Atypical Hemolytic–Uremic Syndrome: A Clinical Review

Ali Nayer, MD1 and Arif Asif, MD2*

Atypical hemolytic–uremic syndrome (HUS) is a rare life-threatening disorder characterized by microangiopathic hemolytic anemia, thrombocytopenia, and ischemic injury to organs, especially the kidneys. Microvascular injury and thrombosis are the dominant histologic findings. Complement activation through the alternative pathway plays a critical role in the pathogenesis of atypical HUS. Genetic abnormalities involving complement regulatory proteins and complement components form the molecular basis for complement activation. Endothelial cell dysfunction, probably because of the effects of complement activation, is an intermediate stage in the pathophysiologic cascade. Atypical HUS has a grave prognosis. Although mortality approaches 25% during the acute phase, end-stage renal disease develops in nearly half of patients within a year. Atypical HUS has a high recurrence rate after renal transplantation, and recurrent disease often leads to graft loss. Plasma therapy in the form of plasma exchange or infusion has remained the standard treatment for atypical HUS. However, many patients do not respond to plasma therapy and some require prolonged treatment. Approved by the Food and Drug Administration in the treatment of atypical HUS, eculizumab is a humanized monoclonal antibody that blocks cleavage of complement C5 into biologically active mediators of inflammation and cytolysis. Although case reports have shown the efficacy of eculizumab, randomized clinical trials are lacking. Therapeutic strategies targeting endothelial cells have demonstrated promising results in experimental settings. Therefore, inhibitors of angiotensin-converting enzyme, HMG-CoA reductase, and xanthine oxidase as well as antioxidants, such as ascorbic acid, may have salutary effects in patients with atypical HUS.

Keywords: atypical hemolytic–uremic syndrome, complements, eculizumab, kidney, endothelial cells

INTRODUCTION

Hemolytic–uremic syndrome (HUS) is a life-threatening disorder caused by a thrombotic microangiopathy resulting in microangiopathic hemolytic anemia, thrombocytopenia, and ischemic injury to organs, especially the kidneys.1–4 HUS occurs in 2 different clinical settings.1–4 In the majority of patients with HUS, a bacterial infection such as hemorrhagic gastroenteritis due to Shiga toxin-producing Escherichia coli (usually serotypes 0157:H7 and 0104:H4) precedes the onset of the disease (STEC-HUS).

In approximately 10% of patients with HUS, a preceding bacterial infection is not identified [so-called atypical infection (aHUS)]. Accumulating evidence indicates that complement activation through the alternative pathway causes aHUS.1–4 Intensive investigations are being conducted to explore the molecular basis of complement abnormalities. Strategies to halt complement activation hold promise in the treatment of aHUS.1–5 Furthermore, the role of endothelial cell dysfunction in aHUS and potential therapeutic strategies to promote endothelial cell health are discussed.6 This review summarizes the clinical and morphologic features and pathogenesis of aHUS. In addition, current and prospective therapeutic interventions for aHUS are highlighted.

PATHOLOGY

Histologically, aHUS is indistinguishable from HUS caused by toxin-producing bacteria. Although aHUS can affect various vascular beds, pathologic features in
tem, complements represent a first-line defense system. An evolutionary conserved part of the immune system may contribute to the development of aHUS. Emerging evidence suggests that endothelial cell dysfunctions involving soluble or membrane-bound proteins that regulate complement activity. In addition, emerging evidence suggests that endothelial cell dysfunction may contribute to the development of aHUS.

**PATHOGENESIS**

The pathogenesis of aHUS remained obscure for decades. Altered serum complement levels (ie, reduced C3 and normal C4) in some patients led to the hypothesis that complement activation through the alternative pathway might be involved in the pathogenesis of aHUS. Subsequent investigations demonstrated that approximately half of patients with aHUS have hereditary defects involving soluble or membrane-bound proteins that regulate complement activity. In addition, emerging evidence suggests that endothelial cell dysfunction may contribute to the development of aHUS.

