Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome

Phillip I Tarr, Carrie A Gordon, Wayne L Chandler

Most cases of diarrhoea-associated haemolytic uraemic syndrome (HUS) are caused by Shiga-toxin-producing bacteria; the pathophysiology differs from that of thrombotic thrombocytopenic purpura. Among Shiga-toxin-producing *Escherichia coli* (STEC), O157:H7 has the strongest association worldwide with HUS. Many different vehicles, in addition to the commonly suspected ground (minced) beef, can transmit this pathogen to people. Antibiotics, antimotility agents, narcotics, and non-steroidal anti-inflammatory drugs should not be given to acutely infected patients, and we advise hospital admission and administration of intravenous fluids. Management of HUS remains supportive; there are no specific therapies to ameliorate the course. The vascular injury leading to HUS is likely to be well under way by the time infected patients seek medical attention for diarrhoea. The best way to prevent HUS is to prevent primary infection with Shiga-toxin-producing bacteria.

22 years ago today, Karmali and colleagues published a report on sporadic cases of haemolytic uraemic syndrome (HUS) associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. This paper not only was a seminal contribution to microbiology, but also clarified aetiological thought about the problem of postdiarrhoeal HUS. Karmali and colleagues found a toxin lethal to cultured African green monkey (Vero) kidney cells in stools of children with postdiarrhoeal HUS. This toxic property was attributed to *E coli* of various serotypes, including O157:H7. Such cytotoxin-producing organisms had previously been isolated from human beings and foods. A week after that paper was published, Riley and co-workers described two clusters of patients with painful bloody diarrhoea, linked by the common consumption of undercooked hamburgers; many of the patients in these clusters had *E coli* with a rare serotype (O157:H7) in their stools. Soon afterwards, O’Brien and colleagues associated the toxic property of *E coli* O157:H7 with that of *Shigella dysenteriae* serotype 1. These observations formed the basis for our current understanding of postdiarrhoeal HUS and have prompted multidisciplinary efforts to prevent and treat infections caused by this virulent group of *E coli*.

HUS causes acute renal failure in children worldwide. In the form most commonly encountered in children, HUS follows gastrointestinal infection with Shiga-toxin-producing *E coli* (STEC), the group of organisms incriminated by Karmali and colleagues. HUS is a thrombotic disorder, characterised by microvascular thrombi (figure 1) and swollen endothelial cells. STEC expressing somatic (O) antigen 157 and flagellar (H) antigen 7 are the serotype most frequently isolated from human beings, and the serotype with the strongest and most enduring aetiological association with HUS. However, at least during certain periods, non-O157:H7 STEC appear to be more common causes of HUS in Australia, Germany, and Austria.

This review focuses on the historical features, epidemiology, microbiology, pathophysiology, and treatment of STEC-associated HUS. Most data pertain predominantly to *E coli* O157:H7 infections, because detailed clinical data on HUS caused by non-O157:H7 strains are scarce. We emphasise diagnostic and therapeutic options during the phase preceding HUS, because management of the HUS phase remains largely supportive, and address differences between STEC-associated HUS and other thrombotic microangiopathies.

History

The early history of HUS has been well described. The term was first used by von Gasser and colleagues in a paper published in 1955, which described a case-series of five children with small-vessel renal thrombi, thrombocytopenia, and non-immune (ie, Coombs-negative) haemolytic anaemia. However, the prodromal phases were not sufficiently characterised to allow assessment of whether STEC were the likely precipitants. We have also found a report about a British soldier who died after dysenteric symptoms in Salonika, Greece, in 1918; his histopathological lesions could conceivably have been caused by HUS associated with Shiga-toxin-producing bacteria.

As early as 1965, Barnard and Kibel proposed that enteric *E coli* infections might precipitate HUS (though,

Search strategy and selection criteria

We searched the PubMed database with the terms: “haemolytic uraemic syndrome and pathogenesis”, “haemolytic uraemic syndrome and pathophysiology”, “haemolytic uraemic syndrome and epidemiology”, and “O157”. We included only studies that, in our joint opinion, met the following criteria: the HUS was plausibly or definitely caused by an STEC; the assays to examine the abnormalities were appropriate; sufficient numbers of participants were included for conclusions to be drawn; and the studies were, ideally, based on the general population. Personal observations, selected instructive historical articles, and microbiological, in-vitro, animal, and pathophysiological studies are also included, based on our knowledge of their existence.
In 1975, Kaplan and colleagues reported intrafamilial synchronous clustering of HUS cases in endemic areas and inferred an environmental, presumably infectious, precipitant. The following general statements can be made about STEC and associated HUS. First, most cases of postdiarrhoeal HUS are caused by STEC or, in regions such as south Asia, S dysenteriae serotype 1. Other shigellae do not cause HUS because virtually none produce Shiga toxin. Second, E coli O157:H7 is the most common, even predominant, cause of HUS in most of the world, the STEC most likely to cause epidemics, and the STEC serotype most commonly associated with human gastrointestinal infection. Third, a subset of non-O157:H7 STEC are pathogens, but strategies to detect these strains in clinical practice and the implications of finding them remain incompletely defined. Fourth, ground (minced) beef is by no means the only vehicle that can transmit E coli O157:H7. Finally, HUS treatment remains supportive, probably because the pathophysiological insult that triggers this disorder occurs early in illness and is not sustained, and the precise cellular mechanisms leading to renal injury are undefined. Fortunately, most children recover from HUS.

