Assessment, Treatment and Prevention of Atypical Hemolytic Uremic Syndrome

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ABSTRACT

Hemolytic uremic syndrome (HUS) is a heterogeneous group of hemolytic disorders. Different terminologies have been described in HUS, which are as follows: (1) D+ HUS: Presentation with a preceding diarrhea; (2) typical HUS: D+ HUS with a single and self-limited episode; (3) atypical HUS (aHUS): Indicated those with complement dysregulation; (4) recurrent HUS: Recurrent episodes of thrombocytopenia and/or microangiopathic hemolytic anemia (MAHA) after improvement of hematologic abnormalities; and (5) familial HUS: Necessary to distinct synchronous outbreaks of D+ HUS in family members and asynchonous disease with an inherited risk factor. aHUS is one of the potential causes of end-stage renal disease (ESRD) in children. It has a high recurrence after renal transplantation in some genetic forms. Therefore, recognition of the responsible mechanism and proper prophylactic treatment are recommended to prevent or delay the occurrence of ESRD and prolong the length of survival of the transplanted kidney. A computerized search of MEDLINE and other databases was carried out to find the latest results in pathogenesis, treatment, and prevention of aHUS.

Keywords: Atypical hemolytic uremic syndrome, children, pathogenesis, prevention, treatment

INTRODUCTION

Hemolytic uremic syndrome (HUS) is an important cause of acute renal failure in children, accounting for 4.5% of pediatric chronic renal replacement therapy. It was first introduced by Conrad Von Gasser in 1955. HUS consists a heterogeneous group of hemolytic disorders and thrombotic microangiopathies (TMA), characterized by the prominent endothelial cell damage.

Bacterial toxins, drugs, systemic disorders, complement dysregulation, and Von Willebrand factor cleaving protease (VWFcp) deficiency are the main causes of TMA, which is classified into diarrhea associated, familial atypical, non-familial atypical, and thrombotic thrombocytopenic purpura.

Clinical manifestations of HUS include a non-immune coombs-negative microangiopathic hemolytic anemia with
fragmented red blood cell (RBC), increased serum lactic dehydrogenase (LDH) and reticulocytosis, thrombocytopenia (usually less than 60,000 platelets), and acute renal failure (serum creatinine more than 97% for age or glomerular filtration rate (GFR) less than 80 ml/min/1.73 m²) in 55-70% of patients.[7,8] Microvascular lesions consist of endothelial swelling, subendothelial protein and debris accumulation, vascular wall thickening, and platelet fibrin thrombi.[7]

Diarrhea dependent HUS (typical HUS) is the most common cause of acute renal failure in children, consisting of more than 90% of HUS.[8,9] It is more common during the summer months, in children less than 5 years. Glomerular involvement occurs in the early phase. Majority of glomeruli are intact and 15-20% become sclerotic. Renal function improves in most cases (up to 70%). Almost 50% of patients require temporary dialysis and 3-5% die during the acute phase. Severity of acute symptoms, especially the central nervous system involvement, and dialysis are predictors of poor prognosis.

Atypical HUS (aHUS) consists of 5-10% of patients. There is no seasonal variation; however, most cases occur during the spring and winter seasons.[7,10] Arteriole and interlobular artery involvements are prominent pathologic lesions.[9] Hypertension and end-stage renal disease (ESRD) are more common in this group of patients.[8] Up to 50% of these cases progress to ESRD and 25% die during the acute phase.[7,9] It may occur in sporadic or familial forms, at different ages, more frequently in adulthood.[7,8]

Sporadic aHUS occur in the following conditions:

