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# Atypical hemolytic uremic syndrome

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## Abstract

Atypical hemolytic uremic syndrome (aHUS) is a thrombotic microangiopathy (TMA) characterized by microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure. Most cases of aHUS are caused by uncontrolled complement activation due to genetic mutations in the alternative pathway of complement. More recently, mutations in the gene of coagulation system have also been identified in patients with aHUS. In Japan, the recent studies of aHUS have identified the unique genetic characteristics in our country and enabled us to revise the diagnostic criteria. In this article, we review the classification of TMAs and describe the pathophysiology, diagnosis, and management of aHUS. We also highlight current progress in clinical and basic research of the patients with aHUS in Japan.

**Keywords:** Atypical hemolytic uremic syndrome, Complement, Alternative pathway, Complement factor H, Complement component C3, Eculizumab

## Background

Thrombotic microangiopathy (TMA) is defined by a histological region characterized by microvascular changes including thrombosis, which results in microangiopathic hemolytic anemia, thrombocytopenia, and organ failure. TMAs are caused by a variety of hereditary or acquired etiologies, and now broadly classified into four categories; hemolytic uremic syndrome caused by Shiga toxin-producing *Escherichia coli* (STEC) infection (STEC-HUS), atypical hemolytic uremic syndrome (aHUS), thrombotic thrombocytopenic purpura (TTP), and secondary TMA.

Historically, HUS was classified into two forms by the presence of diarrhea. Ninety percent of HUS arises from the infection of STEC with severe diarrhea; thus, this form of HUS was previously named "D (diarrhea) (+) HUS", presumably STEC-HUS. On the other hand, the remaining 10% of HUS was named "D (diarrhea) (−) HUS", because it was caused without the infection of STEC. In 1975, several research groups reported that this form of HUS could be familial, implicating the presence of hereditary D(−) HUS [1]. Thus, the term "atypical HUS (aHUS)" was used to describe the patients with D(−) HUS and hereditary D(−) HUS. However, since 1980s, various clinical and experimental studies have convincingly shown that most cases of aHUS result from uncontrolled complement activation due to genetic mutations or acquired autoantibodies in the alternative pathway of complement. These new findings of aHUS led to differentiate complement-mediated aHUS from other types of TMA. According to these observations, currently, the term of aHUS is generally used to describe complement-mediated aHUS [2–4]. Other TMAs associated with a variety of causes including infection, drug, transplant, and pregnancy are now named "secondary TMA" or etiology-based denomination (e.g., pregnancy TMA).

Recent advances in understanding pathogenesis of aHUS have clearly led to differentiate aHUS from other TMAs and changed the therapeutic approach for aHUS [5, 6]. However, making a solid diagnosis of this disease is still not easy due to poor penetrance and lack of method for identifying excess complement activations. Moreover, recent studies have also found that the mutations in the gene of coagulation system predispose to aHUS leading investigators to reconsider the definition of aHUS. Our aim in this review is not only to describe the pathogenesis, diagnosis, and management of aHUS but also to improve the evidence-based practice of this disease.

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## Classification of thrombotic microangiopathies

HUS due to Shiga toxin-producing bacteria is most frequently formed and is generally called STEC-HUS (or HUS or typical HUS). Although STEC-HUS occurs at any age, children can be predominantly affected. Severe abdominal pain, diarrhea, and bloody stools are common findings, which appeared several days after taking contaminated foods. The progression to HUS is related to the binding of Shiga toxin to the target cell surface via globotriaosylceramide, which leads to cytotoxic effect via inhibition of protein synthesis and apoptosis. Moreover, the presence of Shiga toxin also induces the secretion of unusually large von Willebrand factor (VWF) from endothelial cells [7]. Prognosis of STEC-HUS is favorable with 90% of child cases being recovered, but 1–2% of patients die during acute phase, and 12% of patients, who recovered from STEC-HUS once, die or progress to end-stage renal disease (ESRD) in long-term follow-up [3, 8].

TTP results from severe deficiency of a disintegrin-like and metalloprotease with thrombospondin type 1 motif, 13 (ADAMTS13), which is a specific cleaving protease of VWF. ADAMTS13 deficiency leads to the secretion of unusually large VWF from vascular endothelial cells, thus leading to the VWF-dependent platelet adhesion in small vessels. Homozygous or compound heterozygous mutations of *ADAMTS13* are the cause of hereditary TTP [9, 10]. Moreover, acquired TTP arises from autoantibodies against ADAMTS13 [11].

