

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

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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.¹ 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.² A week after that paper was published, Riley and co-workers³ 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 1.⁴ 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.⁵ 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.¹ HUS is a thrombotic disorder, characterised by microvascular thrombi (figure 1) and swollen endothelial cells.⁶⁻¹⁰ 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,¹¹ Germany, and Austria.¹²

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.¹³ 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.¹⁴ 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.¹⁵

As early as 1965, Barnard and Kibel¹⁶ proposed that enteric *E coli* infections might precipitate HUS (though,

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Search strategy and selection criteria

We searched the PubMed database with the terms:

"h(a)emolytic ur(a)emic syndrome and pathogenesis", "h(a)emolytic ur(a)emic syndrome and pathophysiology", "h(a)emolytic ur(a)emic 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.

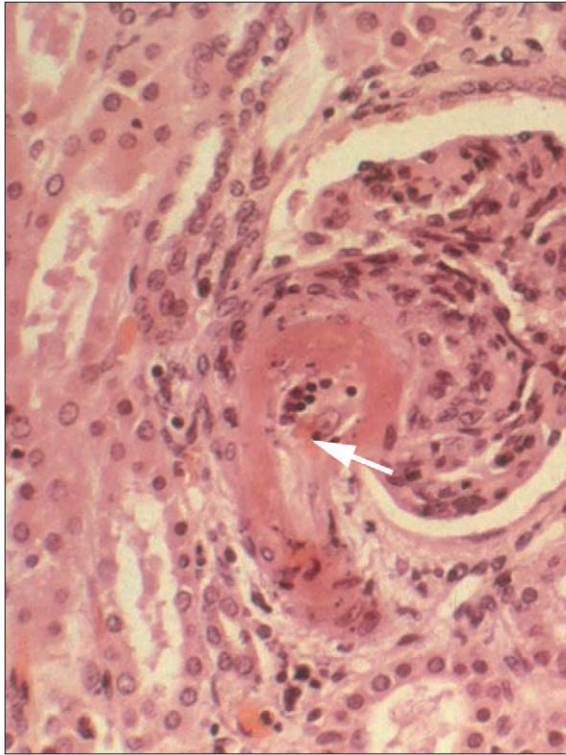


Figure 1: Renal lesion in fatal HUS
Thrombus (arrow) in a glomerular arteriole in a child who died with fulminant postdiarrhoeal HUS.

of course, without knowledge of the existence of STEC). In 1975, Kaplan and colleagues reported intrafamilial synchronous clustering of HUS cases in endemic areas and inferred an environmental, presumably infectious, precipitant.¹⁷

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 1.¹⁸ 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 $150 \times 10^9/L$; and serum creatinine above the upper limit for age,^{7,28-30} 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.³¹ 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,³² and inheritable abnormalities in complement regulatory proteins³³⁻³⁶ 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.^{37,38} This protease was recently identified as a particular member of the ADAMTS family—namely ADAMTS13.³⁹ 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

Type of Shiga toxin	Definition
Shiga toxin (Stx)	Main extracellular cytotoxin produced by <i>S dysenteriae</i> serotype 1 ¹⁹ and rarely by other shigellae. ²⁰
Shiga toxins 1 and 2	Families of toxins produced by <i>E coli</i> . Members of Stx1 family are highly homologous to Stx. Stx2 is 58% identical to Stx1 at the amino acid level and 56% identical at the nucleotide level. ^{19,21} 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) ^{22,23} or five (Stx2, Stx2c, Stx2d, Stx2e, and Stx2f) allelic variants. ²⁴⁻²⁷ These allelic variants of Stx could have different pathogenic potentials.
STEC	<i>E coli</i> that have genes encoding one or more Shiga toxins. Synonymous with verotoxigenic, verocytotoxin-producing, or verocytotoxigenic <i>E coli</i> (VTEC).
Enterohaemorrhagic <i>E coli</i> (EHEC)	Subset of STEC that are pathogenic to human beings. Most carry an <i>eae</i> allele, encoding intimin.

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

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 ago⁴⁰ 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 (panel 1).^{41–52} 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^{53–56} and rates of isolation of *E coli* O157:H7.⁵⁷ The correlation is not absolute; for example, Scotland has a high incidence of HUS and *E coli* O157:H7 infections,⁵⁸ but Denmark does not.⁵⁹ In the southern hemisphere, Buenos Aires in Argentina has a very high incidence of HUS.⁶⁰

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.⁶¹ Visits to dairy farms have been implicated as likely acquisition

sites for infections in Finland⁶² and the USA.⁴⁷ Transmission from cattle to people might be airborne.⁵² North American seroepidemiological surveys^{63,64} 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^{65,66} 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.^{12,17,54,57,67,68} Non-O157:H7 STEC infections in Australia¹¹ and Montana, USA,⁶⁹ had similar seasonality but this pattern did not occur in Seattle, USA.⁶⁷ The incidence of HUS probably increased in several regions during the 1970s and 1980s,^{70,71} but increasing or decreasing trends have not been proven unequivocally, and one population-based study found stable incidence during the 1990s.⁵³ A reported lower likelihood that children of African descent would have HUS^{16,55} was not confirmed in recent series from Natal⁷² and North America.^{54,73} 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.⁷⁴ 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,^{67,75} 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.⁷⁶

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,^{40,57,77} laboratory workers commonly fail to perceive blood in the submitted samples.^{57,67} 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*

Panel 1: Vehicles of transmission of *E coli* O157:H7, and examples of seminal, large, or well-documented clusters

Ground beef³
 Municipal water⁴¹
 Swimming water⁴²
 Deer jerky⁴³
 Unpasteurised milk⁴⁴
 Salami⁴⁵
 Lettuce⁴⁶
 Bovine contact⁴⁷
 Radish sprouts⁴⁸
 Unpasteurised apple cider⁴⁹
 Daycare centres⁵⁰ and person-to-person contact⁴⁰
 Salmon roe⁵¹
 Airborne transmission⁵²

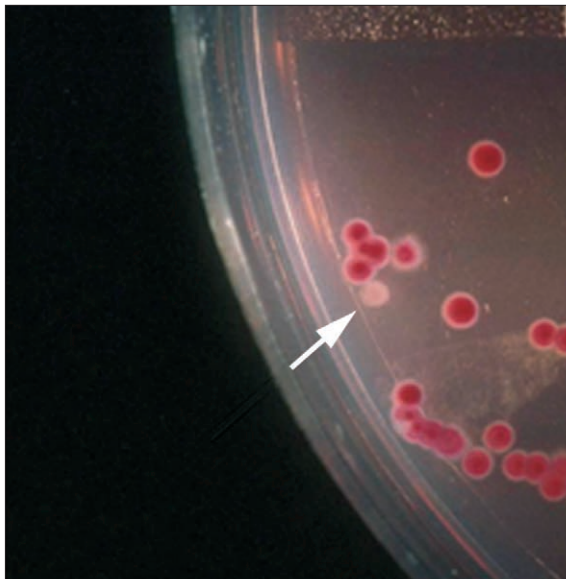


Figure 2: *E coli* O157:H7 on a sorbitol-MacConkey agar plate
Arrow indicates distinctive colourless *E coli* O157:H7 colony.

