"Children on the Frontline Against *E.coli*": Typical Hemolytic-Uremic Syndrome

HEIDI ANDERSEN

A thirteen-month old infant presented with classical hemolytic-uremic syndrome (HUS), but with negative cultures for *Escherichia coli (E. coli)* 0157:H7. HUS is commonly linked to infection with *E. coli* 0157:H7; however, traditional culture has demonstrated poor sensitivity. Pathogenesis of the organism in HUS involves the production of a Shiga-like toxin (STX), resulting in a triad of symptoms. An early and accurate differential diagnosis, based on patient presentation with acute renal failure, hemolytic anemia, and thrombocytopenia, is critical for supportive treatment and improved prognosis. Patient prognosis is related to the duration of renal failure and dialysis treatment. Research is aimed at improved detection of *E. coli* 0157:H7 or the STX produced, and future vaccination to eliminate typical HUS.

ABBREVIATIONS: CDC = Centers for Disease Control and Prevention; HUS = hemolytic-uremic syndrome; STX = shiga-like toxin; TTP = thrombotic thrombocytopenic purpura.

INDEX TERMS: hemolytic-uremic syndrome.

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Heidi Andersen MT(ASCP) *works at St John's Hospital, Anderson IN and at Indiana University Hospital, Indianapolis IN.*

Address for correspondence: *Heidi Andersen MT(ASCP), 2007 B Parsons Drive, Indianapolis IN 46224. (317) 459-3023. handerse@iupui.edu*

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CASE STUDY

In January, a thirteen-month-old Caucasian male presented with progressive diarrhea over a period of two weeks. During this time, medical attention was sought, and the infant was diagnosed with a common childhood diarrhea, suspected to be due to a rotavirus. However, the infant continued with progressive diarrhea and began showing signs of pallor, dehydration, petechiae on his thighs, and decreased appetite. The infant began experiencing episodes of acute abdominal pain with intermittent periods of lethargy. As the diarrhea worsened, one sixteenth of a tablet of Imodium® was given to the infant and he was brought to the local emergency department (ED).

The infant presented in the ED with signs of edema in the extremities from oliguria and acute renal failure. He was catheterized, treated with Lasix® to stimulate kidney function, and given nutritional IV support. Vitals revealed hypertension with a blood pressure of 132/55 (normal blood pressure of a 1-year-old Caucasian male is 102/57) and a temperature of 103.5 °F. As a result, the infant was given Hydralazine® and Tylenol® to reduce his elevated blood pressure and temperature respectively.

The initial laboratory results indicated a diagnostic triad of thrombocytopenia, hemolytic anemia, and acute renal failure (Table 1). Subsequently, the infant was diagnosed with hemolytic-uremic syndrome (HUS). Also, it is important to note that the infant's increased white cell count and fever supported the diagnosis of the onset of the inflammatory reaction that occurs in typical HUS, with *E. coli* 0157:H7 suspected as the cause.

QUESTIONS TO BE CONSIDERED

- What is the typical presentation of HUS and the patient population at risk?
- What is the pathogenesis and pathophysiology of typical HUS?
- What is the differential diagnosis of typical HUS?

- What is the treatment and long-term prognosis of typical HUS?
- What are preventive measures that can be taken to protect children from HUS?

INTRODUCTION TO HUS

The syndrome now known as HUS, was described in 1955 by Gasser. In 1982, Riley isolated pathogenic Shiga-like toxin producing *E. coli* (STEC) serotype 0157:H7 from contaminated hamburger. Then, Karmali linked HUS to *E. coli* 0157:H7 in 1985. Connection of *E. coli* 0157:H7 to HUS was a major breakthrough in differentiating the mechanisms of the often-confused conditions of thrombotic thrombocytopenic purpura (TTP) and HUS. HUS is currently accepted as the most common cause of acute renal failure in children in the U.S. and is primarily caused by *E. coli* 0157:H7. The Centers for Disease Control and Prevention (CDC) estimate that with 73,000 *E. coli* 0157:H7 infections per year in the U.S., 2% to 7% of *E. coli* 0157:H7 infections result in HUS with the majority of cases occurring in Caucasians less than five years of age.[1] The elderly, adults, and older children have higher mortality rates from HUS than occur in younger children.[2,3] Interestingly, HUS caused by infection with *E. coli* 0157:H7 is less common in African-Americans.[4] Overall, *E. coli* 0157:H7 infections costs about 60 lives and $660 million dollars each year, and is now an endemic cause of HUS in the U.S.[1,5,6]

