Management of hemolytic uremic syndrome

Chantal Loirat1, Jeffrey Saland2, Martin Bitzan3

1. Assistance publique–Hôpitaux de Paris, Hôpital Robert-Debré, Nephrology Department, 75019 Paris, France
2. The Mount Sinai School of Medicine, Department of Pediatrics, NY 10029, New York, United States of America
3. McGill University and Montreal Children’s Hospital, Division of Pediatric Nephrology, Montréal, Quebec H3H 1P3, Canada

Correspondence: Chantal Loirat, Hôpital Robert-Debré, service de néphrologie, 48, boulevard Serurier, 75019 Paris, France. chantal.loirat@rdb.aphp.fr

Available online: 27 January 2012

Summary

2011 has been a special year for hemolytic uremic syndrome (HUS): on the one hand, the dramatic epidemic of Shiga toxin producing E. coli – associated HUS in Germany brought the disease to the attention of the general population, on the other hand it has been the year when eculizumab, the first complement blocker available for clinical practice, was demonstrated as the potential new standard of care for atypical HUS.

Here we review the therapeutic options presently available for the various forms of hemolytic uremic syndrome and show how recent knowledge has changed the therapeutic approach and prognosis of atypical HUS.

Hemolytic uremic syndrome (HUS) is defined by the triad of mechanical intravascular hemolytic anemia with schistocytosis (microangiopathic hemolytic anemia), thrombocytopenia and acute renal failure (ARF). The underlying lesion is thrombotic microangiopathy (TMA) affecting arteriole and capillary walls, with endothelial cell swelling and detachment, subendothelial accumulation of proteins and cell debris, and fibrin and platelet-rich thrombi obstructing lumina. TMA predominates in the renal microvasculature but the brain, heart, lungs and intestinal tract can be involved. Ninety per cent of HUS in children is caused by Shiga-toxin (Stx) producing Escherichia coli (STEC). Some non STEC-HUS are secondary, primarily in children, to Streptococcus pneumoniae infection or methyl-malonic aciduria, an exceptional genetic disorder of cobalamin, or, primarily in adults, to a variety of causes (human immunodeficiency virus (HIV) infection, malignancy, cancer chemotherapy, calcineurin inhibitors, sirolimus and anti-vascular endothelial growth factor agents, bone marrow transplantation, systemic disease or pregnancy) [1]. However, in most cases, non STEC-HUS presents as a primary disease, historically called atypical HUS.

1 Since the submission of the manuscript, the U.S. Food and Drug Administration (September 23, 2011) and the European Commission (November 29, 2011) have extended the therapeutic indication of Soliris® to include the treatment of pediatric and adult patients with aHUS.
(aHUS), now demonstrated to be a disease of complement dysregulation. Here we focus on the therapeutic management as of 2011 of STEC-HUS, pneumococcal-HUS and cobalamin-HUS, the most frequent forms in children, and aHUS, a disease both of adults and children.

**Supportive treatment for hemolytic uremic syndrome patients**

All HUS patients require careful supportive treatment and surveillance. Progress in intensive care and dialysis techniques has contributed to the decrease of mortality, especially in young children. Although the scope of this review is not management of ARF, some particularities related to HUS warrant being indicated [2]:

- any patient suspected of having HUS needs to be transferred to a specialized centre (Nephrology or Critical Care unit) where supportive therapy for ARF and hypertension as well as dialysis and plasma exchange (PE) are daily practice;

- as multivisceral involvement may occur in all forms of HUS and ARF/oliguria/volume overload/hypertension may also induce cardio-respiratory failure and neurologic complications, patients require diligent monitoring of vital functions, especially for neurologic, cardiac (cardiac failure and/or ischemia, pericarditis) and respiratory deterioration. Repeated surgical advice may be necessary in case of intestinal complications which may require surgery;

- packed red blood cell (PRBC) for transfusions are indicated when hemoglobin level is less than 0.8 g/L. Erythropoietin treatment may reduce the need for transfusions [3];

- platelet transfusions are contraindicated in HUS patients as they might worsen the TMA process, unless the patient is bleeding (rare) or when a surgical procedure at risk of being hemorrhagic is scheduled in a severely thrombocytopenic patient (< 30 000/mm³) [4];

- vascular access most often relies on a central catheter allowing hemodialysis (HD) and (therapeutic) plasma exchange (PE). Choice of the vein (internal jugular, subclavian or femoral) depends on the patient’s age and local practice. Choice of the double lumen catheter (gauge and length) and its percutaneous insertion have to be performed by trained physicians. Protection of peripheral and central veins (no ligation) is of utmost importance in HUS patients who may need long term vascular access for HD and PE;

- calorie intake may be limited in patients with HUS, due to intestinal involvement, abdominal pain and nausea, uremia, or peritoneal dialysis (PD). Nasogastric feeding or total parenteral nutrition may become necessary in young children and in patients requiring critical care support;

- indications for dialysis are severe electrolyte imbalance (hyperkalemia) and acidosis that cannot be corrected medically, fluid overload in the oligoanuric patient, and symptomatic azotemia. The choice of dialysis modality (HD or PD) depends on patient’s age and size, local preference and experience, especially when dialysing children and infants. Factors favouring HD over PD are previous abdominal surgery or severe intestinal symptoms (e.g., partial obstruction in STEC-HUS). Factors favouring PD are young age, the continuous nature of dialysis, no (systemic) anticoagulation, no procedure-associated blood loss [2]. HUS complicated by multiorgan failure, severe fluid overload, cardiovascular instability, with or without sepsis, are indications to consider extracorporeal continuous renal replacement therapy (CRRT) (by continuous venovenous hemofiltration or hemodialfiltration) or slow HD generally in a critical care setting. In the acute stage, HD and CRRT can be performed with no or tight heparinization. Regional, citrate-based anticoagulation offers an alternative to heparinization, specifically in patients with cerebral stroke or hemorrhage, or after surgery.

**STEC-hemolytic uremic syndrome**

**Background**

**Epidemiology**

Since the discovery of the association between infections by verocytotoxin (Shiga toxin)-producing *Escherichia coli* (VTEC, STEC) and childhood HUS by Karmali et al. [5], the work of numerous investigators firmly established that the majority of cases of HUS in children (also called post-diarrheal or enteropathic HUS [eHUS]) in many parts of the world is triggered by STEC (VTEC) infections [6]. While other bacterial enzymes and toxins may contribute to systemic disease [7,8], their link with human pathology is not well documented. *E. coli* O157:H7 is the most frequently isolated STEC serotype from patients with HUS. However, non-O157:H7 human pathogenic

---

**Glossary**

- aHUS: atypical hemolytic uremic syndrome
- ARF: acute renal failure
- CRRT: continuous renal replacement therapy
- eHUS: enteropathic HUS
- ESRD: end-stage renal disease
- FFP: fresh frozen plasma
- HD: hemodialysis
- HIV: human immunodeficiency virus
- HUS: hemolytic uremic syndrome
- PD: peritoneal dialysis
- PE: plasma exchange
- pnHUS: pneumococcal HUS
- PI: plasma infusions
- PNH: paroxysmal nocturnal hemoglobinuria
- PRBC: packed red blood cell
- RBC: red blood cell
- RRT: renal replacement therapy
- Stx: Shiga-toxin
- TMA: thrombotic microangiopathy
- TTP: thrombotic thrombocytopenic purpura
STEC strains, e.g. O26, O55, O91, O103 and O111 are endemic in many countries, including France. In May 2011, a rare, hybrid enterohaemorrhagic, Stx-producing pathotype, *E. coli* O104:H4, caused a large outbreak, originating in Northern Germany, with 3816 infected persons, 845 HUS cases and 54 deaths [9]. Of note, infection with *Shigella dysenteriae* type 1, which produces Stx, is the main cause of HUS in endemic regions like Bangladesh or Africa [10].