**Complement system**

An evolutionary conserved part of the immune system, complements represent a first-line defense system against invading pathogens. Initially recognized for their complementary bactericidal activity, complements are positioned in the heart of an intricate network of biological systems that regulate innate and adaptive immunity, waste disposal, angiogenesis, regenerative processes, and lipid metabolism. Complements are activated through the classical, lectin, or alternative pathways (Figure 2). The classical pathway is strongly activated by immune complexes, which are recognized by the versatile pattern recognition molecule C1q. Carbohydrates such as those present on microbial surfaces activate the lectin pathway. After target recognition, proteolytic cleavage of C4 and C2 results in generation of the classical and lectin pathway C3 convertase (C4b2b). The alternative pathway is activated by complex polysaccharides such as those present on the surface of microorganisms. Factors B and D and C3 participate in generation of the alternative pathway C3 convertase (C3bBb), which is stabilized by factor P (also known as properdin). C3 cleavage by the C3 convertases and subsequent C5 cleavage by the C5 convertases results in the formation of C5a and C5b. The latter participates in the assembly of the membrane attack complex (MAC; C5b-9, terminal complement complex). In the context of an immune response, anaphylatoxins C3a and C5a trigger proinflammatory signaling and attract neutrophils, monocytes, and macrophages to the site of complement activation. Opsonin C3b facilitates phagocytosis, whereas MAC mediates target cell activation, injury, or lysis in a dose-dependent manner. Regardless of origin, all surface-bound C3 convertases can induce activation of the alternative pathway. Therefore, the alternative pathway plays a dominant role in the total complement activity.

The activity of complements is kept in check by several soluble and membrane-bound regulators (Figure 2). Soluble complement regulatory proteins include factor I (Fl), factor H (FH), and C4-binding protein (C4BP). Synthesized mainly in the liver, Fl is a serine protease that suppresses complement activity by breaking down fluid-phase and cell-bound C3b and C4b. A 155-kDa glycoprotein synthesized mainly in the liver, FH serves as a cofactor for Fl and facilitates Fl-mediated C3b degradation. By removing Bb from C3bBb, FH also accelerates decay of the alternative pathway C3 convertase. C4BP has similar effects on the classical and lectin pathway C3 convertase. Complement regulatory proteins are also found on the surface of most human cells. Membrane cofactor protein (MCP; CD46) is a membrane protein expressed by all cells except erythrocytes. MCP binds C3b and C4b and serves as a cofactor for Fl. Complement receptor 1 (CR1, CD35, C3b/C4b-receptor) serves as a cofactor.
FIGURE 1. Renal thrombotic microangiopathy in aHUS. (A, B) Microthrombi (arrows) in glomerular capillaries. (C) Immunofluorescence micrograph revealing fibrin deposition (green signal) along peripheral capillary walls of a glomerulus. (D, E) Thrombosis (arrows) of a small artery. (F) Immunofluorescence micrograph revealing fibrin deposition (green signal) in a small artery. (G) Electron micrograph demonstrating a patent (arrow) and a thrombosed (asterisk) glomerular capillary. (H) Electron micrograph demonstrating a thrombosed (asterisk) glomerular capillary and endothelial cell denudation (arrows). (I) Electron micrograph demonstrating accumulation of electron-lucent material in the subendothelial zone (bar). (J, K) Irregular thickening of glomerular capillary walls because of deposition of basement membrane material (black arrows) and cell interposition (white arrows). (L) For comparison, normal glomerular basement membrane is shown. (M) Concentric laminations in the fibrotic intima of a small artery known as “onion skin” pattern. Tissue sections were stained with hematoxylin and eosin (A, D), Masson trichrome (B, E), periodic acid–Schiff (J, K), and antiserum against fibrin/fibrinogen (C, F). Courtesy of Xu Zeng, Nephrocor Bostwick Laboratory.

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for FI and accelerates decay of convertases. Thrombo-
modulin, a membrane glycoprotein with anticoagulant
activity, also facilitates FI-mediated C3b inactivation.

**Complement abnormalities in aHUS**

Genetic mutations involving complement regulatory
proteins and complement components are found in
40%–60% of patients with aHUS (Table 1).10–12 Loss-of-
function and inactivating autoantibodies directed against
FH have also been associated with aHUS. In addition,
some patients with aHUS demonstrate gain-of-function
mutations involving factor B and C3. A recent study
demonstrated that single and combined genetic muta-
tions were present in 41% and 3% of patients with aHUS,
respectively.12 Although combined mutations occurred
in only 8%–10% of patients with FH, C3, or FB muta-
tions, 25% of patients with MCP or FI mutations had
a combination of mutations. Approximately 50% of
patients with combined MCP mutations developed
end-stage renal disease within 3 years. It is conceivable
that there are additional complement abnormalities asso-
ciated with aHUS.

Many patients with complement abnormalities
remain asymptomatic for decades before developing
aHUS. Similarly, STEC-HUS develops only in a subset
of patients infected with Shiga toxin-producing E. coli.
Therefore, elusive factors other than known triggers
(infection, trauma, surgery, pregnancy, etc.) may pre-
dispose individuals to the development of HUS.