Table 1. Initial laboratory testing of patient on day 1 of hospitalization

Tests	Results	Reference ranges
Albumin	33	30.8 - 46.6 g/L
BUN	32	1.8 - 7.1 mmol/L
Chloride	102	95 - 105 mmol/L
CO2	17	22 - 27 mmol/L
Creatinine	336	17.7 - 61.9 mol/L
Glucose	5.7	2.2 - 11.1 mmol/L
Hematocrit	0.258	0.340 - 0.480
Hemoglobin	85	96 - 156 g/L
Platelet #	64	150 - 450 x 10^9/L
Potassium	5.3	3.5 - 5.5 mmol/L
Sodium	131	135 - 145 mmol/L
Total bilirubin	11	0 - 10 g/L
WBC #	21.5	5.5 - 17.5 x 10^9/L

Classical HUS is defined as a thrombotic microangiopathy with a diagnostic triad of acute hemolytic anemia with schistocytes, thrombocytopenia, and acute renal failure. However, classical HUS does not always present with a complete diagnostic triad. A major indicator of typical HUS from *E. coli* 0157:H7 is presentation with a one to eight day acute gastroenteritis prodrome and bloody diarrhea, unless the infection is acquired by a urinary tract infection or a respiratory infection.[7,8] The kidney is the major organ target in classical HUS, but other organs such as pancreas, heart, lungs, and brain may also be involved.[9-12] Other factors supportive of a diagnosis of HUS due to *E. coli* 0157:H7 include its predominance in the summer months and its potential to occur in outbreaks.

HUS, as noted by its name, is merely a syndrome, and therefore, has many known etiologies. Typical (infectious) HUS is caused by bacterial and viral infections such as *E. coli* 0157:H7, some non-0157 *E. coli* strains, *Shigella dysenteriae* 1, *Streptococcus pneumoniae*, *Salmonella typhi*, *Campylobacter jejuni*, and HIV. Non-infectious, atypical HUS may be secondary to (but not limited to) pregnancy and postpartum, organ transplant, glomerulonephritis, systemic lupus erythematosus, or treatment with drug therapies such as tacrolimus (FK506), quinine, and mitomycin. However, it is estimated in the U.S. that 90% of cases of HUS in children are primary infections caused by *E. coli* 0157:H7 with increasing findings of non-0157:H7 *E. coli* cases, which may cause higher incidences of HUS in other countries.[13,14]

Infection with *E. coli* 0157:H7 can be acquired from several sources. Enterohemorrhagic *E. coli* (EHEC) is carried asymptomatically in the intestines of cattle, where Globotriaosylceramide (Gb_3) receptors are found throughout the intestinal tract, but cattle lack Gb_3 receptors in the vasculature.[15,16] These findings may play a significant role in colonization. However, the reason cattle are asymptomatic carriers of *E. coli* 0157:H7 is still being studied. During slaughter, the surface of EHEC contaminated meat is ground into and spread throughout the hamburger. Only 50 to 100 viable organisms of *E. coli* 0157:H7 are required to cause infection.[7] Other food products, besides ground beef, that can be a reservoir for EHEC after contamination with cattle feces include: unpasteurized milk products, apple juice, water, and vegetables. There is seasonality to the shedding of EHEC from cattle, where there is an increased amount of organism shed in the summer months, which correlates with an increase in products with fecal contamination, *E. coli* 0157:H7 infections, and HUS in the warmer season.[17] The infant in the case study, however, presented with HUS in January.