**Shiga toxin biology and pathophysiology**

All Shiga toxins (Stx1, Stx2 and Stx2 variants) consist of a pentameric binding subunit (B₅) and an enzymatically active A subunit. They avidly bind to and kill cultured microvascular endothelial cells, including glomerular capillary and brain endothelial cells, epithelial and neuronal cells [11–13]. Stx A subunit-mediated enzymatic cleavage of the (mammalian) ribosomal RNA (fig. 1) leads to a ribotoxic stress response. Dependent on the affected tissue, ribotoxic stress induces apoptosis [13], with loss of endothelial anti-thrombotic properties [14,15] or the production of a variety of inflammatory (cytokines, chemokines) and vasoactive mediators (endothelin, tissue factor) [12,16,17]. Tissue injury may be caused by Stx directly, via apoptosis, or indirectly, due to thrombosis and ischemia. The cascade of events leading to HUS is schematically depicted in fig. 2. Multiorgan injury, affecting brain, pancreas, heart and liver with fulminant HUS, is thought to be due to excessive toxin load.

**Diagnosis and prognosis**

The onset of HUS is evidenced by sudden clinical deterioration after seeming improvement of the diarrhea (fig. 3). The clinical diagnosis is usually straightforward and based on complete blood cell count and smear, serum creatinine and urinalysis (if urine output is maintained). Additional details are listed in table 1. The etiological diagnosis is important, particularly in patients with “atypical” presentation, because prognosis and therapy of aHUS and eHUS differ [18]. It should be remembered that diarrhoea, albeit usually not bloody, occasionally heralds an aHUS.

While ARF is part of the definition of the “tripod” of HUS, only 40–60% of all children with eHUS require dialysis. Often children improve visibly during the first dialysis sessions. The North American Synsorb® trial (see below) controlled indications and timing of dialysis initiation. The trial protocol mandated that dialysis be delayed until 72 h of diagnosis, if clinically acceptable [19]. Under these conditions, 39% of the 49 placebo-treated patients were dialyzed, for a mean of 3.6 days. The prognosis of STEC-HUS is favourable in the majority of children. Mortality is 1 to 5%, mostly due to central nervous system involvement [20–22]. In contrast, STEC are particularly lethal for the elderly – close to 50% in patients > 65 years old in the 1996 Lanarkshire outbreak [23]. A similar trend was noted in the 2011 Hamburg outbreak [9]. Up to 20% of children with HUS suffer long-term sequelae, including chronic kidney disease, arterial hypertension, neurological impairment, or diabetes mellitus [20,21,24].

**Current treatment**

**Volume treatment**

Ake et al. [25] postulated that the oligoanuria of HUS results from renal parenchymal hypoperfusion and ischemia. In a retrospective cohort study of 29 unselected children with *E. coli* O157:H7-HUS, the authors showed that patients who became oligoanuric and needed dialysis had received significantly less intravenous fluid during the first four days of diarrhea than those who had with preserved urine output and who were not dialysed. The authors concluded that early parenteral volume expansion before the onset of HUS attenuates ARF and reduces the need for dialysis. Intravascular volume depletion and renal hypoperfusion are certainly not good for the kidneys and conceivably aggravate ARF in the setting of incipient HUS. While advocating diligent volume replacement,
there is still a lot more to be learned about the mechanisms of renal injury in HUS.

**Plasma therapy**

PE or plasma infusion, recommended for patients with aHUS or thrombotic thrombocytopenic purpura (due to ADAMTS13 deficiency), have no proven role in the treatment of patients with eHUS. While some centres have pheresed patients with severe, life-threatening eHUS as “rescue” therapy [2,26,27], its comparative benefit to other therapies is difficult to prove without adequate controls.

**Antithrombotic and antiinflammatory agents, diuretics**

Anticoagulation with heparin, urokinase or dipyridamole has failed to ameliorate the course of HUS. On the contrary, increased bleeding risks were noted in at least two trials [28,29].

---

**Figure 2**

Schematic diagram of cellular and vascular pathogenetic events underlying Shiga toxin-mediated Hemolytic uremic syndrome (HUS)

- ARF: acute renal failure
- A/E: attachment/efacement
- CNS: central nervous system
- EC: endothelial cells
- LPS: lipopolysaccharide
- N: neutrophils
- Plt: platelets
- STEC: Shiga toxin-producing E. coli
- Stx: Shiga toxin

**Figure 3**

Schematic diagram of the development of diarrhea and Hemolytic uremic syndrome (HUS) due to Shiga toxin-producing E. coli

STEC: Shiga toxin-producing E. coli; Stx: Shiga toxin; TMA: thrombotic microangiopathy; HUS: hemolytic uremic syndrome.
**Table 1**

**Laboratory diagnostic of STEC infection and enteropathic Hemolytic uremic syndrome (HUS)**

<table>
<thead>
<tr>
<th>STEC disease stage</th>
<th>Material</th>
<th>Test</th>
<th>Details</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea/colitis</td>
<td>Stool or rectal swab</td>
<td>Free Stx or Stx genes</td>
<td>Stx ELISA Vero cell tissue culture assay PCR</td>
<td>Fresh stool preferred for Elisa and vero cell tissue culture assay (decay of the toxin)</td>
</tr>
<tr>
<td></td>
<td>E. coli O157:H7</td>
<td>Sorbitol/tellurite MacConkey agar (or comparable) culture media PCR for virulence genes in isolated colonies</td>
<td>Preserve stool sample and colony sweep or broth at −80°C for specialized laboratory</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-O157:H7 STEC strains</td>
<td>Usually sorbitol fermenting in culture PCR for virulence genes in isolated colonies Serological (agglutination) or molecular testing (hybridization, PCR) of bacterial isolates or colonies</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serum</td>
<td>Anti-LPS IgM, IgG and IgA</td>
<td>Elisa, immunoblot</td>
<td>Screening for anti-LPS antibodies against the most frequent local STEC-serotypes</td>
</tr>
<tr>
<td></td>
<td>Blood</td>
<td>CBC, smear</td>
<td>Baseline hemoglobin and platelets; presence of schistocytes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urine</td>
<td>Protein/creatinine ratio Cytology</td>
<td>Baseline/early changes</td>
<td>Microscopic hematuria may be present with colitis or sign of incipient HUS Proteinuria indicates renal involvement</td>
</tr>
<tr>
<td>Acute HUS</td>
<td>Stool/rectal swab/blood</td>
<td>STEC</td>
<td>See above (unless obtained during the diarrhea phase)</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>CBC, smear</td>
<td></td>
<td>Evolution of hemolytic anemia (schistocytes) and thrombocytopenia as markers of TMA Polynuclear leukocytes &gt; 20 × 10⁹/L marker of TMA severity</td>
<td>May consider coagulation profile</td>
</tr>
<tr>
<td>Biochemistry</td>
<td>Creatinine, electrolytes, albumin LDH, haptoglobin Liver enzymes Amylase, lipase, blood glucose Troponine Optional: CRP (or other acute phase reactant) Optional: C3, C4 (most often normal)</td>
<td></td>
<td>AST and indirect bilirubin elevation generally indicate vigorous hemolysis Troponine elevation indicates myocardial ischemic lesions Detailed complement analysis and/or genetic work-up only if diagnosis of eHUS uncertain and aHUS suspected</td>
<td></td>
</tr>
<tr>
<td>Blood bank</td>
<td>Cross &amp; type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>Protein/creatinine ratio, microalbuminuria</td>
<td></td>
<td>During recovery and follow-up: abnormal protein/creatinine ratio or microalbuminuria indicate ongoing TMA or residual kidney disease</td>
<td></td>
</tr>
</tbody>
</table>

The techniques for Stx and STEC identification vary according to countries. Reference laboratories available in most countries. aHUS: atypical HUS; AST: aspartate transaminase; C3, C4: third and fourth complement component; CBC: complete blood count; CRP: C-reactive protein; eHUS: enteropathic HUS; ELISA: enzyme-linked immunosorbent assay; LDH: lactate dehydrogenase; PCR: polymerase chain reaction; Stx: Shiga toxin; STEC: Stx producing _E. coli_; TMA: thrombotic microangiopathy
Based on the observation that Stx can induce inflammatory cytokines, it seems plausible to resort to glucocorticoids. The only reported randomized placebo-controlled trial indeed showed a faster decline of serum creatinine levels in the treatment group without translating into shortened oligoanuria or decreased dialysis needs [30]. In practice, glucocorticoids are not recommended in eHUS. Challenge with high-dose loop diuretics early in HUS has been proposed by some investigators to maintain urine flow [31]. However, as repeatedly demonstrated in clinical trials in patients with ARF of various etiologies, diuretics do not improve survival, shorten the recovery period, or prevent ARF [32].