**Endothelial cell dysfunction**

Endothelial cell injury and dysfunction, probably
because of the effects of complement activation, rep-
resent an intermediate stage in the pathophysiologic
cascade culminating in aHUS.6 Nitric oxide plays a piv-
otal role in the biology of endothelial cells. Released
from endothelial cells, nitric oxide causes relaxation of
vascular smooth muscle and vasodilation. Nitric oxide
has also anti-inflammatory properties by suppressing
leukocyte recruitment and inhibiting mast cell func-
tions. In addition, nitric oxide exerts antithrombotic
effects by reducing platelet adhesion and aggregation.
Anti-inflammatory and anti-thrombotic effects of nitric oxide are in part mediated by reduced release of P-selectin and von Willebrand factor from endothelial cells. Of note, mice deficient in endothelium-derived nitric oxide are prone to develop a thrombotic microangiopathy. Vascular endothelial growth factor (VEGF) plays a pivotal role in vasculogenesis and angiogenesis. Reduced VEGF expression in podocytes is linked to the development of a thrombotic microangiopathy. Because VEGF is a major regulator of nitric oxide production, it is conceivable that reduced VEGF expression results in reduced nitric oxide bioavailability, thus promoting vascular coagulation. Finally, replacing nitric oxide and VEGF is shown to protect against thrombotic microangiopathy in animal models. Taken together, these observations highlight the importance of endothelial cell health and indicate potential targets in the management of patients with thrombotic microangiopathies, including aHUS.

**CLINICAL MANIFESTATIONS**

Atypical HUS can occur in any age. Although usually abrupt in onset, the presentation can be insidious in nearly 20% of patients. Clinical manifestations of aHUS reflect the sequelae of microvascular injury and thrombosis as well as ischemic injury to various organs. A hemoglobin concentration below 10 g/dL, a platelet count below 150,000/μL, and impaired renal function are often found on presentation. Laboratory tests also disclose features of intravascular hemolysis, including elevated lactate dehydrogenase (LDH) and reduced haptoglobin levels in the blood. Fragmented erythrocytes (schistocytes) and reticulocytes are seen on blood film. Serum C3 level may be reduced. Prothrombin and partial thromboplastin times are normal. The Coombs test is usually negative. The kidney is frequently involved in aHUS. The most frequent renal manifestations are azotemia, hypertension, proteinuria, and hematuria. Renal function is usually severely impaired, necessitating renal replacement therapy. Hypertension can be severe as a result of hyperreninemia and volume expansion. Proteinuria is usually mild, but nephrotic-range proteinuria can occur. Hematuria is usually microscopic. Extrarenal manifestations occur in approximately 20% of patients. Neurologic manifestations are present in approximately 10% of patients and include altered mental status from drowsiness to coma, focal neurologic deficits, and seizure. Cardiac and distal limb ischemia may occur. A catastrophic presentation because of the involvement of multiple organs is observed in approximately 5% of patients.

**DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS**

The diagnosis of aHUS relies on the demonstration of absolute or relative thrombocytopenia defined as a platelet count below 150,000/μL or a 25% decline from the baseline, respectively, microangiopathic hemolytic anemia and end-organ damage (Table 2). Microbiologic tests for toxin-producing bacteria are negative. Some patients with aHUS demonstrate low C3 and normal C4 concentrations in the serum. However, the presence of low serum C3 concentration alone does not constitute the diagnosis of aHUS. Furthermore, the demonstration

<table>
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<th>Table 2. Diagnostic criteria, precipitating factors, and interventions for atypical HUS.</th>
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<td><strong>Diagnostic criteria</strong></td>
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<td>Absolute: platelet count &lt;150,000/μL</td>
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<td>Relative: 25% decline from the baseline</td>
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<td><strong>Microangiopathic hemolysis</strong></td>
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<tr>
<td>• Schistocytes on blood film</td>
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<td>• Decreased hemoglobin concentration</td>
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<td>• Decreased serum haptoglobin concentration</td>
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<td>• Increased serum lactate dehydrogenase concentration</td>
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<td><strong>Target organ injury</strong></td>
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<td>(1 or more of the following)</td>
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<td>• Kidney (elevated creatinine, abnormal urinalysis)</td>
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<td>• Central nervous system (altered mental status, focal neurologic deficits, seizure)</td>
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<tr>
<td>• Gastrointestinal tract (diarrhea with or without blood, nausea, vomiting, abdominal pain, gastroenteritis)</td>
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<td><strong>Precipitating factors</strong></td>
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<td><strong>Potential therapeutic interventions</strong></td>
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<tr>
<td><strong>Plasma therapy, eculizumab, ACE-inhibitors, ascorbic acid, HMG-CoA reductase inhibitors, xanthine oxidase inhibitors, liver and kidney transplantation</strong></td>
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of normal serum C3 concentration does not exclude aHUS. Finally, even though many individuals demonstrate an abnormality in complement proteins, not all develop aHUS.\(^3\) Consequently, the demonstration of specific complement abnormalities can be important but are not required for the diagnosis of aHUS.\(^3,10\) Although serologic and genetic tests uncover complement abnormalities in majority of patients with aHUS, a subset of the patients with STEC-HUS demonstrate complement abnormalities as well.\(^25\)

