Management of Shiga toxin-associated Escherichia coli-induced haemolytic uraemic syndrome: randomized clinical trials are needed

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The production of Shiga toxin by a bacterial strain is necessary for induction of enteropathogenic haemolytic uraemic syndrome (HUS). Only strains that produce the toxin are associated with HUS. These include enterohemorrhagic Escherichia coli, Shigella dysenteriae and rarely Citrobacter freundii [1, 2]. All strains associated with haemorrhagic colitis and HUS are Gram-negative, thus producing lipopolysaccharide (LPS), which may also play a role in the pathogenesis of disease. The recent large outbreak of E. coli O104:H4, a strain with combined virulence factors characteristic of enteroaggregative E. coli as well as enterohemorrhagic E. coli, provides additional evidence that, regardless of the bacterial background, acquisition of the bacteriophage-encoded gene for Shiga toxin 2 enhances the virulence of the strain causing haemorrhagic colitis and HUS. In this issue of NDT, Kielstein et al. [3] present a comprehensive summary of the outcome of this outbreak and the treatment options offered in 491 patients. Appropriate management of Shiga toxin-induced disease is contingent on an understanding of the pathogenesis of the disease as depicted in Figure 1. After ingestion, the bacteria may bind to the terminal ileum and follicle-associated epithelium of Peyer’s patches [4]. Colonization is further enhanced by quorum sensing, a form of communication with other strains in the intestinal microflora, as well as induction by the host hormonal response including epinephrine and norepinephrine, which would presumably be secreted during haemorrhagic colitis [5]. These signals enhance bacterial colonization and the release of virulence factors. There is no evidence of bacteremia during infection with Shiga toxin-producing E. coli. Following intestinal colonization, the disease is mediated by the systemic spread of bacterial virulence factors, of which Shiga toxin is of major importance.

Shiga toxin binds to the globotriaosylceramide Gb3 receptor on Paneth cells [6] and is translocated through the intestinal epithelium [7]. The toxin has been shown to induce dysentery [8] and intestinal apoptosis [9]. The inflammatory host response in the intestine is important for the clearance of bacteria from the gut. A reduced intestinal response increases the bacterial burden and thus enables more circulating bacterial virulence factors, as demonstrated in mice [10].

Bacterial virulence factors gain access to the circulation after passing through the intestinal mucosa and damaging the intestinal endothelium. During HUS, Shiga toxin, as well as LPS, circulate bound to platelets, monocytes and neutrophils as well as aggregates between these blood cells [11–14]. Both Shiga toxin and O157LPS induce the formation of platelet and monocyte microparticles bearing tissue factor and complement [14, 15]. These circulating microparticles were also detected during the recent E. coli O104 outbreak [16]. Thus, blood cell activation may contribute to the thrombotic process.

It is unknown whether and how circulatory toxin is transferred from blood cells to Gb3-expressing target organ cells in vivo. It has been suggested that this transfer of toxin may be related to a higher affinity for the glycolipid receptor on endothelial cells in the kidney [17, 18]. Shiga toxin induces glomerular endothelial cell injury and secretion of tissue factor as well as chemokines promoting leukocyte adhesion to endothelial cells [19, 20]. This scenario enables thrombus formation and release of leukocyte proteases and cytokines. In addition, Shiga toxin induces injury and apoptosis of renal cortical tubular cells [21], and the combined effect on glomeruli and tubuli will lead to destruction of the nephron. The brain is also a target organ in Shiga-toxin-induced disease and studies have shown that the toxin injures endothelial cells as well as neurons [22].

To date, there is no specific treatment for Shiga-toxin-mediated infection. In light of the known pathogenesis of disease, various management approaches can be selected (Table 1). In the study by Kielstein et al. [3], three treatment strategies were compared, best supportive care, and plasma exchange with, or without, eculizumab.

