Review

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Atypical hemolytic uremic syndrome: from diagnosis to treatment

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Abstract: Thrombotic microangiopathy (TMA) is a relatively rare condition but a medical urgency requiring immediate intervention to avoid irreversible organ damage or death. Symptoms on presentation include microangiopathic haemolytic anaemia, thrombocytopenia and organ damage. The most frequent direct causes of TMA are thrombotic thrombocytopenic purpura (TTP) and haemolytic uremic syndrome (HUS). The most common form of HUS is related to Shiga toxin producing *Escherichia coli* (STEC) infection while approximately 10% of cases are due to dysregulation of the complement pathway (atypical haemolytic uremic syndrome, aHUS). Optimal treatment regimens differ depending on the underlying cause; however, differential diagnosis may be difficult. The most accurate method of diagnosis is based on exclusion and should consider, beyond the symptoms common to TMA, ADAMTS13 activity levels and STEC infection status. For the management of TTP, plasma exchange (PE) is the most important acute intervention and is associated with lower mortality and better outcomes than plasma infusion. In most patients with STEC-HUS, the course of disease is self-limiting although management of acute kidney injury is often required. Until recently, the management of aHUS consisted of early and intensive PE, although this was mostly ineffective in protecting from subsequent organ damage. Eculizumab, an inhibitor of the alternative complement pathway, produces a rapid and sustained inhibition of the TMA process, with significant improvements in long-term clinical outcomes. Due to the significant improvement achieved, eculizumab has subsequently been approved as first-line therapy when an unequivocal diagnosis of aHUS has been made.

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Introduction

Thrombotic microangiopathy (TMA) is a relatively rare disorder which may be initiated by numerous causes and manifests as microangiopathic haemolytic anaemia, thrombocytopenia and organ damage. The condition is life-threatening and requires immediate management to avoid irreversible organ damage or death. In recent years major improvements have been made in understanding the nature of TMA, thus opening the door for specific, more effective treatment [1].

The most common causes of TMA are thrombotic thrombocytopenic purpura (TTP) and haemolytic uremic syndrome (HUS). In both TTP and HUS, TMA is an integral part of the disease. TMA may also present in other contexts, e.g., malignancy, chemotherapy or bone marrow transplantation [2], but the medical history of the patient can usually rule out these causes.

Here, I will first review the pathophysiology, epidemiology and clinical presentation of TMAs. Then, I will describe in more detail how recent advances in diagnostic and therapeutic processes can help differentiate amongst TMAs and describe how the use of eculizumab has transformed the treatment paradigm for atypical haemolytic uremic syndrome (aHUS).

Pathophysiology

TMA is defined by thickened arterioles and capillaries, swollen and detached endothelial cells, widened subendothelial spaces and accumulation of proteins and cell debris. Blood vessel obstruction occurs due to aggregated platelets, along with haemolysis, and blood smears show fragmented or distorted erythrocytes [3]. This causes

widespread thrombosis and organ ischaemia giving rise to the classical clinical features of TMA and organ failure in TTP, aHUS and Shiga toxin producing *Escherichia coli* (STEC)-HUS.

Thrombotic thrombocytopenic purpura

In TTP, deficiency of plasma ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) activity causes the accumulation of ultra-large multimers of von Willebrand Factor (VWF) on the endothelium. Under normal physiological conditions, ADAMTS13 cleaves the ultra-large VWF released from activated endothelial cells or following vascular injury. This process is crucial to control VWF activity and maintain equilibrium of haemostasis and thrombosis in plasma. Severely reduced ADAMTS13 activity (<5%–10%) results in aggregation of platelets on the ultra-large VWF and widespread microthrombi formation in small arterioles and capillaries throughout the body – the characteristic pathological feature of TTP [4].

There are two variants of TTP; the hereditary form, also called Upshaw-Schulman syndrome, with mutations in the ADAMTS13 gene and the acquired form caused by autoantibodies directed against ADAMTS13. Patients with both congenital and acquired TTP have been shown to lack ADAMTS13 in the plasma or have autoantibodies severely reducing its function, respectively [5–7].