- Drugs: Multiple drugs such as quinine, mitomycin, cisplatin, bleomycin, gemcitabine-1, cyclosporine, tacrolimus, IFN, OKT3, and anti-platelets (ticopidin, clopidogol)[7] result in aHUS by direct toxicity or immunologic mechanisms.
- Post-transplantation (TP): Denovo, drug (calcineurine inhibitors), humoral rejection, and recurrent diseases are the leading causes of aHUS.
- Pregnancy: Increased procoagulant factors and decreased fibrinolytic and ADAMTS13 activity in HELP syndrome (pre-eclampsia, severe thrombocytopenia, MAHA, renal failure, and liver involvement) are the major mechanisms.
- Post-partum: HUS may occur during the first 3 months of labor with poor prognosis and 50-60% mortality rate. Renal dysfunction and hypertension are late complications.
- VWFcp deficiency: Mutation of ADAMTS13 gene and VWFcp deficiency or acquired autoantibodies may present as TTP or rarely, HUS.[9,11]
- Infection: Invasive pneumococcal infection in the form of meningitis, hepatitis, or empyema has been reported in 40% of aHUS and in 4.7% of all patients with HUS. Despite the severe short-term clinical course, renal survival is favorable without residual renal failure.[7-10]

Drugs, opportunistic infections, and tumors are the leading causes of HUS in advanced HIV infection.

- Inborn error of vitamin B12 metabolism: Neurologic, metabolic, and developmental derangements, methylmalonic aciduria, homocystinuria, and aHUS are the clinical manifestations of this rare metabolic disorder.
- Systemic disorders: HUS may be a clinical manifestation of SLE, scleroderma, and anti-phospholipid syndrome.
- Hematopoietic stem cell TP: Radiation, drugs, GVHD, and CMV infection may result in aHUS.
- Tumors: HUS has been reported in prostatic, gastric, and hepatic adenocarcinoma.[9]
- Idiopathic: There is no clear predisposing factor in 50% of sporadic aHUS patients.

Familial aHUS consists of less than 3% of patients with HUS.[7] Autosomal recessive inheritance characterized by early onset disease, with a high incidence of ESRD and 60-70% mortality rate. Autosomal dominant inheritance is less common, begins during adulthood with 50-90% mortality, or requires renal replacement therapy.[7,11] Genetic transmission may occur without previous familial history.[12] Complement dysregulation might be the responsible mechanism in familial or sporadic (mainly idiopathic) aHUS,[7] which is discussed in the next section.

**COMPLEMENT SYSTEM**

A complement system consists of more than
30 types\textsuperscript{13} of plasma and membrane-bound proteins. It is the main part of the innate and acquired immune system, enhances cellular immunity, and protects from bacterial invasion.

Other functions of this system include chemotactic substance production, tissue mast cell degranulation, immune complex obliteration, bacterial and host cells lysis, augmentation of phagocytic bacteria, activation of inflammatory cells, and increased vascular permeability.\textsuperscript{8}

There are three pathways of complement activation: Classic, alternative, and lectin pathways.\textsuperscript{3} The classic pathway activates by C1q adhesion to the antigen–antibody complex and the lectin pathway activates by lectin adhesion to the mannose molecule on the pathogenic surface, independent of antibodies. Alternative complement pathway is an antibody-independent defense mechanism, activated by C3b covalent binding to the pathogens.\textsuperscript{14,15}

C3 is the main component of the complement system. C3 cleavage by C3 convertase produces C3a and C3b. C3a is an anaphylatoxin with antimicrobial activity. C3b deposition on the bacterial surface results in opsonization for phagocytosis by polymorphonuclears (PMN) and macrophages.

In addition, C5 convertase produces C5a and C5b by C3b interaction. C5b initiates the production of C5b-9 (MAC), which induces inflammation, bacteriolysis, and organ damage.\textsuperscript{7,8}

There are two groups of complement regulatory proteins: Membrane bound (CD59, membrane cofactor protein (MCP) MCP, DAF, and CR1) and fluid phase (CFH, CFI). It prevents inappropriate complement activation and self-tissue damage by two major mechanisms: Decay accelerating activity (DAA) and cofactor activity. Alternative complement pathway activation in the absence of membrane-bound or fluid-phase regulators induces cellular damage by alternative pathway convertase.\textsuperscript{17,14}

Subendothelial exposure to collagen, Von Willebrand factor VWB, and fibrinogen results in pro-coagulation activity, platelet adhesion, and matrix accumulation. Complement deposition on normal platelets cause platelet adhesion, thrombin and fibrin polymer production, thrombocytopenia, and small-vessel obstruction.\textsuperscript{7,16}