Although cattle are the major source of *E. coli* 0157:H7, it has been found in other animals, e.g., sheep, goats, deer, and even a small percentage in cats, dogs, horses, and flies.[18,19] *E. coli* 0157:H7 has been shown to survive ten months or longer in contaminated sources that resulted in human infection.[20] Additionally, *E. coli* 0157:H7 can readily be transmitted from person to person, causing concern to families, nursing homes, daycare facilities, and other crowded living conditions.[21,22] After September 11, 2001, *E. coli* 0157:H7 was considered to be a potential agent of bioterrorism.[23] In the case of this thirteen-month old child, no other children or adults with whom he had contact were infected.

Pathogenesis and pathophysiology

The pathogenesis of typical HUS begins with the hardiness of *E. coli* 0157:H7. *E. coli* 0157:H7 can easily live through freezing at –80 °C for as long as nine months. Its acid resistance increases survival in acidic foods (conditions that are normally used to preserve food from bacterial contamination) because acidity decreases the nutritional competition with other non-acid-resistant organisms. *E. coli* 0157:H7 can remain viable in a pH environment less than 2.5.[24]

Once *E. coli* 0157:H7 enters the body orally, it survives the pH of the stomach, and colonizes in the mucosa of the colon. The exact mechanism of *E. coli* 0157:H7 pathogenicity is under extensive research. A proposed mechanism is that the lipopolysaccharide and STX from the organism are responsible for activating the phosphatidylinositol cascade, increasing mobilized ionized calcium that allows adherence of the organism to the epithelial cells of the colon and promotes lesion formation. Lesions change the permeability of the epithelial cell membrane to water and electrolytes, causing the prodromal diarrhea, and initiate an inflammatory response partially responsible for the microangiopathic thrombosis.[25] The inflammatory response activates neutrophils that release cytokines, platelet activating factor (PAF), and tissue necrosis factor alpha. These play a role in increasing activated neutrophils (leukocytosis), platelet aggregation,[26] and up-regulating Globotriaosylceramide (Gb_3) receptors on kidney and brain endothelial cells,[12] respectively. Close proximity of the injured epithelial cells to microvasculature results in hemorrhagic colitis (HC) and bloody diarrhea in the typical HUS.

STX access to circulation following HC allows for direct and indirect mechanisms of pathogenicity. STX reaches the kidney and brain through the circulatory system on the surface of platelets, neutrophils, and monocytes.[27-29] STX bound to platelets induces platelet aggregation directly, and STX prevents apoptosis in neutrophils, resulting in leukocytosis and increased inflammation.[27,30]

The proposed primary target of STX from the circulation is the distal convoluted tubular epithelium in children (Gb_3 receptors may not be present in adults).[31] The injured renal epithelial cells also initiate an inflammatory response and release endothelin, tissue plasminogen activator inhibitor-1 (PAI-1), and PAF. Endothelin increases production of PAF, white blood cell activation that results in leukocytosis, and may somewhat increase blood pressure early on in typical HUS. PAI-1 inhibits fibrinolysis of microvascular thrombosis. PAF-activated platelets release thromboxane hence promoting platelet aggregation by vasoconstriction-induced high shear stress. Thromboxane is regulated by prostacyclin released from injured epithelial cells through negative feedback. However, in typical HUS, platelet activation outnumbers production of prostacyclin resulting in thrombosis.[32]

Microvascular endothelial cells that have Gb_3 receptors (in children and adults), particularly those of the glomerulus, are extremely sensitive to STX. Injury to the endothelial cells by bound STX is key to the pathogenesis of typical HUS.[7,32] STX causes extensive microangiopathic thrombosis, hypoxia, and ischemia in the glomerular endothelial cells, and similar damage can be found in the cerebral endothelial cells of the blood-brain barrier.[33] Injury to the endothelial cells is marked by elevated thrombomodulin levels. Lesions from STX involve the glomerular capillaries; thickening of the capillary wall near the glomerulus decreases the glomerular filtration rate.[34] Both ischemia and thickened capillaries elevate blood pressure. Progressive damage to the kidneys occurs via hyperfiltration of the functional nephrons that remain after the acute phase.[35] Damage to cerebral endothelial cells causes encephalophy, coma, stroke, and cerebral infarcts.[33]