Haemolytic uraemic syndrome

The most common form of HUS (90% of cases) is that caused by a prodromal bacterial infection (Shiga toxin *Escherichia coli* – STEC). The link to Shiga toxin was first proposed in 1983 [8]. Destruction of endothelial cells is likely key to the pathogenesis of STEC-HUS. Cellular damage is believed to be mediated through the transport of Shiga toxins produced in the bowel to capillary beds where the toxin initiates cell death via inhibition of protein synthesis [9]. Shiga toxin has also been found to directly activate complement and interact with complement factor H (CFH) in vitro, suggesting a mechanistic link between complement regulation and STEC infection. Damage of endothelial cells would in itself activate complement [10]. Together the platelet and complement activation and endothelial damage leads to platelet aggregation, small vessel obstruction, mechanical haemolysis and TMA, mainly in the kidney but also in other organs, notably the central nervous system (CNS) and gastrointestinal tract [9, 11, 12].

Atypical haemolytic uraemic syndrome

In aHUS, the pathophysiological consequences result directly from damage caused by the uncontrolled activation of the alternative complement pathway leading to excessive complement activation on cell membranes [13, 14]. A link between complement and aHUS was first reported in 1981 in two brothers who had a deficiency of CFH and mutations in the gene encoding CFH were recognised to be associated with aHUS in 1998 [15, 16]. Since then, mutations in multiple other factors that facilitate uncontrolled complement activation by the alternative pathway have been identified in patients with aHUS [17].

A brief overview of the complement cascade showing the central role of the C3 convertase in the generation of the final membrane attack complex (C5b-9) is shown in Figure 1. The different roles of cleavage products in microbial defence and removal of dead or damaged cells is also shown. Mutations in complement system genes have been identified in 50%–60% of patients with aHUS [13, 14]. The mutations identified impair regulation in the alternative pathway at the level of the C3 convertase and Figure 2 describes these mechanisms.

Mutations in patients with aHUS have also been identified in the coagulation pathway (thrombomodulin and plasminogen), linking these two pathways to the development of aHUS [18, 19]. Recently, mutations in diacylglycerol kinase ε (DGKE) have been found to cause HUS-like

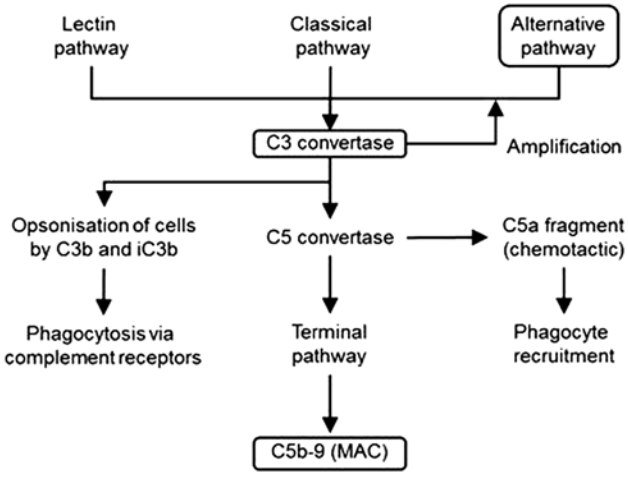

Figure 1: A simplified overview of the complement cascade. The alternative pathway involves the constitutive low level activation of C3 and the subsequent deposit of C3b on cell membranes, ultimately leading to the generation of the membrane attack complex, (MAC), C5b-9. Regulation of the complement cascade is described in Figure 2.