There is little information about the correlation between complement activation and RBC disintegration in aHUS. RBC interaction with the patient’s plasma and not the healthy one stimulates annexin adhesion, seramide production, increased cytosolic calcium activity, and RBC apoptosis, characterized by the exposure of phosphatidylinerse on the RBC surface and cellular shrinkage.\textsuperscript{17}

Complement dysregulation consists of 50% aHUS, 30% CFH, 10% MCP, and 2-5% CFI.\textsuperscript{18,19}

**Complement factor H**

It is one of the main alternative pathway regulators produced by endothelial cells, platelets, and fibroblasts.\textsuperscript{9} Complement regulation resides in the N-terminal region.\textsuperscript{7} The C-terminal region is responsible for host recognition, N-terminal exposure, and protection against complement activation.

Its major functions are mediating C3b cleavage by CFI, inhibition of C3 activation, competition with factor B for C3b binding, limitation of complement consumption in fluid phase, prevention of complement-mediated host cellular damage, production of thrombaxone and PGE2, stimulation of blastogenesis in mouse splenocytes, inhibition of B cell proliferation, monocytic release of IL1beta, and increased monocytic oxidative metabolism.\textsuperscript{9,16,20}

The CFH gene resides in ch 1q23.\textsuperscript{9} More than 80 mutations have been reported in this gene.\textsuperscript{3} Mutation occurs in 40% of familial and 13-17% of sporadic forms\textsuperscript{7} from infancy to mid-adulthood, rarely in the neonatal period.\textsuperscript{21} Altered CFH plasma level, with protein secretion blockage, and protein dysfunction are the two major mutations.\textsuperscript{3}

Fluid-phase activity remains normal in CFH mutation, characterized by normal cofactor activity in C3b fluid-phase proteolysis. However, complement regulation is impaired on the cellular surface, resulting in RBC dysregulation, diminished CFH adhesion to basement membrane glycosamine glycan (GAG) and endothelial cells, and increased C3b adhesion to the endothelial cells.\textsuperscript{7,9,15}

Most mutations are heterozygotes and reside in the C-terminal region with incomplete penetration and partial deficiency (50%).\textsuperscript{7,18} C3, CFB, and C5-9 levels are normal or deficient.\textsuperscript{3,20} Homozygote mutation is rare, which progressed to early renal failure in an involved patient.\textsuperscript{22}
CFB, and C5-9 reduce in homozygote patients. Different disorders such as membranoproliferative glomerulonephritis types 2 and 3 (homozygote mutation), aHUS (C-terminal missense mutation), age-related macular degeneration (AMD) and type 3 collagen glomerulopathy occur in CFH gene mutation.

Acquired CFH: An autoimmune disease has been reported in patients with anti-CFH antibody, which inhibits complement regulatory effects on the cellular surface by blocking the C-terminal recognition sites, similar to C-terminal CFH mutation.

Antibody titer has no significant effect on the severity of cellular lysis. Antibody formation without CFH mutation has been reported in a few (6-10%) patients.

Membrane cofactor protein (MCP)
It is a membrane-bound glycoprotein on all human cells, except in the RBC, and it prevents complement-mediated damage by CFI cofactor activity and C4b proteolysis.

Richards found its mutation in three families at 2003. More than 20 mutations in MCP protein with 54% penetration consist of 13% of aHUS. Most of the patients are heterozygotes and less are homozygotes or compound heterozygotes. are heterozygotes and 25% are homozygotes or compound heterozygotes.

MCP surface expression reduces in more than 75% of mutations (severe reduction in homozygotes or compound heterozygotes, 50% in heterozygotes). Ligand binding (C3b/C4b) and cofactor activity reduce in the remaining mutations with normal serum level.

aHUS with recurrent course and gradual progression to renal failure occurs in MCP mutation.

Complement factor I
This is a specific serine protease, synthesized in the liver. It cleavages α chain from C3b and C4b in the presence of its cofactors (CR1, C4 binding, MCP, CFH), prevents C3, C5 convertase, and down-regulates alternative and classic complement pathway activation. Its gene is located on the 4q25 chromosome. Heterozygote CFI mutation with incomplete penetration is a rare cause of aHUS (3% and 12% in some references). Less than 20 types of mutations have been reported in these patients. Protein synthesis is impaired in the majority of mutations; however, there is a dysfunctional factor with normal level in a few patients.

