Novel aspects of atypical haemolytic uraemic syndrome and the role of eculizumab

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ABSTRACT

The haemolytic uraemic syndrome (HUS) is part of a spectrum of thrombotic microangiopathies. The most common etiologies of HUS are the ones seen in childhood caused by an infection of Shiga toxin-producing Escherichia coli, HUS caused by an infection with Streptococcus pneumoniae and HUS due to abnormalities in the alternative pathway of the complement system. In the past decade, enormous progress has been made in understanding the pathogenesis in the latter group of patients. The analysis of genes that encode for complement regulatory proteins and the development of assays for measuring the activity of ADAMTS13 and the detection of antibodies against factor H contributed significantly to the diagnostic tools in patients with HUS. These assays have made it possible to clearly differentiate between thrombotic thrombocytopenic purpura and various forms of HUS. With the introduction of eculizumab, a monoclonal anti-C5 inhibitor, in the clinical arena as effective treatment of most complement-mediated forms of HUS, a new era of treatment in HUS has begun. We review the recent advances in HUS, with the focus on treatment. We discuss unsolved questions, which should be addressed in future studies.

Keywords: atypical haemolytic uraemic syndrome, complement, eculizumab, renal transplantation

INTRODUCTION

Thrombotic microangiopathy (TMA) is a histopathological entity, defined by the presence of fibrin and/or platelet thrombi in the microcirculation. TMA is typically seen in kidney biopsies of patients diagnosed with haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). Clinical features of patients with TMA are non-immune haemolytic anaemia, thrombocytopenia and related organ damage in kidney or brain. For many years, physicians have considered HUS and TTP as variable manifestations of one, pathogenetically similar, entity [1]. Clinically, there were subtle differences, renal failure being most prominent in HUS, whereas neurological symptoms and severe thrombocytopenia prevailed in TTP. With the discovery of an inherited or acquired deficiency of the plasma protein ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) as the main cause of TTP, diagnostic tools became available to differentiate between HUS and TTP [2].

THE HUS

In 1955 the term HUS was coined by Gasser et al. [3] who described children with a severe illness and characterized by haemolytic anaemia, thrombocytopenia and acute renal failure. HUS is typically a disease of children (>90% of the cases present in childhood) and is associated with bloody diarrhoea, caused by an infection with Shiga toxin-producing Escherichia coli (STEC). However, in ~10% of patients, HUS is not associated with a STEC infection. Such cases of non-STEC-HUS, nowadays called atypical haemolytic uraemic syndrome (aHUS), can occur at any age and are in fact the major cause of HUS in adults. The first reports of aHUS were actually published before the discovery of STEC as cause of HUS and also concerned children [4–6]. Already at that time, it was evident to the authors and clinicians that non-STEC–HUS differed from the usual form of HUS in children, with the absence of...
the prodromal phase of bloody diarrhoea. Careful observations revealed sporadic as well as familial occurrence of aHUS and also showed that the disease could relapse. In some patients with aHUS, hypocomplementemia was observed during active disease as well as after recovery suggesting involvement of the complement system. In 1981, Thompson and Winterborn [7] were the first to document an inherited deficiency of a complement regulatory protein as cause of HUS. The patient was an 8-month-old boy who presented with HUS. Complement C3 levels were consistently low. His unaffected brother also had low C3 levels. Further studies revealed low levels of complement factor H (CFH). In the past decade, it has become evident that dysregulation of the complement system is the major cause of aHUS. The recent introduction of eculizumab as effective treatment of complement-mediated forms of aHUS has stimulated further research in this area and brought hope of cure for patients with this disease [8, 9].