Nomenclature and syndromic definitions
Confusing nomenclature has developed surrounding diarrhoeagenic E coli. Definitions for E coli capable of causing HUS and other diseases via production of Shiga toxin are summarised in table 1.

We have defined HUS stringently, by use of the following criteria: packed-cell volume less than 30% with evidence of erythrocyte destruction on peripheral-blood smear; platelet count less than 150 × 10^9/L; and serum creatinine above the upper limit for age, in patients without other reasons for coagulopathy, such as septicaemia. In postdiarrhoeal HUS, circulating fibrinogen concentrations are normal or high, and the prothrombin time is only slightly prolonged, unlike classic disseminated intravascular coagulation. We do not believe that abnormal results of urine analysis, in the absence of azotaemia, should be used to define HUS. As it pertains to this review, HUS is a complication of infection with Shiga-toxin-producing bacteria, although other infections, such as pneumococcal pneumonia, and inheritable abnormalities in complement regulatory proteins can cause the disorder. Idiopathic thrombotic thrombocytopenic purpura (TTP) includes thrombotic microangiopathies in which there is an acquired inhibitor of the von Willebrand factor metalloprotease. This protease was recently identified as a particular member of the ADAMTS family—namely ADAMTS13. Mutations in ADAMTS13 also cause an autosomal recessive, congenital form of TTP, also known as Upshaw-Schulman syndrome. Although the cause of thrombotic microangiopathy might not be immediately apparent from the clinical presentation, HUS, congenital TTP, and idiopathic TTP are caused by distinct pathological mechanisms. The imprecise term HUS/TTP should be abandoned, because current evidence indicates that TTP and HUS differ in pathogenesis, and they do not

Table 1: Definitions of Shiga toxins and the organisms that produce them

<table>
<thead>
<tr>
<th>Type of Shiga toxin</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shiga toxin (Stx)</td>
<td>Main extracellular cytotoxin produced by S dysenteriae serotype 1 and rarely by other shigellae.</td>
</tr>
<tr>
<td>Shiga toxins 1 and 2</td>
<td>Families of toxins produced by E coli. Members of Stx1 family are highly homologous to Stx. Stx2 is 58% identical to Stx1 at the aminoacid level and 56% identical at the nucleotide level. Stx1 and Stx2 are synonymous with Shiga-like toxins 1 and 2, verotoxins 1 and 2, and verocytotoxins 1 and 2, respectively. Human STEC produce Stx1 and Stx2 with one (Stx1c) or five (Stx2, Stx2c, Stx2d, Stx2e, and Stx2f) allelic variants. These allelic variants of Stx could have different pathogenic potentials.</td>
</tr>
<tr>
<td>STEC</td>
<td>E coli that have genes encoding one or more Shiga toxins. Synonymous with verotoxigenic, verocytoxin-producing, or verocytotoxigenic E coli (VTEC).</td>
</tr>
<tr>
<td>Enterohaemorrhagic E coli (EHEC)</td>
<td>Subset of STEC that are pathogenic to human beings. Most carry an eae allele, encoding intimin.</td>
</tr>
</tbody>
</table>

Figure 1: Renal lesion in fatal HUS
Thrombus (arrow) in a glomerular arteriole in a child who died with fulminant postdiarrhoeal HUS.
encompass all forms of thrombotic microangiopathies. When the underlying cause of disease is uncertain, the term thrombotic microangiopathy is sufficient, appropriate, and preferable.

Epidemiology

A massive outbreak of *E coli* O157:H7 infections caused by consumption of poorly cooked ground beef at many outlets of a fast-food restaurant chain in the western USA 12 years ago raised public and medical awareness of STEC. However, this outbreak and others produced two misconceptions: that *E coli* O157:H7 chiefly causes large epidemics, and that ground beef is its predominant vehicle. In fact, most cases are sporadic or occur in small clusters, and ground beef is commonly not the vehicle. In fact, most cases are sporadic or occur in small clusters, and ground beef is commonly not the vehicle. (panel 1). Exposure inquiries do not aid management of patients, owing to the variety and ubiquity of possible sources of this pathogen. Moreover, such questioning can potentially introduce biases that complicate subsequent interviews by public-health officers. The best policy, we believe, is to report cases to local disease-investigation officers expeditiously and to defer source tracing to them.

In the northern hemisphere, there is a rough correlation between distance from the equator and frequency of HUS and rates of isolation of *E coli* O157:H7 infections, but Denmark does not. In the southern hemisphere, Buenos Aires in Argentina has a very high incidence of HUS. The incidence of diagnosed *E coli* O157:H7 infections in the USA is greater among rural than urban populations (Mead P, Centers for Disease Control and Prevention; personal communication). In Scotland, rural residents have been thought to be at greater risk of exposure to *E coli* O157:H7 than urban residents because of greater exposure to animals or animal excreta. Visits to dairy farms have been implicated as likely acquisition sites for infections in Finland and the USA. Transmission from cattle to people might be airborne. North American seroepidemiological surveys have shown higher frequencies of antibodies to the O157 lipopolysaccharide among residents of rural areas than among people who live in urban areas.

Rates of *E coli* O157:H7 infections in countries lacking diagnostic capabilities are probably underestimated. For example, several reports from Africa have shown that large outbreaks can occur; without diagnostic capabilities, these epidemics can be overlooked.