A link between complement system and aHUS has been highlighted since the 1970s [12], and subsequent study identified that genetic mutation of *complement factor H* (*CFH*), a major complement regulatory factor, is associated with pathophysiology of aHUS [13]. Since then, various genetic mutations in multiple complement factors belonging to alternative pathway have been found in 60% of patients with aHUS. Two types of variants are associated with this disease; one is loss-of-function mutation of the complement regulatory factors, and the other is gain-of-function mutation of complement itself or complement activation factors. Of note, autoantibodies against CFH also predispose to aHUS. These genetic or acquired defects result in excess complement activation on the cell surface, leading to endothelial damage, inflammation, and thrombosis formation. More recently, a link between coagulation system and aHUS has also been highlighted.

Secondary TMA arises from diverse factors and diseases (metabolic disorder, infection, drug, pregnancy, autoimmune disease, systemic disease, hematopoietic stem cell transplantation/solid organ transplantation, malignant hypertension, and malignancy). In pediatric cases, both *Streptococcus pneumoniae* infection and Cobalamin C deficiency are highly related to the cause of secondary TMA. In contrast to STEC-HUS, aHUS, and TTP, the pathogenesis of secondary TMA is still unclear. Especially, the distinction between aHUS and secondary TMA is sometimes not clear. In fact, complement genetic mutations or anti-CFH autoantibodies have been found in a part of patients with post-transplant-mediated TMA [14] and patients with hemolysis, elevated liver enzyme, low platelet count (HELLP) syndrome [15, 16]. It is unclear whether aHUS is underlying the disease or not in these cases; thus, more studies are needed to confirm these findings.

In Japan, the classification of TMAs was originally described in a diagnostic criteria for aHUS proposed by the Joint Committee of the Japanese Society of Nephrology and the Japan Pediatric Society in 2013 [17, 18]. In this criterion, aHUS was defined as TMA excluding STEC-HUS and TTP; therefore, TMA caused by a variety of etiology, now named secondary TMA, was also included in the category of aHUS. This classification has led to the early diagnosis of aHUS and timely initiation of treatment. However, the term of aHUS is now generally accepted to describe only complement-mediated aHUS as described above. To address this situation, the Joint Committee developed a novel guideline for aHUS including recommendation for treating this disease in 2016 [19, 20]. This guideline redefined aHUS as TMA caused by complement dysregulation, and the exclusion of secondary TMA is needed for diagnosing aHUS in addition to the exclusion of STEC-HUS and TTP (ADAMTS13 <10%). Current problem of this field is that there are some cases of aHUS among secondary TMA [14, 21]; however, there are no crucial clinical characteristics and laboratory parameters to distinguish them. To overcome this problem, further research regarding both clinical characteristics and laboratory biomarkers and the establishment of rapid genetic diagnostic system for aHUS are required.

We hope that this novel guideline will improve the understanding of physiopathology and diagnosis of individual TMA, leading to a rapid and correct diagnosis, and more judicious treatment for individual cases in Japan.

## Epidemiology

aHUS is considered a rare disease, but its incidence is not known precisely. The annual incidence of aHUS is estimated to be two cases per million in the USA [22], and the prevalence are reported to be 3.3 per million among patients below the age of 18 [5]. More recently, the research group from France reported an incidence of 0.23 cases per million [23]. aHUS affects at any age, and approximately half of this disease usually occurs before the age of 18, without sex difference [24]. Although, the epidemiology of patients with aHUS in Japan has not been well clarified, based on our cohort study, 100 to 200 patients seem to have been diagnosed.

## Pathophysiology

The complement system is an essential component of innate immunity for protecting host from invading pathogens. Complement system, which consists of over 30 proteins, can be mostly present as inactivated forms, and the activation of it is caused through three main pathways; classical pathway, alternative pathway, and lectin pathway. These three pathways promote the formation of C3 convertase, which degrades C3 into C3a and C3b. The C3b molecule functions as a major opsonin, and binds covalently to pathogens or to any surface. The binding of C3b to Gram-positive bacteria leads to the phagocytosis by neutrophils, macrophages, and monocytes. In the case of Gram-negative bacteria, bound C3b induces the progression of complement cascade and the generation of the lytic membrane attack complex. The activation of the classical and the lectin pathways is initiated by the recognition of invading microorganisms via the Fc moieties of antigen-bound antibody or mannose-binding lectin, respectively. On the other hand, the activation of alternative pathway needs no specific initiator.