**Stx binders**

**Stx receptor analogues**

Predicated of its elective toxin binding in vitro and a reduction of the fecal toxin load in experimentally infected mice [33], synthetic globotriaosyl (Gb3) Stx receptor linked to an inert, non-resorbable carrier (Synsorb-Pk®) has been studied in a randomized controlled North American (US/Canada) trial [19]. The investigators hypothesized that the agent, administered orally soon after the diagnosis of HUS, would diminish continued toxin absorption and result in disease amelioration. Primary endpoints were decreased rates of death, serious extrarenal events and dialysis frequency. The trial was stopped when the interim analysis revealed no difference between treatment and placebo groups. Indeed, the agent may not be able to interfere with the toxin delivery by tightly adherent bacteria directly across the epithelial barrier [34]. New, multi-branched, high-capacity oral and systemic Gb3 analogs and genetically modified Gb3-expressing *E. coli* and (other) probiotics are being developed, but no new trials have been announced.

**Stx antibodies**

In murine models of STEC-HUS, infusion of toxin-specific monoclonal antibodies up to 3 days after orogastric infection protects against haematological and renal disease, but efficacy decreases rapidly with delayed antibody administration [35,36]. No important adverse effects were noted when the anti-Stx2 antibody TMA-15 (Utrozumab) was infused in children with documented STEC-colitis [37]. Another phase 2/3 trial with an Stx1/Stx2 monoclonal antibody combination (Shigamabs®) [38,39] is currently underway in South America. As apparent from fig. 3, the window for meaningful intervention is narrow. Once patients develop HUS, vascular injury has already occurred and the utility of Stx-directed antibody is diminished, although this has never been tested in humans.

**Is the STEC Hemolytic uremic syndrome paradigm changing?**

Recent in vitro studies suggest that Stx may directly bind to complement factor H and interfere with its regulatory function [40]. Studying archived serum samples donated to the Synsorb-Pk trial [19] from patients with eHUS, Thurman et al. [41] detected elevated serum levels of C5b-9 complex and activated factor B (Bb) in early phase samples (days 1–4). Their normalization within 4 weeks after disease onset suggests that complement activation occurs and may have a pathological role in STEC-HUS. Lapeyraque et al. [42] now described three patients with severe STEC HUS, who were treated with the anti-C5 monoclonal antibody eculizumab because of neurological deterioration despite dialysis and PE and, in one patient, low C3 level. The authors witnessed a full recovery after the antibody infusion with normalization of platelet counts and LDH activity. This experience, combined with new laboratory findings [15,40,43,44] has the potential of challenging our current concept of the pathogenesis of STEC disease and HUS. PE and eculizumab were used intensely during the recent *E. coli* O104:H4-mediated HUS outbreak in Germany. Detailed accounts of this experience will undoubtedly be published.

**Pneumococcal hemolytic uremic syndrome**

**Background**

Infection by *S. pneumoniae* can lead to a unique form of HUS, referred to as pneumococcal HUS (pHUS). It differs in clinical presentation, treatment and outcome from STEC-HUS and aHUS caused by complement dysregulation. Pneumococcal infections account for approximately 5% of all cases of HUS, but for 40–50% of non-STEC-HUS cases [45–48]. pHUS is a disease of infants and young children (peak around 1 year of age). The incidence of HUS following invasive pneumococcal disease is 0.4–0.6%. Adults are rarely affected [46,48–51]. Most patients with pHUS (70%) present with pneumonia, often complicated by pleural empyema. Approximately 20–30% present with pneumococcal meningitis, the remainder with isolated pneumococcal bacteremia, sinusitis or acute otitis media [46,48,50,51]. HUS becomes manifest about a week (range 3–18 days) after the onset of the infection [45,51]. The diagnosis of pHUS is based on the triad of hemolytic anemia with schistocytes thrombocytopenia and ARF with rising serum creatinine in association with proven or suspected (invasive) *S. pneumoniae* (box 1). Relevant diagnostic laboratory tests to confirm the diagnosis and monitor a patient with (suspected) pHUS are listed in table II. The direct cause of hemolytic anemia and HUS is a pneumococcal toxin, *N*-acetyl neuraminidase (sialidase). Pneumococcal neuraminidase cleaves neuraminic (sialic) acid (*N*-acetyl neuraminic acid) from glycoproteins and glycolipids. Removal of the terminal sialic acid from the most abundant sialylated glycoprotein of the human red blood cell (RBC) membrane, glycopherin A, exposes the residual Galβ1-3GalNAcα/β1 moiety (α-galactose (1-3)-*N*-acetylα-galactosamine), known as Thomsen-Friedenreich “crypt” antigen (T- or TF-antigen) (fig. 4). The residual digalactosyl sugar is specifically recognized by the peanut lectin.
Box 1

Pneumococcal-Hemolytic uremic syndrome (HUS) case definition

Definitive diagnosis of pneumococcal-HUS

1. HUS triad
   a. Hemolytic anemia (Hb < 100 g/L, elevated LDH, decreased haptoglobin)
   b. Thrombocytopenia < 150 × 10^9/L
   c. Acute kidney injury (creatinine > ULN)
2. Confirmed, invasive S. pneumoniae infection
3. Positive direct agglutination test (DAT, direct Coombs) or Thomsen-Friedenreich antigen detection

Probable diagnosis of pneumococcal or neuraminidase HUS

1. HUS triad
   a. Hemolytic anemia (Hb < 100 g/L, elevated LDH, decreased haptoglobin)
   b. Thrombocytopenia < 150 × 10^9/L
   c. Acute kidney injury (creatinine > ULN)
2. Confirmed or probable, invasive S. pneumoniae infection
3. Positive direct agglutination test (DAT, direct Coombs test) or Thomsen-Friedenreich antigen detection
4. Evidence of influenza virus infection
5. Evidence of infection by other N-acetyl neuraminidase-producing organism

DAT: direct agglutination test; Hb: hemoglobin; LDH: lactate dehydrogenase; ULN: upper limit of normal

(Based on [46,48,51])

Arachis hypogea (hence the lectin-agglutination method for T-antigen detection). Influenza A, including the epidemic A(H1N1) strain, has also been associated with HUS [52], likely due to viral neuraminidase production [47] (box 1). It should be remembered, however, that Influenza virus can pave the way for S. pneumoniae infection with its own HUS risk. Patients with pnHUS do not have a genetic complement abnormality, nor does the disease recur.

The prognosis of pnHUS was considered poor, based on early reports, with mortality up to 50%. Two thirds of the survivors developed chronic kidney disease and/or hypertension [47,49]. Studies after 1990 described mortality rates between 0 and 12% [45,46,48–51]. PnHUS patients die from cerebral hemorrhage and infection, mostly in the context of meningitis, or from complications of sepsis and prolonged critical care. Renal outcome, too, has improved, but remains less favourable than in STEC-HUS [45,50].

**Treatment**

Invasive S. pneumoniae infections are potentially life-threatening and require adequate antibiotic and supportive therapy.

Increased resistance of pneumococcal isolates to penicillin warrants empiric treatment with 3rd generation cephalosporins and vancomycin in the absence of antimicrobial sensitivities. Pleural effusions and empyema, if clinically significant, must be drained for therapeutic and diagnostic purposes. Adjuvant treatment of pneumococcal meningitis with dexamethasone (or glycerol) is not expected to prevent or ameliorate HUS. Patients may demonstrate a mild serum creatinine increase or anuric renal failure with severe renal cortical necrosis [49]. Sixty to 85% of the patients require renal replacement therapy (RRT) during the acute HUS [46,48,51]. Since pnHUS typically affects infants and young children, many centers prefer PD as the initial approach [51]. Sepsis and hemodynamic instability favour CRRT over intermittent HD to minimize rapid blood pressure changes and allow for better fluid balance and nutrition.

