REVIEW ARTICLE

Atypical haemolytic-uraemic syndrome: reflecting over the old and new

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◼ ABSTRACT

Atypical haemolytic uraemic syndrome is a rare disease characterised by microangiopathic haemolytic anaemia, thrombocytopaenia and predominant renal impairment in the absence of Shiga toxin-producing bacteria. For long time it has been difficult to distinguish it from other thrombotic microangiopathies, but in the last decade advances have been made in understanding the pathogenesis of atypical haemolytic uraemic syndrome as a disorder of alternative pathway of the complement system. Knowledge of mutations and polymorphisms in the genes encoding the complement regulatory proteins revealed clinical importance in the management of the patients, altering not only the transplantation perspective but also leading to the search for new drugs, something that will potentially change the poor prognosis of these patients.

This article reviews the differential diagnosis of this thrombotic microangiopathy to reflect on current treatment options and discuss new therapies.

Key-Words:
Alternative complement pathway; atypical haemolytic-uraemic syndrome; eculizumab; plasmapheresis; transplantation.

◼ INTRODUCTION

The term thrombotic microangiopathy (TMA) has been used to describe a histopathological entity characterised by the presence of fibrin and/or platelet thrombi in the microcirculation of various organs[1]. It includes two main syndromes, the haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopaenic purpura (TTP). They both present clinically with microangiopathic haemolytic anaemia and thrombocytopaenia and histologically with vascular abnormalities, namely glomerular endothelial damage, swelling of the endothelium, endothelial detachment of the basement membrane, intima fibrosis and thrombosis. For a long time they were only distinguished by clinical aspects: HUS characterised by predominant renal involvement, i.e. acute renal failure, and TTP by predominant neurological involvement. In many patients, however, the clinical presentation overlaps, making a precise diagnosis impossible.

In the last few years, two important turning points have allowed the distinction between these two entities. The first was the identification that ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type 1 repeats) deficiency is more likely to present with the insidious or fluctuating neurological signs of adult idiopathic TTP[2-5]. The second was the finding that abnormal control of the alternative complement pathway is a risk factor for atypical HUS (aHUS)[6,7]. Since then, the discrimination between these two TMA is made by measuring ADAMTS13 activity, which is greatly reduced (5 to 10% of normal) in TTP. This is mostly due to autoantibodies against ADAMTS13 because congenital TTP, caused by mutations in the ADAMTS13 gene, is an extremely rare autosomal recessive disease (incidence 1:1,000,000)[8].

HAEMOLYTIC-URAEMIC SYNDROME

It is important to distinguish the disease triggered by an infection with Shiga-like toxin producing *Escherichia coli* (STEC), which accounts for 90%, from those which are not, as management and prognosis of these patients is completely different.

STEC-HUS, also called typical or diarrhoea-associated HUS (D+HUS), is a disease especially affecting young children between two and six years old who are infected by enterohaemorragic *Escherichia coli* serotype 0157:H7 or in some tropical regions *Shigella dysenteriae* type 1. Three to eight days after contamination, the patient develops abdominal pain with watery and/or bloody diarrhoea, followed within 24 hours by haemolytic anaemia, thrombocytopaenia and acute renal failure. In 2011 the world's largest STEC outbreak occurred in Germany, affecting mostly adults above 20 years old and predominantly females. It was attributed to changes in the microbial characteristics of the bacteria (STEC O104:H4)[9], indicating that changes in the bacterial characteristics can lead to changes in host profile.

STEC-HUS resolves spontaneously, and no other intervention such as plasmapheresis has shown to be superior to supportive management of renal failure, anaemia, hypertension and fluid-electrolyte imbalance[10].

Mortality in children with STEC-HUS is 3 to 5% during the acute phase of the disease[11]; about 75% of the patients recover completely after a STEC-HUS episode[12]. In the German outbreak, although a greater proportion of patients infected with STEC O104:H4 eventually developed HUS[9], both clinical course of individual patients and mortality (~4%) seemed to be comparable with historic reports[13].

