. A urine culture was negative. A throat swab for group A beta-hemolytic streptococcus was negative and the antistreptolysin O titre was negative. Stool cultures were not sent, but serology for Shiga toxins 1 and 2 were negative by neutralization assay. After antibiotic susceptibilities were known, antibiotic treatment was changed to intravenous penicillin G.

His course in hospital included five washed, packed red blood cell transfusions, four washed platelet transfusions and 10 days of hemodialysis. He required nasal cauterization and packing; the clinical course was further complicated by empyema, hypertension, pericarditis and an incidental finding of Wolff-Parkinson-White syndrome diagnosed after an episode of tachycardia in the perioperative period.

At nine months postadmission, his renal function was normal, with a creatinine of 33 $\mu\text{mol/L}$ and a calculated creatinine clearance of 132 mL/min/1.73 m².

The *S pneumoniae* isolates from both cases were unique on PFGE analysis. Neither organism showed evidence of Shiga toxin production nor evidence of Shiga toxin-encoding genes.

DISCUSSION

The presumed mechanism of pathogenesis for HUS associated with *S pneumoniae* differs from Shiga toxin-related disease (1,33). The Thomsen Friedenreich antigen (T antigen) is a normal structure on the surface of erythrocytes and renal capillary endothelial cells that is obscured by neuramic acid (1,19,34). The neuraminidase produced by *S pneumoniae* (35,36) circulates in serum (6,19,37) and removes neuraminic acid from glycoproteins, revealing the T antigen on cell surfaces (1,6,35). This leads to binding with T antigen-specific immunoglobulin (Ig) M, which is present in all human serum by the age of six months (1,19,34,35), resulting in hemolysis (1,35), agglutination (27) and microangiopathy in the kidneys (1,35) with secondary thrombocytopenia. In such cases, circulating serum anti-T antigen IgM levels may be reduced to zero as a result of consumption (19).

This activated T antigen is also present on hepatocytes and may contribute to the hepatic dysfunction noted in some case series (10,16); as well, T antigen found in the choroid plexus in the brain may contribute to the pathogenesis of meningitis (38,39). Patients with *S pneumoniae*-associated HUS have demonstrable neuraminidase activity, while those with invasive *S pneumoniae* disease without HUS do not (6).

Early recognition of *S pneumoniae*-associated HUS is important – plasma or plasma-containing blood products may worsen the clinical course, because most healthy individuals contain anti-T IgM in their serum (6,19,40). Fluorescein-labelled peanut agglutinin (*Arachis hypogaea*) confirms the presence of T antigen on tested cells or tissues (35,41). Although this assay is a straightforward test, it is not routinely performed in many laboratories (Dr D Easton, Calgary Laboratory Services, Calgary, Alberta August 2002, personal communication). The direct Coombs test is another test that detects (auto-) antibody binding to red blood cells, and a previous, brief review of cases of HUS associated with *S pneumoniae* noted that when performed in these cases, the direct Coombs test is usually positive (42). It has been suggested that this test, which is more readily available, could be useful to detect HUS early in cases of invasive *S pneumoniae* disease (42). However, there are no data on the specificity of the direct Coombs test in these cases.

It is uncertain how often HUS occurs in association with *S pneumoniae* infections. A review of cases from Atlanta found that HUS occurred after 0.6% of invasive *S pneumoniae* infections (3). In contrast, HUS develops after 5% to 14% of *E coli* O151:H7 infections (43,44).

There was significant mortality in the published cases of *S pneumoniae*-associated HUS, with 15 deaths among 72 cases (21% of the total of the 70 cases previously reported in the literature and the two cases reported in the present study) (1,3,6,7,11,19,20,32). These deaths were reported in publications from 1977 to 2001, and the cause of death was not always described, but when described, it was related to acute or chronic renal disease, or to severe acute *S pneumoniae* infection. Thus, it cannot readily be determined whether there is a trend in the incidence of case fatalities related to changes in the care provided to these children. The use of washed blood products has been shown to improve outcome (3-7); earlier treatments included exchange transfusion (19,35).

Although our two patients recovered normal renal function, among reported survivors, there are a considerable number of children with ongoing renal problems; many went on to end-stage renal failure (2,4,5,10,11,15,16,18,31).