Most *E coli* O157:H7 infections and HUS occur in the summer and autumn, but increasing or decreasing trends have not been proven unequivocally, and one population-based study found stable incidence during the 1990s. A reported lower likelihood that children of African descent would have HUS was not confirmed in recent series from Natal and North America. We emphasise, however, that the risk of developing HUS relates also to consumptions and behaviours leading to acquisition of infection, so demographic differences in incidence might reflect demographic differences in exposure to the causative agent, rather than differences in genetic propensity to develop HUS once infected.

Diagnostic and molecular microbiology

The predominant cause of HUS in most of the world is *E coli* O157:H7, which can best be detected by plating of fresh faeces on sorbitol-MacConkey agar. This agar has sorbitol, not lactose, as a carbon source. Unlike most human faecal *E coli*, O157:H7 strains cannot ferment sorbitol after overnight incubation on sorbitol-MacConkey agar, and they therefore appear as colourless colonies (figure 2). Commercial tests for the identification of these and other STEC include direct detection of Shiga toxin, which has the advantage of leading to the identification of non-O157:H7 STEC, and an immunodiffusion card that detects *E coli* O157:H7 at the point of care. Diagnostic approaches for STEC need to take into account the risk that the organism identified is a pathogen, and, in that case, whether that organism has a reasonable likelihood of precipitating HUS. The ideal detection method for *E coli* O157:H7 is culture of the stool on sorbitol-MacConkey agar, accompanied by a Shiga-toxin detection assay, done on a broth culture of the stool. Even though most patients with *E coli* infection report grossly bloody stools, laboratory workers commonly fail to perceive blood in the submitted samples. Therefore, visual inspection of the stool in the microbiology laboratory is inadequate for assessment of whether a sample should be tested for STEC; we believe that screening of all submitted stools for *E coli*
Figure 2: E coli O157:H7 on a sorbitol-MacConkey agar plate
Arrow indicates distinctive colourless E coli O157:H7 colony.

O157:H7 by use of sorbitol-MacConkey agar maximises the ability to detect this pathogen.

Use of Shiga-toxin detection as a hierarchical test rather than sorbitol MacConkey agar is appealing, because the former has the potential to detect both O157:H7 and non-O157:H7 STEC. However, we believe such a protocol has several drawbacks. First, detection of Shiga toxin will identify many patients infected with non-O157:H7 STEC in whom the course is less likely to result in HUS, and antigen detection and even cytotoxicity assays might not identify all allelic variants of Shiga toxin.

Second, a sequential approach, in which the pathogenic STEC is sought only after a non-culture test suggests its presence, delays the transfer to outbreak investigators of E coli O157:H7, which will be the causative agent in most of the world. These investigators need to obtain isolates expeditiously to establish or refute epidemiological associations by use of molecular typing. Third, sorbitol-MacConkey agar could be more sensitive than detection of Shiga-toxin antigen for E coli O157:H7.

We emphasise that HUS can, nevertheless, follow non-O157:H7 STEC infections and that such infections are almost certainly underdetected. There have been several reports on the clinical course of HUS after non-O157:H7 STEC infections. In the one study that compared cases of HUS after O157:H7 and after non-O157:H7 STEC infections, the former group had a more severe course. However, an unknown proportion of sorbitol-fermenting enterohaeorrhagic E coli, which would be overlooked on a SMAC agar plate, probably has pathogenic potential similar to that of E coli O157:H7.

Sorbitol-fermenting E coli O157:H7 exemplify the challenge of detecting non-O157:H7 STEC. This serotype, though closely related to E coli O157:H7 that does not ferment sorbitol, necessitates complex detection methods, because it is not distinguishable on sorbitol-MacConkey agar, and because existing data suggest that these organisms are no less virulent than E coli O157:H7. Identification of such an organism in a microbiology laboratory would necessitate toxin assays or detection of unique genetic loci.

The ability to produce Shiga toxin is the key virulence trait of STEC. Shiga toxins are A,B toxins. The B subunit binds to a glycosphingolipid on the surface of eukaryotic cells, and the A subunit is an N-glycosidase, which inhibits protein synthesis and disrupts the large eukaryotic ribosomal subunit in a similar way to ricin. Shiga toxins also induce apoptosis in human renal cells and tissue. They have additional and diverse effects on endothelial and other eukaryotic cells. Most E coli O157:H7 carry the gene encoding Stx2, and about two-thirds have the gene encoding Stx1.

Enterohaemorrhagic E coli produce factors other than Shiga toxin that could plausibly injure human hosts. Intimin, through which E coli mediates intimate attachment to epithelial cells in vitro and in animal models, is one of the best characterised and most important non-Shiga-toxin virulence traits. Other non-Shiga-toxin molecules that might cause some of the manifestations of STEC infections include StcE, which inhibits C1 esterase inhibitor, subtilase cytotoxin produced by E coli O113:H21, and cytolethal distending toxin. As newly discovered genes in STEC are characterised, additional non-Shiga-toxin virulence traits are likely to come to light.

Clinical course and postulated mechanisms of pathogenesis

Figure 3 shows the aggregate clinical course of E coli O157:H7 infections. The interval between ingestion of a contaminated vehicle and the onset of diarrhoea ranges between 2 days and 12 days. In a well-analysed epidemic, the mean incubation period before the first loose stool was 3-7 days and the median 3 days. Typically, E coli O157:H7 infections cause 1-3 days of non-bloody diarrhoea after which the diarrhoea becomes bloody. The bloody diarrhoea, which occurs in about 90% of cases, is generally the sign that prompts patients or their families to seek medical attention. The colon can be quite severely affected (figure 4). However, mild infections can occur, and E coli O157:H7 have been recovered from the stools of patients with HUS who have no diarrhoea. Postsymptomatic shedding of E coli O157:H7 could lead to community spread of infection, but the greatest period of transmissibility is probably during the acute diarrhoea phase.