C4b binding protein
It is regulates alternative complement pathway and inhibits the classic and lectin pathways.

Decay accelerating factor
DAF accelerates the decay of C4b2a or C3bBb and C5 convertase without cofactor activity. A mutation analysis showed nearly no protein dysfunction.

Complement Receptor 1 (CR1; CD35)
It is a membrane-bound complement regulator with cofactor activity in the classic and alternative complement pathways. Its mutation has not been reported.

Complement factor H related genes
There are five CFH-related genes (CFHR1-5). Deletion of two genes (CFHR1, 3) with no intrinsic cofactor activity and decay accelerating activity (DDA) results in complement dysregulation, impaired RBC protection, and aHUS.

A relation between CFH antibody and low-CFH-related proteins (CFHR1,3) has been reported. The affected patients require to intensive immune suppression and plasmaphoresis.

Complement factor B (CFB)
It carries the catalytic site of alternative pathway convertase. Increased C3bBb convertase production, resistance to inactivation with complement regulators, low C3 level, and persistent alternative complement pathway activation occur in rare CFB mutation, accounting for 0-3% of aHUS.

C3
It is a critical part of all three complement pathways activations. C3 convertase resistance, with low C3 level and normal terminal components, hypertension, micro-hematuria, and chronic kidney disease, occurs in the C3 gain of function mutation.

Others
Properdin gene mutation has been recently reported in aHUS. Synthesis or cellular
presentations of polyanion molecules, which are involved in CFH acquisition into the endothelial cells, are defective in this mutation.[3]

Thrombomodulin mutation with low C3b inactivation has been reported in aHUS. It is an endothelial cell glycoprotein with anticoagulant and anti-inflammatory effect; it inactivates C3b in the presence of CFH or C4b-binding protein and accelerates C3a and C5a inactivation and complement cellular damage in 5% of patients.[29]

In addition, 10% of patients with aHUS have mixed CFI with CFH, MCP, CFB, or C3 mutations.[12]

**ASSESSMENT**

Clinical approach depends on the measurement of:

- C3: Serum C3 level might decrease in CFH and CFI mutation and it is generally normal in MCP mutation. Serum level is usually normal in heterozygote mutation and is the lowest in homozygotes.[3,12] There is a group of patients with low C3 level (30%) and no mutation. Therefore, measurement of C3 level is not a sensitive screening test and normal C3 level does not exclude complement disorder.[3,12] Tests such as increased C3d/C3 plasma level and renal C3 deposition are more sensitive tests.

- CH50, CFB: Decreased levels of CH50 and CFB have been reported in some patients with CFH or MCP mutations.[7]

- Serum measurement of CFH, CFI, and CFB by the ELISA method[3] and MCP by FACS in peripheral monocytes document 75% of their mutations. Serum level is preferable to the plasma level. The specimens must be kept refrigerated, and the measurement is indicated before or at least 2 weeks after plasma infusion.

- Serum levels of CFH and CFI are age and not gender dependent, because they reach the adult level up to 1 year old. Cord blood contains 61% of the adult level, which increases to 83% up to 6 months. Therefore, interpretation of age-dependent serum level is recommended.[18,20] Patients with CFH and CFI mutation are younger compared to those with MCP mutation. Early-onset aHUS (less than 3 months) with decreased C3 level strongly suggests CFH or CFI mutation. MCP mutation has not been reported in patients less than 1 year old.[12,19]

- The serum level of CFI is deficient in 40% of mutations.[9,18] Therefore, measurement of CFH and CFI serum levels can detect only less than 50% of mutations.[3]

- In addition, screening for alternative pathway activity (C3d),[4] ANA, lupus anticoagulant, antiphospholipid antibody, serum homocysteine, serum and urine methyl malonic acid, ADAMTS13 level, and MMACHC gene can help diagnose aHUS disease.