Table 1. Diagnostic tools in HUS and TTP according to aetiology

<table>
<thead>
<tr>
<th>Classification</th>
<th>Aetiology</th>
<th>Diagnostic tools</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTP</td>
<td>Low ADAMTS13 activity (acquired or congenital)</td>
<td>ADAMTS13 activity assay</td>
<td>First measurement of ADAMTS13 activity. If &lt;10%, check for antibodies, if no antibodies present, perform mutation analysis ADAMTS13 gene</td>
</tr>
<tr>
<td>HUS secondary to complement</td>
<td>DNA mutations in complement proteins involved in an alternative pathway</td>
<td>DNA mutation analysis CFH, CFI, MCP, CFB, C3, THBD genes, Plasma C3, C4, C3d, CFH, CF-I, expression of MCP on leucocytes (FACS-analysis), DNA mutation analysis CFH, MCP</td>
<td>Be aware that normal C3, CFH or CFI levels in plasma or normal expression of MCP on leucocytes do not rule out abnormal complement activation on endothelium</td>
</tr>
<tr>
<td>dysregulation</td>
<td>At-risk haplotypes (CFH&lt;sub&gt;1&lt;/sub&gt; or the MCP&lt;sub&gt;1&lt;/sub&gt; or the MCP&lt;sub&gt;2&lt;/sub&gt;)</td>
<td>Autoantibodies against CFH, ELISA method using coated purified human CFH [10]</td>
<td>Associated with deletion CFHR-1/CFHR-3. Mostly seen in children and young adults Repeat stool culture, use SMAC plate to detect the most detected serotype O157</td>
</tr>
<tr>
<td>HUS secondary to underlying</td>
<td>STEC infection</td>
<td>Stool culture, PCR Shiga toxins 1 and 2, detection serological antibodies against O-antigens (especially O157) Blood/liquor culture</td>
<td>Mostly in young children and elderly; plasmatherapy may be harmful!</td>
</tr>
<tr>
<td>causes</td>
<td>Streptococcus pneumonia infection</td>
<td>Serology, PCR</td>
<td></td>
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<td></td>
<td>Viruses: CMV and HIV</td>
<td>History</td>
<td></td>
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<tr>
<td></td>
<td>Use of drugs such as: CNIs, mTOR inhibitors, vincristine, quinine, oral contraceptives, ticlodipine, clopidogrel, mitomycin, bleomycin, bevacizumab</td>
<td>DNA mutation analysis of MMACH gene</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Deficiency in cobalamin metabolism</td>
<td>Elevated serum levels of homocysteine and methylmalonic acid</td>
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<td></td>
<td>Associated with systemic diseases: SLE, antiphospholipid syndrome, scleroderma, malignant hypertension, malignancy</td>
<td>DNA mutation analysis of MMACH gene</td>
<td></td>
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<tr>
<td></td>
<td>Associated with pregnancy: pre-eclampsia, HELLP</td>
<td>History, ultrasound kidney/heart, ECG (LVH), antinuclear antibody, antiphospholipid antibodies, lupus coagulant</td>
<td></td>
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<tr>
<td></td>
<td>Bone marrow transplantation: graft versus host disease</td>
<td>History</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DGKE mutation</td>
<td>DNA mutation analysis DGKE</td>
<td>aHUS presentation before age 1 year (n = 9)</td>
</tr>
</tbody>
</table>

For details of assays, see [11].

TTP, thrombotic thrombocytopenic purpura; ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; CFH, complement factor H; CFI, complement factor I; CFB, complement factor B; C3 complement component 3; MCP, membrane cofactor protein; THBD, thrombomodulin; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; PCR, polymerase chain reaction; HELLP, haemolysis, elevated liver enzymes and low platelets syndrome; DGKE, diacylglycerol kinase epsilon; LVH, left ventricular hypertrophy; CMV, cytomegalovirus; HIV, human immunodeficiency virus; mTOR, mammalian target of rapamycin; MMACH, methylmalonic aciduria (cobalamin deficiency); cbC type, with homocystinuria; SLE, systemic lupus erythematosis; SMAC, Sorbitol MacConkey agar.
age, clinical presentation and laboratory parameters will mostly point to a certain aetiology, some pitfalls need to be addressed. In 6% of patients with STEC–HUS, there is no prodromal phase of diarrhoea [12]. In contrast, diarrhoea (most frequently non-bloody diarrhoea) was a presenting feature in 15–39% of patients with aHUS [13–15]. Although neurological symptoms are one of the main characteristics of TTP, central nervous system involvement can occur in 8–30% of patients with HUS [16, 17]. These neurological symptoms can vary and include visual disturbances, alterations in consciousness, seizures and hemiparesis as well as brainstem symptoms [16]. Normal levels of serum complement C3 and complement regulatory proteins do not exclude a diagnosis of complement-mediated aHUS [13, 14, 18].

**FOCUS ON COMPLEMENT-MEDIATED HUS**

Most cases of otherwise unexplained aHUS are caused by abnormalities in the complement system. The human complement system is part of innate immunity and consists of >40 plasma and membrane-associated proteins (Figure 1 [19]). The complement system is involved in the recognition of pathogens (opsonization), the activation and chemotaxis of leucocytes and the induction of cell lysis. Three activation pathways are recognized: the classical pathway, the mannos-binding lectin pathway and the alternative pathway. The latter is always active with the continuous formation of small amounts of C3bBb at the surface of membranes. To prevent continued and unopposed complement activation, and resulting cell damage, the complement system is tightly regulated. The key regulators of the alternative pathway are CFH, complement factor I (CFI) and membrane cofactor protein (MCP or CD46). These complement regulatory proteins are either constitutively present on the endothelial cell membrane (MCP), or circulate in the blood and are active at the cell membrane by binding to the endothelial glycocalyx (CFH, CFI). They prevent local complement activation by binding C3. Next to the inhibitory regulators, the alternative pathway also includes proteins that activate complement, such as complement factor B and complement factor D (Figure 1). Vascular endothelium that lacks MCP or is not able to bind CFH and CFI is prone to ongoing complement activation on the cell surface leading to the final formation of the C5b-9 membrane attack complex (MAC). This will cause endothelial cell injury and contributes to TMA. There are many causes for