Toxins are freely permeable to the glomerulus, and the large capillary surface area enhances toxin pathogenicity. The concentration of toxin is increased by countercurrent roles of vasculature and tubules in the kidney.[36] STX has one enzymatic subunit A and five receptor-specific B subunits. Subunit A invades and kills the renal endothelial cells by endocytosis and inhibiting translation. Subunit B activates neutrophils, releasing proteases and hydrogen peroxide that irreversibly damage renal cells.[37] Oxidative substances and fibrin-platelet aggregations occluding the microvasculature fragment red cells, producing schistocytes on the peripheral blood smear.

Differential diagnosis

It is critical to differentiate typical HUS from atypical HUS, thrombotic thrombocytopenic purpura (TTP), and disseminated intravascular coagulation (DIC) for both treatment and prognosis (Table 2). For example, typical HUS requires supportive therapy while TTP mandates plasma exchanges and possibly immunosuppressive treatment with glucocorticoids. Misdiagnosis could be life threatening; a significant number of deaths from TTP occur within forty-eight hours of presentation.[38]

Atypical causes of HUS are associated with a high mortality rate and recurrences. These causes include complement deficiency involvement, neurological involvement, an inborn error in the metabolism of cobalamin, or a factor H deficiency. Most secondary causes of atypical HUS can be ruled out with patient history. Generally, atypical HUS does not present with leukocytosis or GI prodrome and bloody diarrhea, and is seen more frequently in adults than young children, perhaps simply because adults are likely to have secondary causes.

TTP, a completely separate disease from HUS, is the result of a decreased level of von Willebrand Factor (vWF)-cleaving enzyme (ADAMTS-13) due to a deficiency of ADAMTS-13 or antibodies against it.[39,40] The classical presentation of TTP includes fever, central nervous system involvement, and the diagnostic triad seen in classical HUS. However, 25% of typical HUS cases have nervous system involvement and many present with fever.[41] Unlike typical HUS, TTP does not generally involve gastrointestinal inflammation, or leukocytosis.[42] TTP is usually systemic with severe thrombocytopenia resulting in platelet counts below 20 x 10^9/L, where typical HUS is primarily localized to the kidney with platelet counts between 30 x 10^9/L and 150 x 10^9/L. Risk for TTP is not targeted to a specific age group, like typical HUS. In the future, differentiating TTP from HUS may be easier by measuring the functional ADAMTS-13 level in the plasma.[43]

DIC is a systemic activation of both the coagulation cascade and fibrinolysis, and is commonly associated with pregnancy-related complications like atypical HUS. The easiest most reliable way to differentiate DIC is with routine coagulation testing. Prothrombin time (PT), partial thromboplastin time (PTT), and D-dimer levels are abnormal in DIC, but are normal in patients with typical and atypical HUS. However, vitamin K deficiency may prolong the PT, and D-dimer levels may be elevated with a high concentration of fibrin in the microangiopathic thrombus in patients with typical HUS.[44]

Table 2. Differential diagnosis of typical HUS including patient case study results

Laboratory and clinical findings	Patient	Typical HUS	Atypical HUS	TTP	DIC
Abnormal kidney tests	Y	Y	Y	Y	Y/N
Abnormal liver tests	N	N	N	N	Y/N
Abnormal PT, PTT, and D-dimer	N	N	N	N	Y
CNS involvement	N	Y/N	Y/N	Y	Y
GI prodrome/ bloody diarrhea	Y	Y	N	N	N
Hemolytic anemia	Y	Y	Y	Y	Y
Hypertension	Y	Y	Y	Y	Y
Leukocytosis	Y	Y	N	Y/N	Y
Positive Coombs test	N	N	N	N	N
Recurrences	N	N	Y/N	Y	N
Renal failure	Y	Y	Y	Y/N	Y/N
Thrombocytopenia	Y	Y	Y	Y	Y