Several clues help clinicians differentiate E coli O157:H7 infections from colitis caused by other bacteria. Most patients with E coli O157:H7 are afebrile when investigated in a medical setting, even though about half of all infected patients report fever before
Seminar

Leucocytes are found in only about half of examined faecal samples and are rarely described as abundant if they are present. The abdominal pain is greater than is generally seen in other forms of bacterial gastroenteritis, physicians commonly note abdominal tenderness during their examination, and defecation tends to be painful.

Management of patients with bloody diarrhoea and possible or definite E coli infection

Patients with acute bloody diarrhoea, and especially those in whom no fever is documented in a medical setting, and those with very painful diarrhoea should be considered to have possible E coli O157:H7 infection. We encourage hospital admission of such patients if for no other reason than for infection control; application of contact precautions to acutely infected inpatients, while allowing other infected patients to remain in the community where the risk spreading this potentially lethal pathogen is much higher, is inconsistent.

We believe that intravenous rehydration and maintenance fluid provide optimum nephroprotection, as shown by the association between parenteral volume expansion before the development of HUS, and attenuated renal injury during HUS. Standard rehydration protocols, which consist of volume repletion followed by replacement of continuing stool losses, and provision of maintenance fluid with hypotonic solutions, might be inadequate for STEC infections. There is likely to be vascular leakage, the extent of which cannot be accurately taken into account in assessment of fluid requirements. We observe oedema in many infected patients, whether or not their infection progresses to HUS, and the oedema can confound attempts to assess hydration status. For these reasons, we infuse more sodium during the diarrhoeal phase than is generally used to support volume during gastroenteritis, but we also insist that infected patients are admitted to institutions that can monitor closely for signs of cardiovascular overload (panel 2).

Antibiotics should not be administered to patients with definite or possible enteric STEC infections. In the 1993 outbreak in Washington state, antibiotics administered early in illness were not associated with a diminished risk of development of HUS. In a prospective study in the Pacific Northwest, children infected with E coli O157:H7 who were treated with antibiotics had a higher rate of HUS. Antibiotics might also increase the risk of HUS in adults. The mechanisms by which antibiotics increase the risk of HUS remain unknown, but they

Figure 3: Progression of E coli O157:H7 infections in children

About 3 days after ingestion of the organism, the patient develops diarrhoea, abdominal pain, fever, and vomiting. The diarrhea becomes bloody 1–3 days later, rarely on the first day. In 80–90% of infected children with positive cultures, visible blood is present in the stools. When bloody diarrhoea first develops, the patient has a normal platelet count, creatinine concentration, and packed-cell volume, with no red-cell fragmentation. However, if studies of the coagulation and fibrinolytic systems are done early in the illness, there is evidence that thrombin generation is increased, fibrin deposition is occurring, and plasminogen activation is suppressed.

Figure 4: Radiographic features of E coli O157:H7 infection

A: radiograph after barium enema with thumbprinting appearance of mucosa (arrow), suggesting colonic oedema, in a patient who subsequently developed HUS. B: CT of the pelvis of an 8-year-old boy on day 8 of an E coli O157:H7 infection. Note severely thickened colon (circled). This infection did not progress to HUS.
Panel 2: Management of patients with suspected or confirmed E. coli O157:H7 infections

Do not give antibiotics, antimotility agents, narcotic opioids, or non-steroidal anti-inflammatory drugs.

Bolus with intravenous normal saline, 20 mL/kg, on presentation, if there is no evidence of cardiopulmonary overload.

Continue intravenous maintenance fluid in the form of isotonic crystalloid (normal saline, normal saline with 5% dextrose, or lactated Ringer’s solution), and not hypotonic crystalloid.

Potassium can be added to the intravenous fluids if the serum potassium concentration is normal or low.

Most patients can eat or drink ad libitum, though appetite tends to be diminished during the acute infection.

Repeat boluses of normal saline (10–20 mL/kg) if there is any question of diminished urine output, and the patient is not showing signs of central volume overload.

Daily laboratory tests should include complete blood count, electrolytes, and serum urea nitrogen and creatinine concentrations.

The patient should be admitted to an institution skilled in the age-appropriate monitoring of fluid status.

The HUS risk period is past when the platelet count rises, or if the platelet count is stable, and symptoms are resolved or resolving. We also repeat laboratory tests 1 day after discharge.

As the creatinine concentration rises, patients should be monitored even more assiduously for hypertension or signs of cardiopulmonary overload and transferred, if necessary, to a centre where acute renal failure can be managed and treated.

could involve bacterial lysis, thereby liberating Shiga toxin, or induction of bacteriophages on which stx genes are located, with subsequently increased production of the toxin.

A 2002 meta-analysis implied that antibiotic therapy of E. coli O157:H7 infections might not be harmful. We believe that conclusion was flawed because the inferred beneficial effect of antibiotics depended largely on a single study that associated fosfomycin administration, at selected times in the illness, with a lessened risk of development of HUS. In that particular study, fosfomycin was compared only with other antibiotics, but the meta-analysis mischaracterised fosfomycin as being superior to no antibiotics.

Similarly, we recommend that antimotility agents, or narcotics in any form, should not be given to patients with bloody diarrhoea or with definite STEC infections, because these agents have also been associated with an increased risk of development of HUS or neurological complications of the disorder. We also advise against use of non-steroidal anti-inflammatory agents, because they can diminish renal blood flow.