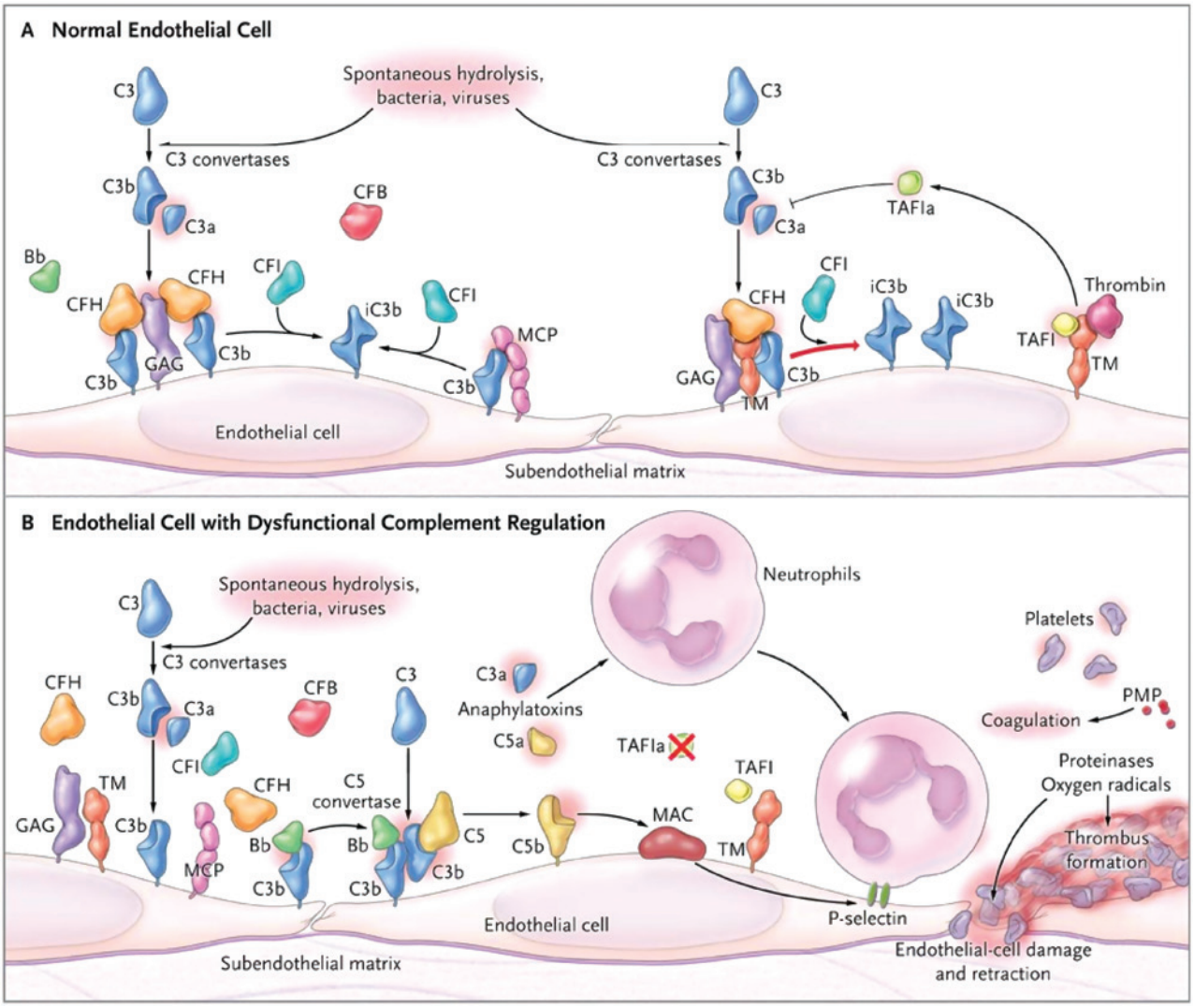

Figure 2: Model for the mechanisms leading from impaired regulation of the alternative pathway to thrombotic microangiopathy. In a normal endothelial cell (A), complement factor H (CFH) binds to the endothelial surface and to C3b and together with membrane cofactor protein (MCP) acts as a cofactor for cleavage of C3b, which is mediated by complement factor I (CFI), a process that prevents its interaction with factor B. CFH also dissociates the C3 convertase of the alternative pathway (C3b). Thrombomodulin (TM) enhances CFI-mediated inactivation of C3b in the presence of CFH and promotes activation of the thrombin-activatable fibrinolysis inhibitor (TAFIa), which degrades C3a and C5a. In patients with loss-of-function mutations in complement regulatory genes [CFH, CFI, MCP, and THBD (the gene encoding thrombomodulin)] (B), C3b is not degraded efficiently and forms the C3 and C5 convertases of the alternative pathway. A similar situation applies to patients with gain-of-function mutations in CFB and C3. Mutant CFB forms a superconvertase that is resistant to dissociation by CFH. Mutant C3b does not bind CFH and MCP and is resistant to degradation by CFI. From Noris et al. [14]. Reprinted with permission from Massachusetts Medical Society.

disease. Currently, it has only been described in paediatric patients younger than 1 year [20] and has also been associated with a membranoproliferative glomerulonephritis (MPGN)-like syndrome in nine patients from three families [21]. Some of these patients with mutations in DGKE have been found to carry additional mutations in complement genes, and therefore the involvement of complement dysregulation in patients with DGKE mutations remains unclear [18].