![Figure 1: Activation of the complement system via the classical, lectin or alternative pathway leads to production of C3 convertases, which can cleave C3 in C3b and the anaphylatoxin C3a. Additional formation of the C5 convertases initiates the production of the MAC that can cause cell lysis. The regulators of the alternative complement pathway, which play an important role in the pathogenesis of aHUS, are indicated in italic (CFH, CFI, MCP), mutations in these genes are loss of function mutations. Mutations in CFB and C3 lead to an increased complement activation and are called gain of function mutations. Properdin is a positive regulator of the alternative pathway. It can stabilize the alternative pathway of C3 convertase and it may also activate complement. Eculizumab is a humanized IgG antibody that binds to C5 and prevents the formation of C5a and C5b, thereby inhibiting the formation of MAC. CFH, complement factor H; CFI, complement factor I; MCP, membrane cofactor protein; CFB, complement factor B; MAC, membrane attack complex. Figure was adapted from Westra et al. [11] with permission from Van Zuiden Communications, publisher of the Neth J Med.](https://academic.oup.com/ndt/article-abstract/29/suppl_4/iv131/1909798)
dysregulation of the complement system. The so-called loss of function mutations in CFH, CFI and MCP prevent the binding to the endothelial surface or to C3 and thus the inactivation of the C3 convertase. Gain of function mutations in CFB and C3, respectively, decrease the decay and increase the formation of the C3 convertase. In some patients, aHUS is associated with the presence of autoantibodies against CFH, which prevent proper function of factor H [10]. From a genetic viewpoint, all abnormalities result in increased activity of the C3bBb convertase and the ensuing formation of the C5b-9 MAC complex. Mutations in the thrombomodulin (THBD) gene are also associated with the development of aHUS. THBD is a membrane-bound protein, known for its role in the coagulant cascade. However, this protein also affects the complement system by enhancing the CFI-mediated inactivation of C3 and by interfering with thrombin-mediated activation of C5 [20]. Small amounts of THBD circulate in the plasma.

Table 2 lists the known abnormalities in complement-regulating proteins that are associated with aHUS and their prevalence rate. The variation between studies is explained by the inclusion of variable numbers of children (42–100%) and familial cases (20–49%) [14, 15, 30, 31], factors which may influence the prevalence of genetic abnormalities (more frequent in familial cases and children) [30]. Moreover, registry data may be biased by selective referrals [28], and older studies by nature have not analysed all possible abnormalities [30]. In a recent report that summarizes the data of a reasonably complete paediatric registry from the Netherlands, disease-causing abnormalities were observed in 44% of patients [14]. A nationwide study in France reported complement-related disease in more than 66% of patients, with no major difference between children and adults [15]. Thus, abnormalities of the complement system may be the underlying cause in up to two-thirds of patients with unexplained aHUS [29, 32].

Although abnormalities in complement regulatory proteins are a major cause of HUS, it is important to realize that these abnormalities mostly influence the complement regulation at the surface of endothelial cells and do not necessarily affect systemic, fluid-phase complement regulation. Thus, although low levels of C3 are suggestive of complement-mediated aHUS, levels of C3 are often normal, dependent on the underlying abnormality (Table 2) [30].

Detailed studies in families of patients with aHUS and a documented mutation in one of the complement genes have shown that only 50% of mutation carriers develop active disease [19, 20, 30]. This incomplete penetrance points to the role of additional mutations, disease-associated polymorphisms or environmental triggers.

Case studies already reported the presence of combined mutations in some patients with aHUS [14, 15, 33]. A recent study analysed four independent cohorts totalling 795 patients with aHUS [31]. Combined mutations were found in 27/795 (3.4%) of patients and many consisted of combined MCP/CFI and MCP/CFH mutations. Genetic studies have also pointed to a role for CFH-related proteins 1–5 (CFHR1–5), which genes are located downstream of the gene for CFH on chromosome 1q32 [34]. By using multiplex ligation-dependent probe amplification (MLPA), a CFH/CFHR1 hybrid gene has been reported in 1–3% of patients with aHUS [28]. The resulting hybrid protein CFH/CFHR1 lacks proper cell binding thus exposing cells to continuous complement activation [34, 35]. Notably, routine mutation analysis will not disclose the presence of this hybrid gene. The development of CFH autoantibodies is strongly associated with the presence of homozygous polymorphic deletion of the genes that encode factor H-related protein 1 and protein 3 factor (CFHR1/CFHR3). Antibodies to CFH bind to epitopes of the C-terminal region of CFH, thereby reducing the regulatory function of CFH. Anti-CFH autoantibodies were observed in 59–82% of patients with a deletion of CFHR1 and in 0–6% in patients without a deletion [36–38]. This association of antibody formation with a genetic background likely explains the rather interesting finding that antibody formation is mainly observed in children, with anti-CFH antibodies being observed in 10–25% of paediatric patients and in 3.2% of adults [15].