We have noted that volume repletion with intravenous isotonic crystalloids can, in many cases, ameliorate abdominal pain during the diarrhoea phase.

An appealing approach is consideration of oral therapy against Shiga toxin to prevent the development of HUS in infected patients, but recent evidence suggests that such an intervention would be futile. First, most infected children do not have a demonstrable reservoir of toxin in stool, despite having about 10⁷ viable E. coli O157:H7 per g of stool. Second, by day 4 of illness, vascular injury is well under way, and much toxin-related vascular damage has probably already occurred. Third, orally administered toxin binder failed to attenuate the severity of HUS when administered to children presumed to be infected with STEC.

Onset of HUS

The risk that a child younger than 10 years with a diagnosed E. coli O157:H7 infection will develop HUS is about 15%. The case definition of HUS is typically attained between days 5 and 13 of illness, with day 1 being the first day of diarrhoea; the median is about a week after the onset of diarrhoea. As E. coli O157:H7 infections evolve into HUS, thrombocytopenia is the first abnormality in most patients. Haemolytic-uraemic syndrome, presumably from physical injury to erythrocytes from passing through nascent thrombi in small vessels, generally precedes azotaemia, but in some cases we have observed that the creatinine concentration begins to rise a day or two before the packed-cell volume decreases.

We rarely request urine analyses during E. coli O157:H7 infections; because a cleanly voided specimen can be very difficult to obtain during diarrhoea, especially in children. We are also very concerned about bladder catheterisation because of the risk of introducing STEC into the urinary system. Moreover, the finding of urinary abnormalities, even if genuine, would not be clinically helpful. A raised creatinine concentration, in combination with clinical monitoring, is sufficient to guide management before and at the onset of HUS.

Some risk factors, such as raised white-blood-cell count, early presentation to care, antibiotic administration, use of antimotility agents, and age under 10 years, are associated with increased risks of development of HUS. However, there are no formulae or factors that can be used to exclude the possibility of this outcome in assessment of infected patients of any age. Therefore, all infected patients must be considered at risk of HUS, until the platelet count is definitely rising. In almost all children studied sequentially during the first week of illness, we observe a fall in the platelet count. This decrease is possibly related to volume expansion, or, more likely, to consumption of thrombocytes, because prothrombotic processes occur in many patients even without the subsequent development of HUS. In most cases, an unequivocal reversal of the falling platelet count will occur within a week of the onset of diarrhoea. At that point, administration of parenteral fluids should be discontinued and discharge considered, unless other issues require a continued stay in hospital. A few patients have stable (ie, not rising) platelet counts as diarrhoea abates, but discharge can safely be considered in these patients also. We do, however, attempt to obtain a blood count and chemistry results on the day after discharge in
all patients, to confirm that improvement is sustained. If the platelet count is stable or increasing, mild post-discharge increments in serum urea or creatinine concentrations can be overlooked. There is rarely a need to undertake laboratory tests after an improving trend in the platelet count is clearly shown, even if the interval of risk is not yet past.

In our experience, the rate of development of partial or incomplete HUS is roughly the same as that of complete HUS among infected children. Such children have thrombocytopenia, with or without anaemia (defined as above), but the serum creatinine concentration remains normal. We have occasionally needed to transfuse erythrocytes into patients with symptomatic anaemia but no renal insufficiency.

**Pathophysiology of HUS**

Enteric STEC infections are almost never accompanied by bacteraemia. Presumably, systemic complications, such as HUS, arise from lesions caused by circulating Shiga toxin. In fact, early in the course of these non-bacteraemic infections, there are prothrombotic coagulation abnormalities similar to those observed several days later when HUS develops. Furthermore, in animals, colitis can be caused by parenterally administered Shiga toxin. Perhaps the bloody diarrhoea, and possibly even the diarrhoea, are caused by mesenteric ischaemia initiated by circulating Shiga toxin, rather than by direct STEC injury of the intestinal epithelium. Interactions between Shiga toxin and circulating leucocytes and platelets could also have roles in pathogenesis.

Shiga toxins bind to the glycosphingolipid globotriaosylceramide, which occurs on renal glomerular endothelial, mesangial, and tubular epithelial cells. Shiga toxin has been identified bound to renal sections taken after death from infected children who died of HUS, and cellular differences in expression of globotriaosylceramide might underlie organ-specific responses to circulating Shiga toxin. The lack of a suitable animal model of HUS after enteric challenge with an STEC, which possibly relates to inter-species differences in expression of globotriaosylceramide, poses challenges to investigators attempting to elucidate these early cellular events after enteric colonisation. For this reason, infected human beings must be studied for characterisation of the cascade leading from gastrointestinal infection to renal impairment.

The profound haematological abnormalities during and before HUS and histopathological analyses show that the basis of HUS is thrombotic, not vasculitic. Even if vascular occlusion is not the underlying major lesion in HUS, thrombosis-independent thrombin-mediated host mechanisms could cause renal injury after *E coli* O157:H7 infections. The plasma of patients with HUS shows fibrinolysis inhibition manifest by increased activity of plasminogen activator inhibitor 1 (PAI-1), presumed increased intravascular generation of fibrin, as shown by high concentrations of D-dimers and generation of thrombin, as inferred from raised concentrations of fragment 1 + 2. On or before day 4 of diarrhoea, many patients infected with *E coli* O157:H7 have similar abnormalities (figure 5), even those who do not develop HUS.