Epidemiology and clinical presentation

Interestingly all three clinical entities have variable penetrance. Although onset of the hereditary form of TTP is in the neonatal period or early childhood, approximately 10%–20% remain asymptomatic until over 20 years of age [22]. It is not clear what determines this variability, but

further environmental and possible genetic triggers may be involved [17, 23].

A similar situation is true for STEC-HUS where not all patients with STEC infection develop STEC-HUS. In the German outbreak in 2011, 19% of patients infected with STEC developed STEC-HUS [24].

Penetrance is also incomplete in aHUS, and seems to be 40%–50% among carriers of CFH, membrane cofactor protein (MCP), and CFI mutations [25]; healthy carriers of C3 and CFB mutations have also been described [26, 27]. There are numerous reports describing the onset of aHUS symptoms in association with an environmental trigger. Infectious diseases have been associated with 22%–55% of clinical cases of patients with mutations in CFH, MCP, CFI or C3[25] and diarrhoea (including STEC diarrhoea) may also precede aHUS in as many as 30% of cases [28, 29]. Pregnancy, drugs, malignancy, connective tissue disorders and specific metabolic defects are also known triggers [30]. The full range and function of environmental parameters associated with onset of aHUS is not completely understood.

Thrombotic thrombocytopenic purpura

The first known description of what is now considered as TTP was by Moschcowitz in 1924. He described a 16-year-old girl presenting with pallor, weakness, purpura and haemiparesis who died within 2 weeks [31]. It is now well understood that most cases in adults are caused by the acquired form with autoantibodies to ADAMTS13. Classically TTP is described by widespread VWF and platelet rich microthrombi classically affecting the CNS, but TMA can occur in any organ.

The hereditary form of TTP is rare and only accounts for 2%–4% of all TTP cases [32, 33]. It occurs primarily in neonates and children, but adult onset cases have been reported [33, 34]. Acquired TTP occurs mostly in adults [35]. While it is a rare disease (approximately six cases per million per year in the UK) [36], the prognosis if untreated is very poor and today mortality remains at 10%–20% in acute TTP [17, 37].

HUS: STEC-HUS and aHUS

The term HUS was first used in 1955 by Gasser et al. to describe five children, aged 2 months to 7 years, with acquired haemolytic anaemia, unusual poikilocytes and renal insufficiency, three of whom had thrombocytopenia; all the patients died [38]. The pathological hallmarks of HUS are platelet-fibrin rich microthrombi causing small vessel thrombosis, schistocytosis, and TMA in the kidney and also other organs [39].

Overall, approximately 90% of HUS cases are due to STEC, which although predominantly considered a paediatric disease, is well known to also affect adults [24]. The frequency of STEC-HUS in North America has been reported as approximately two or three cases per 100,000 in children under 5 years of age [9]. Worldwide the reported incidence varies widely, and it should also be noted that large, localised outbreaks occur due to the ingestion of contaminated food or water [9, 24]. For example, the outbreak in Germany in 2011 resulted in almost 4000 people being infected with a particularly virulent enteroaggregative *E. coli* strain, leading to 854 cases of HUS of which 54 (6.3%) died [24]. A pooled analysis of almost 3500 patients from 49 studies of STEC-HUS found that overall the incidence of death or end-stage renal disease (ESRD) was 12% and clinically significant renal damage occurred in 25% of patients [11].

Generally, the prognosis of STEC-HUS is favourable in the majority of children, although serious systemic complications, such as haemorrhagic colitis and CNS involvement, can occur and mortality is between 1% and 5% [40–42]. In patients aged over 65 years, a lethal outcome occurs in up to 50% of cases [43]. Long-term consequences affect up to 20% of children with STEC-HUS and include arterial hypertension, neurological impairment, chronic kidney disease, or diabetes mellitus [40, 41, 44].

The atypical form of HUS is a very rare disease with a very poor prognosis. In the US, aHUS is estimated to have an annual incidence rate of ~1–2 cases/million inhabitants [45] and in Europe, a recent international, multicentre study reported an incidence of 0.11 cases/million inhabitants between the ages of 0 and 18 years [13]. Age of initial onset of aHUS is approximately equal in adults and children [28, 29] and distribution is similar between males and females, although there is a slight predominance in females among adult onset patients [28]. Prognosis is poor with a mortality up to 25% and over half of patients developing ESRD after the first presentation of aHUS [12, 17, 25, 46, 47].