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,^{57,78} and antigen detection and even cytotoxicity assays might not identify all allelic variants of Shiga toxin.^{22,24} 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.^{79,80} Third, sorbitol-MacConkey agar could be more sensitive than detection of Shiga-toxin antigen for *E coli* O157:H7.⁶⁷

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.^{11,12,81-83} 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.¹² However, an unknown proportion of sorbitol-fermenting enterohaemorrhagic *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:H⁻ 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:H7.⁸⁴ Identification of such an organism in a microbiology laboratory would necessitate toxin assays or detection of unique genetic loci.⁸⁵

The ability to produce Shiga toxin is the key virulence trait of STEC. Shiga toxins are A₁B₂ 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.⁸⁶ Shiga toxins also induce apoptosis in human renal cells and tissue.^{87,88} They have additional and diverse effects on endothelial and other eukaryotic cells.⁸⁹ Most *E coli* O157:H7 carry the gene encoding Stx2, and about two-thirds have the gene encoding Stx1.^{57,67}

Enterohaemorrhagic *E coli* produce factors other than Shiga toxin that could plausibly injure human hosts. Intimin,⁹⁰ through which *E coli* mediates intimate attachment to epithelial cells in vitro and in animal models,⁹¹ 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,⁹² subtilase cytotoxin produced by *E coli* O113:H21,⁹³ and cytolethal distending toxin.⁹⁴ 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.^{3,40} In a well-analysed epidemic, the mean incubation period before the first loose stool was 3.7 days and the median 3 days.⁴⁰ Typically, *E coli* O157:H7 infections cause 1-3 days of non-bloody diarrhoea after which the diarrhoea becomes bloody.⁷⁷ 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,⁹⁵ and *E coli* O157:H7 have been recovered from the stools of patients with HUS who have no diarrhoea.^{96,97} Postsymptomatic shedding of *E coli* O157:H7 could lead to community spread of infection,³⁰ 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,³⁰ even though about half of all infected patients report fever before

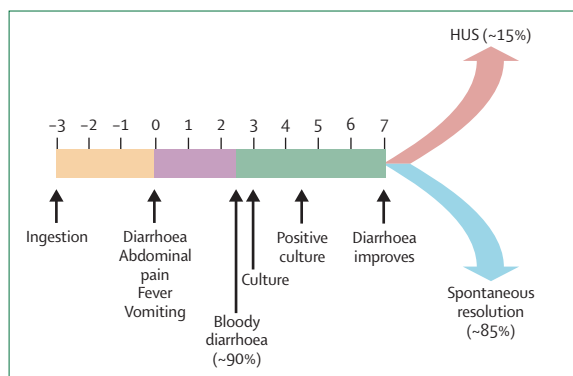


Figure 3: Progression of *E coli* O157:H7 infections in children

About 3 days after ingestion of the organism, the patient develops diarrhoea, abdominal pain, fever, and vomiting. The diarrhoea 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.²⁹

assessment. Leucocytes are found in only about half of examined faecal samples and are rarely described as abundant if they are present.^{57,67} The abdominal pain is greater than is generally seen in other forms of bacterial gastroenteritis, physicians commonly note abdominal tenderness during their examination,^{57,67} 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 precautions⁹⁸ 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;⁹⁹ we recommend use of isotonic crystalloid for volume expansion and maintenance.¹⁰⁰ 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).^{99,100}

Antibiotics should not be administered to patients with definite or possible enteric STEC infections. In the 1993 outbreak in Washington state,¹⁰¹ 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.³⁰ Antibiotics might also increase the risk of HUS in adults.¹⁰² The mechanisms by which antibiotics increase the risk of HUS remain unknown, but they

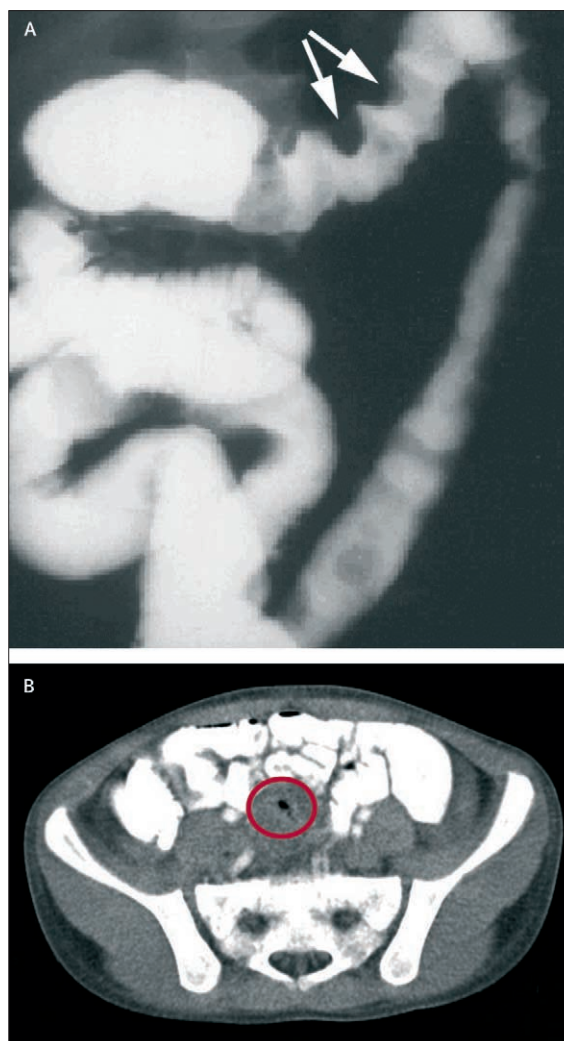


Figure 4: Radiographic features of *E coli* O157:H7 infection

A: radiograph after barium enema with thumbprinting appearance of mucosa (arrows), 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,¹⁰³ or induction of bacteriophages on which *stx* genes are located, with subsequently increased production of the toxin.¹⁰⁴

A 2002 meta-analysis implied that antibiotic therapy of *E coli* O157:H7 infections might not be harmful.¹⁰⁵ 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.¹⁰⁶ In that particular study, fosfomycin was compared only with other antibiotics, but the meta-analysis mischaracterised fosfomycin as being superior to no antibiotics.¹⁰⁷

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.^{101,108} We also advise against use of non-steroidal anti-inflammatory agents, because they can diminish renal blood flow.¹⁰⁹ 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.²⁸ Second, by day 4 of illness, vascular injury is well under way,^{29,110} 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.¹¹¹

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%.^{29,30,77,101,112} 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.^{29,30} As *E coli* O157:H7 infections evolve into HUS, thrombocytopenia is the first abnormality in most patients. Haemolysis, 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.^{30,101,113} 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^{112,114} 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 abnormalities²⁹ similar to those observed several days later when HUS develops.^{115–118} Furthermore, in animals, colitis can be caused by parenterally administered Shiga toxin.^{119–121} Perhaps the bloody diarrhoea, and possibly even the diarrhoea,¹²² 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.^{123–128}

Shiga toxins bind to the glycosphingolipid globotriaosylceramide,¹²⁹ which occurs on renal glomerular endothelial, mesangial, and tubular epithelial cells.^{130–133} Shiga toxin has been identified bound to renal sections taken after death from infected children who died of HUS,¹³⁴ and cellular differences in expression of globotriaosylceramide might underlie organ-specific responses to circulating Shiga toxin.¹³⁵ 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,¹³⁶ 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.^{6–10,137} Even if vascular occlusion is not the underlying major lesion in HUS, thrombosis-independent thrombin-mediated host mechanisms¹³⁸ 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),^{29,115,118} presumed increased intravascular generation of fibrin, as shown by high concentrations of D-dimers,^{29,117,118,139} and generation of thrombin, as inferred from raised concentrations of fragment 1+2.^{29,115} On or before day 4 of diarrhoea, many patients infected with *E coli* O157:H7 have similar abnormalities (figure 5),²⁹ 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.²⁹ 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.⁷ 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.²⁹ 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,^{140,141} which can similarly mediate thrombotic responses.¹⁴² Common host prothrombotic alleles do not seem to have major roles in the development of HUS.¹⁴³

Patients do not seem to undergo systemic inflammatory responses before HUS develops. They are rarely febrile once bloody diarrhoea begins and are not hypotensive, acidotic, or in shock. Nonetheless, there is a strong possibility that at the cellular level proinflam-