Table 2. Complement-mediated aHUS

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Type of mutation</th>
<th>Prevalence in aHUS (%)</th>
<th>Decreased C3 concentration (%)</th>
<th>Risk of recurrence after Tx</th>
<th>Year of first publication</th>
<th>Risk of death or ESRD at first episode or within &lt;1 year (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFH</td>
<td>Loss-of-function</td>
<td>20–30</td>
<td>30–50</td>
<td>High (68%)</td>
<td>1998 [21]</td>
<td>50–70</td>
</tr>
<tr>
<td>MCP (CD46)</td>
<td>Loss-of-function</td>
<td>10–15</td>
<td>0–27</td>
<td>Low (20%)</td>
<td>2003 [22]</td>
<td>0–6</td>
</tr>
<tr>
<td>CFI</td>
<td>Loss-of-function</td>
<td>2–12</td>
<td>20–30</td>
<td>High (78%)</td>
<td>2004 [23]</td>
<td>50</td>
</tr>
<tr>
<td>CFB</td>
<td>Gain-of-function</td>
<td>1–2</td>
<td>100</td>
<td>High (100%)</td>
<td>2007 [24]</td>
<td>50</td>
</tr>
<tr>
<td>Complement component 3 (C3)</td>
<td>Gain-of-function</td>
<td>10</td>
<td>70–80</td>
<td>High (78%)</td>
<td>2008 [25]</td>
<td>60</td>
</tr>
<tr>
<td>Anti-CFH antibodies (associated with homozygous deletion CFHR1–CFHR3)</td>
<td>May occur isolated or associated with mutation CFH, CFI or MCP</td>
<td>5–10</td>
<td>40–60</td>
<td>Moderate (33%)</td>
<td>2005 [10]</td>
<td>30–40</td>
</tr>
<tr>
<td>DGKE</td>
<td>Recessive mutation and loss of DGKE results in prothrombotic state</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
<td>2013 [27]</td>
<td>Unknown</td>
</tr>
<tr>
<td>Combined mutation</td>
<td>2–9%</td>
<td>30</td>
<td>30–50%</td>
<td>2013 [14, 28, 29]</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>None of the above-mentioned mutations</td>
<td>40–50</td>
<td>20</td>
<td>Moderate</td>
<td>20–40</td>
<td>28, 30</td>
<td></td>
</tr>
</tbody>
</table>
some adult patients with aHUS, CFH autoantibodies were present in addition to mutations in other complement genes [14, 39]. Of note, there are many patients with a CFHR1 deletion who do not develop antibodies against CFH, suggesting that besides the genetic background other factors must play a role in stimulating antibody formation.

Genetic susceptibility factors included risk haplotypes (polymorphisms) in the CFH and MCP genes [40]. The identified risk haplotypes are $\text{CFH}_{\text{TGTGT}}$ and $\text{MCP}_{\text{GGAAC}}$. The presence of these risk haplotypes contributes to the development of aHUS. Fremeaux-Bacchi and colleagues observed that 33% of aHUS patients were homozygous for the $\text{MCP}_{\text{GGAAC}}$ haplotype, 22% were homozygous for the $\text{CFH}_{\text{TGTGT}}$ haplotype and 12% were homozygous for both (for comparison the prevalence in controls was 8, 5 and 0%, respectively). They calculated that the presence of an at-risk haplotype increased the risk of aHUS by a factor 3–4 [31].

Based on these data, it has recently been suggested that aHUS patients probably have a specific genetic complement profile, a so-called complotype, which makes them vulnerable to develop aHUS, especially in the presence of certain triggers [41].

Examples of environmental triggers are hypertension, infections, pregnancy, drugs, surgery or haematopoietic stem cell transplantation [28, 42, 43]. The role of these environmental triggers deserves special note. In an individual patient, it is impossible to discern if the underlying condition is the sole cause of aHUS. It may also be the trigger for the development of aHUS in a patient with an underlying abnormality in the complement system. Fakhouri et al. [44] studied 100 patients with aHUS. In 21 patients, disease onset was related to pregnancy and occurred mostly in the postpartum period. More than half of these patients had had an uneventful previous pregnancy. A complement abnormality was found in 85%. Thus, it must always be considered to evaluate the complement system in patients with otherwise ‘explained’ HUS. We suggest that this should be done in patients with an unexpected course or bad outcome. An intriguing example is the patient with proven STEC–HUS, who developed end-stage renal disease (ESRD). Recurrent disease caused loss of his first kidney graft, and only subsequent analysis revealed a mutation in CFI [45].

Although a complement abnormality can be found in almost two-thirds of patients with aHUS, the disease remains unexplained in a significant number of patients. It is likely that in the next decade, with new genetic techniques, new causative mutations in other complement proteins will be found. We can also expect new discoveries related to other proteins. Last year, by using exome sequencing in two families with autosomal recessive aHUS in infancy, homozygous mutations were detected in the gene encoding for diacylglycerol kinase-epsilon (DGKE). This mutation was encountered in infants with proteinuria, haematuria and hypertension [27]. The DGKE protein is present in podocytes and endothelial cells and is suggested to affect the coagulation pathway. There is no involvement of the complement pathway.

A recent review proposed that other mechanisms might be involved in the pathophysiology of aHUS. The authors speculated that some cases of aHUS might be related to defective binding of complement components such as factor H to the endothelium, not because of a change in the CFH protein, but rather due to changes in the endothelial cell surface and particularly the endothelial glycocalyx [46].