Best supportive care would include volume replacement, parenteral nutrition and dialysis. Volume expansion with isotonic solutions administered during the
In the first 4 days of infection and pre-HUS was found to decrease the rate of oligo-anuria and the need for dialysis [23]. Rehydration may mitigate toxin-mediated tubular injury as well as the formation of microvascular clots. Likewise, diuretics plus rehydration could potentially have a beneficial effect on kidney perfusion, as long as there is residual tubular function, although this is debatable [24]. The need for dialysis, and time at which to commence, is comparable with other conditions associated with acute renal failure [25]. In children, peritoneal dialysis is usually the preferred modality of treatment, whereas haemodialysis was the chosen modality for most adult patients during the O104 outbreak. Of note, most children with HUS during the O104 outbreak received supportive care only (67/90, 74%) and the short-term outcome was similar to previous outbreaks of Shiga toxin-producing E. coli [26].

The issue of antibiotic treatment to eradicate the bacterial strain has previously been addressed, most recently in a multi-centre investigation of children with E. coli O157:H7 [27]. Regardless of the antibiotic given, children treated with antibiotics during the diarrheal phase more frequently developed HUS than those who did not. This has been attributed to toxin release from bacteria during the early phase of treatment, due to bacteriophage lysogenesis. In mice, the bacterial burden, and presumed toxin release, could be correlated to the severity of symptoms [9, 10]. Thus, the presence of a viable bacterium in the intestine, continuously releasing toxin, could affect the course of disease. Whether antibiotic treatment during HUS has a beneficial or deleterious effect remains to be evaluated. The E. coli O104 strain associated with the recent outbreak had an extended-spectrum beta lactamase phenotype. The German Society for Infectious Diseases recommended antibiotic treatment under certain circumstances, such as invasive intestinal infection, eradication of meningococci when eculizumab was used, or for reduction of the intestinal bacterial count if colonization was persistent (www.ehec-register.de). Patients who received antibiotics during the outbreak exhibited fewer seizures, lower morbidity, required no abdominal surgery and excreted the E. coli strain for a shorter time [28]; thus, antibiotic treatment during ongoing HUS may be beneficial.

Plasma exchange has been attempted in patients with HUS. The rationale would be to remove bacterial toxin, prothrombotic factors, inflammatory mediators and/or blood cell-derived microparticles and to replenish coagulation and complement factors. The amounts of free Shiga toxin in the circulation are negligible [12] but toxin may

![Fig. 1. Schematic presentation of the pathogenesis of Shiga toxin-induced disease.](https://academic.oup.com/ndt/article-abstract/27/10/3669/1831322/3670/Nephrol-Dial-Transplant-2012-Editorial-Comment)
be bound to blood cells or microparticles in the circulation [14, 16]. These microparticles possess tissue factor and complement factors on their surfaces [14, 15]. If they are not phagocytosed they may be prothrombotic. Thus, their elimination could theoretically be beneficial. A study carried out in 47 patients during the recent *E. coli* O104 outbreak showed that leukocyte-derived microparticles were removed by plasma exchange [16]. There is, however, insufficient evidence that plasma exchange is beneficial in *Shiga* toxin-mediated disease. In children, plasma does not provide an additive effect to supportive care alone [26, 29], but there is anecdotal evidence for a certain effect in adults [30, 31], although these studies were not controlled. During the O104 outbreak most adult patients with HUS were treated with plasma exchange, with or without eculizumab [3, 28]. Patients treated with