**Transfusion of blood products**

In many centers, plasma-containing blood products are avoided, out of concern they may aggravate the hemolytic process. This concern is based on the observation that virtually all adult plasma contain naturally occurring IgM class antibodies to the TF crypt antigen [47]. Hence, RBCs and platelets are washed prior to transfusion, and no plasma is given. However, very few authors reported that the administration of plasma or potentially plasma-containing (unwashed) blood cell products in patient with T activation was followed by aggravated hemolysis [52]. In a disorder characterized by rapid hemolysis and potentially poor outcome and in the absence of controlled trials, the precise cause of clinical deterioration is difficult to determine in an individual case. In contrast, most published series where unwashed RBCs or plasma had been administered, did not report worsening of hemolysis or organ function [46,50,53,54]. Furthermore, agglutination of TF-transformed RBCs by antihuman globulin (Coombs test) is strongest at 4° C and generally absent at 37° C. Experimental work showed that RBCs become fragile and are removed from the circulation by the mononuclear phagocyte system upon quantitative desialylation, without the presence of anti-TF antibodies. Therefore some authors propose that plasma and unwashed RBC and platelet transfusions can be given in patients with evidence of T activation [55]. However, although not well founded, most authors still recommend to avoid plasma and unwashed cells products for children with pnHUS or where T activation tests, if available, are positive [46,47,56,57].

**Plasmapheresis and plasma therapy**

The rationale for PE is the removal of anti-TF antibodies and plasma neuraminidase activity. Neuraminidase may also be neutralized by donor plasma which may have enzyme-neutralizing capacity [53]. However, experience with PE for pnHUS is limited [46,50,51,58]. Six of 43 patients with pnHUS described by Waters et al. [46] received PE, two were exchanged against “low-titre anti-TF” fresh frozen plasma.
(FFP) and three against albumin. All six pheresed patients survived with renal and neurological outcomes similar to the remainder of the surveyed cohort. Full recovery was also noted in a young child with DAT and T-antigen positive pnHUS who received 13 PE against 5% albumin [58]. Six of 12 patients reported by Brandt et al. [50] (three Coombs test positive) received FFP infusions without discernible effect on platelet counts following treatment. Two of 11 patients reported by Prestidge et al. [51] received FFP and one cryoprecipitate, without apparent adverse effects. In summary, although PE against FFP has been described in few patients with pnHUS without reported adverse effects [46], the efficacy of this approach is uncertain and it seems prudent to use albumin replacement [58].

**Exchange transfusion**

Newborns and infants with severe hemolytic anemia or HUS due to invasive pneumococcal disease or necrotizing enterocolitis due to other neuraminidase producing bacteria have

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Tests</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microbiology/Infectious Diseases</strong></td>
<td>Bacterial culture of blood, pleural or cerebrospinal fluid, tympanic aspirate etc.</td>
<td>Cultures may be negative in the absence of pleural effusion or empyema, and after initiation of antibiotic therapy</td>
</tr>
<tr>
<td></td>
<td>Pneumococcal polysaccharide antigen testing of urine, cerebrospinal fluid, other body fluids</td>
<td>Useful in cases of prior antibiotic therapy Rapid procedure</td>
</tr>
<tr>
<td></td>
<td><em>S. pneumoniae</em> 16S ribosomal RNA (rRNA) sequencing (various material)</td>
<td>Highly sensitive, useful when bacterial culture is negative Genetic characterization of infection strain in absence of bacterial isolate possible</td>
</tr>
<tr>
<td></td>
<td>C-reactive protein (serum)</td>
<td>Monitoring of infection (inflammation)</td>
</tr>
<tr>
<td><strong>Hematology</strong></td>
<td>CBC and blood smear</td>
<td>Often elevated WBC count with band forms, anemia, reticulocytosis and fragmented RBC (schistocytes)</td>
</tr>
<tr>
<td></td>
<td>Coombs test (DAT)</td>
<td>Positive in majority of patients during early stages Becomes negative within days</td>
</tr>
<tr>
<td></td>
<td>PT/INR, aPTT, fibrinogen, d-dimers, lactate</td>
<td>Differentiation between HUS and sepsis-induced organ injury</td>
</tr>
<tr>
<td><strong>Biochemistry</strong></td>
<td>Renal function</td>
<td>Serum creatinine &gt; ULN</td>
</tr>
<tr>
<td></td>
<td>Liver enzymes</td>
<td>AST often elevated due to RBC lysis. Liver injury is a rare complication</td>
</tr>
<tr>
<td></td>
<td>Amylase, lipase, blood sugar Troponine</td>
<td>Rare pancreas injury or myocardial failure</td>
</tr>
<tr>
<td></td>
<td>LDH (haptoglobin)</td>
<td>Monitoring of hemolysis.Hp depletion due to Hb binding and uptake, normalizes slowly after cessation of hemolysis</td>
</tr>
<tr>
<td><strong>Imaging</strong></td>
<td>Chest X-ray</td>
<td>Confirm pneumonia, pleural fluid accumulation, heart size (pericardial effusion)</td>
</tr>
<tr>
<td></td>
<td>Abdominal ultrasononography</td>
<td>For suspected pancreatitis, liver injury. Kidneys will appear swollen and/or show decreased corticomedullary differentiation</td>
</tr>
</tbody>
</table>

CBC: complete blood count; DAT: direct agglutination test; Hb: hemoglobin; Hp: haptoglobin; LDH: lactate dehydrogenase; PT: prothrombin time; aPTT: activated partial thromboplastin time; RNA: ribonucleic acid; WBC: white blood cells
been treated with blood exchange transfusions with the aim of eliminating circulating neuraminidase and TF-transformed RBC prone to hemolysis [53,55]. For example, Poschmann and Fischer performed exchange transfusions with heparinized fresh blood in four infants with T activation due to S. pneumoniae or C. perfringens infection and reported an impressive beneficial effect [53].

**Future aspects**

In view of shifts in the epidemiology of invasive pneumococcal serotypes, novel preventive and therapeutic strategies have been proposed, targeting virulence and disease-associated proteins including neuraminidases. One attractive option is the early administration of pooled intravenous immunoglobulin preparations (IVIG) with high neuraminidase neutralizing antibody titres.

**Cobalamin–hemolytic uremic syndrome**

HUS may complicate the neonatal form of methyl-malonic aciduria with homocystinuria, Cblc type, an uncommon hereditary disorder of intracellular vitamin B12 (cobalamin) metabolism. Failure to thrive, feeding difficulties, hypotonia, lethargy, developmental delay and leukopenia in preceding days/weeks suggest the diagnosis. Mortality is extremely high once HUS has developed, due to multivisceral failure [59,60]. Diagnosis relies on plasma/urine amino acid and urine organic acid chromatography, showing hyperhomocysteinemia, hypomethioninemia and methylmalonic aciduria with homocystinuria. Identification of mutations within the MMACHC gene confirms diagnosis and allows prenatal diagnosis. Early parenteral hydroxycobalamine therapy (+ oral carnitine, betaine and folic acid) may allow survival, but neurological involvement and visual complications impair prognosis despite treatment. However three cases of mild methylmalonic aciduria without neurological involvement have been reported, revealed by HUS at age four to 12, who had a favourable outcome under continuous cobalamin supplementation [61,62]. This justifies the recommendation to perform biological screening for this potentially treatable disease in all children with aHUS, whatever their age.
Atypical hemolytic uremic syndrome

Background

Major progress has been made during the last decade in the understanding of aHUS, now demonstrated to be a disorder of complement alternative pathway regulation. Complement alternative pathway is constitutively permanently activated to ensure defence against infectious agents. It is normally tightly regulated so as to prevent host endothelial cell surface attack secondary to C3b deposits on endothelial cells and the subsequent cascade of C3 convertase activation down to the membrane attack complex. More than one thousand aHUS patients screened for complement mutations have been reported from five European series [63–69] and one from the USA [70]. Mutations in the genes encoding regulatory proteins factor H (CFH), membrane cofactor protein (MCP), factor I (CFI) or thrombomodulin (THBD) have been demonstrated in 20–30%, 5–15%, 4–10% and 3–5% of patients respectively, and mutations in the genes encoding C3 convertase proteins, C3 and factor B (CFB), in 2–10% and 1–4% respectively [63–73]. Up to 12% of patients have various combinations of two or more mutations. In addition, 6–10% of patients, mainly children around adolescence, have anti-CFH antibodies [74]. A familial incidence of the disease is observed in approximately 20% of pedigrees. Penetration of the disease is only approximately 50%, as half of family members with the same mutation as the proband are healthy. Onset is from the neonatal period to adult age and the disease is equally frequent in adults and children. Most patients have an acute onset of hemolytic anemia with schistocytes, high lactate dehydrogenase and undetectable haptoglobin plasma levels, thrombocytopenia and ARF, and 20% have extrarenal manifestations, mainly central nervous system involvement. Two to 10% of patients die and at least one third progress to end-stage renal disease (ESRD) at the first episode. Half have relapses. A relapsing course, often triggered by infections, is particularly frequent (70–90% of patients) in MCP-HUS. CFH-HUS has the worst prognosis as 60–70% of patients either die or progress to ESRD within the year of onset. CFI-HUS is more severe when the CFI mutation is associated with another complement anomaly [69]. C3-HUS and CFB-HUS are nearly as severe as CFH-HUS. Conversely, most MCP-HUS patients have preserved renal function at least during the first 5 years of the disease [63,64]. One third of patients with anti-CFH HUS progress to ESRD within 3 years follow-up but recent data show a more favourable prognosis if treatment is started early [74] (Table III).