Y = Yes, N = No

LABORATORY FINDINGS AND CLINICAL COURSE

Upon diagnosis, the thirteen-month-old infant was transferred via ambulance to a major referral childrens hospital. Albumin, total bilirubin, and hemoglobin from the initial laboratory tests (Table 1) were near normal supporting the beginning of the acute phase of HUS. Hemoglobin drops as hemolysis increases during the acute

phase, and total bilirubin rises as hemoglobin from lysed red cells is broken down, following degradation of the hemoglobin porphyrin ring. A type and screen (T&S) and indirect Coombs were ordered for type-specific supportive transfusions. The indirect Coombs was negative, supporting a non-immune-mediated cause of hemolysis. Clinically, hemolysis is monitored by the hemoglobin level, but LDH and AST tests supplement evaluation of hemolysis. LDH is more specific to hemolysis; however, extremely elevated LDH also reflects tissue necrosis (Table 3).[45] Since there is extensive red cell destruction in HUS, most of the haptoglobin is bound to free hemoglobin, giving lower detectable levels of haptoglobin in the serum. Coagulation studies were found to be normal ruling out DIC in the differential diagnosis (Table 3).

Recovery of a small urine sample was obtained from the Foley catheter of the infant for urinalysis (Table 4) which confirmed proteinuria, acidosis, and hemolysis. Red cell casts were not found in this sample, but are commonly found with careful examination in patients with typical HUS.[46] The infant began peritoneal dialysis on day 3 (Figures 1, 2, and 3), was given nasogastric tube feedings for nutritional support, and was placed in barrier isolation to prevent transmission of suspected *E. coli* 0157:H7.

In addition to the urinalysis, stool cultures were ordered for growth of *E. coli* 0157:H7, *Shigella*, *Yersinia*, *Salmonella*, and *Campylobacter*. Cultures are done to confirm a causative agent of HUS and for epidemologic purposes such as tracking of the contaminated food product and disease control. Sorbitol MacConkey agar is generally the medium of choice for isolating the non-sorbitol-fermenting *E. coli* 0157:H7. Most non-pathogenic *E. coli* found in the normal flora of the GI tract ferment sorbitol. After successive negative stool cultures for STX-producing organisms, the infant was released from barrier isolation. A negative culture does not rule out a typical HUS diagnosis, and is commonly seen due to the transient shedding of *E. coli* 0157:H7, culturing too late in the course of the infection, or poor culture sensitivities. The best time to obtain a positive culture for the infectious organism is during the GI prodrome.[14]

Throughout the infant's 32-day hospitalization, HUS was monitored with daily basic metabolic panels and cell blood counts. Monitoring hemoglobin, platelets, creatinine, and albumin throughout the acute phase of HUS is indicative of severity and duration of hemolysis, microthrombi, and kidney dysfunction, respectively. An increase in catabolism, during the acute phase of the infection, and the change in distribution of volume to albumin in the serum resulted in hypoalbuminemia, reaching a low of 1.7 mg/dL. Because no platelets were transfused or any other therapy given that would directly affect the platelet count, the rise in platelet count on day-11 marked the initiation of the recovery phase (Figure 2). Day-15 marked the end of the acute phase of the illness with stabilization of the platelet count, hemoglobin, and creatinine (Figures 1-3). After day-15, supportive transfusions were no longer necessary, and on day-20, recovery was confirmed by discontinuation of dialysis with continued stabilization of creatinine levels (Figure 3).