### Table 1. Treatment options for *Shiga* toxin-producing *E. coli* infection

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rationale</th>
<th>Comment</th>
<th>Outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume expansion</td>
<td>Kidney perfusion during the first days after onset of diarrhoea</td>
<td>Isotonic solutions preferred for volume replacement</td>
<td>Treatment pre-HUS prevents oligoanuria</td>
<td>[23, 40]</td>
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<tr>
<td>Diuretics</td>
<td>Kidney perfusion during acute renal failure</td>
<td>A single study showed a beneficial effect on diuresis</td>
<td>Platelet transfusions may worsen the clinical course</td>
<td>[41]</td>
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<tr>
<td>Blood transfusion</td>
<td>Packed red blood cells for severe anaemia Hb&lt;60 g/L</td>
<td>Platelet transfusions should be reserved for patients undergoing surgery or during major bleeding episodes</td>
<td>Early initiation of dialysis has no proven benefit</td>
<td>[25, 44]</td>
</tr>
<tr>
<td>Dialysis</td>
<td>Management of hypervolaemia, hyperkalaemia, severe acidosis and uraemia</td>
<td>Choice of dialysis modality is dependent on the centre and if the patient has undergone abdominal surgery. Anti-coagulation should be tightly monitored</td>
<td></td>
<td></td>
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<tr>
<td>Antibiotics</td>
<td>Antimicrobial effect to reduce the bacterial load</td>
<td>Antibiotic treatment in the pre-HUS stage may increase the risk of developing HUS</td>
<td>The effect of antibiotics after HUS-onset may be beneficial</td>
<td>[27, 28, 45]</td>
</tr>
<tr>
<td>Plasma exchange</td>
<td>Removal of toxin, inflammatory mediators and/or blood cell degradation products</td>
<td>Risk of allergic reactions</td>
<td>No benefit shown in children or adults. Controlled studies required</td>
<td>[25, 26, 28–30, 46]</td>
</tr>
<tr>
<td>Immunoabsorption</td>
<td>Removal of a presumptive antibody</td>
<td>STEC-mediated HUS has not been shown to be antibody-mediated</td>
<td>Benefit shown in a single study. Controlled studies required</td>
<td>[47]</td>
</tr>
<tr>
<td>Eculizumab</td>
<td>Blockade of the terminal complement pathway</td>
<td>Patients should be vaccinated against meningococci or treated with antibodies</td>
<td>Benefit in single paediatric cases. No clear benefit in the German <em>E. coli</em> O104 outbreak. Controlled studies required</td>
<td>[3, 26, 28, 33]</td>
</tr>
<tr>
<td>Anti-coagulation/anti-thrombosis(^a)</td>
<td>Prevention of thrombotic microangiopathy</td>
<td>Treatment is costly</td>
<td>No benefit</td>
<td>[48–50]</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>Reduction of the inflammatory response</td>
<td>Risk of bleeding</td>
<td>No effect on haematological, neurological, or nephrological parameters</td>
<td>[28, 51]</td>
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<tr>
<td>Potential future treatments</td>
<td></td>
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<td>Monoclonal antibodies to Shiga toxin</td>
<td>Neutralize Stx1 and/or 2</td>
<td>Well tolerated in healthy volunteers</td>
<td>Urtoxazumab was tested in infected children. Phase 2/3 trial of ShigaMabs is ongoing</td>
<td>[25, 52–54]</td>
</tr>
<tr>
<td>Gb3 receptor analogues</td>
<td>Bind Stx</td>
<td>Tested in mice</td>
<td>Not yet tested in humans</td>
<td>[55, 56]</td>
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<td>Manganese</td>
<td>Blocks intracellular trafficking of Stx</td>
<td>Tested <em>in vivo</em> in mice and <em>in vitro</em></td>
<td>Not yet tested in humans</td>
<td>[57]</td>
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<td>Vaccines</td>
<td>Directed to:</td>
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<td><em>E. coli</em> O157:H7 O-specific polysaccharide</td>
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<td>Stx 2</td>
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<td></td>
<td>Stx 2B-1B fusion protein</td>
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<tr>
<td></td>
<td>EspA, intimin and Stx2</td>
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<td></td>
<td>Stx2 A2 and B subunits</td>
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Stx, *Shiga* toxin; EspA, *E. coli* secreted protein A.

\(^a\)This recommendation is for children; adults may develop symptomatic anaemia and require transfusion at higher haemoglobin levels.

\(^b\)References refer to trials or cases testing heparin, heparin plus dipyramidole and heparin plus urokinase.
plasma presented worse symptoms and signs of disease at onset, compared with those treated with supportive care, which may have affected the degree of residual symptoms and higher creatinine levels at discharge [3]. Although a comparison between best supportive care and therapeutic plasma exchange was not possible, the authors suggested that better general medical care, rather than frequent plasma exchange, may have accounted for the good outcome during this outbreak [3]. A recent study validating the treatment strategies given during the E. coli O104 outbreak reported no benefit of plasma exchange [28] and similar conclusions were drawn in a review of interventions in HUS [32].