The activation process of alternative pathway is illustrated in Fig. 1. In the alternative pathway, C3 is easily converted to $C3(H_2O)$ and rapidly reacted with complement factor B (CFB) and complement factor D (CFD). This reaction leads to the formation of $C3(H_2O)Bb$, which works as an initial fluid phase C3 convertase and prompt to generate more C3b. This spontaneous activation system called "tick over" is potentially dangerous; thus, it is strictly controlled by complement regulatory factors including CFH, complement factor I (CFI), and membrane cofactor protein (MCP). Generally, $C3(H_2O)Bb$ or C3b is rapidly and proteolytically inactivated by CFI collaborated with CFH in the fluid phase. On the self-cell surface like endothelial cells, CFH also acts as a complement regulator by binding to sialic acids or sulfated glycosaminoglycans on self-surface via its C-terminal basic domains. A transmembrane protein MCP helps to inactivate C3b by CFI. Thrombomodulin (THBD), a transmembrane protein, which generates anticoagulant active protein C to reduce blood coagulation, is also associated with the downregulation of alternative pathway by accelerating CFI-mediated inactivation of C3b. On the other hand, once C3b binds to pathogens lacking complement regulatory proteins, bound C3b preferentially reacts with CFB and CFD, which results in formation of a C3 convertase and generating more C3b. The C3 convertase can recruit another C3b to form C3bBbC3b (C5 convertase), which generates a potent anaphylatoxin C5a through cleavage of C5. The binding of C5b to C6, C7, C8, and C9 causes the formation of membrane attack complex (C5b–9) for eliminating pathogens.

Several research groups have shown that approximately 60% of patients with aHUS have mutations in complement regulatory or complement factors in the alternative pathway (CFH, CFI, MCP, THBD, C3, and CFB). Loss of function mutations in complement regulatory factors (CFH [13, 25–27], CFI [28, 29], MCP [30, 31], and THBD [32]) cause the impairment of inactivating C3b both on the cell surface and fluid phase. On the other hand, gain of function mutations in complement activation factors, C3 and CFB reduce the binding affinity for CFH and/or MCP leading to impaired CFI-mediated inactivation [33–36]. These aHUS-associated mutations cause excess complement activation on host-cell surface leading endothelial damage. Moreover, combined mutations in the foregoing six genes seem to increase susceptibility to aHUS [37]. Acquired autoantibodies against CFH have been identified in 5–10% of patients with aHUS [38–40]. These antibodies are highly associated with the alteration in *CFH-related* (*CFHR*) genes. The genes encoding the five CFHR (CFHR1-5)

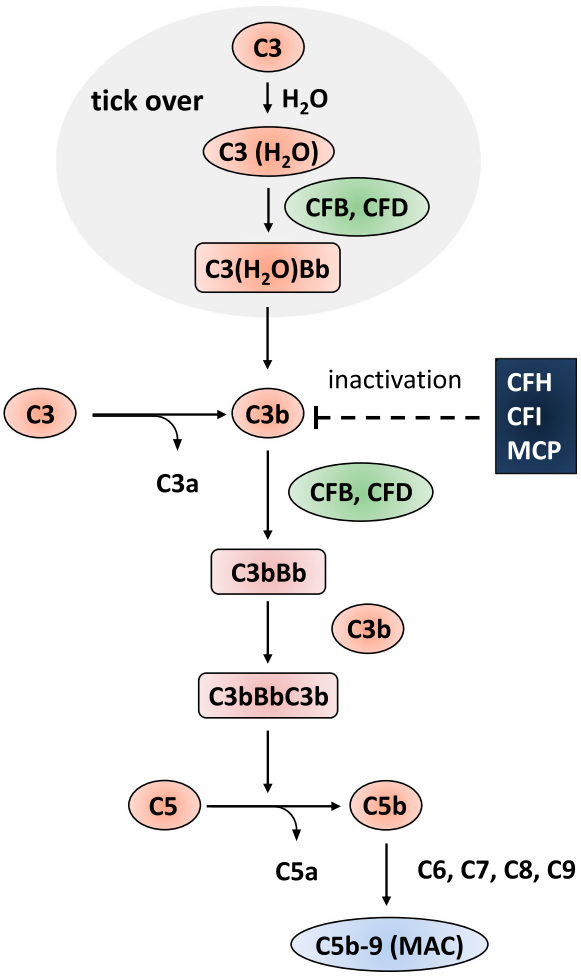


**Fig. 1** Alternative pathway of complement. *CFH* complement factor H; *CFI* complement factor I; *MCP* membrane cofactor protein; *C3* complement component C3; *CFB* complement factor B; *CFD* complement factor D; *MAC* membrane attack complex