Why one infected patient develops HUS and another does not is unknown. Prothrombotic abnormalities occur in many infected children, whether or not HUS develops. Also, some infected children, even those who do not develop HUS, have degraded von Willebrand factor in plasma early in illness, probably representing shear stresses on this molecule from thrombi in one or more vascular beds. However, children with higher concentrations of fragment 1 + 2, D-dimer, and PAI-1 when they initially present with diarrhoea have an increased risk of developing HUS several days later, even though their packed-cell volumes, platelet counts, and serum creatinine concentrations are normal early in illness. Thrombin generation, as reflected in concentrations of fragment 1 + 2, does not increase greatly during this interval. Though there is overlap between values in children who develop HUS and those whose infections resolve without complication, we hypothesise that the development of HUS is related to the degree of prothrombotic activation early in infection, and to the intensity of the coagulation response that subsequently develops. The endothelium is a likely target of the absorbed Shiga toxin, but in-vitro data raise the possibility that the toxin also injures monocytes, which can similarly mediate thrombotic responses.

Common host prothrombotic alleles do not seem to have major roles in the development of HUS. Patients do not seem to undergo systemic inflammatory responses before HUS develops. They are rarely febrile once bloody diarrhoea begins and are not hypotensive, acidic, or in shock. Nonetheless, there is a strong possibility that at the cellular level proinflam-
matory cytokines and chemokines act synergistically with Shiga toxins to injure host cells.\textsuperscript{144} Evidence pertinent to the pre-HUS phase of illness comes from studies showing that Shiga toxins can activate various C-X-C chemokines in intestinal epithelial cells.\textsuperscript{145} STEC flagellin also induces this response,\textsuperscript{146} and activates epithelial-cell mitogen-activated protein (MAP) kinase and nuclear factor-κB pathways, resulting in secretion of interleukin 8 by intestinal epithelial cells.\textsuperscript{147} \textit{E coli} O157:H7 abrogate Stat1-mediated interferon-γ signal transduction in cultured human intestinal epithelial cells.\textsuperscript{148} Increased circulating concentrations of growth-related oncogen α, macrophage inflammatory protein 1β, and monocyte chemotactic protein 1 are observed in \textit{E coli} O157:H7 infections, and concentrations of granulocyte colony-stimulating factor are especially high whereas those of epithelial-cell-derived neutrophil-activating protein 78 are low during HUS.\textsuperscript{149}

The Shiga-toxin genotype of the infecting strain is a potential determinant of infection outcome, with organisms that produce both Stx1 and Stx2 being paradoxically less virulent than those that produce Stx2 but not Stx1;\textsuperscript{150} however, the association is far from absolute.\textsuperscript{150} Also, expanding knowledge of allelic variants of Stx2 make such analyses quite complex.\textsuperscript{149,151}

**Management of HUS**

As azotaemia develops in infected patients, the challenge of management is to maintain renal perfusion while avoiding deleterious fluid overload. A rising serum creatinine concentration in an infected patient who is still urinating might reflect a process that is destined to result in renal shutdown, presumably acute tubular necrosis, in which case fluid restriction is advisable, and dialysis is probably inevitable. Alternatively, further volume expansion might yet counteract the effects of a thrombotic process where there is almost certainly renal hyperperfusion. Furthermore, accurate assessment of intravascular volume and achievement of a normal volume are elusive goals in the face of vomiting, diarrhoea, poor oral intake, hypoalbuminaemia, and thrombosed, probably hyperpermeable, blood vessels.

Patients in all phases of HUS should be monitored carefully and continuously for signs of fluid overload, because their renal and vascular statuses are in flux, and volume overload or depletion could exacerbate injury. The weight and fluid balance of HUS patients should be carefully measured daily. However, in the early stage of HUS, weight gain does not necessarily mean intravascular overload, because hypoalbuminaemia and vascular leakage cause a diffusely oedematous state.

Technical features of the management of established HUS have been well reviewed.\textsuperscript{152-155} At the first indication of hypertension or cardiopulmonary overload, fluids should be restricted. Diuretics, sometimes given during early HUS, rarely avert anuria. If their use does lead to urine production, intravascular volume depletion might be inadvertently exacerbated, and thrombus development facilitated; these processes could further compromise renal blood flow. Use of diuretics should be restricted to severe clinically consequential central volume overload, but dialysis is likely to be more effective. Vasodilators\textsuperscript{156} are the preferred agents for the treatment of hypertension. We avoid inhibitors of angiotensin-converting enzyme because of our concern that they might exacerbate kidney injury by diminishing renal perfusion.\textsuperscript{157} Most patients with early renal insufficiency and a diminishing urine output who do not respond to boluses of isotonic crystalloid progress to oligoanuric renal failure. HUS patients whose hourly urine output remains below 0.5 mL/kg beyond day 10 of illness (with the first day of illness being the first day of diarrhoea) generally do not become anuric.

Nephrotic medications should be stopped (they should avoided, if possible, during the diarrhoea phase), and the doses of drugs that are renally excreted should be adjusted accordingly. Narcotics should be used with caution, because their metabolites could cause seizures during renal failure.\textsuperscript{157} Some non-renal complications of HUS should be anticipated;\textsuperscript{158} neurological complications are the most ominous and are important determinants of morbidity and mortality.\textsuperscript{158-160} Irritability, lethargy, and confusion could be caused by fatigue, cerebral microvascular thrombi, ischaemia–hypoxia, or the direct neuronal effects of Shiga toxin; stroke (thrombotic or haemorrhagic), seizures, and coma occur in about 10% of patients.\textsuperscript{157} Cranial imaging should be used to assess any true neurological complications.