When STEC-HUS progresses to end–stage renal disease (ESRD), kidney transplantation is an option, with no recurrence of the disease[14].

Non-STEC-HUS or aHUS is only seen in 5 to 10% of all HUS cases[15]. It differs from STEC-HUS in that it can appear at any age; patients have a poor prognosis with a high mortality and morbidity in the acute phase of the disease; and progression to ESRD occurs in 50% of cases[16,17].

Many causes of aHUS have been identified, such as associations with non-enteric infections (especially

Table I

Causes of thrombotic microangiopathy

Infectious
– Infection with Shiga-like toxin producing Escherichia coli (STEC)
– Infection with neuraminidase producing Streptococcus pneumonia
– Human immunodeficiency virus (HIV)
– Other viruses: Cytomegalovirus, Parvovirus
Complement Dysregulation
– Genetic abnormalities in complement (regulating) proteins
– Acquired defects (auto antibodies against CFH)
ADAMTS 13 deficiency
– Genetic abnormalities
– Auto antibodies against ADAMTS 13
Clinical associations but unknown aetiology
– Defective cobalamin metabolism
– Systemic diseases: SLE, antiphospholipid syndrome
– Medication: ticlopidin, mitomycin, bleomycin, cysplatin, quinine, tacrolimus, ciclosporin, rifampicin, clopidopogrel, oral contraceptives
– Malignancies: chemotherapy
– Transplantation: calcineurin inhibitors, rejection
– Bone marrow transplantation: radiation, medication, graft vs host disease
– Pregnancy: pre-eclampsia, HELLP syndrome
– Glomerulopathies: MPGN type II

Streptococcus pneumoniae infections, called neuroaminidase associated-HUS), viruses, malignancies, drugs, bone marrow and kidney transplantation, pregnancy, and systemic diseases. The recently recognised disorders of complement regulation will be outlined in this review.

The European Pediatric Research Study group for HUS proposed a new classification to TMA based on its cause (Table I)[18,19] which distinguishes different TMAs, allowing guided investigation into its diagnosis and treatment.

aHUS: A DISEASE OF ALTERNATIVE COMPLEMENT PATHWAY DYSREGULATION

The human complement system is part of the innate immunity. Three activation pathways are recognised: the classical pathway, the mannose binding lectin pathway, and the alternative pathway. In aHUS, the alternative pathway is mostly affected.

Since 1974, reduced serum levels of complement fraction C3 with normal levels of C4 have been

reported in patients with aHUS[20-23] but it was only in the last decade that a clear link was demonstrated between aHUS and genetic abnormalities in complement (regulating) genes. The most frequently reported mutations (50-60%) are in the gene encoding complement factor H (*CFH*), a plasma protein synthesised by the liver which downregulates alternative pathway activation[24]. More than 100 mutations have been described[25]. Many patients with heterozygous *CFH* mutations have normal CFH protein level but abnormal function.

Less frequently mutations in the genes encoding membrane cofactor protein (*MCP*) and complement factor I (*CFI*) are present. MCP or CD46 is a widely expressed transmembrane glycoprotein that inhibits complement activation in host cells by serving as a membrane-bound cofactor for CFI to cleave C3b and C4b. About 35 different mutations have been described, mostly heterozygous. They are responsible for 10 to 15% of patients presenting with aHUS[26,27].

CFI is a 2-chain serine predominantly synthesised by the liver that downregulates the alternative pathway by cleaving C3b, but it is efficient only in the presence of cofactor proteins (i.e. CFH and MCP). Between 5% and 13% of aHUS patients have an *CFI* mutation, and approximately 25 different mutations have been reported, all heterozygous[26,28-31].

More recently, a subgroup of aHUS patients without mutations in the genes encoding complement regulatory proteins but showing persistent activation of the alternative pathway with very low serum levels of C3 and normal or elevated serum levels of C4 have been identified. They were called gain-of-function mutations because they result in enhanced formation of C3bBb convertase or increased resistance to inactivation by complement regulatory proteins[32]. Mutations in complement factor B (*CFB*) and complement C3 (*C3*) were proposed and are reported in 1 to 2% and in 4 to 10% respectively of aHUS patients, with heterozygous mutations, usually with low C3 levels [26,33].