Differential diagnosis of aHUS includes thrombotic thrombocytopenic purpura (TTP), which is another form of thrombotic microangiopathies characterized by microangiopathic hemolytic anemia, thrombocytopenia, and ischemic injury to organs.\(^2,3\) Although fever and neurologic manifestations frequently dominate the clinical picture in patients with TTP, most patients with aHUS suffer from renal disease. TTP is usually caused by a deficiency of ADAMTS13 (A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), a plasma metalloproteinase that cleaves von Willebrand factor. Plasma activity of ADAMTS13 is less than 5% of normal in most patients with TTP. However, a substantial percentage of patients with recurrent or familial HUS also demonstrate ADAMTS13 deficiency. Other forms of thrombotic microangiopathies, such malignant hypertension, scleroderma renal crisis, and antiphospholipid syndrome, should be considered and excluded.

**NATURAL COURSE AND TREATMENT**

Atypical HUS has a grave prognosis. Although approximately 25% of patients succumb to their illness during the acute phase, nearly half develop end-stage renal disease within a year.\(^1,2\) Patients are also plagued by a high recurrence rate after renal transplantation.\(^17\) Recurrent aHUS often leads to graft loss.\(^17\)

Plasma therapy is considered the standard treatment for aHUS. Considering the role of complement activation in the pathogenesis of aHUS, therapeutic strategies to suppress complement activity are being developed. In addition, interventions that promote endothelial cell health are gaining therapeutic significance. Finally, renal and liver transplantation has been undertaken in patients with advanced and irreversible organ damage.

**Plasma therapy**

Plasma therapy in the form of plasma exchange or infusion has remained the standard treatment for aHUS.\(^1,2\) Although plasma infusion replenishes deficient regulatory proteins, plasma exchange has the additional benefit of removing factors that inhibit the function of regulatory proteins. Plasma therapy has been associated with reduced mortality from aHUS from 50% to 25%. However, many patients do not respond to plasma therapy and some require prolonged treatment to induce or maintain a remission.

**Complement inhibition**

Eculizumab is a humanized monoclonal antibody that blocks cleavage of C5 and formation of the MAC.\(^18\) Because C5 is a terminal complement component, eculizumab can be effective regardless of the type of complement abnormality.\(^2,4,18\) Eculizumab is approved for the treatment of patients with aHUS by the US Food and Drug Administration.\(^18\) Case reports demonstrated beneficial effects of eculizumab in the treatment of aHUS in patients who failed plasma therapy.\(^26–30\) Three open-label multicenter clinical trials (studies 1–3) were recently conducted to evaluate the safety and efficacy of eculizumab in patients with aHUS.\(^2,4,18\) The follow-up period in these studies was 26 weeks. Efficacy indicators included increased platelet count, improved estimated glomerular filtration rate, and thrombotic microangiopathy event-free status. The latter was defined by no decrease of the platelet count by greater than 25% from the baseline, no plasma therapy, and no new dialysis for at least 12 consecutive weeks.

Study 1 was a single-arm prospective study involving 20 adults with progressive aHUS defined by a platelet count below 150,000/µL, elevated LDH, and elevated serum creatinine concentration.\(^18\) A genetic mutation involving a complement component or an autoantibody was identified in 60% of patients. Ten patients (50%) had advanced chronic kidney disease (stages 4–5). All patients received plasma therapy for a median duration of 10 months. Eight patients (40%) received 1 or more kidney transplants. Sixteen patients (80%) achieved thrombotic microangiopathy event-free status. Eighteen patients (90%) had normalization of both the platelet count and the LDH. All patients discontinued plasma therapy and required no new dialysis. Renal function improved by greater than 1 chronic kidney disease (CKD) stage in 7 patients (35%).