However, the disease is heterogeneous and unpredictable and the clinical characteristics of patients with aHUS and the risk of TMA after renal transplant seem to vary based on specific complement mutation and environmental factors (Table 1). Factor H is the most common mutation which also has the worst prognosis leading to renal impairment, ESRD or death in up to 79% of patients at 3 years post-initial presentation. Although lesions typically affect

Table 1: Genetic abnormalities and clinical outcome in patients with aHUS.

Complement abnormality	Main effect	Frequency, %	Death or ESRD within one year of first presentation, %	TMA post-transplantation, %
Factor H mutations	Increased activity of C3 convertase (decreased inhibition)	20–30	50–60	75–90
Factor I mutations	Decreased C3b inactivation	2–12	42–50	45–80
C3 mutations	C3 convertase resistant to inhibition	5–10	43–63	40–70
Factor B mutations	C3 convertase stabilisation (increased activity)	1–2	50	100
Thrombomodulin (THBD) mutations	Reduced C3b inactivation	0–5	50	1/1
Membrane cofactor Protein (MCP) mutations	Increased activity of C3 convertase (decreased inhibition)	3–15	0–63	≤20
Factor H antibodies	Inactive factor H (increased activity of C3)	6–10	30–40	Greater with elevated antibody levels

Adapted from Noris and Remuzzi [14], Campistol et al. [13], Zuber et al. [48] and Fremeaux-Bacchi et al. [46].

the kidney, in approximately 20% of patients extra-renal symptoms are present and can involve the CNS, cardiovascular system, lungs, skin, skeletal muscle and gastrointestinal tract [49].

Diagnosis

As discussed, TMA can be due to several causes and may be further precipitated by other underlying diseases, drugs, bone marrow transplant, or pregnancy [13]. In these cases, intervention to discontinue the event which initiated the onset of symptoms (if possible, e.g., stopping a drug treatment) may lead to resolution of TMA. If TMA persists, the event may have been the precipitating factor exposing an underlying predisposition to aHUS in the patient.

TTP may potentially be accurately differentiated from aHUS by severe deficiency of the ADAMTS13 protease [13, 50] and, if the facilities are available, ADAMTS13 activity can be assessed within 2 days. It is important to take samples for analysis before any plasma has been given. An analysis of serum creatinine and platelet count at initial presentation was performed in patients with severe ADAMTS13 deficiency versus patients with aHUS to develop a predictive score [51]. Although less definitive, a diagnosis of severe ADAMTS13-deficiency (TTP) can almost be excluded based on a serum creatinine level above 150–200 μmol/L or a platelet count $>30\times10^9$/L at presentation [51, 52].

Clinical presentation does not always accurately differentiate the aetiology of TMA. Although STEC-HUS is more common in children and TTP is more common in adults there are frequent exceptions and patients with aHUS can present at any age. So age is not considered a reliable guide to the cause of TMA.

Sometimes renal versus CNS involvement has been proposed to distinguish between STEC-HUS/aHUS and TTP. While aHUS and STEC-HUS almost always have renal involvement and while severe renal injury requiring haemodialysis is less common in patients with severe ADAMTS13 deficiency, acute renal failure has been reported in up to 10% of TTP patients [50, 53]. CNS involvement has been considered a hallmark of TTP, with neurologic injury reported in 25%–79% of patients at presentation, it is also the most frequent extra-renal symptom in aHUS, occurring in 10%–48% of patients [49] and can occur in patients with STEC-HUS [41]. Thus the type of organ involvement cannot be used as a reliable diagnostic tool.

If the patient has a history of gastrointestinal symptoms, STEC-HUS may be thought more likely than aHUS and testing for the presence of Shiga toxin genes and serum reactivity by PCR and ELISA, respectively, and STEC culture for *E. coli* strains is indicated. Again, however, a caveat exists in that prodromal diarrhoea does not define STEC-HUS as up to 30% of cases of aHUS include a previous diarrhoea episode [28, 29] and not all patients with STEC-HUS report diarrhoea.