There is a growing list of the mutations, polymorphisms and other complement abnormalities that alone or in combination account for about 10% of all aHUS, especially of *CFI* gene mutations with either *CFH* or *MCP* gene mutations. Mutations in the gene *THBD* encoding thrombomodulin, a membrane-bound glycoprotein with anticoagulant properties that

modulates complement activation on cell surfaces, have been very also associated to aHUS[34]. These mutations may be acquired as autosomal recessive cases or autosomal dominant but the prognosis is not influenced by the inheritance[11]. The absence of a familiar history does not exclude the possibility of a genetic transmission because of an incomplete penetrance of the disease. The penetrance of disease among carriers of mutations in *CFH*, *CFI*, and *MCP* is approximately 50 to 60%[35]. This indicates that the genetic aberrations are probably important for the development of aHUS, but not the sole cause; an environmental factor, such as a complement trigger, is probably needed to develop the disease[36].

Antibodies against factor H (αFH) have also been observed in patients with aHUS, and in most cases in association with homozygous deletion of the genes encoding complement factor H-related proteins 1 and 3 (*CFHR1* and *CFHR3*)[37].

■ INVESTIGATION IN AHUS PATIENTS

The screening for all mutations mentioned above takes time and does not influence the initial treatment which should be started as soon as possible, but the analysis of the specific aHUS predisposing defects may help to establish differential diagnosis when the clinical presentation is ambiguous. It also influences long-term clinical management of affected patients, namely when considering transplantation[38-41]. STEC infection has to be ruled out because the classification of patients as STEC-associated or aHUS may be difficult: firstly, 10% of the patients do not present with diarrhoea[42], and STEC-HUS can occur in adults as well, as in the German outbreak. Such patients, if not tested for STEC, could erroneously be classified as aHUS. Secondly, STEC infection criteria (positive PCR for Stx genes in stools and/ or circulating anti-lipopolysaccharides antibodies) are negative in approximately 15% of STEC-HUS patients[43], leaving the physician concerned that the patient might in fact have aHUS. Thirdly, gastroenteritis was the triggering event in up to 28% of aHUS patients, including patients with *CFH, IF,* or *MCP* mutations, in the French paediatric series[44].

To select patients who should be analysed for complement mutations, the European Pediatric

Research Study group for HUS suggests all patients who present with atypical features, regardless of whether they have typical signs such as a prodromal diarrhoeal illness[41,45]. Mutational screening should be performed in the complement genes that have been associated with aHUS (*CFH, CFI, MCP, C3, CFB,* and *THBD*), irrespective of serum C3, CFH, or CFI levels because their deficiency is not enough to exclude the diagnosis. It must be stressed that most assays measure the presence of the protein and not the activity. Moreover, abnormalities in complement regulation may only occur at the level of the endothelial cell surface, and not systemically. Therefore, serum levels may be normal in patients with complement dysregulation[16,38]. Moreover, in 40% of patients no mutation is found (un-genotyped patients).

Both aFH and ADAMTS13 should be searched for, the latter to exclude TTP. The possibilities of a rare cause of aHUS, such as HIV infection, pregnancy, or cobalamin deficiency, should be considered and investigated at presentation[36].

An overview of the investigations to be performed in patients with aHUS is shown Table II.

■ TREATMENT OPTIONS

■ Plasma therapies

For a long time, the results of studies into the role of plasmapheresis in HUS have been controversial, because trials did not distinguish between STEC-HUS (where plasmapheresis seems not to change prognosis) from aHUS. Based on cohort studies that showed a 25 to 50% decrease in mortality rate since the introduction of plasma therapies[36], guidelines propose the beginning of plasmapheresis within 24 hours of diagnosis[41,45,46].