**OUTCOME IN THE PRE-ECULIZUMAB ERA**

Without treatment, the prognosis of aHUS was considered miserable with half of the patients progressing to ESRD and up to 25% dying during the acute phase of the disease [42, 47]. Although these figures are repeatedly mentioned in all reviews and manuscripts, it is important to realize that these outcome data are mainly based on studies that included a heterogeneous patient group, likely combining patients with TTP, aHUS, typical HUS and secondary forms of TMA. In the early 1980s, plasmapheresis (plasma infusion and/or plasma exchange) became the therapy of choice in aHUS [48]. Theoretically, plasma infusion replaces the defective complement components. Plasma exchange has the additional advantage of removing any defective dominant-negative complement protein and/or antibodies against complement components. For obvious reasons, information of the underlying genetic cause was lacking in these older studies. It is now evident that prognosis critically depends on the type of genetic mutation. Patients with a mutation in CFH have the worst prognosis and long-term outcome is bad, with a very high risk of ESRD or death (70–80%). Outcome is only slightly better in patients with CFI, C3, CFB and THBD mutations [30, 49]. In contrast, patients with a MCP mutation have, in general, a much better prognosis. The majority of patients will remit after the first episode, and although many patients will have a relapsing course, the long-term outcome is relatively favourable with only 20–25% of patients progressing to ESRD (Table 2).

The first description of the use of plasmapheresis in patients with HUS dates back to 1979 [50]. Hollenbeck et al. [51] studied adults with TMA. They compared the outcome in 14 patients treated with intensive plasmapheresis and in 14 patients not given plasmapheresis. Prognosis was definitely better with plasmapheresis, one patient dying and three developing ESRD. Outcome in the delay between disease onset and start of plasmapheresis. In 46% of the cases, the disease was complicated by ESRD within the first year after onset of disease [15, 28, 30], and 5–19% of aHUS episodes were complicated by death [15, 52].

Most recent literature data suggest that the outcome of aHUS may have further improved over the last decade, especially in children. Table 3 summarizes these studies, showing a
relatively low rate of ESRD and death in children treated in the last decade. This improvement in prognosis occurred in the era before the introduction of eculizumab and may have been related to better, more rapid start, and more intensive plasmapheresis. In addition, conservative therapy may also have improved, including better dialysis techniques, more adequate blood pressure control, better anti-infectious therapy, etc. Somewhat unexpected, outcome in adult patients with aHUS has not improved in parallel, with almost half of the adult patients treated in the period 2000–08, and using high-intensity plasmapheresis, progressing to ESRD [15].

Despite these uncertainties, expert opinion proposes that plasmapheresis should be considered effective, especially in patients with a mutation in CFH, and possibly CFI, factor B and C3. No effect is expected in patients with a mutation in MCP. Plasmapheresis is important for removing antibodies in patients with aHUS related to anti-CFH antibodies, although in such cases the production of these antibodies should be suppressed by additional immunosuppression [53]. Current guidelines advise that plasmapheresis should be started as soon as the diagnosis is suspected. A practical advice is to exchange 1.5 times (60–70 mL/kg) the plasma volume with fresh frozen plasma or virus-inactivated pooled plasma as replacement or to perform plasma infusion of 20–30 mL/kg (after initial infusion of 30–40 mL/kg) of body weight when plasmapheresis is not immediately possible [54].

Most studies that evaluated outcome in patients with aHUS have focused on death and renal outcome such as acute kidney injury and ESRD. In a recent review, attention was given to the development of cardiovascular complications in patients with aHUS [55]. Cardiac complications occur in 3–10% of patients with aHUS. Moreover, these patients may also be at increased risk for thrombosis and stenosis of the medium and large arteries, with some suffering from ischaemia of the extremities or brain infarction.

### KIDNEY TRANSPLANTATION AND aHUS

Approximately 50–60% of patients with aHUS develop recurrent disease after kidney transplantation [56]. Median time to disease recurrence is 1–3 months and graft failure occurs in 60–100% of patients with recurrent disease. Risk factors for disease recurrence are age, low C3 levels, the use of mammalian target of rapamycin (mTOR) inhibitors and possibly calcineurin inhibitors (CNIs), rejection and the type of mutation [56, 57]. The recurrence rate is highest in patients with a mutation in CFH, CFI and CFB (Table 2) [45]. In patients with established recurrent disease, plasmapheresis is not effective [56]. In contrast, it is suggested that intensive plasmapheresis may prevent disease recurrence [28, 31, 56]. Le Quintrec et al. reported nine patients who were treated with prophylactic therapy. In six patients, there was no evidence of recurrence of the disease. In statistical analysis, prophylactic plasmapheresis reduced the risk of recurrent disease by 66%, although in multivariable analysis the effect was not significant (RR: 0.34, 95% CI: 0.10–1.13; P = 0.079) [56]. Noris and Remuzzi [45] reviewed the current literature data and described 25 patients who received prophylactic plasma therapy. Recurrent disease developed in eight patients. The protocols that are used for prophylaxis are quite variable with respect to the frequency and volumes of plasmapheresis and the overall duration. In general, plasmapheresis or plasmapheresis is done daily in the first week after transplantation, with gradual tapering thereafter, and continued at one or two weekly intervals for months–years. In several patients, recurrent disease developed after interrupting plasmapheresis [45].

