Shiga Toxin-Producing *Escherichia coli*-Associated Kidney Failure in a 40-Year-Old Patient and Late Diagnosis by Novel Bacteriologic and Toxin Detection Methods

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Infection with Shiga toxin-producing *Escherichia coli* (STEC) is the most common cause of kidney failure in children. High morbidity is also associated with infections in the elderly. We describe STEC-associated kidney failure in a 40-year-old patient, including the methods used to identify STEC a month after disease onset.

CASE REPORT

A 40-year-old African-American male with a 10-year history of hypertension presented at a community hospital with several days of abdominal cramps, vomiting, and loose stool with interspersed blood. Upon admission, he was found to be in acute renal failure with anemia and occult blood in the stool. He was afibrile, with an elevated blood pressure of 243 over 172 mm Hg and an otherwise normal physical examination that included neurologic, skin, and abdominal examinations. Admission laboratory results included an abnormal hematocrit of 27.4%, a platelet count of 69,000/mm3, a blood urea nitrogen level of 83 mg/dl, and a creatinine level of 9.5 mg/dl. Coagulation parameters were normal.

On hospitalization day 5, a microangiopathic hemolytic process was considered on the basis of a peripheral blood smear that showed occasional schistocytes, polychromasia, normal platelets, helmet cells, and no spherocytes. Three days later, bone marrow biopsy results indicated a left shift of erythroid precursors, increased megakaryocytes with enlargement and cytoplasmic vacuolization, and increased stainable iron. At that time, the patient’s anemia persisted with a hematocrit of 58.5%; a reticulocyte count of 69,000/mm3, a lactate dehydrogenase level of 588 (normal, 120 to 190) U/liter, and a creatinine level of 12.3 mg/dl; haptoglobin, less than 10 (normal, 30 to 200); lactate dehydrogenase level, 855 U/liter; reticulocyte count, 6.1%. The patient’s peripheral blood smear revealed normal platelets, anisocytosis, poikilocytosis, occasional schistocytes, and helmet cells. A renal ultrasound showed normal-size kidneys with slightly increased echogenicity. Total plasma exchange was attempted for 2.5 days but was interrupted by an anaphylactoid reaction on day 3. One week after the last total plasma exchange, a test for von Willebrand’s factor (vWf) multimeric cleaving protease showed a normal level (vWf protease levels are decreased in TTP but normal in the case of hemolytic-uremic syndrome [HUS]).

On day 30 of illness, a stool sample was obtained and cultured for enteric pathogens, including *Escherichia coli* O157:H7 (no prior stool cultures had been ordered). That culture was reported negative for enteric pathogens after 48 h. Our clinical suspicion of enteric infection-related HUS or TTP was high. Therefore, the same fecal sample that had been cultured by the hospital laboratory was refrigerated and frozen for 1 month. Toxin detection methods (see below for details).

A dietary history was taken from the patient upon his transfer to our hospital. He noted that he had eaten fast food restaurant hamburgers 4 and 6 days prior to the onset of his initial gastrointestinal symptoms. In addition, he reported that he had eaten a steak the evening before the onset of his illness. The steak had been marinated overnight, transported to the desert in an ice chest, and grilled many hours later. After several transfusions, the patient’s laboratory findings were as follows: hematocrit, 35.7%; platelet count, 290,000/mm3; creatinine level, 12.3 mg/dl; haptoglobin, less than 10 (normal, 30 to 200); lactate dehydrogenase level, 855 U/liter; reticulocyte count, 6.1%. The patient’s peripheral blood smear revealed normal platelets, anisocytosis, poikilocytosis, occasional schistocytes, and helmet cells. A renal ultrasound showed normal-size kidneys with slightly increased echogenicity. Total plasma exchange was attempted for 2.5 days but was interrupted by an anaphylactoid reaction on day 3. One week after the last total plasma exchange, a test for von Willebrand’s factor (vWf) multimeric cleaving protease showed a normal level (vWf protease levels are decreased in TTP but normal in the case of hemolytic-uremic syndrome [HUS]).

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The patient remains hemodialysis dependent 4 months after the onset of symptoms. The clinical impression is that of HUS or TTP precipitated by Shiga toxin-producing *E. coli*. Indicators for ongoing microangiopathic hemolysis have subsequently improved. While we favor a diagnosis of HUS, we realize that the normal vWf protease level may be a result of the antecedent plasmapheresis.

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On the day the fecal sample was referred to our laboratory, it was screened for the presence of preformed Shiga toxin by the Vero cell cytotoxicity assay (4, 11). In addition, an investigational 10-min chromatographic immunoassay for Stx1 or Stx2 detection was performed. This handheld Shiga toxin detection (HHSTD) system, manufactured at the Naval Medical Research Center (Silver Spring, Md.), incorporates rabbit polyclonal anti-Stx1 or anti-Stx2 antibody immobilized on filter paper to capture Stx-Stx1 or Stx2 and Stx2 toxin variants, respectively, as they migrate across the membrane in sample buffer. Colloidal-gold-labeled toxin-specific monoclonal antibodies (described elsewhere [10, 12]) were also used to impregnate the filter paper but not immobilized. The monoclonal antibodies bind the antigen, if it is present, and form a visible band where the capture antibodies are fixed to the filter paper. We tested the sensitivity of the kits with purified toxin and stool specimens seeded with known numbers of toxin-producing organisms. The level of detection was 3 to 6 ng of toxin (for Stx2 and Stx1, respectively) or approximately 10^7 CFU of Shiga toxin-producing E. coli (STEC). The Stx2 kit was less sensitive for the Stx2 variant Stx2c or Stx2d and detected 20 ng of toxin or 10^4 CFU. The kits were specific for toxin type and showed no cross-reactivity between Stx1 or Stx2 and its variants or with negative human stool components in our trials.