At the time of the outbreak of E. coli O104, a publication in New England Journal of Medicine described the use of eculizumab, a monoclonal anti-C5 antibody (Soliris, Alexion), in three paediatric cases of HUS associated with other strains of Shiga toxin-producing E. coli [33]. This prompted the German Society of Nephrology to recommend the use of eculizumab for the sickest patients during this outbreak. The patients suitable for this treatment were defined as having an infection with Shiga toxin-producing E. coli or bloody diarrhoea, as well as neurological symptoms and/or acute kidney injury stadium III and/or venous or arterial thromboembolic events. In the paper by Kielstein et al. [3], 193/491 patients were treated with eculizumab in addition to plasma exchange. Eculizumab is approved for treatment of the complement-mediated diseases paroxysmal nocturnal haemoglobinuria and atypical HUS. The rationale for its use in enteropathogenic HUS is that complement activation via the alternative pathway occurs during this form of HUS [15, 34, 35] although this appears to be a secondary phenomenon and not necessarily related to the degree of renal injury [35]. The results presented thus far from the E. coli O104 outbreak do not support the use of eculizumab in adults or children with HUS [3, 26, 28]. Treatment did not affect levels of platelet-derived microparticles but seemed to increase the numbers of circulating dead leukocytes [16]. Randomized clinical trials are needed to determine whether certain patients with severe symptoms may benefit from this treatment. Treatment with an antibody that blocks the terminal complement pathway may have deleterious effects and prolong bacterial survival in the gut. The complement system is active in the colon [36, 37] and blocking its function would be expected to increase the bacterial burden. As a consequence, patients treated with eculizumab may have prolonged toxin release if not treated with antibiotics to eradicate the strain. It is thus important that future studies also address the issue of bacterial survival.

During the E. coli O104 outbreak, 3842 individuals were infected in Germany, 855 developed HUS and 54 patients died (1.4% mortality) [28, 38]. The study presented in this issue [3] also included patients treated in Sweden and the Netherlands in which a total mortality rate of 4.1% was described for 491 HUS patients. Importantly, not all cases of death occurred in HUS patients. The authors provide the causes of death in HUS patients showing that many patients died of severe complications not necessarily related to the degree of renal failure, as 7/20 patients who died were not on dialysis, and that mortality occurred in older patients. The mortality rate raises the question whether this strain is hyper-virulent in comparison with other Shiga toxin-producing E. coli strains. Although 26% of affected children developed neurological symptoms, in similarity to previous outbreaks [26], the percentage of neurological symptoms in affected adults was higher (69%) and the neurological symptoms occurred in a biphasic manner after signs and symptoms of HUS were improving [39], thus presenting an unexpected course of disease. This emergent strain of Shiga toxin-producing E. coli differs in its clinical presentation in adults. Although the short-term outcome in children was similar to previous outbreaks [26], the course of disease in adults may require management strategies different from those used over the years in children. The efficacy of available and future treatments should therefore be evaluated by randomized clinical trials in order to establish which treatment strategies are most beneficial to patients.

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References


Pulmonary hypertension in dialysis patients: a prevalent, risky but still uncharacterized disorder

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Pulmonary hypertension (PH) is a complex hemodynamic alteration which may result from disparate causes. In 1973 at a conference endorsed by the World Health Organization (WHO), a classification based on two categories only (primary and secondary PH) was proposed [1]. In 2001, a new classification establishing five categories of PH supplanted the first classification [2]. Since then, minor modifications were made and the last (2008) WHO classification maintains five diagnostic categories (Table 1) [3]. In the 2008 classification by the WHO and in more recent guidelines by the European Society of Cardiology (ESC) [4], for the first time attention was given to PH in dialysis patients which was classified in the fifth category, i.e. in a limbo category gathering various forms of PH ‘with unclear or multifactorial etiology’. At that time only one survey in dialysis patients was available [5] and this report showed an unexpectedly high prevalence of PH which was mainly attributed to high cardiac output secondary to the presence of arterio-venous fistula [6], anemia and/or fluid overload and to left ventricular (LV) disorders. During the last 5 years, PH in patients with kidney diseases has attracted increasing attention and over 100 original or review articles dealing with PH in dialysis patients or in predialysis chronic kidney disease (CKD) and in transplant patients are now deposited in PubMed. In this issue of NDT, Rajiv Agarwal reports on the largest study performed so far in

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