Exchange 1.5 times the expected plasma volume (60 to 75 ml/kg) and replace plasma with fresh frozen plasma or virus-inactivated pooled plasma is suggested. Plasmapheresis should be performed daily for five days, then five sessions a week for two weeks, and then three times a week for two weeks[18]. The total treatment time is not determinate, but recommendations state that treatment should be continued for at least two days after complete remission has been achieved. The dose and frequency may be reduced to weekly or biweekly intervals if plasma therapy appears to be successful. The parameters proposed to define patient remission are platelet count and lactate dehydrogenase levels in serum, since haptoglobin levels often remain decreased after achieving haematological remission. Some aHUS patients will remain plasma dependent and need chronic plasma treatment to stay in remission. It is also suggested that intercurrent infections and vaccinations can trigger a relapse of aHUS[47,48] for which plasmapheresis should be started again or intensified.

For patients with isolated *MCP* mutation, plasma therapy has limited value since MCP is a membrane-bound protein, meaning the defect cannot be substituted by plasma therapy. In these cases, remission is achieved in 80 to 90% of these patients' plasma therapy[36]. However, it is important to stress that by the time of first presentation, it is not known which

Table II

Investigations that should be performed in patients presenting with HUS

Infectious
STEC infection:
– Stool Culture
– PCR
– serology, anti-O157 antibody
Streptococcus pneumoniae infection:
– Culture
– PCR
Human immunodeficiency virus:
– HIV serology
Complement Dysregulation
– C3 and C4 levels
– C3d
– CFH and CFI
– Surface expression MCP
– Mutational screening CFH, CFI, MCP, C3, and CFB
ADAMTS 13 deficiency
– Auto antibodies against ADAMTS 13
Clinical associations but unknown aetiology
Defective cobalamin metabolism:
– Homocysteine levels
– Methylmalonic acid levels
– Mutational screening MMACHC methylmalonic aciduria and homo-cystinuria type C protein
Systemic diseases:
– Antinuclear antibody
– Lupus anticoagulant
– Antiphospholipid antibody
Pregnancy:
– Pregnancy test
– Liver enzymes (HELLP syndrome)

complement genes are involved in the pathogenesis of aHUS and since it is recognised that combined mutations can occur, plasma therapy remains the first choice for treatment.

Plasmapheresis is the optimal treatment for patients with aFH, associated with steroids and immunosuppressive treatment (azathioprine, cyclophosphamide, mycophenolate mofetil, or rituximab) to prevent the redevelopment of antibodies after plasmapheresis cessation[49,50].

If plasmapheresis is not available or cannot be applied immediately in the acute phase, plasma infusion should be started[25]. When plasma infusion is used instead of plasmapheresis, the suggested dosage is 30 to 40 ml/kg initially and 10 to 20 ml/kg per day thereafter[16] because of the risks associated with the infusion of volume in patients who are already hypertensive and overloaded due to renal impairment. It can also be used after the initial period of plasmapheresis unless the tests have demonstrated that antibodies are the cause of the disease.

Plasmapheresis also plays an important role in transplantation, as stated above.

In addition to plasmapheresis, avoiding triggers of endothelial injury, such as hypertension and hypercholesterolemia, by adequate blood pressure control and the use of statins are important treatment options in the acute phase of the disease and should be maintained once in remission[51].

■ Transplantation

Kidney transplantation

Transplantation has also been controversial in aHUS because of the elevated risk of graft loss in the first year from 38%[44] to 83%[52] depending on the studies. This is attributed to an elevated risk of recurrence but also to a higher proportion of acute rejections[53].

The underlying genetic defect predicts a different risk of recurrence. The biggest risk is seen in patients with a *CFH* mutation with a recurrence rate of 75 to 90%, followed by patients with a *CFI* with 45 to 80% and *C3* mutation with 40 to 70%. Recurrences have been reported in patients with *CFB* and *THBD* mutations, as well[54]. Mutation in the gene encoding the membrane-bound MCP is at the lowest risk of developing a disease recurrence in the graft (<20%)[36]. As the graft has normal MCP levels, it would be expected that the risk would be zero. Nevertheless, cases have been reported[26,55] probably because of the association of other mutations[26].