The results of the HHSTD immunoassays and the conventional cytotoxicity assay of the primary stool sample were negative. However, we reasoned that very low numbers of STEC bacteria would be shed at this late point after disease onset. We also suspected that if O157:H7 organisms were present, their appearance would be masked by the predominant normal flora seen when stool is plated directly onto sorbitol MacConkey (SMAC) agar (3). Similarly, toxin production would be below detectable levels. Two approaches were used to enhance STEC recovery and increase toxin detection. First, in order to observe greater numbers of organisms in the stool, we suspended approximately 1 ml of sample in 10 ml of brain heart infusion broth (Remel, Lenexa, Kans.) and incubated the mixture overnight at room temperature to slow the growth of normal fecal flora, as is done when culturing Yersinia enterocolitica. The enrichment broth was diluted in serial 10-fold increments on the next day, and 100 μl of each dilution was spread plated onto Difco Luria-Bertani (LB) agar and SMAC agar (Becton Dickinson, Sparks, Md.) and incubated overnight. Clearly discernible individual sorbitol-negative and -positive colonies were seen in the 10^-6 and 10^-7 dilutions plated on SMAC agar. Sweeps of growth from the corresponding dilutions of overnight broth plated on LB agar were collected with a sterile cotton swab and mixed in 1 ml of the HHSTD kit buffer, and 300 μl was tested for toxin (isolates from SMAC agar were not used owing to the concern that false-positive reactions in the HHSTD kit might occur because of carryover of neutral red from the MacConkey base). The HHSTD kit gave positive reactions for Stx1 and Stx2 on the mixed bacterial sweeps, which prompted us then to screen the sorbitol-positive and -negative colonies on SMAC agar for toxin production.

A Shiga toxin gene probe was used to identify individual toxin-producing organisms by colony blot hybridization. One hundred fifty colonies of both the sorbitol-positive and -negative phenotypes were transferred with sterile toothpicks onto three LB agar plates (50 colonies each) in a grid pattern. The colonies were blotted onto nitrocellulose membranes after overnight incubation. The membrane-bound colonies were lysed, and the DNA was denatured with saturation with 0.5 N NaOH. We used colony blot hybridization. The sorbitol-negative phenotype correlated 100% with hybridization to the Stx2 gene probe. The sorbitol-negative colonies were identified as E. coli with an API 20E strip (BioMérieux, Durham, N.C.), and the serotype was determined by agglutination with O157 and H7 antisera (Centers for Disease Control and Prevention, Atlanta, Ga.). Clari-

fied overnight culture lysates of the E. coli O157:H7 isolate (designated WR30) produced 10^5 50% cytotoxic doses/ml by Vero cell assay and were positive for both Stx1 and Stx2 when tested with the HHSTD kit.

At the same time that the broth enrichment stool culture was inoculated, a second broth culture was prepared to enhance toxin expression by induction of lysogenic toxin-converting bacteriophages. One milliliter of the fecal sample was diluted in 10 ml of brain heart infusion broth to which 5 μl of mitomycin C (Sigma, St. Louis, Mo.), an alkylating agent known to induce the lytic cycle of lambdoid phages, was added (1, 6). After overnight incubation at 37°C with shaking, the broth culture supernatant was tested for Shiga toxin with the investigational test kit. A weak positive reaction for Stx2 was obtained, whereas a similarly prepared mitomycin-free broth culture was negative.

In E. coli O157:H7 strain EDL933, the Stx2-converting phage is readily induced but the phage that encodes Stx1 in that strain appears to be defective (9). Such differences in the associated toxin-converting phages may account for increased expression of Stx2 but not Stx1 with mitomycin C treatment in strain WR30.

HUS follows infection with STEC in approximately 10% of infected children under the age of 10 years and produces a mortality rate of 5% in that age group (13). STEC infections in elderly adults have also been associated with high morbidity and mortality (2, 5). Although individuals between the ages of 10 and 65 years seldom develop HUS, it is important to consider STEC infection in the diagnosis of acute renal failure in adults, as this case illustrates. Culture confirmation of STEC-associated kidney failure may be problematic because of the low numbers present in the postdiarrheal phase of the disease. We found that isolation of STEC late in infection was facilitated by broth culture enrichment, followed by serial dilution. The investigational immunochromatographic Shiga toxin detection kit we used was helpful in the quick identification of Shiga toxin-producing colonies on the dilution culture plates, although the sensitivity of the test remains to be analyzed with clinical specimens. We speculate that commercially available rapid antibody-based toxin detection kits could be used for the same purpose. Mitomycin C induction further increased the sensitivity of our antibody-based Stx2 detection kit, a strategy that has been suggested by others (1, 6). Antibiotics such as ciprofloxacin that have been associated with increased toxin expression due to bacteriophage induction may also prove use-
ful for amplifying toxin expression in clinical samples (14, 15). The methods described for toxin detection would be practical for detection of non-O157:H7 STEC strains. Such sorbitol-fermenting organisms are responsible for an estimated 36,000 cases of hemorrhagic colitis in the United States annually, but they are not detectable with the widely used SMAC agar screening procedure (7, 8).

The contents of this report are solely our responsibility and do not necessarily represent the official views of the Department of the Army and the Department of Defense.

REFERENCES