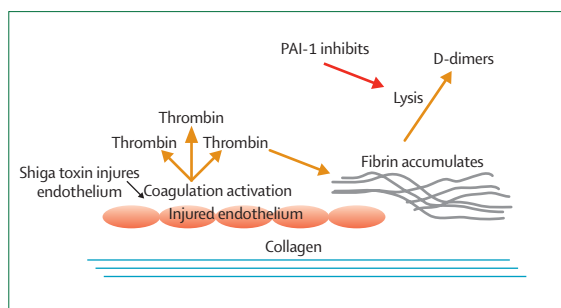


Figure 5: Proposed model for pathological coagulation response leading to HUS

Shiga toxin injures endothelial cells during the first few days of infection, possibly even before bloody diarrhoea occurs. Endothelial injury generates thrombin, and fibrin is deposited in the microvasculature. Concentrations of PAI-1 rise. PAI-1 blocks fibrinolysis, further accelerating the accumulation of fibrin in vessels, and exacerbating the thrombotic injury. There may be additive injurious effects of Shiga toxin on renal tubular cells.

matory cytokines and chemokines act synergistically with Shiga toxins to injure host cells.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.¹⁴⁵ STEC flagellin also induces this response,¹⁴⁶ and activates epithelial-cell mitogen-activated protein (MAP) kinase and nuclear factor κ B pathways, resulting in secretion of interleukin 8 by intestinal epithelial cells.¹⁴⁷ *E coli* O157:H7 abrogate Stat1-mediated interferon- γ signal transduction in cultured human intestinal epithelial cells.¹⁴⁸ Increased circulating concentrations of growth-related oncogen α , macrophage inflammatory protein 1 β , and monocyte chemotactic protein 1 are observed in *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.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;⁷⁷ however, the association is far from absolute.¹⁵⁰ Also, expanding knowledge of allelic variants of Stx2 make such analyses quite complex.^{24,69,151}

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 hypoperfusion. 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.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. Vasodilators¹⁵⁴ 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.¹⁵⁶ 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 above 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.

Nephrotoxic medications should be stopped (they should be 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.¹⁵⁷

Non-renal complications of HUS should be anticipated;⁹⁷ neurological complications are the most ominous and are important determinants of morbidity and mortality.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.^{97,159} 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 *E coli* O157:H7 in the western USA,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.¹⁶¹ The most common pulmonary consequence is fluid overload and pleural effusions, but adult respiratory distress syndrome can also occur.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.¹⁶² 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.¹⁶³

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.^{71,97} 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);¹⁶⁴ 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; oligouria or anuria as a limiting factor for nutritional support; and the need for blood transfusions in patients with poorly controlled hypertension.¹⁵⁴ 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,¹⁶⁵ 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

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 illness²⁹ 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 ciclosporin. 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

Panel 3: Ineffective approaches in typical HUS

- Corticosteroids¹⁶⁵
- Heparin¹⁶⁶
- Aspirin¹⁶⁷
- Dipyridamole¹⁶⁷
- Urokinase/streptokinase¹⁶⁶
- Plasmapheresis or plasma infusion^{168–171}
- Synsorb Pk¹¹¹

Feature	Shiga-toxin-related HUS	Idiopathic TTP
Epidemiology	Endemic areas (commonly, not exclusively)	Endemic regions not reported
Similar cases in family	If yes, synchronous	If yes, separated in time and space
Recurrences	Rare	Common
Gastrointestinal prodromes	Painful diarrhoea, frequently bloody.	Non-diarrhoeal abdominal symptoms predominate, but not as prodrome ¹⁷⁷
von Willebrand factor profiles	Increased degradation to smaller multimers	Ultralarge forms (variably detectable, assay is not universally available and is technically difficult); depletion of large and ultralarge forms in advanced stage
ADAMTS13 activity	Normal	Deficient (<0.1 U/mL)
Characteristics of intravascular thrombi	Fibrin predominates	von Willebrand factor antigen predominates
Endothelial-cell appearance	Swollen	Not swollen
Response to plasma therapy	Not demonstrated	Yes
Diagnosis	Isolation of an STEC; antibody response to O157 lipopolysaccharide antigen	ADAMTS13 activity; inhibitors of ADAMTS13 activity; genetic analysis for mutations of ADAMTS13 gene*

*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.

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

the evolution of the thromboses in STEC-associated HUS.⁷ In contrast to idiopathic TTP, in which cleavage of von Willebrand factor is impaired and pathogenetic multimers cause platelet aggregation and thrombi, 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.⁷ 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 illness¹⁷⁸ 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.³¹ Third, HUS related to *E coli* O157:H7 can recur,¹⁷⁹ 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,³⁶ which attenuates host injury from the alternative pathway of complement,¹⁸⁰ and factor I, which is a serine proteinase that inhibits the alternative complement pathway amplification loop, by cleaving the C3b a chain,³⁵ or the complement regulator, membrane cofactor protein (CD46), which inhibits membrane bound C3b and C4b.³⁴

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.¹⁸¹

Conflict of interest statement

We declare that we have no conflict of interest.

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References

- 1 Karmali MA, Steele BT, Petric M, Lim C. Sporadic cases of haemolytic-uraemic syndrome associated with faecal cytotoxin and cytotoxin-producing *Escherichia coli* in stools. *Lancet* 1983; **1**: 619–20.
- 2 Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. *Infect Immun* 1977; **18**: 775–79.
- 3 Riley LW, Remis RS, Helgerson SD, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N Engl J Med* 1983; **308**: 681–85.
- 4 O'Brien AO, Lively TA, Chen ME, Rothman SW, Formal SB. *Escherichia coli* O157:H7 strains associated with haemorrhagic colitis in the United States produce a *Shigella dysenteriae* 1 (SHIGA) like cytotoxin. *Lancet* 1983; **1**: 702.
- 5 Williams DM, Sreedhar SS, Mickell JJ, Chan JC. Acute kidney failure: a pediatric experience over 20 years. *Arch Pediatr Adolesc Med* 2002; **156**: 893–900.
- 6 Upadhyaya K, Barwick K, Fishaut M, Kashgarian M, Siegel NJ. The importance of nonrenal involvement in hemolytic-uremic syndrome. *Pediatrics* 1980; **65**: 115–20.
- 7 Tsai HM, Chandler WL, Sarode R, et al. von Willebrand factor and von Willebrand factor-cleaving metalloprotease activity in *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. *Pediatr Res* 2001; **49**: 653–59.
- 8 Inward CD, Howie AJ, Fitzpatrick MM, Razaat F, Milford DV, Taylor CM. Renal histopathology in fatal cases of diarrhoea-associated haemolytic uremic syndrome. *Pediatr Nephrol* 1997; **11**: 556–59.