**Genetic analysis**

Genotype–phenotype correlation has been identified in complement regulator mutations. Genetic analysis of complement proteins is indicated in any patient, even in those with normal serum level. It has no clinical value in healthy family members, except for TP donors. HPLC and single-strand polymorphism are non-sensitive procedures in mutation analysis; however, PCR sequence has been found to be the most sensitive method.[12,14,18]

- There are two types of mutations: (1) loss of function (CFH, CFI, and MCP) and (2) gain of function mutation (C3, CFB).[30]

- Incomplete penetration has been documented in all three complement regulator mutations (approximately 50%), which is predisposing rather than the primary cause.[18] Therefore, precipitating events, such as infection (70%, drug use and pregnancy (4%) in CFH, pregnancy (40%), infection (60%) in CFI, and infection in 100% of MCP mutations, are necessary for inducing complement deficiency and endothelial damage in these patients. aHUS has been preceded by BCG, MMR, and pneumococcal vaccination in patients with CFH deficiency.[14,21]

- Screening of family members and avoidance of predisposing factors during high-risk periods (pregnancy and infection) are recommended in these patients.[14]

**TREATMENT**

- Long-term prognosis depends on the early
diagnosis and treatment of the first episode of aHUS. Treatment is symptomatic and experimental. Determination of predisposing factors is necessary for specific and aggressive treatment. Supportive therapy including blood transfusion in Hgb less than 5-7 g/dl, platelet infusion in active bleeding or surgery, maintaining fluid and electrolyte balance, peritoneal or hemodialysis in patients with serum urea more than 200 mg/dl, acidosis, hyperkalemia, edema, and anuria with antihypertensive drugs in hypertensive patients is recommended. [8]

• Despite the absence of a prospective randomized controlled trial, plasma therapy (infusion and exchange) is recommended as the first line of treatment in aHUS, which decreases the mortality rate from 50% to 25%. [9,12] It is recommended during the first 24 h of presentation. [7]

• Plasma exchange argues as the first line of treatment, especially in limited plasma infusion, such as renal or heart failure. [7,15] Its major benefits are the removal of toxic substances such as anti-CFH and ADAMTS13 autoantibodies and circulatory mutant complement proteins. [12,15]

Large volume of plasma has a favorable effect in treatment and in the prevention of dysfunctional CFH. [31] According to MCP localization, plasma therapy has no significant effect in isolated MCP dysfunction. [15]

Usually one plasma volume (35-40 cc/kg) is exchanged per session. The initial recommended dose of plasma infusion is 30-40 cc/kg/day, followed by 10-20 cc/kg/day afterwards. Treatment must continue for at least 2 days after the complete remission. [9] Twice daily plasma exchanges with one plasma volume is the selective treatment in resistant patients. [7] Remission usually occurs in 7-10 days and continues until the normalization of platelets, with cessation of hemolysis and serum LDH below 400 IU/L. Serum creatinine and urine output generally improve after the increment of platelets. Every other day, plasma treatment is recommended in recurrent thrombocytopenia lower than 100,000/mm³. [33]

Plasma treatment aggravates the progression of pneumococcal infection, and hence is not recommended. [9]

Steroids and immunosuppressive drugs such as rituximab prevent antibody formation after plasma discontinuation. [12]

Synthetic complement regulators such as CFH have been recommended in patients with CFH abnormalities to prevent complications of plasma treatment. [31]

Monoclonal antibody (anti-C5a), a new treatment of aHUS, prevents terminal complement activation. [7] Pexelizumab is a single-chain short-acting anti-C5 antibody, administered for reperfusion after myocardial infarction. Eculizumab is a long-acting humanized monoclonal antibody, blocking terminal complement activation by C5 cleavage and prevention of C5a and C5b-9 production. It has proved useful in the treatment of paroxysmal nocturnal hemoglobinuria, rheumatoid arthritis, and membranous glomerulopathy with favorable results. [8,13]

Soluble complement inhibitors (CR1) are complement blocking agents, and it prevents complement-mediated renal damage and complications such as liver dysfunction in combined liver–kidney TP. [7] Gene therapy is a new challenging option. [3]

Splenectomy is limited to plasma-resistant and relapsing HUS with high plasma volume requirement, which may increase the mortality rate. [3,8]

Bilateral nephrectomy has been effective in refractory HUS with diffuse microvascular thrombosis, severe hypertension, hypertensive encephalopathy, continuous hemolysis, or thrombocytopenia. [7] Nephrectomy does not decrease the recurrence rate after renal TP.