A high proportion of vascular thrombosis has also been observed, probably because of the thrombogenic role of complement dysregulation[26]. The French paediatric series[44] reported that of the 24 renal transplants performed in 15 aHUS children, 16 (67%) failed, and 66% of patients had at least one graft failure. Of the 16 graft failures, eight (50%) were due to graft vascular thrombosis 0-45 days after surgery.

Prevention of post-transplant aHUS recurrence

In the attempt to decrease recurrence of the disease, several hypotheses have been studied. Bilateral nephrectomy has been proposed because of a French cohort of non-genotyped adult patients who reported an increased risk of post-transplant recurrence in patients who still had their native kidneys compared with those who haven't[55]. It was not observed in another series[32]: among genotyped aHUS patients, although 93% (14/15) of the patients in a French paediatric group[44] had bilateral nephrectomy for hypertension before transplantation, the post-transplant recurrence rate was not lower than that reported in other genotyped cohorts[38,51]. Therefore, it is doubtful that pretransplant bilateral nephrectomy is beneficial in preventing post-transplant recurrence.

The avoidance of calcineurin inhibitors as also been proposed since *de novo* TMA have been reported with the use of these drugs however it was not associated with an increased incidence of HUS recurrence[14,53,57]. Further, HUS recurrence has been reported in aHUS patients treated by sirolimus, including one with MCP mutation[54]. In the consensus recommendation of 2009[46] no specific guidance is given about immunosuppression protocols, meaning aHUS was not considered *per se* a specific contraindication for treatment with calcineurin inhibitors.

Furthermore initial immunosuppression with mammalian target of rapamycin inhibitors is not encouraged

in the absence of clear clinical advantages because of the possible association with impaired wound healing and delayed graft function[58].

Finally, treatment with IVIG has occasionally been reported as efficient in aHUS patients, for instance in one child with non-genotyped aHUS[59], or to treat post transplant recurrence in a child with *CFI* and *CFB* mutation (1 g/kg every three weeks for five and a half years[43] or in a patient with *MCP* mutation[54]. Neutralisation of C5a complement activation product may be one of mechanism of action of IVIG[60].

Living-related kidney transplantation

aHUS has been considered a contraindication for living related kidney transplantation, not only for the high risk of aggressive relapse in graft[61] but also because of the risk for the related donor who could himself develop HUS sometime after kidney donation. It was reported in four donors aged 21-31 years old, who donated a kidney to one of their children or siblings and had HUS three weeks to 10 months after donation[62-64]. It was also demonstrated in one patient who had HUS shortly after unilateral nephrectomy secondary to a traffic accident and was discovered to have a *CFH* mutation. The haemodynamic changes induced by unilateral nephrectomy could be the trigger for HUS in the donor with a predisposing genetic anomaly[52]. Even when no mutation is found, donation is not recommended because some polymorphisms have been linked to aHUS, as well as still unknown mutations.

Combined liver-kidney transplantation

The fact that the most frequent mutations occur in two circulating proteins synthesised by the liver, CFH and CFI, meant the advent of liver transplantation created great expectations. It would theoretically restore normal complement regulation and prevent disease recurrence. However, the first three combined liver-kidney transplantations in children with CFH deficiency occurred between 2002-2005 were disappointing, as the three died soon after. Autopsy of the liver revealed diffuse thrombotic and ischaemic lesions, most likely due to the thrombogenic effect of complement activation products deposited on the microvasculature of the liver after transplantation. Taking into account that liver transplantation might trigger intense local complement activation, it was suggested that liver transplantation should be performed under intensive pre- and perioperative plasma therapy to correct complement dysregulation. The first successful combined liver-kidney transplantation was done under such protocol and was reported in 2006[46].

This increased the bioavailability of functional CFH during the critical period needed for the liver graft to recover synthetic functions and, at the same time, removed the endogenous mutant CFH. In addition, posttransplant anticoagulation with low-molecular-weight heparin at prophylactic dosages and low-dose aspirin was used in each of the successful procedures[46]. This knowledge led a consensus group to propose protocols to make isolated kidney and combined-kidney transplantation[46].