Table 3. Laboratory evaluation of patient hemolysis and confirmation of typical HUS

Tests	Patient results	Reference ranges
AST	176	25 - 45 U/L
LDH	10240	425 - 975 U/L
Lipase	160	25 - 120 IU/L
PT/INR	12.8 sec/1.05	NA/0.9 - 1.1
PTT	32.5	24.7 - 33.4 sec

Table 4. Patient urinalysis results on day-2 of hospitalization

Urinalysis and microscopic	Patient results	Reference range
Appearance	cloudy	clear
Bacteria	many	negative
Bilirubin	negative	negative
Color	pink	colorless-dark yellow
Glucose	negative	negative
Hemoglobin	250 Ery/µL	negative
Ketones	5 mg/dL	<75 mg/dL
Nitrite	negative	negative
pH	5.0	5.0-8.5
Protein	500 mg/dL	negative
Red blood cells	663/HPF	0-3/HPF
Specific gravity	1.016	1.003-1.030
Urobilinogen	negative	0.1-1.0 Ehrlich's units
WBC esterase	100 LEU/µL	negative
White blood cells	66/HPF	0-5/HPF

As commonly seen in classical typical HUS, the WBC differentials showed a left shift with few myelocytes and metamyelocytes along with leukocytosis and thrombocytopenia. Cell morphology included moderate hypochromic, microcytic red cells with a few schistocytes, nucleated red cells, ovalocytes, polychromasia, and giant platelets. Reticulocyte counts would likely be increased, while the infant attempted to compensate for the anemia with increased red cell production. A bone marrow aspirate is certainly not necessary and would be risky given the presence of thrombocytopenia. However, cellular morphology of the bone marrow would be expected to show normal to increased megakaryocytes (depending on platelet consumption), and erythroid hyperplasia.

As often occurs with HUS, this infant had several complications. On day-2, the infant developed tachycardia, cardiac arrythmia, and periorbital edema. Cardiac arrhythmias in HUS are due to cardiac ischemia, which can also result in myocardial infarction in typical HUS.[10] On day-4, slight involvement of the pancreas was seen with elevated serum lipase (Table 3). Both lipase and amylase are markers for pancreatitis. Since lipase is not elevated by as many conditions as amylase, it is a more specific indicator for pancreatitis. After the onset of abdominal pain, lipase levels rose within 12 hours and rapidly fell to normal range within a few days. In addition, the infant developed peritonitis when the dialysis catheter was compromised, which was reflected by the increasing creatinine levels at day-9 (Figure 3). A dialysate culture revealed coagulase-negative *Staphylococcus sp.* and *Streptococcus pyogenes*, and a dialysate sample revealed a neutrophil count of 1830 x 10^9/L with 75% neutrophils and 23% monocytes with toxic granulation. The infant was treated with vancomycin and serum levels were monitored. On day-25, the infant spiked a fever of 103 °F and was treated with cefotaxime and vancomycin for possible septicemia. Both blood and urine cultures were obtained, and both were found to be negative for growth.

Figure 1. Hemoglobin vs. Time

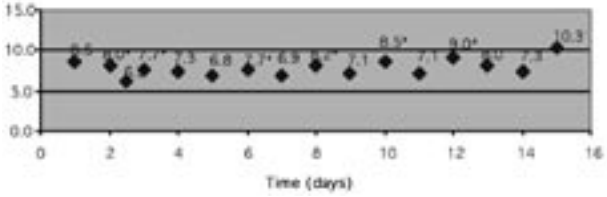


Figure 2. Platelet Count vs. Time

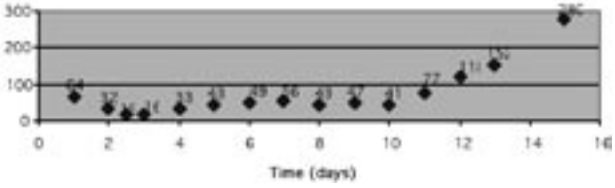
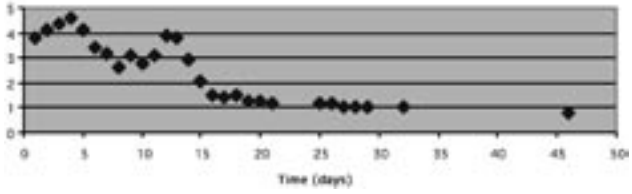


Figure 3. Creatinine vs. Time



After conclusion of the acute phase, a urinalysis still showed significant proteinuria along with a few granular casts and renal epithelial cells, but the hemoglobin levels and red cell numbers supported the cessation of hemolysis. The infant was discharged on day-32 with resolution of the fever and the acute phase of typical HUS. The infant continues to be closely monitored for long-term prognosis.