Avoidance of calcineurin inhibitors does not reduce recurrent HUS. [12]

Steroids, aspirin, heparin, antioxidants, anti-platelet agents, prostacycline, intravenous immune globuline (IVIG) or fibrinolytic agents have no consistent therapeutic effect in aHUS. [3,8,9] Steroid and IVIG have been effective in a patient without plasma treatment. Intravenous immune globuline prevents membrane attack complex production, and activated C3b, C4b binding and tissue damage. [33]

A few patients with persistent alternative complement pathway activation and low C3 level (CFB or CFH homozygote deficiency) must be considered immune deficient. Continuous prophylactic antibiotics (penicillin or macrolids in
beta lactamase allergy) with meningococcal and pneumococcal vaccine are recommended in these patients.[12]

PROGNOSIS

Long-term prognosis has not been satisfactory in aHUS. Almost 50% progress to ESRD (50% in sporadic and 60% in familial form) and 25% die during the acute phase.[7]

Renal survival has been unfavorable in all groups, except in MCP mutation.[9] CFH has the worst prognosis; 60-70% progress to renal failure or die during the first year. In addition, prognosis is also unfavorable in CFI; 50-60% progress to ESRD and 50% see improvement.[12]

Relapsing course with complete resolution, even without plasma treatment, is common in MCP and in some undefined mutations. Renal failure occurs in 38% of MCP mutations over several years.[12,18]

Serum creatinine at the first episode determines renal survival in the 1st year.[19] Familial or sporadic occurrence, age and C3 level are not responsible for the final prognosis.[12]

PREVENTION

Prophylactic fresh frozen plasma (FFP) (exchange and infusion) would maintain native and allograft kidney function by treating and preventing recurrent episodes of aHUS.[34]

Around 10-20 cc/kg of plasma infusion either as weekly or in the every second week has been successful in patients with low CFH level. Therapeutic effects of large plasma volume have been documented, even, in dysfunctional CFH mutation.[31]

In addition, alternative treatments such as hydroxyl cobalamin, folic acid, and betain have been recommended in the management of cb1C.[9]

Prophylactic eculizumab treatment maintained chronic suppression of complement activity and reduced graft failure in a patient with CFH mutation and recurrent HUS.[35]

TRANSPLANTATION

A genotype–phenotype correlation has been documented in complement regulatory gene mutations and in the prognosis of renal TP. Therefore, donor and recipient genotyping of CFH, MCP, CFI, and CFB genes is necessary in live-donor TP.[9,14]

Prognosis is unfavorable in all groups, except in MCP mutation.[3,8] Recurrent disease occurs in 80% with CFH, 100% with CFI, and in less than 10% with MCP mutation.[21] TP may be a favorable primary option in mixed heterozygote mutations (CFI/MCP) owing to MCP expression in renal TP.[30]

TP is a high-risk procedure in recurrent familial aHUS (recurrence up to 100%), and requires careful consideration.[2]

Live related donor(LRD)TP is not recommended in aHUS, especially in familial or recurrent disease. Graft failure has been reported in more than 70% of patients. The mechanisms responsible for this are renal thrombosis and recurrent disease.[4,15]

One-seventh of the second TP is successful; however, it is not recommended in recurrent disease. Prolonged interval between clinical manifestation and TP does not reduce the recurrence rate.[2] Preemptive administration of eculizumab and plasmaphoresis has been advocated in recurrent disease.[36]

Combined liver/kidney TP: According to the hepatic synthesis of CFH and CFI, combined liver and kidney TP seems reasonable.[9] However, primary results have not been satisfactory for increased thrombotic complications with a high death rate. Therefore, it has not been recommended in CFH mutation, except in life-threatening events. Preliminary plasma exchange has been recommended with better results.[4]

CONCLUSION

According to a widespread complement pathway dysregulation, recognition of the related mechanism with appropriate treatment and proper preventive strategy is recommended to lengthen the native and transplanted kidney survival in aHUS.

REFERENCES


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