However, risks associated with this procedure still remain, and assessment of the risk/benefit ratio requires careful and individual attention. In addition, in the absence of a noted mutation, comprising a sizable fraction of patients with aHUS, liver-kidney transplantation should be avoided[46].

■ Emerging new therapies

Concentrated CFH

In patients with genetic *CFH* abnormalities it seems obvious to give normal CFH. A human plasma-derived CFH concentrate has been developed commercially with that intention, and it received the European orphan drug designation in January 2007. Substitution with CFH concentrate is a therapeutic option for patients with quantitative and functional CFH deficiency but it will have to be taken into account that such commercial concentrates have a short half-life. The same rationale applies to aHUS associated with *CFI* gene mutations but a CFI concentrate is still not available[34].

Eculizumab

Eculizumab is a monoclonal antibody that inhibits the production of the terminal complement components C5a and the membrane attack complex C5b-9 by binding to complement protein C5. It has been approved since March 2007 for the treatment of patients with paroxysmal nocturnal haemoglobinuria (PNH). In September 2011 the U.S. Food and Drug

Administration (FDA) also approved it for the treatment of aHUS patients based on its ability to prevent formation of C5a and the terminal complement complex inhibiting complement-mediated thrombotic microangiopathy.

By March 2012, 21 cases were reported in the literature of aHUS treated with eculizumab, including eight cases in recurrence in transplanted kidney, and three in prophylactic treatment after kidney transplant. Eighteen patients went into complete or partial remission with functional kidney recovery and no need for subsequent renal replacement therapy. The longest period of remission has been observed in a patient treated with eculizumab for 28 months with no evidence of aHUS recurrence. Relapses after eculizumab treatment have only been seen when the treatment was discontinued or in patients who received a single dose[65].

In addition to the above case reports, two prospective clinical trials have been conducted to evaluate the safety and efficacy of eculizumab use in aHUS[66,67]. In one study, seventeen patients with aHUS resistant to or intolerant of plasma therapy were treated with eculizumab for a minimum of 26 weeks. These patients showed decreased signs of TMA activity, including improvement in platelet counts and eGFR. In a second study, twenty patients with aHUS undergoing chronic plasma exchange or plasma infusion therapy were treated with eculizumab for a minimum of 26 weeks.

Adverse effects that were most frequently reported were hypertension, upper respiratory tract infection and diarrhoea. Meningococcal disease has been reported from PNH use and it was attributed to the impaired capacity for opsonization and clearance of encapsulated organisms[68]

Patients should be vaccinated at least two weeks before the start of the treatment. As vaccination does not protect against all serotypes, both patients and physicians should be aware of early signs of meningococcal infection[69]. Attention also has to be paid to patients treated with immunosuppressive drugs, as these therapies can reduce the response upon vaccination.

In STEC-HUS patients, eculizumab is not indicated as a treatment option.

Eculizumab is administered as an intravenous infusion. The recommended dosing for adult patients with aHUS is 900 mg weekly for the first four weeks, followed by 1200 mg weekly one week later, and 1200 mg every two weeks thereafter. The dosage regimen for paediatric patients is based upon body weight[70].

Turning to the use of eculizumab in transplantation, a protocol has been proposed in pre-emptive use for isolated kidney transplantation[71], which introduces some changes to previous protocols performed before eculizumab approval, but more studies are required to support the evidence of its use.

■ CONCLUSION

aHUS will always be a challenge to diagnose because it overlaps other TMAs. A differential diagnosis is crucial to the management of the entity. The knowledge of mutations associated to the regulators of alternative pathway was an important step in moving forward. Patients who should be studied have to be selected before starting treatment. Plasmapheresis is still the first choice but until now eculizumab has not been considered. This drug may represent a new hope for the treatment of primary disease and for recurrence after renal transplantation. Furthermore, although eculizumab is very expensive, it is expected to free dependent patients from plasmapheresis which is also an expensive treatment, with the advantage of granting a better quality of life.

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