proteins are characterized by several large genomic repeat regions having a high sequence identity. Thus, these regions occur nonallelic homologous recombination leading to the deletion or duplication within *CFH* and *CFHR* genes. Notably, homozygous gene deletion of *CFHR1* is specifically linked with the production of autoantibodies against CFH [39–41]. A majority of these antibodies recognize the C-terminal region of CFH and inhibit the complement regulatory function of CFH on the cell surface [42]. Moreover, nonallelic homologous recombination of *CFHR* gene region predisposes to the formation of hybrid gene of *CFH-CFHR*, which causes aHUS [6].

Currently, several research groups have shown the correlation between coagulation system and aHUS. In 2013, Lemaire et al. have identified that the homozygous or compound heterozygous mutations in the *diacylglycerol kinase epsilon* (*DGKE*) gene in 13 aHUS patients who belong to nine unrelated families by whole-exome sequencing [43]. DGKE is a lipid kinase family protein, and is expressed in endothelium, platelets, and podocytes. The underlying pathology of DGKE-associated aHUS is still unclear, but one possibility is that the loss of function of DGKE results in upregulation of prothrombotic factors and platelet activations. It is still debatable whether DGKE-associated aHUS is linked with complement activation or not.

More recently, one published paper has identified four genetic variants in the gene of plasminogen in the patients with aHUS, and three of these variants were known plasminogen deficiency mutations [44]. Miyata et al. have shown that one genetic variant p.Ala620Thr in plasminogen, which is commonly observed in the northeast Asian populations including Japanese and causes dysplasminogenemia, is not predisposing variant for aHUS [45]. Further studies are required to confirm whether the coagulation system is additional pathogenesis for aHUS or not.

In addition to disease-associated mutations mentioned above, some environmental factors are related to increase the risk of developing aHUS. The onset of aHUS seems to be facilitated by various triggers such as infection and pregnancy [14, 24]. One retrospective study has shown that 21 of 100 adult female patients with aHUS developed pregnancy-associated TMA mainly in postpartum period [46].

## Diagnosis of aHUS

### Clinical diagnosis of aHUS

Rapid diagnosis of aHUS is critical for early initiation of treatment that prevents the patient's kidney from ESRD. Clinically, initial diagnosis of aHUS is made by microangiopathic hemolytic anemia (Hb <10 g/dL, negative direct Coombs test, elevated LDH, decreased haptoglobin, and the presence of schistocytes), thrombocytopenia (platelet count $<150 \times 10^9$/L), and renal failure (elevated serum creatinine, low glomerular filtration rate, proteinuria, and hematuria). Other diseases which show similar clinical presentation to TMA such as disseminated intravascular coagulation or heparin-induced thrombocytopenia should be carefully excluded. In patients with aHUS, the major target organ is the kidney, but the heart, lungs, gastrointestinal tract, pancreas, and brain can also be affected. Although the presence of diarrhea is a representative manifestation of STEC-HUS, it has also been identified in 10–30% of aHUS patients [14, 24].

To differentiate aHUS from TTP, the activity of ADAMTS13 should be measured before the initiation of plasma therapy. Severe deficiency of ADAMTS13 activity (<10%) is common findings in patients with TTP. One study of 214 patients with TMA has shown that severe low platelet count ($<30 \times 10^9$/L) and serum creatinine (<2.26 mg/dL) were commonly detected in 157 of 160 patients with patients having severe ADAMTS13 deficiency [47] implicating that kidney damage is not generally severe in TTP. Of note, these laboratory data do not always differentiate aHUS from TTP. The confirmation of STEC-HUS needs the direct detection of Shiga toxins in feces and anti-lipopolysaccharide immunoglobulin M antibody measurements. Moreover, the screening of various diseases or specific laboratory data associated with TMA is critical for diagnosing secondary TMA.