Recommended investigations to confirm diagnosis of atypical hemolytic uremic syndrome

Table IV shows biological investigations recommended in patients suspected of having aHUS, to eliminate STEC-HUS, pHUS, thrombotic thrombocytopenic purpura (TTP), methylmalonic aciduria and, in adults, HIV infection and systemic disease. Complement investigation is mandatory in all aHUS patients and must be performed in a reference laboratory [18,75–77] (list of laboratories in [18,76]). Blood sampling must be performed before starting plasmatherapy or eculizumab (except for MCP expression on peripheral leucocytes and genetic screening). As complement mutations have been demonstrated in 86% of pregnancy-HUS [78], 36% of HELLP (Hemolysis, elevated Liver enzymes and Low Platelet count) syndrome [79] and 29% of de novo HUS after kidney transplantation [80], all these patients also need complement investigations.

Table III

Frequency of the various complement abnormalities among patients with atypical Hemolytic uremic syndrome (HUS), outcome of the disease and risk of post-transplant recurrence according to complement abnormality.

<table>
<thead>
<tr>
<th>Gene or subgroup</th>
<th>Frequency in aHUS</th>
<th>Risk of death or ESRD at 1st episode or within &lt; 1 year</th>
<th>Risk of relapses</th>
<th>Risk of recurrence after renal transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFH</td>
<td>20–30%</td>
<td>50–70%</td>
<td>50%</td>
<td>75–90%</td>
</tr>
<tr>
<td>CFI</td>
<td>4–10%</td>
<td>50%</td>
<td>10–30%</td>
<td>45–80%</td>
</tr>
<tr>
<td>MCP</td>
<td>5–15%</td>
<td>0–6%</td>
<td>70–90%</td>
<td>&lt; 20%</td>
</tr>
<tr>
<td>C3</td>
<td>2–10%</td>
<td>60%</td>
<td>50%</td>
<td>40–70%</td>
</tr>
<tr>
<td>CFB</td>
<td>1–4%</td>
<td>50%</td>
<td>3/3 not in ESRD</td>
<td>3/3</td>
</tr>
<tr>
<td>THBD</td>
<td>3–5%</td>
<td>50%</td>
<td>30%</td>
<td>1 patient</td>
</tr>
<tr>
<td>Anti-CFH antibodies</td>
<td>6%</td>
<td>30–40%</td>
<td>40–60%</td>
<td>Yes if high antibody titer</td>
</tr>
</tbody>
</table>

aHUS: atypical hemolytic uremic syndrome; CFH/1/B: complement factor H/1/B; MCP: membrane cofactor protein; THBD: thrombomodulin; ESRD: end-stage renal disease
**Table IV**

<table>
<thead>
<tr>
<th>Investigations recommended in patients suspected of having atypical Hemolytic uremic syndrome (HUS)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>STEC infection</strong>&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Pneumococcal infection</strong>&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>ADAMTS13 deficiency</strong> (inherited or acquired)</td>
</tr>
<tr>
<td><strong>Cobalamin metabolism:methyl malonic aciduria</strong></td>
</tr>
<tr>
<td><strong>HIV</strong></td>
</tr>
<tr>
<td><strong>Pregnancy, HELLP syndrome</strong></td>
</tr>
<tr>
<td><strong>Miscellaneous</strong></td>
</tr>
</tbody>
</table>

ADAMTS13: A Desintegrin And Metalloproteinase with a Thrombospondin type 1 motif, member 13; ELISA: enzyme-linked immunosorbent assay; HIV: human immunodeficiency virus; HELLP: Hemolysis, Elevated liver enzymes, Low Platelet count; MCP: membrane cofactor protein; FACS: fluorescence-activated cell sorter; MMACHC: methylmalonic aciduria and homocystinuria; PCR: polymerase chain reaction; STEC: Shiga-toxin producing *Escherichia coli*; Stx: Shiga-like toxin; THBD: thrombomodulin

<sup>1</sup>See table I for further details

<sup>2</sup>See table II for further details

Adapted from Ariceta et al. [18]

---

**Caution towards triggers of relapses**

Physicians must be aware that infections, which trigger HUS relapses, should be treated if indicated and justify intensification of biological controls to detect relapse early and resume or intensify therapy. Influenza A (seasonal or epidemic [A/H1N1] type) is a strong trigger of relapses. Therefore, vaccination is recommended. Women and their obstetricians have to be informed of the risk of HUS relapse in case of pregnancy, most often during the post-partum, so that early treatment can be initiated.

**Plasmatherapy in atypical hemolytic uremic syndrome**

**Rationale for plasmatherapy**

Plasmatherapy was proposed to treat aHUS long before its logic was understood, when its efficiency was demonstrated in TTP. It became first line treatment of aHUS approximately one decade ago and remained so until now, empirically or based on expert opinion, not on clinical trials [17,75–77,81]. Virion-activated FFP brings normal amounts of CFH, CFI, CFB and C3, but no MCP, a non-circulating protein anchored in cell membranes. PE removes mutant CFH, CFI, CFB, C3 and anti-CFH antibodies, and possibly inflammatory/thrombogenic factors that participate in endothelial injury and platelet hyperaggregability. In addition, PE, through volume restitution with FFP, replenishes missing or dysfunctional complement proteins, without the risk of volume overload, hypertension and cardiac failure. It also prevents hyperprotidemia which develops when plasma infusions (PI) (10–20 ml/kg) are repeated several times per week.

**Clinical experience with plasmatherapy**

Data from the Italian Registry show that overall approximately 70% of HUS episodes (50% of patients) respond to plasmatherapy (PI or PE). Complete or partial remission (hematological response, but renal sequelae) was observed in 63, 25, 57 and 88% of patients with CFH, CFI, C3 and THBD mutation respectively. However, the percentage of complete renal recovery under plasmatherapy in the same groups was only 5, 12.5, 43 and 62% respectively, while death or ESRD occurred in 37, 75, 43 and 13% [64]. Lack of prospective data greatly limits analysis of registry-related therapy responses since poor renal outcomes could be related to delayed and/or insufficient plasmatherapy given the uncontrolled setting. Conversely, case reports – about a dozen – mainly in children with CFH mutations, have shown that intensive plasmatherapy (PE rather
than PI), started early (when serum creatinine was moderately elevated) and maintained daily until all criteria of TMA were improving (not only normalized platelet count, but also resolution of hemolysis with normal LDH and stabilized hemoglobin and improvement of renal function) can rescue HUS. These reports also showed that long term maintenance plasmatherapy may prevent relapses and development of ESRD, at least during the 1 to 6 years follow-up under plasmatherapy reported [81–85]. However two patients developed ESRD after 4 and 7 years, suggesting continued (subclinical) TMA [86,87]. Nonetheless, empiric maintenance prophylaxis appears superior to therapy driven by clinical events: most CFH-mutated patients who received plasmatherapy only during acute episodes died or progressed to ESRD within less than one year [81,84]. A few patients with CFI, C3 or CFB mutations who were clearly plasma responsive have also been reported [81]. In contrast, as MCP is not a circulating protein, the expectation that plasmatherapy should not be of benefit in MCP-HUS is supported by the observation that 90% of episodes resolved whether the patients received plasmatherapy or not [63,64]. HUS due to anti-CFH antibodies is a clear indication for PE, which removes the pathological antibodies. The antibody titre often rebounds after the cessation of PE, with a high relapse risk. Therefore steroids and immunosuppressive drugs should be prescribed. Intravenous cyclophosphamide, mycophenolate mofetil or rituximab have all been used successfully. Duration of PE and choice and duration of immunosuppressive therapy are best guided by the evolution of anti-CFH antibody titres [74,88–90].