### ECULIZUMAB IN COMPLEMENT-MEDIATED HUS

Eculizumab is a humanized monoclonal antibody that binds with high affinity to the C5 component of the complement cascade thus blocking the generation of the C5b–C9 MAC. Eculizumab therapy was approved in 2008, for the treatment of patients with paroxysmal nocturnal haemoglobinuria, which is characterized by complement-mediated haemolysis [58]. In 2009, the first case-reports were published that demonstrated the potential beneficial effects of eculizumab in patients with aHUS [8, 9, 59–61]. In 2011, Eculizumab was approved for treatment of aHUS by the Food and Drug Administration (USA) and the European Medicine Agency (EMA). Approval was based on the results of two recently published prospective open label phase 2 trials and a retrospective analysis of a cohort of treated children [62, 63]. The prospective studies included 37 aHUS patients who were treated with eculizumab. The first study included 17 patients with renal failure and progressive TMA (low platelet counts) who were resistant to plasma exchange or infusion. The second trial evaluated 20 patients with aHUS who were in remission but dependent on continuous plasmapheresis or plasma infusion. In all patients, eculizumab was started and plasmapheresis was stopped [64].

Overall results were impressive (Table 4). Signs of TMA activity disappeared in most patients and plasmapheresis therapy could be stopped permanently in 88% (trial 1) and 100% (trial 2). In both studies, estimated glomerular filtration rate (eGFR) increased. Dialysis could be discontinued in four of the five patients who required dialysis at the time of initiation of eculizumab. There was an inverse relation between improvement of eGFR and time to start of eculizumab, indicating that

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<tbody>
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<td>Number</td>
<td>795</td>
<td>45</td>
<td>89</td>
<td>125</td>
</tr>
<tr>
<td>Children (%)</td>
<td>54</td>
<td>100</td>
<td>100</td>
<td>0</td>
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<tr>
<td>Male gender (%)</td>
<td>51</td>
<td>49</td>
<td>53</td>
<td>26</td>
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<td>Familial (%) versus sporadic</td>
<td>49</td>
<td>24</td>
<td>27</td>
<td>14</td>
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<tr>
<td>Plasmapheresis (%)</td>
<td>54</td>
<td>60</td>
<td>39</td>
<td>80</td>
</tr>
<tr>
<td>ESRD or death (%)</td>
<td>32</td>
<td>18</td>
<td>17</td>
<td>46</td>
</tr>
</tbody>
</table>

Table 3. Outcome of aHUS in the twenty-first century

*Contains patients from four international registries, including a French cohort which is also included in [15].

The period of patient inclusion is not defined.
Table 4. Eculizumab in aHUS

<table>
<thead>
<tr>
<th>First author/study number</th>
<th>Legendre [62]</th>
<th>C09–001R [63]</th>
<th>Zuber [65]</th>
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<tbody>
<tr>
<td><strong>Study</strong></td>
<td>Prospective</td>
<td>Retrospective</td>
<td>Retrospective</td>
</tr>
<tr>
<td><strong>n</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Inclusion</strong></td>
<td>17</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Progressive TMA under PE</td>
<td>28 (17–68)</td>
<td>28 (13–63)</td>
<td>New aHUS, aHUS recurrence or post-transplantation aHUS &lt;12</td>
</tr>
<tr>
<td>Stable platelet count under PE</td>
<td>41</td>
<td>40</td>
<td>9 (6–41)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>29</td>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>41 (0.2–3.7)</td>
<td>40</td>
<td>69 (0.03–14)</td>
</tr>
<tr>
<td><strong>Male gender (%)</strong></td>
<td>29</td>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td><strong>Previous kidney transplantation (%)</strong></td>
<td>41</td>
<td>69</td>
<td>69</td>
</tr>
<tr>
<td><strong>Interval in months from disease onset to start eculizumab</strong></td>
<td>0.8 (0.2–3.7)</td>
<td>8.6 (1.2–45.0)</td>
<td>0.8 (0.03–14)</td>
</tr>
<tr>
<td><strong>Serum creatinine (μmol/L) at start eculizumab</strong></td>
<td>256 (124–787)</td>
<td>234 (106–893)</td>
<td>NA</td>
</tr>
<tr>
<td>Five patient on dialysis</td>
<td>eGFR 19 (5–59)</td>
<td>eGFR 28 (6–72)</td>
<td>NA</td>
</tr>
<tr>
<td>eGFR &lt;15 or dialysis</td>
<td>41% eGFR &lt;15 or dialysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Estimated GFR (mL/min/1.73 m²)</strong></td>
<td>218 (105–421)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Platelet × 10⁹/L count at start eculizumab</strong></td>
<td>118 (62–161)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td>TMA event-free status 15/17 (88%) in week 26</td>
<td>TMA event-free status 16/20 (80%) in week 26</td>
<td>93% platelet normalization Complete TMA response in 47%, 53% with &gt;15 mL/min/1.73 m² increase eGFR</td>
</tr>
<tr>
<td>Mean increase eGFR 32 (95% CI: 16–47) mL/min/1.73 m²</td>
<td>9 (95% CI: 4–14) mL/min/1.73 m²</td>
<td>n = 8 recurrence free post-transplantation course</td>
<td></td>
</tr>
<tr>
<td><strong>n</strong></td>
<td>16/20 (80%) in week 26</td>
<td>93% platelet normalization Complete TMA response in 47%, 53% with &gt;15 mL/min/1.73 m² increase eGFR</td>
<td>n = 1 arterial thrombosis of graft</td>
</tr>
<tr>
<td><strong>Mean increase eGFR</strong></td>
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<td>9 (95% CI: 4–14) mL/min/1.73 m²</td>
<td>n = 8 recurrence free post-transplantation course</td>
</tr>
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<td>n = 1 arterial thrombosis of graft</td>
</tr>
</tbody>
</table>