### Complement assessment in aHUS

To confirm the clinical diagnosis of aHUS, a variety of specific diagnostic tests are recommended as follows: quantification of complement components (C3 and C4), regulators (CFH, CFI, MCP, and CFB), and complement activity (CH50 for classical pathway, AP50 for alternative pathway), screening of anti-CFH autoantibodies, and genetic test of candidate gene (*CFH*, *CFI*, *MCP*, *C3*, *CFB*, *THBD*, and *DGKE*) [5]. The low level of C3, but not C4, may reflect the excess activation of the alternative pathway, but only 30–40% of patients with aHUS show a low level of C3 [14, 23]. Thus, a normal C3 level does not exclude the diagnosis of aHUS. Similarly, normal levels of abovementioned complement factors do not rule out the diagnosis of aHUS, because a majority of mutations cause functional impairment instead of quantitative defects. The hemolytic assay can be used to assess the CFH function [48–50]. In this assay, patient specimens are incubated with sheep red blood cells (RBCs) that have been known as "non-activating cells" leading no amplification of C3b on its cell surface. CFH is a major complement regulatory factor in the alternative pathway, and consists of 20 short consensus repeats (SCRs) with each about 60 amino residues. CFH is capable of protecting sheep RBCs from complement-mediated lysis via

binding of CFH SCRs19-20 to sialic acids richly expressed on surface of sheep RBCs. Thus, *CFH* mutations or anti-CFH autoantibodies having a defect in the protection of cell surfaces lyse the sheep RBCs [50]. The anti-CFH autoantibodies are generally measured by ELISA. Although several ELISA methods have been reported, Watson et al. have recommended the use of the Paris method, which is the most robust, cost-effective, and easy to establish [51].

Various biomarkers have been studied to reveal the underlying complement perturbations of aHUS to differentiate it from other TMAs. The significantly increased levels of C5a and soluble C5b-9 (sC5b-9), markers of terminal complement activation, have been identified in the acute phase of aHUS compared with TTP or healthy controls [52, 53]. Moreover, urine C5a, sC5b-9, and alternative pathway activation marker Ba are elevated in acute phase of aHUS, and these markers are decreased by eculizumab administration, with the exception of Ba [54]. However, Noris et al. has reported that plasma C5a and sC5b-9 were not suitable markers for diagnosing aHUS, because these values were normal in 9 out of 19 cases even during the acute phase [55]. Recent study has described a new technique for detecting excess complement activation by using modified HAM test, which is classically used to diagnose the patients with paroxysmal nocturnal hemoglobinuria (PNH). Gavriilaki and their colleagues have established the GPI-anchored protein-deficient cells, that is, PNH-like reagent cells [56]. When this cells are treated with the serum from TMA patients, significantly reduced cell viability are found in aHUS, compared to TTP. This novel method might be used for rapid diagnosis for aHUS to differentiate it from other TMAs.

Degradation of C3 spontaneously occurs after blood collection, which leads to altered complement state in patient specimens. To avoid pre-analytical errors, blood samples should be immediately centrifuged, and serum or plasma should be stored at −80 °C. EDTA plasma is generally used for measuring C5a, sC5b-9, and Ba but cannot be used for measuring ADAMTS13 activity, which can be only determined by using citrated plasma. Storage of EDTA plasma, citrated plasma, and serum before therapy is critical for accurate hemolytic assessment of underlying complement profile in patients suspected with aHUS.

### Genetic test

Genetic screening is needed to confirm the clinical diagnosis of aHUS. Direct sequencing of six candidate genes (*CFH*, *CFI*, *MCP*, *C3*, *CFB*, and *THBD*) should be performed to reveal the predisposing mutations associated with aHUS. The screening of *DGKE* mutations is also recommended for the patients with onset of aHUS before the age of 1–2 years. Multiple ligation-dependent probe amplification is generally used to identify the copy number variations of the genes encoding CFHR proteins. As shown in the recent finding of *DGKE* mutations, next-generation sequencing analysis is helpful to revealing the undiscovered disease-associated genes in aHUS.