**Guidelines for plasmatherapy**

Guidelines have been published in 2009, based on expert opinion and experience [18,77] (box 2). Plasmatherapy should be started as early as possible, without waiting for results of biological investigations, i.e. within 24 hours after admission, as irreversible renal damage can develop within a few days. PE with FFP replacement is preferred to PI. PE should be performed daily until all TMA criteria are under control, i.e. platelet count > 150 × 109, hemoglobin stabilized, LDH normalized and renal function improving (decrease of serum creatinine). Some patients respond within less than a week, but many need daily PE for longer periods. Thus, criteria for tapering PE are different from those in TTP. Appropriate control of TTP is defined by normalization of platelet count after five daily PE (allowing tapering of PE) and failure of plasmatherapy by persistent thrombocytopenia after five daily PE (an indication to switch the patient to rituximab). In aHUS patients, even if platelet count has normalized, lack of improvement of renal function and/or hemolysis after three to five daily PE has been regarded as criterium for uncontrolled TMA, an indication to maintain daily PE (no tapering) or, since recently, to switch the patient to eculizumab. When disease activity (TMA criteria) is controlled

**Box 2**

**Recommendations for plasmatherapy in the acute phase of atypical hemolytic uremic syndrome and during the first month**

2011 is a transition year, as eculizumab is on the way of becoming the new standard of care for aHUS. Many pediatricians propose that aHUS in a child can now be considered as an indication for eculizumab without previous plasmatherapy. Incomplete response to 3-5 daily PE, occurrence of a relapse at plasmatherapy tapering or cessation, intolerance to plasmatherapy or vascular access difficulties are or will be indications to eculizumab if or when available/reimbursed. See text for further details.

**When must plasmatherapy be started?**

- as soon as possible: within 24 hours after onset
- as soon as the patient’s condition allows (blood pressure, volemia, hydroelectrolyte equilibrium, anemia corrected)

**Which modality and which volume?**

- PE: 1.5 plasma volume (60–75 ml/kg) with FFP for restitution
- if PE impossible, infuse FFP 10–20 ml/kg (if blood pressure and cardiac function are normal)

**Which frequency during the first month?**

- daily until stable normalization of platelets, cessation of hemolysis and improvement renal function over several days.
- Consider administration of eculizumab if normalization of platelet count, cessation of hemolysis and decrease of creatinine is not achieved after 3 to 5 daily PE.
- if initial plasmatherapy effective, complete 5 sessions per week during 2 weeks, followed by
- 3 sessions per week during up to 2 weeks

**What are the situations which allow not to do PE or to stop early?**

- MCP mutations (PE often performed during HUS episodes, with uncertain benefit, but not preventively)

**Which frequency after the first month?**

- empirical: determine the appropriate modality (PE or PI), threshold dose, interval between sessions and duration for each individual patient
- PE: plasma exchange; PI: plasma infusion; FFP: fresh frozen plasma; MCP: membrane cofactor protein

(Adapted from Ariceta et al. [18] and Taylor et al. [77]).
CFH mutations, and likely also for those with CFI, C3 and CFB mutations. However, an attempt to stop plasmatherapy is generally considered when no relapse of HUS has occurred several months (or years) after tapering plasmatherapy to approximately monthly sessions. Of note, interruption of plasmatherapy is often considered much earlier than indicated here, for logistic reasons or technical difficulties. Some patients may do well, but many will relapse and progress to ESRD after discontinuing plasmatherapy.

Limits of plasmatherapy
PE is technically challenging and requires specialized centres, especially for children [91–93]. The most frequent complications are hypotension, hypocalcemia, catheter-related thrombosis and infections, all more frequent in children than in adults (55% of sessions in children vs 4.3 to 28% in adults [92]). Some patients develop severe anaphylactic reactions to FFP which requires cessation of plasmatherapy. Altogether, the overall poor outcome of aHUS presently reported [64], except for MCP-HUS, probably illustrates best the limits of plasmatherapy in many places and acceptability for patients.

Complement blockers in atypical hemolytic uremic syndrome: eculizumab

Rationale for complement blockade
Understanding the role of complement activation dysregulation in aHUS opened the way to a new therapeutic option, the use of complement blockers [87,94,95]. The rationale for this approach has been reinforced by the demonstration of a core role of C5 activation in the development of the disease [96,97]. Eculizumab (Soliris®, Alexion Pharmaceuticals, Cheshire, CT, USA) is a recombinant, humanized, monoclonal anti-C5 immunoglobulin G, which blocks the cleavage of C5 to C5b and thus the generation of the membrane attack complex C5b-9 (fig. 5). It has been approved for the treatment of paroxysmal nocturnal hemoglobinuria (PNH), with several hundreds of patients treated worldwide, some of them since more than ten years [98–100].

Clinical experience with eculizumab for atypical haemolytic uremic syndrome

Today, 17 cases have been published or presented at congresses (available on the net), thereof seven cases with aHUS with native kidneys [101–107] and 10 cases where eculizumab

Figure 5

Blockade of complement activation by eculizumab
Eculizumab, a recombinant, humanized, monoclonal antibody that targets C5, blocks its cleavage to C5a and C5b and thus prevents the formation of the membrane attack complex.
Adapted from [98]
has been used to rescue \( n = 7 \) \cite{108-115} or prevent \( n = 3 \) \cite{116-118} post-transplant recurrence. Ten patients were children (19 months to 18 years of age). In the majority of patients who received eculizumab as salvage therapy after failure of plasmatherapy, increase of platelet count, cessation of hemolysis and improvement of renal function was observed within a few days after the first injection. The response was similar whether the patients had a complement mutation (mostly in \( C^\text{FH} \), some in \( C^3 \) or \( C^F \)) or not. The only patient whose kidney function was not rescued was treated late, after approximately 50 days on dialysis \cite{103}. Patients maintained on long-term eculizumab therapy had preserved native or graft kidney function after up to nearly 3 years follow-up. In contrast, four patients who received a single injection had a subsequent relapse of aHUS and ultimately progressed to ESRD \cite{103,104,108,113} and personal communication from J. Nurnberger [April 2010] and M. Lozano [January 2011] to C. Loirat, with permission).

International Multicenter Prospective phase II trials have been conducted in 2009–2010 in adults and adolescents with aHUS (on native kidneys or post-transplant recurrence), who were resistant to plasmatherapy (17 patients) or received chronic plasmatherapy (20 patients) and were switched from plasmatherapy to eculizumab \cite{119,120}. These studies confirmed that eculizumab stops the TMA process, as indicated by the rapid increase of platelet counts, cessation of hemolysis, improvement or stabilisation of renal function without having to return to PE or initiate dialysis. They also confirmed that response to eculizumab was similar in patients with or without detectable complement mutations. Two new trials have started in 2010, one in adults, one in children (1 month to 18 years of age), including HUS with native kidneys or post-transplant recurrence, who will receive eculizumab as primary therapy or following previous plasmatherapy.

The risk of eculizumab-associated meningococcal infection

Eculizumab, by blocking the complement terminal pathway, induces an increased risk of \textit{Neisseria meningitidis} infection \cite{121}. Therefore, meningococcal quadrivalent conjugate vaccine is mandatory before initiation of eculizumab. However, available vaccines do not protect against \textit{Neisseria meningitidis} B. Therefore, additional (oral) penicillin prophylaxis has been advised in some countries (including France) for patients receiving eculizumab. The availability of \textit{N. meningitidis} B vaccine is eagerly awaited. Precise and repeated information regarding the risk and symptoms of \textit{N. meningitidis} infections must be provided to patients and their physician.