- 9 Richardson SE, Karmali MA, Becker LE, Smith CR. The histopathology of the hemolytic uremic syndrome associated with verocytotoxin-producing *Escherichia coli* infections. *Hum Pathol* 1998; **19**: 1102–08.
- 10 Habib R. Pathology of the hemolytic uremic syndrome. In: Kaplan BS, Trompeter RS, Moake JL, eds. Hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. New York: Marcel Dekker, 1992: 315–53.
- 11 Elliott EJ, Robins-Browne RM, O'Loughlin EV, et al. Nationwide study of haemolytic uraemic syndrome: clinical, microbiological, and epidemiological features. *Arch Dis Child* 2001; **85**: 125–31.
- 12 Gerber A, Karch H, Allerberger F, Verwey HM, Zimmerhackl LB. Clinical course and the role of Shiga toxin-producing *Escherichia coli* infection in the hemolytic-uremic syndrome in pediatric patients, 1997–2000, in Germany and Austria: a prospective study. *J Infect Dis* 2002; **186**: 493–500.
- 13 Gautier E, Siebenmann RE. The birth of the hemolytic uremic syndrome. In: Kaplan BS, Trompeter RS, Moake JL, eds. Hemolytic uremic syndrome and thrombotic thrombocytopenic purpura. New York: Marcel Dekker, 1992: 1–18.
- 14 Gasser C, Gautier E, Steck A. Hamolytisch-uramische syndrome: Bilaterale Nierenrindennekrosen bei akuten erworbenen hamolytischen Anamien. *Schweiz Med Wochenschr* 1955; **85**: 905–09.
- 15 Bamforth J. A case of symmetrical cortical necrosis of the kidneys occurring in an adult man. *J Pathol Bacteriol* 1923; **26**: 40–45.
- 16 Barnard PJ, Kibel M. The haemolytic-uraemic syndrome of infancy and childhood: a report of eleven cases. *Cent Afr J Med* 1965; **11**: 4–11, 31–34.
- 17 Kaplan BS, Chesney RW, Drummond KN. Hemolytic uremic syndrome in families. *N Engl J Med* 1975; **292**: 1090–93.
- 18 Koster F, Levin J, Walker L, et al. Hemolytic-uremic syndrome after shigellosis: relation to endotoxemia and circulating immune complexes. *N Engl J Med* 1978; **298**: 927–33.
- 19 Calderwood SB, Auclair F, Donohue-Rolfé A, Keusch GT, Mekalanos JJ. Nucleotide sequence of the Shiga-like toxin genes of *Escherichia coli*. *Proc Natl Acad Sci USA* 1987; **84**: 4364–68.
- 20 Strauch E, Lurz R, Beutin L. Characterization of a Shiga toxin-encoding temperate bacteriophage of *Shigella sonnei*. *Infect Immun* 2001; **69**: 7588–95.
- 21 Jackson MP, Neill RJ, O'Brien AD, Holmes RK, JWN. Nucleotide sequence analysis and comparison of the structural genes for Shiga-like toxin I and Shiga-like toxin II encoded by bacteriophages from *Escherichia coli* 933. *FEMS Microbiol Lett* 1987; **44**: 109–14.
- 22 Zhang WL, Bielaszewska M, Kuczius T, Karch H. Identification, characterization, and distribution of a Shiga toxin 1 gene variant (*Stx(1c)*) in *Escherichia coli* strains isolated from humans. *J Clin Microbiol* 2002; **40**: 1441–46.
- 23 Paton AW, Beutin L, Paton JC. Heterogeneity of the amino-acid sequences of *Escherichia coli* Shiga-like toxin type-I operons. *Gene* 1995; **153**: 71–74.
- 24 Friedrich AW, Bielaszewska M, Zhang WL, et al. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. *J Infect Dis* 2002; **185**: 74–84.
- 25 Yutsudo T, Nakabayashi N, Hirayama T, Takeda Y. Purification and some properties of a vero toxin from *Escherichia coli* O157:H7 that is immunologically unrelated to Shiga toxin. *Microb Pathog* 1987; **3**: 21–30.
- 26 Newland JW, Strockbine NA, Neill RJ. Cloning of genes for production of *Escherichia coli* Shiga-like toxin type II. *Infect Immun* 1987; **55**: 2675–80.
- 27 Kokai-Kun JF, Melton-Celsa AR, O'Brien AD. Elastase in intestinal mucus enhances the cytotoxicity of Shiga toxin type 2d. *J Biol Chem* 2000; **275**: 3713–21.
- 28 Cornick NA, Jelacic S, Ciol MA, Tarr PI. *Escherichia coli* O157:H7 infections: discordance between filterable fecal Shiga toxin and disease outcome. *J Infect Dis* 2002; **186**: 57–63.
- 29 Chandler WL, Jelacic S, Boster DR, et al. Prothrombotic coagulation abnormalities associated with *Escherichia coli* O157:H7 infections. *N Engl J Med* 2002; **346**: 23–32.
- 30 Wong CS, Jelacic S, Habeeb RL, Watkins SL, Tarr PI. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N Engl J Med* 2000; **342**: 1930–36.
- 31 Katz J, Lurie A, Kaplan BS, Krawitz S, Metz J. Coagulation findings in the hemolytic-uremic syndrome of infancy: similarity to hyperacute renal allograft rejection. *J Pediatr* 1971; **78**: 426–34.
- 32 Brandt J, Wong C, Mihm S, et al. Invasive pneumococcal disease and hemolytic uremic syndrome. *Pediatrics* 2002; **110**: 371–76.
- 33 Perkins SJ, Goodship TH. Molecular modelling of the C-terminal domains of factor H of human complement: a correlation between haemolytic uraemic syndrome and a predicted heparin binding site. *J Mol Biol* 2002; **316**: 217–24.
- 34 Richards A, Kemp EJ, Liszewski MK, et al. Mutations in human complement regulator, membrane cofactor protein (CD46), predispose to development of familial hemolytic uremic syndrome. *Proc Natl Acad Sci U S A* 2003; **100**: 12966–71.
- 35 Fremeaux-Bacchi V, Dragon-Durey MA, Blouin J, et al. Complement factor I: a susceptibility gene for atypical haemolytic uraemic syndrome. *J Med Genet* 2004; **41**: e84.
- 36 Caprioli J, Bettinaglio P, Zipfel PF, et al. The molecular basis of familial hemolytic uremic syndrome: mutation analysis of factor H gene reveals a hot spot in short consensus repeat 20. *J Am Soc Nephrol* 2001; **12**: 297–307.
- 37 Tsai HM, Lian EC. Antibodies to von Willebrand factor-cleaving protease in acute thrombotic thrombocytopenic purpura. *N Engl J Med* 1998; **339**: 1585–94.
- 38 Furlan M, Robles R, Galbusera M, et al. von Willebrand factor-cleaving protease in thrombotic thrombocytopenic purpura and the hemolytic-uremic syndrome. *N Engl J Med* 1998; **339**: 1578–84.
- 39 Levy GG, Nichols WC, Lian EC, et al. Mutations in a member of the ADAMTS gene family cause thrombotic thrombocytopenic purpura. *Nature* 2001; **413**: 488–94.
- 40 Bell BP, Goldoft M, Griffin PM, et al. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers: the Washington experience. *JAMA* 1994; **272**: 1349–53.
- 41 Swerdlow DL, Woodruff BA, Brady RC, et al. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. *Ann Intern Med* 1992; **117**: 812–19.
- 42 Keene WE, McAnulty JM, Hoesly FC, et al. A swimming-associated outbreak of hemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. *N Engl J Med* 1994; **331**: 579–84.
- 43 Keene WE, Sazie E, Kok J, et al. An outbreak of *Escherichia coli* O157:H7 infections traced to jerky made from deer meat. *JAMA* 1997; **277**: 1229–31.
- 44 Keene WE, Hedberg K, Herriott DE, et al. A prolonged outbreak of *Escherichia coli* O157:H7 infections caused by commercially distributed raw milk. *J Infect Dis* 1997; **176**: 815–18.
- 45 Tilden J Jr, Young W, McNamara AM, et al. A new route of transmission for *Escherichia coli*: infection from dry fermented salami. *Am J Public Health* 1996; **86**: 1142–45.
- 46 Ackers ML, Mahon BE, Leahy E, et al. An outbreak of *Escherichia coli* O157:H7 infections associated with leaf lettuce consumption. *J Infect Dis* 1998; **177**: 1588–93.
- 47 Crump JA, Sulka AC, Langer AJ, et al. An outbreak of *Escherichia coli* O157:H7 infections among visitors to a dairy farm. *N Engl J Med* 2002; **347**: 555–60.
- 48 Michino H, Araki K, Minami S, et al. Massive outbreak of *Escherichia coli* O157:H7 infection in schoolchildren in Sakai City, Japan, associated with consumption of white radish sprouts. *Am J Epidemiol* 1999; **150**: 787–96.
- 49 Besser RE, Lett SM, Weber JT, et al. An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *JAMA* 1993; **269**: 883–88.
- 50 Belongia EA, Osterholm MT, Soler JT, Ammend DA, Braun JE, MacDonald KL. Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day-care facilities. *JAMA* 1993; **269**: 883–88.
- 51 Terajima J, Izumiya H, Tamura K, Watanabe H. [PulseNet Japan—network system for the utilization of epidemiological information and the results of pulsed-field gel electrophoresis]. *Nippon Rinsho* 2002; **60**: 1070–76.
- 52 Varma JK, Greene KD, Reller ME, et al. An outbreak of *Escherichia coli* O157 infection following exposure to a contaminated building. *JAMA* 2003; **290**: 2709–12.