Study 2 was a single-arm prospective study involving 17 adults without hematologic manifestations of thrombotic microangiopathy but evidence of chronic kidney disease despite plasma therapy.\(^18\) A genetic mutation involving a complement component or an autoantibody was identified in 60% of patients. A normalization of the platelet count was observed in 87% of patients. An increase in the platelet count was observed as early as 7 days. Thirteen patients (76%) had normalization of both the platelet count and the LDH. Eculizumab therapy reduced the need for plasma therapy and new dialysis in all patients from a median of 6 per week to zero.
Of the 5 patients who were on dialysis, 4 (80%) did not require dialysis at the end of follow-up. Estimated glomerular filtration rate increased by an average of 31 mL/min per 1.73 m² in the remaining patients.

Study 3 was a retrospective analysis involving 19 children with aHUS. A normalization of the platelet count occurred in 17 patients (89%).

Nine patients (47%) demonstrated an increase in estimated glomerular filtration rate by greater than 15 mL/min per 1.73 m². Of the 8 patients on dialysis, 4 (50%) discontinued dialysis. None of the other patients required dialysis. Similarly, plasma therapy was reduced from a median of 2 per week to zero.

The most frequent adverse reactions reported in these studies included headache, hypertension, cough, upper respiratory tract infections, urinary tract infections, nausea, vomiting, diarrhea, and abdominal pain. Eculizumab therapy increases the risk of meningococcal infections. Vaccination against Neisseria meningitidis is recommended at least 2 weeks before starting eculizumab therapy. When eculizumab therapy is to be initiated immediately, vaccination and a 2-week course of antibiotics should be administered.

How long should eculizumab therapy be continued? Is it possible to reset the alternative pathway of complement activation long-term and withdraw eculizumab successfully? It is difficult to answer these questions at the present time. However, it is important to point out that a cohort of 18 patients who discontinued eculizumab therapy for various reasons suffered a relapse. Five of these patients (28%) experienced severe complications after a missed dose. Targeting specific points of dysfunction in the complement system might produce fruitful results in the future. In this context, a recombinant FH showed positive results in experimental settings. A better graft survival for MCP mutations is likely because of the presence of abundant MCP, a membrane protein, in the transplanted kidney. Nonetheless, the presence of combined MCP mutations shortens renal allograft survival. Considering poor outcome, there is growing consensus not to perform living donor kidney transplantation in patients with aHUS. ACE inhibitors have anti-inflammatory and immunomodulatory properties. Therefore, ACE inhibitors are potentially useful in the treatment of aHUS by reducing oxidant stress and increasing nitric oxide bioavailability.

In addition to lipid-lowering properties, statins improve endothelial cell dysfunction through nitric oxide and reduced thrombogenicity. Statins also have anti-inflammatory and immunomodulatory properties. Therefore, statins can potentially be helpful in restoring endothelial cell health in patients with aHUS.

Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and the oxidation of xanthine to uric acid. Reactive oxygen species are byproducts of these reactions. Recent studies demonstrated that allopurinol, an inhibitor of xanthine oxidase, ameliorate endothelial cell dysfunction, probably by reducing oxidative stress. Allopurinol may prove to be a therapeutic agent in the treatment of aHUS.

Ascorbic acid restores endothelium-dependent vasodilation impaired by oxidative stress. Ascorbic acid also increases the bioavailability of nitric oxide by scavenging reactive oxygen species. Ascorbic acid could represent a therapeutic strategy in patients with aHUS through enhanced nitric oxide production and reduced oxidative stress.

Transplantation

The outcome of kidney transplantation in patients with aHUS is poor. The recurrence rate in renal allograft is as high as 50%. In the majority of cases, recurrent aHUS leads to graft loss. Allograft failure rates are up to 80% for FH and FI mutations and nearly 20% for MCP mutations. A better graft survival for MCP mutations is likely because of the presence of abundant MCP, a membrane protein, in the transplanted kidney. Nonetheless, the presence of combined MCP mutations shortens renal allograft survival. Considering poor outcome, there is growing consensus not to perform living donor kidney transplantation in patients with aHUS.

On a hypothetical basis, liver transplantation should correct reduced activity of fluid-phase complement regulatory proteins that are normally synthesized in the liver and released into the circulation. Therefore, combined kidney and liver transplantation has been proposed in the treatment of advanced renal disease caused by FH or FI deficiency. However, limited clinical experience with combined kidney and liver transplantation in the treatment of FH- or FI-associated aHUS has been disappointing.

CONCLUSIONS

Atypical HUS is a devastating disease associated with high morbidity and mortality. Until recently, very few treatment options were available for patients with aHUS.
Emerging evidence supports the concept of complement inhibition as an important therapeutic strategy. Targeting endothelial cell dysfunction is also assuming an important role in the management of aHUS. It is of utmost importance that the diagnosis of aHUS is made in a timely fashion to limit organ injury.

REFERENCES