Ultimately, therefore, differentiating between causes of TMA is a diagnosis of exclusion and an algorithm for differential diagnosis has been proposed by Campistol et al. (Figure 3) [13]. Therefore, to confirm a diagnosis of aHUS, it is necessary that tests for STEC (Shiga toxin) and pneumococcus infections are negative and ADAMTS13 activity is normal (>5%–10%). Measurement of serum

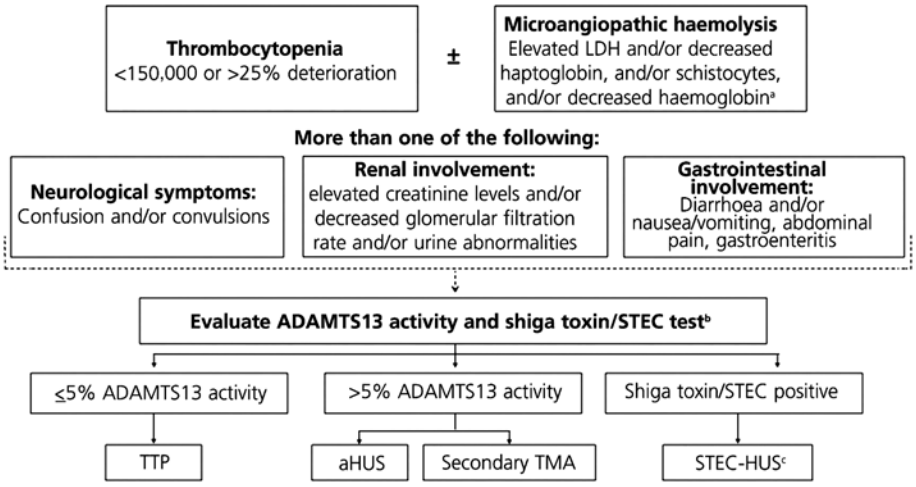

Figure 3: An algorithm for the differential diagnosis of TMA.
ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; aHUS, atypical haemolytic uraemic syndrome; HUS, haemolytic uraemic syndrome; LDH, lactate dehydrogenase; STEC, Shiga toxin producing Escherichia coli; TMA, thrombotic microangiopathy; TTP, thrombotic thrombocytopenic purpura. [a]Negative direct Coombs test; [b]The Shiga toxin test/STEC is indicated when the patient has a history of digestive system involvement or gastrointestinal symptoms; [c]In some patients with aHUS, STEC infection can trigger the underlying disease. From Campistol J, et al. [13]. Reprinted with permission from the Nefrologia Publishing Group.

levels of C3, C4, CFH and CFI, and complement antibody and genetic mutation screening can give an indication [13, 54], although it should be noted that normal complement protein levels or the absence of a mutation does not exclude a diagnosis of aHUS as serum activity does not correlate with complement activity on the endothelial surface [46, 55, 56].

Treatment

Treatment of aHUS

Until Gruppo and Rother published data describing the successful treatment of aHUS with eculizumab [57] management of aHUS consisted of early and intensive PE at high volumes at variable frequency (based upon clinical presentation). While PE initially manages haematological symptoms in some patients, up to 65% of aHUS patients require dialysis, develop permanent kidney damage or die within 1 year despite PE or plasma infusion (PI) [25]. However, haematologic improvement does not mean that TMA is not ongoing in organs, and unlike in the treatment of TTP, it has been demonstrated that the use of PE in aHUS is mostly ineffective [46].

When the diagnosis of aHUS is unequivocal, eculizumab is recommended in both adult and paediatric patients as first-line therapy, in order to protect organ function [13]. Eculizumab is a monoclonal antibody that binds with high affinity to complement protein C5, blocking the formation of the C5b-9 cell membrane attack complex, leaving proximal functions (opsonisation and immune clearance) intact. Two pivotal prospective studies in a total of 37 patients with aHUS have shown that administration of eculizumab produces a rapid and sustained inhibition of the TMA process, with significant improvements in clinical outcomes (haemolysis, long-term platelet counts and renal function), and discontinuation of plasma management and a 80% reduction in need for dialysis [58].

In trial 1, the mean increase in platelet count from baseline to week 26 was 73×10^9/L (primary outcome), and dialysis was discontinued in four of five patients. In the second trial, 80% of patients had TMA event-free status (primary outcome; ≥12 weeks of no reduction in platelet count >25%, no PE/PI and no new dialysis) during eculizumab treatment. Earlier intervention was associated with improved glomerular filtration rate and treatment improved renal function across patient subgroups, including those with long-standing, substantial kidney damage who had previously been managed on chronic plasma exchange or infusion.