- 53 Cummings KC, Mohle-Boetani JC, Werner SB, Vugia DJ. Population-based trends in pediatric hemolytic uremic syndrome in California, 1994–1999: substantial underreporting and public health implications. *Am J Epidemiol* 2002; **155**: 941–48.
- 54 Tarr PI, Hickman RO. Hemolytic uremic syndrome epidemiology: a population-based study in King County, Washington, 1971 to 1980. *Pediatrics* 1987; **80**: 41–45.
- 55 Jernigan SM, Waldo FB. Racial incidence of hemolytic uremic syndrome. *Pediatr Nephrol* 1994; **8**: 545–47.
- 56 Gianviti A, Rosmini F, Caprioli A, et al. Haemolytic-uraemic syndrome in childhood: surveillance and case-control studies in Italy. *Pediatr Nephrol* 1994; **8**: 705–09.
- 57 Slutsker L, Ries AA, Greene KD, Wells JG, Hutwagner L, Griffin PM. *Escherichia coli* O157:H7 diarrhea in the United States: clinical and epidemiologic features. *Ann Intern Med* 1997; **126**: 505–13.
- 58 Thomas A, Chart H, Cheasty T, Smith HR, Frost JA, Rowe B. Vero cytotoxin-producing *Escherichia coli*, particularly serogroup O157, associated with human infections in the United Kingdom: 1989–91. *Epidemiol Infect* 1993; **110**: 591–600.
- 59 Nielsen EM, Scheutz F. Characterisation of *Escherichia coli* O157 isolates from Danish cattle and human patients by genotyping and presence and variants of virulence genes. *Vet Microbiol* 2002; **88**: 259–73.
- 60 Lopez EL, Diaz M, Grinstein S, et al. Hemolytic uremic syndrome and diarrhea in Argentine children: the role of Shiga-like toxins. *J Infect Dis* 1989; **160**: 469–75.
- 61 O'Brien SJ, Adak GK, Gilham C. Contact with farming environment as a major risk factor for Shiga toxin (vero cytotoxin)-producing *Escherichia coli* O157 infection in humans. *Emerg Infect Dis* 2001; **7**: 1049–51.
- 62 Lahti E, Eklund M, Ruutu P, et al. Use of phenotyping and genotyping to verify transmission of *Escherichia coli* O157:H7 from dairy farms. *Eur J Clin Microbiol Infect Dis* 2002; **21**: 189–95.
- 63 Raymond D, Johnson RP, Karmali MA, et al. Neutralizing antibodies to *Escherichia coli* vero cytotoxin 1 and antibodies to O157 lipopolysaccharide in healthy farm family members and urban residents. *J Clin Microbiol* 1996; **34**: 2053–57.
- 64 Haack JP, Jelacic S, Besser TE, et al. *Escherichia coli* O157 exposure in Wyoming and Seattle: serologic evidence for rural risk. *Emerg Infect Dis* 2003; **9**: 1226–31.
- 65 Effler E, Isaacson M, Arntzen L, et al. Factors contributing to the emergence of *Escherichia coli* O157 in Africa. *Emerg Infect Dis* 2001; **7**: 812–19.
- 66 Cunin P, Pedjouka E, Germani Y, et al. An epidemic of bloody diarrhea: *Escherichia coli* O157 emerging in Cameroon? *Emerg Infect Dis* 1999; **5**: 285–90.
- 67 Klein EJ, Stapp JR, Clausen CR, et al. Shiga toxin-producing *Escherichia coli* in children with diarrhea: a prospective point of care study. *J Pediatr* 2002; **141**: 172–77.
- 68 Decludt B, Bouvet P, Mariani-Kurkdjian P, et al. Haemolytic uremic syndrome and Shiga toxin-producing *Escherichia coli* infection in children in France. *Epidemiol Infect* 2000; **124**: 215–20.
- 69 Jelacic JK, Damrow T, Chen GS, et al. Shiga toxin-producing *Escherichia coli* in Montana: bacterial genotypes and clinical profiles. *J Infect Dis* 2003; **188**: 719–29.
- 70 Martin DL, MacDonald KL, White KE, Soler JT, Osterholm MT. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. *N Engl J Med* 1990; **323**: 1160–67.
- 71 Tarr PI, Neill MA, Allen J, Siccardi CJ, Watkins SL, Hickman RO. The increasing incidence of the hemolytic-uremic syndrome in King County, Washington: lack of evidence for ascertainment bias. *Am J Epidemiol* 1989; **129**: 582–86.
- 72 Bhimma R, Rollins NC, Coovadia HM, Adhikari M. Post-dysenteric hemolytic uremic syndrome in children during an epidemic of Shigella dysenteriae in Kwazulu/Natal. *Pediatr Nephrol* 1997; **11**: 560–64.
- 73 Rogers MF, Rutherford GW, Alexander SR, et al. A population-based study of hemolytic-uremic syndrome in Oregon, 1979–1982. *Am J Epidemiol* 1986; **123**: 137–42.
- 74 March SB, Ratnam S. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J Clin Microbiol* 1986; **23**: 869–72.
- 75 Park CH, Gates KM, Vandel NM, Hixon DL. Isolation of Shiga-like toxin producing *Escherichia coli* (O157 and non-O157) in a community hospital. *Diagn Microbiol Infect Dis* 1996; **26**: 69–72.
- 76 Stapp JR, Jelacic S, Yea YL, et al. Comparison of *Escherichia coli* O157:H7 antigen detection in stool and broth cultures to that in sorbitol-MacConkey agar stool cultures. *J Clin Microbiol* 2000; **38**: 3404–06.
- 77 Ostroff SM, Kobayashi JM, Lewis JH. Infections with *Escherichia coli* O157:H7 in Washington State: the first year of statewide disease surveillance. *JAMA* 1989; **262**: 355–59.
- 78 Cohen MB. Shiga toxin-producing *E coli*: two tests are better than one. *J Pediatr* 2002; **141**: 155–56.
- 79 Swaminathan B, Barrett TJ, Hunter SB, Tauxe RV. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. *Emerg Infect Dis* 2001; **7**: 382–89.
- 80 Bender JB, Hedberg CW, Besser JM, Boxrud DJ, MacDonald KL, Osterholm MT. Surveillance by molecular subtype for *Escherichia coli* O157:H7 infections in Minnesota by molecular subtyping. *N Engl J Med* 1997; **337**: 388–94.
- 81 Brooks JT, Bergmire-Sweat D, Kennedy M, et al. Outbreak of Shiga toxin-producing *Escherichia coli* O111: H8 infections among attendees of a high school cheerleading camp. *Clin Infect Dis* 2004; **38**: 190–98.
- 82 Misselwitz J, Karch H, Bielazewska M, et al. Cluster of hemolytic-uremic syndrome caused by Shiga toxin-producing *Escherichia coli* O26: H11. *Pediatr Infect Dis J* 2003; **22**: 349–54.
- 83 McCarthy TA, Barrett NL, Hadler JL, et al. Hemolytic-uremic syndrome and *Escherichia coli* O121 at a Lake in Connecticut, 1999. *Pediatrics* 2001; **108**: E59.
- 84 Karch H, Bielazewska M. Sorbitol-fermenting Shiga toxin-producing *Escherichia coli* O157:H(-) strains: epidemiology, phenotypic and molecular characteristics, and microbiological diagnosis. *J Clin Microbiol* 2001; **39**: 2043–49.
- 85 Friedrich AW, Nierhoff KV, Bielazewska M, Mellmann A, Karch H. Phylogeny, clinical associations, and diagnostic utility of the pilin subunit gene (*sfpA*) of sorbitol-fermenting, enterohemorrhagic *Escherichia coli* O157: H-. *J Clin Microbiol* 2004; **42**: 4697–01.
- 86 Endo Y, Tsurugi K, Yutsudo T, Takeda Y, Ogasawara T, Igarashi K. Site of action of a vero toxin (VT2) from *Escherichia coli* O157:H7 and of Shiga toxin on eukaryotic ribosomes: RNA N-glycosidase activity of the toxins. *Eur J Biochem* 1988; **171**: 45–50.
- 87 Taguchi T, Uchida H, Kiyokawa N, et al. Verotoxins induce apoptosis in human renal tubular epithelium derived cells. *Kidney Int* 1998; **53**: 1681–88.
- 88 Karpman D, Hakansson A, Perez MT, et al. Apoptosis of renal cortical cells in the hemolytic-uremic syndrome: in vivo and in vitro studies. *Infect Immun* 1998; **66**: 636–44.
- 89 O'Loughlin EV, Robins-Browne RM. Effect of Shiga toxin and Shiga-like toxins on eukaryotic cells. *Microbes Infect* 2001; **3**: 493–507.
- 90 Jerse AE, Yu J, Tall BD, Kaper JB. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proc Natl Acad Sci USA* 1990; **87**: 7839–43.
- 91 Donnenberg MS, Tzipori S, McKee ML, O'Brien AD, Alroy J, Kaper JB. The role of the *eae* gene of enterohemorrhagic *Escherichia coli* in intimate attachment in vitro and in a porcine model. *J Clin Invest* 1993; **92**: 1418–24.
- 92 Latham WW, Grys TE, Witowski SE, et al. StcE, a metalloprotease secreted by *Escherichia coli* O157:H7, specifically cleaves C1 esterase inhibitor. *Mol Microbiol* 2002; **45**: 277–88.
- 93 Paton AW, Srimanote P, Talbot UM, Wang H, Paton JC. A new family of potent AB cytotoxins produced by Shiga toxin-producing *Escherichia coli*. *J Exp Med* 2004; **200**: 35–46.
- 94 Janka A, Bielazewska M, Dobrindt U, Greune L, Schmidt MA, Karch H. Cytotoxin distending toxin gene cluster in enterohemorrhagic *Escherichia coli* O157: H- and O157:H7: characterization and evolutionary considerations. *Infect Immun* 2003; **71**: 3634–38.
- 95 Rodrigue DC, Mast EE, Greene KD, et al. A university outbreak of *Escherichia coli* O157:H7 infections associated with roast beef and an unusually benign clinical course. *J Infect Dis* 1995; **172**: 1122–25.