NA, not available.

*Median and range.
treatment should preferably be started within 1 week after disease onset. Efficacy was maintained in the 32 patients who participated in the extension study over a period of 2 years. In the retrospective study in paediatric patients, normalization of thrombocytes was seen in 14/15 patients [63]. A complete TMA disappeared in 53% of the patients [63]. Many uncontrolled studies and case series have confirmed the efficacy of eculizumab therapy [66].

The above-mentioned studies also included a significant number of patients with recurrent aHUS after kidney transplantation, and also in these patients, disease activity disappeared after start of eculizumab therapy. This is an important finding, in view of the dismal outcome of kidney transplantation in patients with aHUS. A recent study confirmed that eculizumab therapy greatly improved outcome after kidney transplantation in patients with aHUS [65]. Zuber et al used eculizumab prophylactically in nine patients with aHUS, and the disease did not recur in any of them. These authors also described successful remission of recurrent aHUS in all 13 patients who started eculizumab therapy after recurrence was noted [65]. Of note, serum creatinine concentrations normalized in patients who received eculizumab within 28 days after onset of disease.

The treatment schedules as advised for the treatment of aHUS in children and adults are given in Table 5 [62] (http://www.ema.europa.eu). Treatment with eculizumab should be started within 1 week after disease onset, and there is no need to have the genetic complement analysis finalized before starting eculizumab. Eculizumab infusions are well tolerated and relatively safe on the short term. Although side effects were reported in 35–50% of patients, consisting of hypertension, upper respiratory tract infections, diarrhoea, vomiting, headache and haematological abnormalities, the relationship with eculizumab is uncertain. The terminal complement pathway is very important for encapsulated bacteria such as meningococci. Therefore, development of meningococcal meningitis is the most feared side effect. It is obligatory that patients receive meningococcal vaccination before the start of eculizumab and/or additional antibiotic prophylaxis. We should keep in mind that the most common serotype (type B) of Neisseria meningitidis is not included in the current vaccine. Recently, a new vaccine that includes serotype B was approved by EMA [67]. Importantly, patients who are treated with eculizumab should be advised to contact the physician immediately when they experience fever or headache. Two recent cases of meningococcal infection have been documented despite early vaccination [63, 68].

Vaccination against Streptococcus pneumoniae and Haemophilus influenzae type b vaccination is required in children. Eculizumab is not a panacea. Some patients with aHUS will have another underlying abnormality and may not respond to eculizumab. As an example, we mention patients with the recently discovered DGKE mutation who have no evidence of complement involvement. Patients with CFH autoantibodies-associated HUS need treatment with B-cell depleting antibodies or alternative immunosuppressive therapies in addition to the use of eculizumab [53].

**UNSOLVED QUESTIONS**

The introduction of eculizumab has dramatically improved the outcome and quality of life of patients with complement-mediated HUS. Most patients with aHUS will no longer need continued treatment with plasmapheresis or plasma infusion, and many will never develop ESRD. Moreover, patients with aHUS who are currently treated with dialysis may now consider kidney transplantation. Obviously, the introduction of eculizumab, a very expensive drug, poses many new questions: who should be treated, when should treatment be started and can treatment be stopped? Are there any precautions when considering kidney transplantation in patients with (suspected) aHUS?

In the following, we will address these questions. In the absence of controlled clinical trials, we cannot make grade A, evidence-based recommendations. We have tried to identify the most important questions and provide our personal view and arguments to support them. We emphasize the need for more rigorous clinical trials and evaluation of treatment protocols.

**PATIENTS WITH aHUS IN THEIR NATIVE KIDNEYS**

An infection with STEC is the underlying cause in the vast majority of patients with HUS, at least in children. Typically, STEC–HUS is a self-limiting disease, with recovery in most patients. Although heavily debated, there is no evidence that treatment with eculizumab improves prognosis in patients with STEC–HUS [69]. Therefore, this and other diagnoses...
such as TTP should be excluded in any new patient presenting with clinical evidence of TMA (see Table 1). For the moment, we suggest to start plasmapheresis therapy in any patient with TMA and suspected HUS, while awaiting test results for STEC–HUS and TTP. Based on the published data, we feel confident that patients can be observed for 4 days with daily plasmapheresis. This not only allows proper diagnostic tests to be performed and reported, but also ensures that patients with early remission will be identified, which would limit the need for eculizumab therapy. If patients do not respond after 4 days, treatment with eculizumab should be started. It is debatable if earlier start of therapy could be more beneficial, and this can be the topic of a future randomized clinical trial (RCT).