Two further trials in 22 paediatric and 41 adult aHUS patients have recently reported data – a total of prospective trial data in 100 patients with aHUS. In paediatric patients, early intervention with eculizumab (median time from diagnosis to treatment of 6 days) was associated with haematological normalisation (platelet and LDH normalisation) in 18 (82%) patients and a complete

TMA response (primary outcome; haematological normalisation and ≥25% improvement in serum creatinine from baseline) in 14 (64%) patients [59]. The trial in adult patients with aHUS recruited a broad population: 30 (73%) patients were newly diagnosed, six (15%) patients had no PE/PI during the current aHUS manifestation, 24 (59%) patients were on dialysis at baseline, nine (22%) patients had a prior kidney transplant and 20 (49%) patients had an identified complement factor mutation [60]. The majority of patients achieved platelet and haematological normalisation as well as a complete TMA response (primary outcome; haematological normalisation and <25% increase in serum creatinine from baseline). Also in these studies plasma management was discontinued and 80% of patients on dialysis at baseline were able to discontinue dialysis.

No cumulative toxicity or unexpected serious infection-related adverse events, were observed through the trial period or the extension phase and survival was 100% in the studies [58, 61]. However, there were two cases of meningococcal infections that were resolved with antibiotic treatment [61]. Due to the mechanism of action of eculizumab, preventative measures (vaccination and if needed prophylactic antibiotics) should be initiated against *Neisseria meningitides* prior to starting treatment [13]. The long-term safety and efficacy of eculizumab is being further studied in a non-interventional global aHUS registry [62].

Kidney transplantation

Due to the high rates of recurrence of TMA and graft loss, aHUS is a contraindication for live kidney donation [63, 64]. Prior to transplant a complete genetic analysis should be performed to detect known complement mutations and anti-CFH antibodies. TMA presents in the transplanted kidney in around 50% of patients who undergo transplantation (ranging from 15% to 100% in patients), and graft failure occurs in 80%–100% of those with TMA [14]. With a lack of treatment guidelines, patients in whom a kidney transplant is considered should be evaluated on an individual basis, based on the risk of graft failure and availability of eculizumab [13, 64].

Plasma administration on occurrence of TMA in aHUS patients with a kidney transplant is of limited value [65, 66]. Prophylactic plasma can decrease graft loss. Of nine patients who received pre-emptive plasma, four had an event-free successful renal transplantation. TMA occurred in three other patients who were successfully treated with eculizumab in each case [66].

As the liver is the source of some complement proteins, combined liver-kidney transplantation could be an option for the prevention of further TMA in aHUS patients with ESRD and a mutated protein produced in the liver. The procedure is associated with a not inconsiderable mortality risk; all patients died when no prevention of complement activation was used. However, when either prophylactic eculizumab or PE was used outcomes were good in 80% with a mortality rate of 15% [64]. In aHUS patients with functioning kidney, isolated liver transplantation should no longer be recommended as the risks related to lifelong immunosuppressive therapy far outweigh those associated with long-term eculizumab therapy [67].

Eculizumab has been used successfully to treat aHUS following transplant in a number of cases and as prophylaxis in transplants at high risk of recurrence. For this group, eculizumab prophylactic therapy has been proposed as preferable to PE for several reasons, including failure of PE to prevent TMA, the unpredictable risk of TMA when PE is tapered and subclinical progression of disease [46, 48, 66, 68].

Conclusions

A patient presenting with TMA needs careful investigation to distinguish between TTP, STEC-HUS and aHUS. Characterisation of aHUS is performed by the presence of non-immune microangiopathic haemolytic anaemia, thrombocytopenia, and organ injury an ADAMTS13 activity (>5%) and no evidence of STEC. TMA is a medical urgency and it is important to initiate specific treatment early to avoid irreversible damage to organs. Multiple organ involvement has to be considered. Assessment of platelet levels and serum creatinine while waiting for STEC and ADAMTS13 test results may give an indication whether TTP or aHUS is present.

In aHUS, mutations and polymorphisms in the genes of certain complement factors that are involved in either the regulation or activation of the alternative complement system pathway have been identified. In 30%–40% of patients no mutation has been identified yet. The prognosis of aHUS is complex but depends upon predisposing factors like the genetic mutation, background of polymorphisms and also precipitating factors like the complement activating environment. Eculizumab is currently the only licensed therapy for aHUS in both the USA and Europe and it is effective in all patients with aHUS whether or not a complement mutation has been identified and whether the patient has a native or transplanted kidney. Consensus

guidelines recommend its early use in all patients with aHUS for optimal outcomes [13, 52].

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