- 96 Miceli S, Jure MA, Saab OA, et al. A clinical and bacteriological study of children suffering from haemolytic uraemic syndrome in Tucuman, Argentina. *Jpn J Infect Dis* 1999; **52**: 33–37.
- 97 Brandt JR, Fouser LS, Watkins SL, et al. *Escherichia coli* O157:H7-associated hemolytic-uremic syndrome after ingestion of contaminated hamburgers. *J Pediatr* 1994; **125**: 519–26.
- 98 Contact precautions for *Escherichia coli*. Red Book 2000: Report of the Committee on Infectious Diseases, 25 edn. Washington DC: American Academy of Pediatrics, 2000: 246–47.
- 99 Ake JA, Jelacic S, Ciol MA, et al. Relative nephroprotection during *Escherichia coli* O157:H7 infections: association with intravenous volume expansion. *Pediatrics* (in press).
- 100 Tarr PI, Neill MA. *Escherichia coli* O157:H7. *Gastroenterol Clin North Am* 2001; **30**: 735–51.
- 101 Bell BP, Griffin PM, Lozano P, Christie DL, Kobayashi JM, Tarr PI. Predictors of hemolytic uremic syndrome in children during a large outbreak of *Escherichia coli* O157:H7 infections. *Pediatrics* 1997; **100**: E12.
- 102 Dundas S, Todd WT, Stewart AI, Murdoch PS, Chaudhuri AK, Hutchinson SJ. The central Scotland *Escherichia coli* O157:H7 outbreak: risk factors for the hemolytic uremic syndrome and death among hospitalized patients. *Clin Infect Dis* 2001; **33**: 923–31.
- 103 Grif K, Dierich MP, Karch H, Allerberger F. Strain-specific differences in the amount of Shiga toxin released from enterohemorrhagic *Escherichia coli* O157 following exposure to subinhibitory concentrations of antimicrobial agents. *Eur J Clin Microbiol Infect Dis* 1998; **17**: 761–66.
- 104 Kimmitt PT, Harwood CR, Barer MR. Toxin gene expression by Shiga toxin-producing *Escherichia coli*: the role of antibiotics and the bacterial SOS response. *Emerg Infect Dis* 2000; **6**: 458–65.
- 105 Safdar N, Said A, Gangnon RE, Maki DG. Risk of hemolytic uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 enteritis: a meta-analysis. *JAMA* 2002; **288**: 996–1001.
- 106 Ikeda K, Ida O, Kimoto K, Takatorige T, Nakanishi N, Tataru K. Effect of early fosfomycin treatment on prevention of hemolytic uremic syndrome accompanying *Escherichia coli* O157:H7 infection. *Clin Nephrol* 1999; **52**: 357–62.
- 107 Wong CS, Brandt JR. Risk of hemolytic uremic syndrome from antibiotic treatment of *Escherichia coli* O157:H7 colitis. *JAMA* 2002; **288**: 3111.
- 108 Cimolai N, Morrison BJ, Carter JE. Risk factors for the central nervous system manifestations of gastroenteritis-associated hemolytic-uremic syndrome. *Pediatrics* 1992; **90**: 616–21.
- 109 Murray MD, Brater DC. Renal toxicity of the nonsteroidal anti-inflammatory drugs. *Annu Rev Pharmacol Toxicol* 1993; **33**: 435–65.
- 110 Smith JM, Jones F, Ciol MA, et al. Platelet-activating factor and *Escherichia coli* O157:H7 infections. *Pediatr Nephrol* 2002; **17**: 1047–52.
- 111 Trachtman H, Cnaan A, Christen E, et al. Effect of an oral Shiga toxin-binding agent on diarrhea-associated hemolytic uremic syndrome in children: a randomized controlled trial. *JAMA* 2003; **291**: 1337–44.
- 112 Rowe PC, Orrbine E, Lior H, et al. Risk of hemolytic uremic syndrome after sporadic *Escherichia coli* O157:H7 infection: results of a Canadian collaborative study. *J Pediatr* 1998; **132**: 777–82.
- 113 Pavia AT, Nichols CR, Green DP, et al. Hemolytic-uremic syndrome during an outbreak of *Escherichia coli* O157:H7 infections in institutions for mentally retarded persons: clinical and epidemiologic observations. *J Pediatr* 1990; **116**: 544–51.
- 114 Lopez EL, Contrini MM, Devoto S, et al. Incomplete hemolytic-uremic syndrome in Argentinean children with bloody diarrhea. *J Pediatr* 1995; **127**: 364–67.
- 115 Nevard CH, Jurd KM, Lane DA, Philippou H, Haycock GB, Hunt BJ. Activation of coagulation and fibrinolysis in childhood diarrhoea-associated haemolytic uraemic syndrome. *Thromb Haemost* 1997; **78**: 1450–55.
- 116 Van Geet C, Proesmans W, Arnout J, Vermynen J, Declerck PJ. Activation of both coagulation and fibrinolysis in childhood hemolytic uremic syndrome. *Kidney Int* 1998; **54**: 1324–30.
- 117 van de Kar NC, van Hinsbergh VW, Brommer EJ, Monnens LA. The fibrinolytic system in the hemolytic uremic syndrome: in vivo and in vitro studies. *Pediatr Res* 1994; **36**: 257–64.