The duration of therapy is also for debate. In the clinical trials, patients were treated for a period of 6 months, with an extension of another 2 years. Literature data do not allow any firm conclusion regarding the optimal duration of therapy. Obviously, many patients with confirmed genetic mutations develop aHUS in adulthood, indicating that it is possible to live without aHUS for a prolonged time period without any therapy. Moreover, many patients with aHUS in the pre-eculizumab era recovered renal function and never relapsed. In a recent report by Ardissino et al. [70], eculizumab was successfully withdrawn in 7 out of 10 patients. We suggest that it should be considered to withdraw eculizumab after 3–12 months of therapy, provided all signs of TMA have disappeared and close follow-up is guaranteed which must be continued lifelong. Future studies should address the issue of treatment duration. Furthermore, it is important to develop biomarkers that predict ongoing endothelial damage as well as relapse. Treatment will also benefit from assays that allow therapeutic drug monitoring in the individual patient.

**KIDNEY TRANSPLANTATION IN aHUS**

Kidney transplantation in patients with ESRD due to aHUS carries a poor prognosis. This is explained by the fact that many factors that can contribute to disease development are present after kidney transplantation. These include genetic susceptibility factors carried by the recipient or the donor. In addition, there are factors such as donor graft injury (related to the donor procurement, cold ischaemia time and reperfusion injury), acute rejection, use of mTOR inhibitors and CNIs, and hypertension and lipid-mediated vascular injury. The detrimental outcome of kidney transplantation in patients with aHUS was a reason for many authors to argue against kidney transplantation as mode of renal replacement therapy.

Although RCTs are lacking, it is evident that eculizumab has significantly improved the prognosis of kidney transplantation in aHUS patients [64, 65]. We suggest that the introduction of eculizumab must lead to a changing view of kidney transplantation in aHUS. Kidney transplantation should be considered in patients with ESRD due to aHUS. Although some authors prefer prophylactic therapy with eculizumab, the available data do not provide evidence that this is necessary. In fact, early start of therapy after onset of recurrent disease resulted in good outcome with complete recovery of renal function [65]. Clinical studies must address the optimal treatment protocol. Furthermore duration of therapy is unknown. It has been shown that treatment could be interrupted for periods of up to 21 months [60]. Our recent experience suggests that living kidney donation can be considered and may even be preferable in patients with aHUS. We reported four patients with ESRD and proven aHUS, all at moderate to high risk for disease recurrence based on the known mutations or a history of previous graft loss due to recurrent disease [71]. The patients received a kidney graft of a living donor, thus reducing ischaemia time and preventing the injury that normally occurs in a brain-death donor. They were transplanted using a protocol that eliminates as much as possible triggers of endothelial damage. Details are presented elsewhere [71]. The protocol included the immediate aggressive treatment of blood pressure and cholesterol, used a low dose of CNI and quadruple therapy to limit the risk of rejection. No plasmapheresis or eculizumab was given prophylactically. Thus far, we did not observe recurrent disease in these four patients during a follow-up that now extends 24–30 months. Oyen et al. [72] also described seven aHUS patients who were successfully transplanted without aHUS recurrence using a CNI-free regimen. Of the seven patients, four had received a kidney from a living donor. Controlled clinical trials should address the possible advantage of prophylactic therapy and study the optimal duration of therapy.

When considering a living kidney transplantation in a patient with aHUS, donor selection is an important issue. If the donor is a close relative, it is important to exclude that the potential donor carries the disease-causing mutation. This can only be tested if the mutation is known in the recipient. In patients with aHUS without identified mutation, the situation is even more complex. We suggest that in such cases, family members may not be used as donor.

**THERAPIES UNDER INVESTIGATION**

Recombinant CFH or a CFH protein concentrate is being developed for the treatment of patients with CFH mutations. Alternative therapies include drugs that target other molecules in the complement pathway such as C3, C1R or the C3/C5 convertase. Thus far, these drugs have not been used clinically [73–75]. In the future, even gene therapy may become a realistic option.

**CONCLUSION**

With increasing knowledge of the involvement of the alternative pathway of the complement system in aHUS, it has become possible to better characterize patients with HUS. It is evident that the complement inhibitor eculizumab is effective and has greatly improved outcome of patients with aHUS. Many unresolved questions remain, such as the underlying cause of aHUS in patients without detectable complement mutations and the optimal treatment schedules.
REFERENCES

32. Loriot C, Fremeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis 2011; 6: 60

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CONFLICT OF INTEREST STATEMENT

N.C.A.J.v.d.K. is a member of the Alexion International Advisory Board of aHUS.
67. Martin NG, Snape MD. A multicomponent serogroup B meningococcal vaccine is licensed for use in Europe: what do we know, and what are we yet to learn? Expert Rev Vaccines 2013; 12: 837–858

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