Cardiac dysfunction was detected in about 10% of children with HUS during the 1993 epidemic of \textit{E coli} O157:H7 in the western USA,\textsuperscript{97} and congestive heart failure seems to be somewhat more common among adults with HUS (PIT, unpublished). Raised concentrations of troponin I during HUS should be attributed to cardiac ischaemia, not azotaemia.\textsuperscript{161} The most common pulmonary consequence is fluid overload and pleural effusions, but adult respiratory distress syndrome can also occur.\textsuperscript{97} Intestinal complications during acute HUS consist of perforation and necrosis. Acidosis that is not easily corrected by dialysis suggests ischaemic or necrotic bowel; strictures and pigment gallstones generally do not become apparent until after resolution of HUS.\textsuperscript{161} Clinically significant pancreatitis and glucose intolerance can occur during HUS, but asymptomatic increases in serum concentrations of amylase and lipase, which are quite common, are not contraindications to enteral nutrition.

Oliguric or anuric patients might need potassium restriction to prevent hyperkalaemia (though this electrolyte abnormality is uncommon despite haemolysis and renal failure) and phosphate restriction and phosphate binders to prevent hyperphosphataemia.
Sodium excess can contribute to oedema in hypoalbuminaemic states and can worsen hypertension. After dialysis is initiated, diets can be less restricted. Patients should receive appropriate calories for their age and size, enterally or parenterally, and those on dialysis should also receive appropriate vitamin supplementation.143

HUS patients can become profoundly and rapidly anaemic, and the usual indications for erythrocyte transfusion (largely cardiovascular or respiratory compromise) apply; about 80% of patients with HUS need erythrocyte transfusions.7,97 Blood transfusions should, however, be administered cautiously, because rapid intravascular expansion can cause hypertension. Blood products should be volume-reduced and depleted of leucocytes, if possible,154 and given slowly with frequent monitoring of vital signs, preferably during dialysis. We discourage platelet transfusions unless there is clinically significant haemorrhage or invasive procedures are being undertaken, because platelets could conceivably exacerbate thromboses. Most patients do not need iron to treat anaemia because the iron from haemolysed cells should remain available for erythropoiesis. Haemolysis can be the most persistent abnormality as HUS resolves.

Indications for dialysis in HUS are similar to those in other forms of acute renal failure: hyperkalaemia (potassium concentration higher than 6.5 mmol/L with electrocardiographic changes); serum urea concentrations higher than 36 mmol/L (although azotaemia by itself as an indicator for dialysis has been questioned);144 persistent acidosis (bicarbonate concentration less than 10 mmol/L); hypertension from volume overload not responding to medical therapies; volume overload leading to cardiac or respiratory compromise; oliguria or anuria as a limiting factor for nutritional support; and the need for blood transfusions in patients with poorly controlled hypertension.145 The choice between haemodialysis and peritoneal dialysis varies among specialists and centres.

Many approaches have been unsuccessful in HUS, and are not commonly used today (panel 3).111,165-171 In the absence of convincing evidence of their efficacy, we discourage use of antithrombotic agents in children with HUS, because the hypertension, thrombocytopenia, and azotaemia might increase the risk of intracranial bleeding. In one study,145 serum creatinine concentrations fell slightly more rapidly in children who were assigned corticosteroids than in those assigned placebo, but the treatment group did not have a diminished rate of renal failure or need for dialysis, and these agents are also not commonly used during HUS. Plasma therapies have also been studied in small groups of children with HUS.168-172 However, interpretation of these studies, which largely did not demonstrate benefit, is difficult because the aetiologies of the HUS cases were not well delineated or the data were collected retrospectively.

Because the precipitating cause of postdiarrhoeal HUS can now be identified in many cases, and infected children

<table>
<thead>
<tr>
<th>Panel 3: Ineffective approaches in typical HUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids</td>
</tr>
</tbody>
</table>

can be studied, emerging data might focus intervention strategies. For example, there is no theoretical justification for plasma therapies in STEC-related HUS, because there is no evidence that the thrombotic microangiopathy results from ADAMTS13 deficiencies,7 or from any other factor that can be removed or replaced by plasma exchange. Also, the failure of Synsorb-Pk, an oral agent that binds Shiga toxin, to ameliorate the course of HUS111 is not surprising in view of the paucity of free faecal toxin in children with HUS after E coli O157:H7 infections.28 An unresolved issue in consideration of antitoxin treatment for STEC infections is whether Shiga toxin is accessible in the gut, on cells, or free in the circulation, after patients present, but before HUS ensues; if the toxin were present and could be neutralised by antibodies or parenteral synthetic toxin binders,173,174 injury might be lessened. However, the presence of coagulation abnormalities early in the illness15 suggests that the extraintestinal injury cascade is already under way by the time of diagnosis, and that antitoxin therapy might be too late. Attenuation of the prothrombotic response before development of HUS is another possible intervention.