- 118 Bergstein JM, Riley BS, Bang NU. Role of plasminogen-activator inhibitor type 1 in the pathogenesis and outcome of the hemolytic uremic syndrome. *N Engl J Med* 1992; **327**: 755–59.
- 119 Richardson SE, Rotman TA, Jay V, et al. Experimental verocytotoxemia in rabbits. *Infect Immun* 1992; **60**: 4154–67.
- 120 Taylor FB Jr, Tesh VL, DeBault L, et al. Characterization of the baboon responses to Shiga-like toxin: descriptive study of a new primate model of toxic responses to Stx-1. *Am J Pathol* 1999; **154**: 1285–99.
- 121 Barrett TJ, Potter ME, Wachsmuth IK. Continuous peritoneal infusion of Shiga-like toxin II (SLT II) as a model for SLT II-induced diseases. *J Infect Dis* 1989; **159**: 774–77.
- 122 Igarashi T, Inatomi J, Wake A, Takamizawa M, Katayama H, Iwata T. Failure of pre-diarrheal antibiotics to prevent hemolytic uremic syndrome in serologically proven *Escherichia coli* O157:H7 gastrointestinal infection. *J Pediatr* 1999; **135**: 768–69.
- 123 Zoja C, Angioletti S, Donadelli R, et al. Shiga toxin-2 triggers endothelial leukocyte adhesion and transmigration via NF-kappaB dependent up-regulation of IL-8 and MCP-1. *Kidney Int* 2002; **62**: 846–56.
- 124 Te Loo DM, van Hinsbergh VW, van den Heuvel LP, Monnens LA. Detection of verocytotoxin bound to circulating polymorphonuclear leukocytes of patients with hemolytic uremic syndrome. *J Am Soc Nephrol* 2001; **12**: 800–06.
- 125 Te Loo DM, Monnens LA, van Der Velden TJ, et al. Binding and transfer of verocytotoxin by polymorphonuclear leukocytes in hemolytic uremic syndrome. *Blood* 2000; **95**: 3396–402.
- 126 Karpman D, Papadopoulou D, Nilsson K, Sjogren AC, Mikaelsson C, Lethagen S. Platelet activation by Shiga toxin and circulatory factors as a pathogenetic mechanism in the hemolytic uremic syndrome. *Blood* 2001; **97**: 3100–08.
- 127 Heuvelink AE, Van de Kar NC, Van Der Velden TJ, Chart H, Monnens LA. Verocytotoxin-producing *Escherichia coli* infection in household members of children with hemolytic-uremic syndrome in the Netherlands. *Pediatr Infect Dis J* 1999; **18**: 709–14.
- 128 Tazzari PL, Ricci F, Carnicelli D, et al. Flow cytometry detection of Shiga toxins in the blood from children with hemolytic uremic syndrome. *Cytometry* 2004; **61B**: 40–44.
- 129 Lingwood CA. Shiga toxin receptor glycolipid binding: pathology and utility. *Methods Mol Med* 2003; **73**: 165–86.
- 130 Boyd B, Lingwood C. Verotoxin receptor glycolipid in human renal tissue. *Nephron* 1989; **51**: 207–10.
- 131 Lingwood CA. Verotoxin-binding in human renal sections. *Nephron* 1994; **66**: 21–28.
- 132 Robinson LA, Hurley RM, Lingwood C, Matsell DG. *Escherichia coli* verotoxin binding to human paediatric glomerular mesangial cells. *Pediatr Nephrol* 1995; **9**: 700–04.
- 133 Takeda T, Dohi S, Igarashi T, Yamanaka T, Yoshiya K, Kobayashi N. Impairment by verotoxin of tubular function contributes to the renal damage seen in haemolytic uraemic syndrome. *J Infect* 1993; **27**: 339–41.
- 134 Uchida H, Kiyokawa N, Horie H, Fujimoto J, Takeda T. The detection of Shiga toxins in the kidney of a patient with hemolytic uremic syndrome. *Pediatr Res* 1999; **45**: 133–37.
- 135 Hughes AK, Ergonul Z, Stricklett PK, Kohan DE, Ergonal Z. Molecular basis for high renal cell sensitivity to the cytotoxic effects of Shigatoxin-1: upregulation of globotriaosylceramide expression. *J Am Soc Nephrol* 2002; **13**: 2239–45.
- 136 Tesh VL, Samuel JE, Burriss JA, Owens JW, Taylor FB Jr, Siegler RL. Quantitation and localization of Shiga toxin/Shiga-like toxin-binding glycolipid receptors in human and baboon tissues. In: Karmali MAGA, ed. Recent advances in verocytotoxin-producing *Escherichia coli* infections. Bergamo, Italy: Elsevier Science, 1994: 189–92.
- 137 Richardson SE, Karmali MA, Becker LE, Smith CR. The histopathology of the hemolytic uremic syndrome associated with verocytotoxin-producing *Escherichia coli* infections. *Hum Pathol* 1998; **19**: 1102–08.
- 138 Cunningham MA, Rondeau E, Chen X, Coughlin SR, Holdsworth SR, Tipping PG. Protease-activated receptor 1 mediates thrombin-dependent, cell-mediated renal inflammation in crescentic glomerulonephritis. *J Exp Med* 2000; **191**: 455–62.