Causality between genetic variants and the development of aHUS should be carefully assessed. A recent important study has shown that 122 of 406 (27%) mutations, which were published as disease-associated mutations in 104 individuals were either common polymorphisms or lacked direct evidence for pathogenicity [57]. Guidelines for genetic analysis have stated the importance of the terms to describe the genetic variants, and recommended the careful assessment to differentiate disease-causing genetic variants from many variants of human genome. To avoid the false-positive reports of causality, various mutations detected in aHUS should be carefully studied, and correctly named by using appropriate nomenclature (e.g., pathogenic, likely pathogenic, uncertain significance, likely benign and benign) instead of simple term like “causative” or “no causative” [58].

Genetic background of aHUS is complicated because of its various hereditary forms (autosomal dominant or autosomal recessive manner) and poor penetrance (about 50%). Therefore, individual and families should be provided an opportunity for genetic consultation by physicians, genetic professionals, and genetic counselors. Patient and patients’ family members should be provided the following information: (1) the hereditary forms of aHUS and the importance of genetic test in the patients’ parents, siblings, and offspring, (2) the risk of aHUS in the patient’s parents, siblings, and offspring, and (3) the risk of aHUS during pregnancy or after delivery.

## Treatment for aHUS

### Plasma treatment

Since 1980s, plasma infusion (PI) or plasma exchange (PE) had empirically been considered as the first choice for the treatment of aHUS. PI with fresh frozen plasma serves functional complement regulatory factors. In addition to this effect, PE is presumed to remove the abnormal complement related factors like mutant proteins or anti-CFH antibodies. There are no prospective clinical trial data, but the introduction of plasma therapy has decreased the mortality of patients with aHUS and achieved hematological remission in 70% of patients with aHUS. On the contrary, plasma therapy resulted in ESRD or death in 48% of pediatric and 67% of adult cases died or reached ESRD within 3-year follow-up [14]. Especially in aHUS patients with *CFH* or *CFI* mutations, complete hematological remission or renal recovery rates was low.

Although some patients do not achieve complete remission by plasma therapy, it is still an important

therapeutic approach for the patients suspected with aHUS. Early initiation of plasma therapy followed by maintenance PI/PE could be effective for attaining hematological remission and preserving renal function. However, evaluation of other TMAs and the diagnosis of aHUS take some times in practical situations. Of note, the efficacy of plasma therapy is uncertain in the treatment for STEC-HUS. In the case of secondary TMA, the necessity and efficacy of plasma therapy depends on the coexisting disease, and PE should be avoided in the cases with *S. pneumoniae*-mediated TMA. Concomitant immunosuppression and plasma therapy may allow better outcomes by reducing antibody titers in the case of anti-CFH antibody positive patients. So far, combined therapy of PE and immunosuppression (steroids with or without immunosuppressants such as cyclophosphamide or rituximab) for the induction and maintenance therapy with steroids and immunosuppresants (mycophenolate mofetil or azathioprine) have been reported to be favorable [5, 59, 60]. Eculizumab also seems to be effective in anti-CFH antibody aHUS patients [5]. However, the optimal treatment combination for anti-CFH antibody positive patients remains to be elucidated in the future. Catheter-related complications should be concerned in pediatric cases, and PI may be recommended when PE is technically difficult to perform. Plasma therapy should be tapered based on increased platelet count, improvement of hemolysis, and lactate dehydrogenase level.

### Eculizumab

Eculizumab, a monoclonal humanized antibody against C5, was originally approved for treating the patients with PNH. This antibody specifically binds to C5 and blocks the terminal complement activation by inhibiting the cleavage of C5 into C5a and C5b. The efficacy and safety of eculizumab for the treatment of aHUS have been reported since 2009 [61–63], and both the USA Food and Drug Administration and the European Medicines Agency approved the indication of aHUS in the treatment of aHUS in 2011. Subsequent reports also showed that the use of eculizumab was effective for the patients with aHUS who underwent the renal transplantation [64].

In pediatric cases, eculizumab administration is recommended as a first-line therapy when the diagnosis of aHUS is made, because these patients have a high risk of catheter-related complications and a lower incidence of secondary TMA than adults. Contrary, in adult patients, the initial choice of PE or eculizumab is often difficult because adults have higher incidence of secondary TMA, some secondary TMA have the indication of PE, and currently eculizumab is not approved for secondary TMA in our country. The authors recommend evaluating STEC-HUS, TTP, and secondary TMA before the initial use of eculizumab. In adult cases, eculizumab administration may be recommended as a first choice in the following situations: (1) the patients have repeated episode of aHUS or family history (especially, one or more family members have renal failure caused by TMA), (2) the patients are already diagnosed with aHUS, and (3) the probability of the diagnosis of aHUS is high. Although the reports are limited, eculizumab treatment for aHUS during pregnancy stabilizes clinical and laboratory markers and shows no overt safety issues [65]. However, further investigations are needed to ascertain the efficacy and safety of eculizumab in the treatment of the patients with aHUS during pregnancy.