Eculizumab in practice in 2011

The therapeutic schedule is similar to that for PNH patients, except for a 30% higher dose to ensure complete blockade of complement activation. In adults, 900 mg are injected intra-

venously over 35 minutes at weekly intervals for 4 weeks. The fifth injection contains 1200 mg, followed by maintenance therapy of 1200 mg every 14 days. Presently, life-long treatment is recommended. Doses for children are adjusted to weight but as experience in children is limited, we recommend to confirm complete complement blockade (CH50 ≤ 10% in a reference laboratory).

Due to the particular challenge of PE in children, it is not unreasonable to suggest eculizumab as first line treatment in children with aHUS, provided it is immediately available. With the possibility of less-invasive peripheral vein access, plasmatherapy remains a less expensive and appropriate initial therapy for older teens and adult patients where it is usually well tolerated. In our opinion, use of eculizumab should be considered for plasmatherapy patients defined by persistent thrombocytopenia and/or ongoing hemolysis and/or lack of improvement of renal function after 3–5 daily PE. 2011 will likely be a transitional year: publication of the prospective trials results is pending and financial responsibility for this expensive biological treatment – which will likely become the new standard of care of aHUS – not yet universally accepted by governmental or private health insurances. Perhaps a sign of the transition, France offers the possibility of reimbursing hospitals for the costs incurred treating patients in such situations, and private insurers in the United States have approved eculizumab on a case-by-case basis.

Transplantation Issues in atypical hemolytic uremic syndrome

Atypical hemolytic uremic syndrome recurrence risk after isolated kidney transplantation

Historically poor renal outcomes of aHUS have been recapitulated in a high rate, over 50%, of graft loss due to aHUS recurrence following kidney transplantation \cite{115,122}. Planning for transplantation after aHUS-induced ESRD requires comprehensive genetic testing, attention to specific clinical features, and detailed surgical evaluation. Current and rapidly evolving transplantation options leave individuals suffering kidney failure from aHUS and those caring for them with complex decisions involving both risk and quality of life. Genotyping of individuals with aHUS helps to estimate the risk of aHUS recurrence following kidney transplantation based on specific mutation \cite{115} (\textit{table III}).

Defects in heptatically-synthesized, circulating complement-related gene products

Individuals with \textit{CFH} mutation suffer a 75–90% risk of aHUS recurrence and graft loss after kidney transplantation. The rate is 45–80% in \textit{CFI} mutation \cite{64,115,122} with the caveat that far fewer cases are described and many of those individuals, perhaps more than half, harbor additional complement-related defects \cite{69}. The risk of recurrence and graft loss among individuals with \( C^3 \) mutation is 40–70% \cite{64,71,114}, while
the risk in patients with CFB mutation is not clearly established (only three patients transplanted, recurrence and graft loss in all [72,73]. Since nearly all expression of CFH, CFI, CFB, or C3 occurs in liver, where synthesis of these circulating factors is accomplished, individuals with CFH, CFI, CFB or C3 defects remain susceptible to aHUS following kidney transplantation. The concept of liver transplantation to cure aHUS related to these defects has been derived from these observations. The “hepatic model” neglects a small amount of extrahepatic production for most circulating complement proteins. Examples of quantitatively minor sites of CFH synthesis include mononuclear cells, adipose and renal tissue [123–125] and renal tissue is also a site for some C3 and CFB synthesis [125,126]. Inflammation and other pathological processes appear to upregulate this limited extra-hepatic production. Whether this limited synthesis could impact post-transplant aHUS recurrence following even liver transplantation is not known.

Defects in complement-related gene products synthesized extra-hepatically

MCP is expressed by nearly all cell types (including cells within the kidney) and its product is a transmembrane protein that functions locally to limit complement activity. Among individuals with MCP mutation, post-transplant recurrence is uncommon (from zero [64] to 15–20% [115]). Thus, it is understood that kidney transplant usually provides sufficient wild-type MCP. At least two mechanisms could explain recurrence in MCP-mutated patients. The first is the presence of undiagnosed, additional complement factor mutations. A second is recognition that over time, microchimerism occurs within donor renal grafts, particularly in the endothelium where two-thirds of grafts demonstrate this phenomenon [127]. Indeed, micro-colonization of graft endothelium by a recipient’s MCP-deficient cells was reported in a case of aHUS recurrence [128]. While testing for microchimerism is not routine during clinical biopsy analysis, this sentinel observation highlights the need for careful monitoring following transplantation even in this group at lower risk of recurrence.

In contrast to MCP, THBD mutation may impart a higher risk of recurrence. Of seven individuals with aHUS related to THBD mutation, two underwent kidney transplantation, and both lost their graft to aHUS within days (this assumes aHUS was the original cause of ESRD in one of those individuals where documentation was not available) [129]. THBD is synthesized by and expressed on the surface of endothelial cells and protease cleavage leads to circulating soluble forms. The rapid time frame of recurrence suggests several possible explanations. First, the tissue mass of donor kidney endothelium may be insufficient to protect against robust peri-operative complement activation. Second, endothelial synthetic function in a fresh transplant may be transiently diminished, leaving the new graft susceptible to uninhibited complement activation. Yet another possibility is that soluble forms of THBD play a role in aHUS, in which case the defective product may persist in the circulation after transplant.

Auto-antibodies impacting complement function

The risk of post-transplant recurrence is not well established in anti-CFH autoantibody-mediated HUS, but is likely related to persistent, high antibody titres. Measures to prevent post-transplant recurrence includes PE, immunosuppression and monitoring (and reduction) of the antibody titre before and after kidney transplantation [74,88,130].

Pre-transplant considerations

Individuals with aHUS require particular attention in several areas. Complete complement investigation including the screening for mutations of all known complement factors associated with aHUS is recommended before considering transplantation [77,131]. Patients with aHUS are often sensitized due to the extensive use of blood products. High-resolution techniques like flow-cross match and single antigen bead detection of antibody to HLA can help estimate the risk of rejection and ameliorate that risk by improved donor selection. Historically, some patients with ESRD due to aHUS have spent many years undergoing hemodialysis. Central venous occlusions and other vascular complications should be actively sought because they greatly impact planning as major surgical risk factors during isolated kidney or liver-kidney transplantation. In addition to routine pre-transplant vaccination recommendations, meningococcal vaccine should be provided to reduce the infectious risk in case eculizumab therapy is utilized. Living-related donation is difficult to recommend despite genetic screening since mutations causing aHUS are often incompletely expressed, found in multiples, and in the current era, still incompletely known. Thus, living related donation may confer HUS risk to both the recipient and the donor.

Specific transplantation strategies

Isolated renal transplantation should not be undertaken without preventative therapy to reduce the risk of recurrence during the perioperative period, except in patients with MCP mutation in whom additional mutations have been excluded [122,131]. Another probable exception is the patient with autoimmune (anti-CFH antibody) HUS, whose disease is quiescent and who has no detectable titre for several years. Conversely, if aHUS recurred following a previous transplant, preventative therapy is required for subsequent transplants, regardless of the defect. Likewise, while the potential risk of yet-to-be-discovered mutations to cause recurrence in patients with isolated MCP mutation appears to be small, individual clinicians may choose to use short-term preventative therapy for that reason, especially if the patient had demonstrated low C3 or other abnormal measures of circulating complement activation.
There are currently two major preventive strategies, as well as the option to pursure liver transplantation to cure aHUS related to heptatically synthesized products.

**Plasmatherapy to prevent post-transplant recurrence**

Consensus recommendations are to provide 1.5 to 2 plasma volumes by PE in the several hours prior to transplantation [131]. A series of prophylactic PE should then be performed after transplantation, with decreasing frequency, from daily to once-weekly or the minimum required to prevent recurrence. Complete discontinuation of prophylaxis is generally achieved in patients with anti-CFH antibodies, noting routine immunosuppression may suffice for protection. For CFH and other heptatically-synthesized proteins, discontinuation of complement-directed therapy may not be feasible, although there are successful examples without recurrence during the reported observation period [132,133]. Importantly, dependence upon plasmatherapy does not assure long-term efficacy. Inherent risks include overt aHUS recurrence despite prophylaxis, slow decline of renal function, and severe infusion reactions [115].