Similar to the present cases, most of the reported *S pneumoniae*-associated HUS cases are linked to focal invasive infections. Sixty-five per cent (47 of 72 cases) were associated with pneumonia and/or empyema (1-6,10,11,15-19,26-28,31), and 31% were associated with meningitis (3,6,7,11,16,20,23,25,29,31). Sepsis was documented in 22% (1,2,18,21-24,28-30,32) and otitis media in 7% of cases (3,19); one case was associated with mastoiditis (3). One study suggested that HUS typically followed pneumonia by approximately one week (18); however, our patients demonstrated both concurrently. Among the described cases, *S pneumoniae* was isolated from several different sites, including blood (61%) (2-6,11,16,18,20-22,24,25,27-30,32), cerebrospinal fluid (27%) (3,7,11,16,20,25,29) and pleural fluid (13%) (10,16,19,26).

Most authors did not comment on the coagulation profiles in their cases. However, some authors did note similar, mild abnormalities in the coagulation profiles seen in their cases, which were similar to the present cases (3,5,16,25,30,31). It is unclear whether these mild abnormalities are part of a systemic inflammatory response to the *S pneumoniae* infection or are a result of HUS.

TABLE 1
Canadian Paediatric Society *Streptococcus pneumoniae*-associated hemolytic uremic syndrome case definitions

Children under the age of 16 years demonstrating the following (need not be present simultaneously):

- 1) Evidence of invasive *S pneumoniae* infection (blood or another normally sterile biological fluid: cerebrospinal, pericardial, articular, peritoneal, pleural), excluding middle ear, sinus and tracheal aspirates
- 2) Both renal and hematological organ failures defined as:
 - a) Acute renal impairment with serum creatinine:
 - >50 µmol/L if younger than five years old
 - >60 µmol/L if five to nine years old
 - >90 µmol/L if 10 to 13 years old
 - >110 µmol/L if older than 13 years old
 - b) Microangiopathic hemolytic anemia (hemoglobin <100g/L with fragmented red cells)
 - c) Thrombocytopenia (<150,000×10⁹/L) in the absence of septicemia, malignant hypertension, chronic uremia, collagen or vascular disorders
- 3) No chronic conditions that are causative for the renal and hematological abnormalities seen

Other organ failures may occur

Definite case	Evidence of thrombotic microangiopathy on renal biopsy or autopsy
Possible case	Distinction between pneumococcal sepsis with secondary organ failures and <i>S pneumoniae</i> -associated hemolytic uremic syndrome will be determined through a Delphi process

Data taken from reference 49

Both of our patients had mild liver function abnormalities. This has been described in several patients as part of their clinical presentation (5,10).

There is variability in the laboratory criteria for reported cases. Of the 70 reported cases, only 16 had clear demonstration of T antigen with agglutination on red blood cells (4-7,10,16,19,27), seven had data about T antigen agglutination on kidney tissue (1,6,15,26,27) and six had data about thrombotic microangiopathy on kidney tissues (11). The presence of T antigen on cell surfaces and its clinical implications warrant further investigation; however, it is not clear what kind of T antigen test may be appropriate as a routine test.

One of our patient's HUS was caused by *S pneumoniae* serotype 14, and the other's was caused by serotype 3. Serotype 14 is the most prevalent serotype causing invasive disease in young children in Canada (28% of invasive isolates); serotype 3 is much less common (1% of invasive cases) (45). Serotype 14 has been previously associated with HUS (3,5), in addition to 6B (3), 9V (6,15), 19 (6,27), 3 (19), 8 (25) and 23F (3). "Nephritic strains" of *S pneumoniae* have been discussed in older literature (46). There are no current data about whether specific serotypes or virulence factors, apart from neuraminidase, are important in the pathogenesis of HUS.

There is no consistent case definition for *S pneumoniae*-associated HUS. The Canadian Paediatric Society has a surveillance definition, and the Centers for Disease Control and Prevention currently have a case definition for postdiarrheal HUS (47), but not for *S pneumoniae*-associated HUS alone. The Canadian Paediatric Surveillance Program case definition is outlined in Table 1. From April 2000 to March 2002, three

possible cases and one definite case were reported in Canada (48) using the above definitions, and debate continues as to which cases truly qualify as *S pneumoniae*-associated HUS (3,8).

We suggest a modification to the Canadian Pediatric Society case definition so that 'possible' cases are considered to be 'probable' cases. Renal biopsy is not usually necessary to make a diagnosis of *S pneumoniae*-associated HUS if all other criteria have been fulfilled and if clinicians are confident that the laboratory changes are not caused by sepsis. The diagnosis can be based on findings consistent with microangiopathic hemolytic anemia on a peripheral blood smear, in addition to the biochemical renal and hematological abnormalities noted in Table 1. In circumstances in which the diagnosis or etiology is unclear, a biopsy could be considered.

If a widely accepted case definition can be determined for *S pneumoniae*-associated HUS, further studies about the clinical course, as well as the therapeutic and prognostic value of measuring neuraminidase activity, T antigen and T antigen antibody should be facilitated.

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