- 139 van de Kar NCAJ, Sauerwein RW, Demacker PNM, Grau GE, van Hinsbergh VWM, Monnens LAH. Plasma cytokine levels in hemolytic uremic syndrome. *Nephron* 1995; **71**: 309–13.
- 140 Foster GH, Tesh VL. Shiga toxin 1-induced activation of c-Jun NH-terminal kinase and p38 in the human monocytic cell line THP-1: possible involvement in the production of TNF-alpha. *J Leukoc Biol* 2002; **71**: 107–14.
- 141 Ramegowda B, Tesh VL. Differentiation-associated toxin receptor modulation, cytokine production, and sensitivity to Shiga-like toxins in human monocytes and monocytic cell lines. *Infect Immun* 1996; **64**: 1173–80.
- 142 Grignani G, Maiolo A. Cytokines and hemostasis. *Haematologica* 2000; **85**: 967–72.
- 143 Sprouse JT, Wong CS, Chandler WL, Williams GD, Watkins SL, Tarr PI. Thrombogenic alleles, *Escherichia coli* O157:H7 infections, and hemolytic uremic syndrome. *Blood Coagul Fibrinolysis* 2001; **12**: 283–88.
- 144 Proulx F, Seidman EG, Karpman D. Pathogenesis of Shiga toxin-associated hemolytic uremic syndrome. *Pediatr Res* 2001; **50**: 163–71.
- 145 Thorpe CM, Smith WE, Hurley BP, Acheson DW. Shiga toxins induce, superinduce, and stabilize a variety of C-X-C chemokine mRNAs in intestinal epithelial cells, resulting in increased chemokine expression. *Infect Immun* 2001; **69**: 6140–47.
- 146 Rogers TJ, Paton AW, McColl SR, Paton JC. Enhanced CXC chemokine responses of human colonic epithelial cells to locus of enterocyte effacement-negative Shiga-toxigenic *Escherichia coli*. *Infect Immun* 2003; **71**: 5623–32.
- 147 Berin MC, Darfeuille-Michaud A, Egan LJ, Miyamoto Y, Kagnoff MF. Role of EHEC O157:H7 virulence factors in the activation of intestinal epithelial cell NF-kappaB and MAP kinase pathways and the upregulated expression of interleukin 8. *Cell Microbiol* 2002; **4**: 635–48.
- 148 Ceonis PJ, McKay DM, Ching JC, Pereira P, Sherman PM. Enterohemorrhagic *Escherichia coli* O157:H7 disrupts Stat1-mediated gamma interferon signal transduction in epithelial cells. *Infect Immun* 2003; **71**: 1396–404.
- 149 Proulx F, Toledano B, Phan V, Clermont MJ, Mariscalco MM, Seidman EG. Circulating granulocyte colony-stimulating factor, C-X-C, and C-C chemokines in children with *Escherichia coli* O157:H7 associated hemolytic uremic syndrome. *Pediatr Res* 2002; **52**: 928–34.
- 150 Jelacic S, Wobbe CL, Boster DR, et al. ABO and PI blood group antigen expression and *stx* genotype and outcome of childhood *Escherichia coli* O157: H7 infections. *J Infect Dis* 2002; **185**: 214–19.
- 151 Eklund M, Leino K, Siitonen A. Clinical *Escherichia coli* strains carrying *stx* genes: *stx* variants and *stx*-positive virulence profiles. *J Clin Microbiol* 2002; **40**: 4585–93.
- 152 Siegler RL. The hemolytic uremic syndrome. *Pediatr Clin North Am* 1995; **42**: 1505–29.
- 153 Kaplan BS, Meyers KE, Schulman SL. The pathogenesis and treatment of hemolytic uremic syndrome. *J Am Soc Nephrol* 1998; **9**: 1126–33.
- 154 Corrigan JJ Jr, Boineau FG. Hemolytic-uremic syndrome. *Pediatr Rev* 2001; **22**: 365–69.
- 155 Loirat C, Taylor CM. Hemolytic uremic syndromes. In: Avner ED, Harmon WE, Niaudet P, eds. *Pediatric nephrology*, 5th edn. Philadelphia: Lippincott Williams and Wilkins, 2004: 887–915.
- 156 Schoolwerth AC, Sica DA, Ballermann BJ, Wilcox CS. Renal considerations in angiotensin converting enzyme inhibitor therapy: a statement for healthcare professionals from the Council on the Kidney in Cardiovascular Disease and the Council for High Blood Pressure Research of the American Heart Association. *Circulation* 2001; **104**: 1985–91.
- 157 Hassan H, Bastani B, Gellens M. Successful treatment of normeperidine neurotoxicity by hemodialysis. *Am J Kidney Dis* 2000; **35**: 146–49.
- 158 Hughes DA, Beattie TJ, Murphy AV. Haemolytic uraemic syndrome: 17 years' experience in a Scottish paediatric renal unit. *Scott Med J* 1991; **36**: 9–12.
- 159 Taylor CM, White RH, Winterborn MH, Rowe B. Haemolytic-uraemic syndrome: clinical experience of an outbreak in the West Midlands. *Br Med J (Clin Res Ed)* 1986; **292**: 1513–16.
- 160 Bale JF Jr, Brasher C, Siegler RL. CNS manifestations of the hemolytic-uremic syndrome: relationship to metabolic alterations and prognosis. *Am J Dis Child* 1980; **134**: 869–72.
- 161 Thayu M, Chandler WL, Jelacic S, Gordon CL, Rosenthal GL, Tarr PI. Cardiac ischemia during hemolytic uremic syndrome. *Pediatr Nephrol* 2003; **18**: 286–89.
- 162 Tapper D, Tarr P, Avner E, Brandt J, Waldhausen J. Lessons learned in the management of hemolytic uremic syndrome in children. *J Pediatr Surg* 1995; **30**: 158–63.
- 163 Brizee L. Nutrition support in *E. coli* induced hemolytic uremic syndrome. *Renal Nutr Forum* 1994; **13**: 1–4.
- 164 Bhimma R, Coovadia HM, Adhikari M, Connolly CA. Re-evaluating criteria for peritoneal dialysis in "classical" (D+) hemolytic uremic syndrome. *Clin Nephrol* 2001; **55**: 133–42.
- 165 Perez N, Spizzirri F, Rahman R, Suarez A, Larrubia C, Lasarte P. Steroids in the hemolytic uremic syndrome. *Pediatr Nephrol* 1998; **12**: 101–04.
- 166 Diekmann L. [Treatment of the hemolytic-uremic syndrome with streptokinase and heparin (author's transl)]. *Klin Padiatr* 1980; **192**: 430–35.
- 167 O'Regan S, Chesney RW, Mongeau JG, Robitaille P. Aspirin and dipyridamole therapy in the hemolytic-uremic syndrome. *J Pediatr* 1980; **97**: 473–76.
- 168 Gianviti A, Perna A, Caringella A, et al. Plasma exchange in children with hemolytic-uremic syndrome at risk of poor outcome. *Am J Kidney Dis* 1993; **22**: 264–66.
- 169 Loirat C, Sonsino E, Hinglais N, Jais JP, Landais P, Fermanian J. Treatment of the childhood haemolytic uraemic syndrome with plasma: a multicentre randomized controlled trial. *Pediatr Nephrol* 1988; **2**: 279–85.
- 170 Rizzoni G, Claris-Appiani A, Edefonti A, et al. Plasma infusion for hemolytic-uremic syndrome in children: results of a multicenter controlled trial. *J Pediatr* 1988; **112**: 284–90.
- 171 Ogborn MR, Crocker JF, Barnard DR. Plasma therapy for severe hemolytic-uremic syndrome in children in Atlantic Canada. *CMAJ* 1990; **143**: 1323–26.
- 172 Brichard B, Vermynen C, Heremans MT, Ninane J, Cornu G. Plasma infusion as treatment for 33 children with haemolytic uraemic syndrome: a good therapy? *Acta Clin Belg* 1993; **48**: 156–63.
- 173 Tzipori S, Sheoran A, Akiyoshi D, Donohue-Rolfe A, Trachtman H. Antibody therapy in the management of Shiga toxin-induced hemolytic uremic syndrome. *Clin Microbiol Rev* 2004; **17**: 926–41.
- 174 Karmali MA. Prospects for preventing serious systemic toxic complications of Shiga toxin-producing *Escherichia coli* infections using Shiga toxin receptor analogues. *J Infect Dis* 2004; **189**: 355–59.
- 175 Brandt JR, Joseph MW, Fouser LS, et al. Cholelithiasis following *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. *Pediatr Nephrol* 1998; **12**: 222–25.
- 176 Garg AX, Suri RS, Barrowman N, et al. Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome: a systematic review, meta-analysis, and meta-regression. *JAMA* 2003; **290**: 1360–70.
- 177 Bell WR, Braine HG, Ness PM, Kickler TS. Improved survival in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: clinical experience in 108 patients. *N Engl J Med* 1991; **325**: 398–403.
- 178 Havens PL, Hoffman GM, Sheth KJ. The homogeneous nature of the hemolytic uremic syndrome. *Clin Pediatr (Phila)* 1989; **28**: 482–83.
- 179 Siegler RL, Griffin PM, Barrett TJ, Strockbine NA. Recurrent hemolytic uremic syndrome secondary to *Escherichia coli* O157:H7 infection. *Pediatrics* 1993; **91**: 666–68.
- 180 Ault BH. Factor H and the pathogenesis of renal diseases. *Pediatr Nephrol* 2000; **14**: 1045–53.
- 181 Starr M, Bennett-Wood V, Bigham AK, et al. Hemolytic-uremic syndrome following urinary tract infection with enterohemorrhagic *Escherichia coli*: case report and review. *Clin Infect Dis* 1998; **27**: 310–15.