**Eculizumab to prevent or rescue post-transplant recurrence**

Eculizumab is promising due to its potential to permit isolated kidney transplantation. It has been used successfully both as prophylaxis [116–118] and as “salvage” [108–115] therapy for post-transplantation HUS recurrence, prophylaxis presumably being a better strategy than salvage treatment. Two prospective trials comprising 15 patients with post-transplant recurrence suggest rescue with eculizumab [119,120]. Dosage and frequency similar to that used in trials for aHUS in native kidneys appeared efficient and was not associated with an increased risk of infectious complications [108–118]. For both plasmatherapy and eculizumab, it is reasonable to assume that most individuals with complement regulator mutations need lifelong treatment. Recurrence risk can be anticipated to rise during complement-activating stresses such as infection, surgery, or pregnancy. In current practice, however, recognizing and responding promptly to triggers is likely to be quite difficult since aHUS can rapidly destroy a transplant and some triggers seem innocuous. The recent report of Weitz et al. is particularly informative, demonstrating that attentive clinical and laboratory monitoring was required to avoid aHUS relapse in the face of infectious triggers [117]. In the future, more widespread and reliable testing for both long-term and imminent risk of recurrence, for example by assays of terminal complement cascade activity or endothelial markers, may be of great use to guide the need for, frequency of, and efficacy of prophylaxis. Currently, in preventing complications of aHUS, indefinite routine prophylaxis is a more reliable approach for most patients, at least for plasmatherapy [81]. Experience with eculizumab is limited, but likely the same logic applies, considering just two post-transplant cases, originally published as successful “salvage” treatment by a single dose of eculizumab [108,113], followed by unpublished recurrence and ultimately graft loss (personal communication from J. Nurnberger, April 2010, and M. Lozano, January 2011, to C. Loirat, with permission).

**Liver transplantation**

Liver transplantation to cure aHUS is known to be effective. There have been, to the authors’ knowledge, 20 such transplants (19 for CFH mutation, one for CFB mutation) [131,134–142] (including 10 unpublished cases, C. Rinat, MA. Cnapaphomchai, L. Milner, H. Jalanko, RA. Cohn, A. Bensman, P. McKiernan, E. Gottlich, personal communications to J. Saland, April-June 2011, with permission, and one patient of author J.Saland). Liver-kidney transplantation was performed for individuals who had already suffered ESRD and were in need of kidney transplantation. A successful procedure including preoperative PE (to protect against complement-mediated liver injury) was introduced in 2004 and allowed a series of successful outcomes [131,137–142]. The four earliest procedures (all in 2002) did not include pre-conditioning with plasmatherapy and were uniformly fatal. However analysis of these cases provided proof of the therapeutic concept [134–136] (4th case, C. Rinat, personal communication to J. Saland, Feb 2011, with permission). In the first case, auxiliary liver transplant (leaving the native liver) was performed and aHUS recurrence could not be excluded nearly a year later during an infection episode that proved fatal [136]. In two of these cases, complement-mediated liver injury leading to hepatic necrosis and death was demonstrated [134,135]. Liver failure and hepatic necrosis also occurred in a 2010 procedure that did not include treatment with plasmatherapy (M. Cnapaphornchai, personal communication to J. Saland, March 2011, with permission). Thus, pre-conditioning appears to be an indispensable part of this procedure, recognizing that eculizumab pre-conditioning might be suitable as well as plasmatherapy. Despite use of preconditioning PE, short-term mortality of the combined transplant approach is significant: two of 14 patients have died within several days not from aHUS, not from complement-mediated hepatic injury due to complement activation, but rather from surgical complications. One had fatal hepatic artery thrombosis within days of transplant, and the other had good function of both grafts but suffered cerebral ischemia related to superior vena cava syndrome during manipulation of the inferior vena cava intraoperatively (data from author J. Saland). This 86% patient survival is intermediate to the 12 month survival for children (< 18 years, n = 46, 91% survival) and adults (n = 1166, 82% survival) undergoing combined organ transplantation (for all causes) between 2001–2005 as reported a large U.S. registry [143]. In one case, a child who was dependent on plasmatherapy underwent successful isolated liver transplantation to maintain native renal function free of aHUS [140]. Without ESRD, this
approach introduces the need for transplant immunosuppres-
sion where none would otherwise be needed. Registry data
from the U.S. shows that the 5-year patient survival for liver
transplant in children is 85–88% [144]. In the current era, this
approach has to be compared not only to chronic plasmath-
ery but also to chronic eculizumab therapy.
After the immediate peri-operative risk period, the long-
term outlook after liver-kidney or isolated liver transplant is good.
There have been no reports of aHUS recurrence with this
approach and routine monitoring is well-established. Simulta-
neous liver-kidney transplantation carries the additional ben-
efit of being protective against kidney rejection compared to
isolated kidney transplantation [145,146]. Thus, following the
immediate transplant period, the ongoing lifetime risk, cost,
and quality of life is (arguably) better than for patients who
would otherwise require intravenous plasmatherapy or eculi-
zumab indefinitely, particularly for those with ESRD who
already require a kidney transplant.

Conclusion and perspectives

2011 is a special year in the HUS domain. On the one hand, the
epidemic of STEC-HUS in Germany has once again brought the
disease to the attention of the general population. At the same
time, eculizumab, the first complement blocker to be reach
clinical application, is most likely on the way to become the
standard of care for aHUS. Gains in the understanding of the
mechanism of aHUS during the last decade have aroused renewed interest in the role of complement in STEC-HUS.
Progress in the development of “biological” therapeutic agents,
including monoclonal antibodies and recombinant
molecules to neutralize Stx and to block the terminal comple-
ment pathway, opens exciting perspectives for a better
understanding and novel treatments both for STEC-HUS and
aHUS. Hopefully, eculizumab will fulfill the expectation that
it can prevent progression to ESRD in patients with aHUS and
offer the possibility of a successful kidney transplant to the
many aHUS patients presently on dialysis. Isolated liver
transplantation is a therapeutic option for eculizumab-
dependent patients without ESRD, and combined liver-kidney
transplantation is an option for those with ESRD. Liver
transplantation carries a significant risk of death in the
short term, but it is a definitive cure that potentially offers
reduced long-term risk and cost, and improved quality of life
without the need for intravenous plasmatherapy or eculizu-

dab. It is likely that the transplantation of patients with aHUS
will remain a patchwork of the various options for some time,
depending on access to various treatments, results of ongoing
studies and observations, and the subjective preferences of
patients as they consider the short and long-term risks,
benefits, and quality of life associated with each of the
available options.

Disclosure of interest: Chantal Loirat has been coordinator for France of the
Alexion trials “Safety and efficacy of Eculizumab in atypical HUS adult
patients resistant to plasma therapy/sensitive to plasmatherapy” C08-002A
and C08-003A, is coordinator of the trials “Eculizumab in adult patients/
pediatric patients with atypical HUS” C10-004 and C10-003, and is on
scientific advisory boards of Alexion Pharmaceuticals and LFB
Biotechnologies. Martin Bitzan serves as clinical advisor to Thalion
Pharmaceuticals, Inc. Jeffrey Saland has no conflict of interest to declare.
Source of supports: None to declare

Acknowledgments: Dr. Blair Whittemore for stimulating discussions; the
members of the Association pour l’information et la Recherche sur les
maladies rénales Génétiques (AIBG) for their continuous support and help.
Many thanks to the patients and their families for their collaboration and
encouragement.

References

5. Karmali MA, Steele BT, Petric M, Lim C. Sporadic cases of haemolytic-uremic syn-
6. Tarr PI, Gordon CA, Chandler WL. Shiga-
toxin-producing Escherichia coli and hae-
7. Karch H, Tarr PI, Bielaszewska M. Enter-
8. Wang H, Paton JC, Paton AW. Pathologic changes in mice induced by subtilase
cytotoxin, a potent new Escherichia coli
10. Oloto AI, Mithwani S, Newton CR. Haemoly-
11. Pijpers AH, Setten PA, Heuvel LP, Assmann KJ, Dijkman HB, Pennings AH et al. Verocyt-
toxin-induced apoptosis of human micro-
Management of hemolytic uremic syndrome


Management of hemolytic uremic syndrome


[143] Source: “1 Month, 3 Month, 1 Year, and 5 Year Patient Survival for Kidney-Liver Transplants from 2001 to 2005” analyses prepared by the Scientific Registry of Transplant Recipients (SRTR), under contract with the U.S. Department of Health and Human Services, Date March 17, 2011.