There are many potential sequelae of childhood HUS, but most long-term concerns relate to renal function. Most survivors of a large outbreak in 1993 had good renal function 5 years after infection.175 The severity of the initial episode of HUS and the need for initial dialysis are risk factors for long-term renal sequelae.176

**Differentiation of Shiga-toxin-related HUS from other thrombotic microangiopathies**

HUS and TTP have been classified as similar disorders because of the common occurrence of thromboses. However, idiopathic TTP is now known to be caused in most cases by deficient ADAMTS13 activity, whereas this activity is normal in HUS associated with E coli O157:H7, when measured with an appropriate assay.7 Idiopathic TTP is distinct from thrombotic microangiopathies, such as those associated with cancer, infections, pregnancy, vasculitis, and use of cyclosporin. Idiopathic TTP can be differentiated from STEC-associated HUS by various criteria (table 2). HUS is more frequently characterised by endothelial swelling than is TTP, and glomerular thrombi in HUS patients contain fibrin but little von Willebrand factor, also suggesting that von Willebrand factor is not involved in
the evolution of the thromboses in STEC-associated HUS. In contrast to idiopathic TTP, in which cleavage of von Willebrand factor is impaired and pathogenetic multimers cause platelet aggregation and thrombosis, during HUS associated with *E coli* O157:H7 there is increased fragmentation of von Willebrand factor, probably because this molecule is sheared as it passes through small vessels containing fibrin thrombi. Plasma therapies are effective in idiopathic TTP because they supply ADAMTS13 activity. Removal of inhibitory antibodies probably also contributes to the efficacy of plasmapheresis in TTP.

Diverse other disorders can resemble HUS, but postdiarrhoeal HUS is actually a homogeneous and recognisable illness that is generally distinguishable from other thrombotic microangiopathies. However, review of criteria that characterise STEC-related HUS is useful. First, in almost all cases of HUS associated with *E coli* O157:H7 there is preceding diarrhoea (though we do recommend seeking faecal and urinary STEC during the first thrombotic microangiopathic episode even if diarrhoea is not present). Second, almost all patients with this disorder have normal or slightly raised fibrinogen concentrations and prolonged prothrombin times. Third, HUS related to *E coli* O157:H7 can recur, so a second postdiarrhoeal episode should not, by itself, initiate a search for an inheritable or autoimmune disorder. However, recurrent or non-synchronous familial thrombotic microangiopathies, especially if diarrhoea is absent, should prompt investigations for precipitants other than an STEC (eg, deficient ADAMTS13 activity or complement abnormalities).

Recurrent or familial HUS has lately been attributed to abnormalities in complement factor H, which attenuates host injury from the alternative pathway of complement, and factor I, which is a serine protease that inhibits the alternative complement pathway amplification loop, by cleaving the C3b a chain, or the complement regulator, membrane cofactor protein (CD46), which inhibits membrane bound C3b and C4b.

However, any association between temporary deficiencies in these factors, or allelic variants, and STEC-associated HUS, is speculative at present. Any child or adult presenting for the first time with a thrombotic microangiopathy, but who does not have diarrhoea, could still be infected with an STEC; such patients should be investigated for the presence of an STEC by microbiological analysis of the stool, as well as of the urine.

Conflict of interest statement
We declare that we have no conflict of interest.

Acknowledgments
We thank Sandra Watkins, Han-Mou Tsai, and J Evan Sadler for critical reading of the review and helpful suggestions, Helge Karch for stimulating discussions, Jennifer Bradley and James Dowd for assistance in preparation of the review, Christine A Musser for artwork, and patient, families, laboratory workers, housestaff, and physicians for assistance in our studies. The work of PIT and WLC has been supported by National Institutes of Diabetes and Digestive and Kidney Diseases grant RO1 DK52081. These funding agencies had no role in the preparation of this seminar.

References

Table 2: Characteristics that differentiate Shiga-toxin-related HUS from TTP

<table>
<thead>
<tr>
<th>Feature</th>
<th>Shiga-toxin-related HUS</th>
<th>Idiopathic TTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidemiology</td>
<td>Endemic areas (commonly, not exclusively)</td>
<td>Endemic regions not reported</td>
</tr>
<tr>
<td>Similar cases in family</td>
<td>If yes, synchronous</td>
<td>If yes, separated in time and space</td>
</tr>
<tr>
<td>Recurrences</td>
<td>Rare</td>
<td>Common</td>
</tr>
<tr>
<td>Gastrointestinal prodromes</td>
<td>Painful diarrhoea, frequently bloody</td>
<td>Non-diarrhoeal abdominal symptoms predominate, but not as prodrome</td>
</tr>
<tr>
<td>von Willebrand factor profiles</td>
<td>Increased degradation to smaller multimers</td>
<td>Ultralarge forms (variably detectable, assay is not universally available and is technically difficult); depletion of large and ultralarge forms in advanced stage</td>
</tr>
<tr>
<td>ADAMTS13 activity</td>
<td>Normal</td>
<td>Deficient (&lt;0.1 U/mL)</td>
</tr>
<tr>
<td>Characteristics of intravascular thrombi</td>
<td>Fibrin predominates</td>
<td>von Willebrand factor antigen predominates</td>
</tr>
<tr>
<td>Endothelial-cell appearance</td>
<td>Swollen</td>
<td>Not swollen</td>
</tr>
<tr>
<td>Response to plasma therapy</td>
<td>Not demonstrated</td>
<td>Yes</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Isolation of an STEC, antibody response to O157 lipopolysaccharide antigen</td>
<td>ADAMTS13 activity; inhibitors of ADAMTS13 activity; genetic analysis for mutations of ADAMTS13 gene</td>
</tr>
</tbody>
</table>

*Obtain and freeze citrated plasma and serum before and after the initiation of plasma therapies; DNA should also be obtained, preferably before any cells are transfused.