TREATMENT

The treatment regimen used in the acute phase of typical HUS is primarily supportive and may include medication to control blood pressure, continuous peritoneal dialysis or hemodialysis, packed red cell transfusions, fluid restriction, diuretics, and very careful maintenance of electrolytes. In rare cases, a kidney transplant may be required. Untreated typical HUS will lead to coma, cerebral infarcts, pancreatic insufficiency, and death.[46] However,

rigorous management of treatment has decreased the mortality rate of typical HUS from 50% to about 5% to 10% throughout the last five decades.[47,48]

There are important contraindications to certain treatments when typical HUS is diagnosed. Platelets are generally not transfused, because they may cause more severe microangiopathic thrombosis. Antibiotics are also not suggested, because killing *E. coli* 0157:H7 may rupture the organism's cell wall, thereby increasing the amount of toxin released, and contributing to the severity of the acute phase.[49] If complications occur, however, during typical HUS, such as peritonitis or sepsis, antibiotics may be necessary. Anti-motility drugs for diarrhea are not advised, because they may increase the risk of typical HUS by extending the exposure time of STX to the patient's cells.[50]

Long-term treatment for patients requires monitoring of blood pressure, creatinine, and urinalysis.[51] Patients exhibiting hypertension and proteinuria are treated to lower the blood pressure with an angiotensinogen-converting enzyme (ACE) inhibitor (blocking the production of renin and consequently, aldosterone) to reduce further damage to the glomerulus, reduce proteinuria, and delay or prevent future chronic renal failure.[52] Two years of post-HUS laboratory testing on this infant confirmed hypertension and proteinuria. After beginning treatment with an ACE inhibitor, the infant showed a decrease in blood pressure and proteinuria.

PROGNOSIS

Although prognosis of HUS remains variable, a recent meta-analysis produced significant data. It is estimated that 58% of HUS patients fully recover, 5% die within the acute phase, 11% develop chronic renal failure or die within four years of the acute phase, and 17% experience long-term residual effects such as hypertension, proteinuria, and a decreased glomerular filtration rate. There is often a period of perceived renal recovery before the onset of long-term residual effects. Surprisingly, even some patients who presented with mild HUS (without dialysis or abnormal urine output) developed renal sequelae.[47] Renal sequelae may result in renal failure more than twenty years after the acute phase of HUS.[53] Thus, it is extremely important that the renal function of any child with a history of HUS be followed for life.

The best practical indicator of prognosis is the duration of oliguria or anuria and consequently, the need for dialysis during the acute phase of HUS.[54] The most sensitive indicator is a kidney biopsy, which shows the degree of renal cortical necrosis. This procedure is impractical and contraindicated in the acute phase, but it is used later to assess long-term prognosis.[53,55] Other factors that may support poor long-term prognosis include: elevated white cell count >20 x 10^9/L, neural involvement, post-HUS proteinuria, and hypertension during the acute phase.[35,56-58] In the case history of the thirteen month old infant, the following poor prognostic indicators were found: oliguria/anuria >10 days, leukocytosis >20 x 10^9/L, post-HUS proteinuria, and hypertension at onset of HUS.

PREVENTION

There are three important levels of prevention when dealing with *E. coli* 0157:H7. On the public level, proper handwashing and safe preparation of food remain the best practices for the prevention of typical HUS. However, too much responsibility has been placed on the public.[59] The state and federal government play critical roles in prevention. They are responsible for keeping the food supply safe and ensuring quality assurance by mandating effective screening of food products for *E. coli* 0157:H7 and other foodborne pathogens. In the past, only a few states required such screening. With the new rapid STX detection methods available, surveillance of *E. coli* 0157:H7 in the beef industry is improving.[60] The Food and Drug Administration has approved the irradiation of ground beef, which can kill at least 90% of *E. coli* 0157:H7 contaminants.[24] A law mandating irradiation of other potentially contaminated food products was approved by the United States Department of Agriculture and is being implemented. Cases involving contaminated water sources have also called attention to enforcing regulations for protecting small water systems that in the past received little attention.[61]

RESEARCH AND THE FUTURE

Current research is aimed at faster and more reliable testing of STX as well as countering the action of STX produced by any organism. The conventional method involving the culture of stool on MacConkey-Sorbitol agar for *E. coli* 0157:H7 is only 40% sensitive and takes up to three days to complete.[62] Further complicating the diagnosis of typical HUS is the usual delay of symptoms for one to two weeks after infection, leaving few organisms in the stool for detection. In addition, more than 100 other shiga toxin-producing *E. coli* (STEC) serotypes are left undetectable by many clinical laboratories because they ferment sorbitol, like normal intestinal strains of *E. coli*.[63]

Improved technology is strongly needed to enhance the sensitivity of culture for *E. coli* 0157:H7 in HUS patients.

In 1996, research showed a success rate of 90% for *E. coli* 0157:H7 if an immunomagnetic separation (IMS) technique was used to isolate the 0157 antigen (using beads with the corresponding antibody) after use of a pre-enrichment culture medium. This method proved to have many advantages over new PCR methods for detecting *E. coli* 0157:H7 including at least a 100-fold increase in sensitivity, and less complex testing.[64] In 1999, the United States Department of Agriculture began implementing IMS to improve the screening of ground beef. To date, many clinical laboratories have not adopted the improved methods for routine screening of *E. coli* 0157:H7 in children suspected of HUS.[65] If a clinical laboratory is unable to implement new technology for detecting *E. coli* 0157:H7 and/or STX, the specimen should be referred to an appropriate reference laboratory.

Alternative rapid tests for non-0157 STEC are directed toward the detection of STX produced instead of the organism. One method uses an EIA technology that requires approximately 18 hours to perform (this includes a 16-hour incubation period for increased sensitivity), while another method is a simple toxin detection tests that only takes three hours to perform. Both methods may significantly shorten the time for diagnosis. In addition, other improved methods include PCR followed by pulse-field gel electrophoresis, impedance technology, and enzyme-linked immunoassays.

Vaccination is another potential area of interest. Healthy persons develop an antibody to the antigenic subunit B of STX after exposure.[66] When mice were immunized with the B subunit of STX, they were able to produce antibodies that neutralized the potent effects of STX.[67] Monkeys have also been successfully immunized and protected by an STX vaccine when challenged with a lethal dosage.[68,69] Immunizations may be administered for both humans and cattle in order to reduce *E. coli* 0157:H7 infections and incidence of typical HUS.

Treatment of patients with monoclonal antibodies, currently in clinical trials, may neutralize the STX in patients presenting with early diagnosis of typical HUS. The monoclonal STX antibody binds STX in the intestine and may render the toxin less able gain access to the circulation. Hence, STX is less likely to affect the kidney or other organs.[42] One remaining concern with this treatment is the remaining lipopolysaccharide (LPS) of the bacteria that also plays an antigenic role in typical HUS, perhaps warranting the need for treatment with another monoclonal specific antibody or a polyvalent antibody to both STX and LPS.[32]

CONCLUSION

As in the case of this thirteen-month-old infant with HUS, it is estimated that *E. coli* 0157:H7 causes 73,000 of the 76 million food-borne illnesses each year. Most food-borne illnesses in the U.S. are self-limiting, but those that cause HUS may lead to death or life-long consequences such as renal sequelae and gastrointestinal complications. Progress has been made in the elimination of food-borne causes of typical HUS via irradiation of food products and improved quality assurance and control in the food industry. Current research is focused on vaccination, improved diagnostic testing methods, and intervening treatments with early diagnosis. "So HUSH all you children and don't you cry, together we will beat the bug they call *E. coli*."[70]

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