The appearance of eculizumab opened a new era for treatment of aHUS; however, it is still unclear how long eculizumab therapy should be continued. Eculizumab treatment requires the patients to visit the hospital once every 2 weeks, which may lead to the impaired quality of life. Moreover, lifelong treatment may cause the compromised vascular access. The extremely high cost of eculizumab is also important limitation of this drug. The current report concerning the eculizumab withdrawal has shown that 24 patients discontinued the treatment, and 6 out of 24 patients (25%) had recurrence at the time of publication [66]. Four of these six patients had *CFH* mutation or anti-CFH antibodies, suggesting that the patients having CFH-related abnormalities were frequently associated with the recurrence of aHUS [5, 66]. On the other hand, patients with MCP mutation, CFI mutation, or no mutation showed no recurrence. Although more studies are needed to confirm these observations, lifelong treatment may not be needed for all patients with aHUS, and clarifying the genetic background may help to discontinue the anti-complement drug [67].

## Prognosis

Before the use of eculizumab for treating aHUS, a poor prognosis of this disease has historically been described, with higher than 50% mortality and ESRD. The clinical outcomes vary depending on the genetic alterations [14, 24]. The worst prognosis is the patients with *CFH* mutations, with over 50% mortality or ESRD rates within 1 year from the first episode of aHUS [14, 24]. On the contrary, the cases of the patients with MCP mutations have a good prognosis; none of the children and only 25% of adults reached to ESRD at first episode although they have a high risk of relapse [2]. Of note, these data come from the historical studies treated with plasma therapy [14, 23, 24]. Recent advances of therapy including early initiation of plasma therapy or eculizumab administration significantly improved the mortality and prognosis of aHUS [62, 68]. In the near future, the data showing prognosis of aHUS patients depending on the genetic background treated with eculizumab will be

clarified. Systemic management including blood pressure control is also important as short- and long-term treatment; however, the effects of different antihypertensive drug classes remain to be elucidated.

## Current studies for the patients with aHUS in Japan

Since the early 2000s, some patients with clinically suspected aHUS have been identified in Japan. In 2008, Mukai et al. reported the predisposing mutation of CFH in a 1-year-old female aHUS patient by using both hemolytic assay and genetic analysis [69]. Further study was performed by Fujimura et al., who have studied Japanese patients with TMA by measuring ADAMTS13 activity since 1998. In 2009, they reported the characteristics of 919 TMA patients and noted that 24 of 919 were suspected to be "congenital aHUS" because of having repeated and familial TMA episodes with ADAMTS13 levels of more than 10% [70]. To reveal the underlying pathogenic mechanisms of these patients, they established the diagnostic system for aHUS (the quantitative hemolytic assay, the screening of anti-CFH antibody, and the genetic tests of *CFH*, *CFI*, *MCP*, *C3*, *CFB*, *and THBD*) [45, 50, 71, 72]. In the hemolytic assay, Yoshida et al. [50] produced inhibitory monoclonal antibodies against CFH and used one of these antibodies as a positive control, which enabled to quantitatively calculate the degree of hemolysis in patient plasma. They revealed that patient plasma with CFH mutation or anti-CFH autoantibodies had >50% hemolysis. In addition, patient plasma with the C3 p.K1105Q mutation positioned at the CFH binding interface also showed >50% hemolysis.

The genetic analysis of 45 patients with aHUS has shown that the frequency of *C3* mutation (43%) was greater than that in Western countries (2–10%). In contrast, only 7% of patients with aHUS carried the *CFH* mutations in Japan, which is much less than the frequency reported by Western countries [50]. Interestingly, about 80% patients with *C3* mutations have the same variants of I1157T, and the patients carrying this variants were only found in an extremely restricted area (Kansai district including Mie, Nara, Kyoto and Osaka prefectures) of West Japan [50, 73]. These observations suggested that the genetic background of aHUS in Japan differs from that of Western countries. However, because study population was small and 60% of the patients with aHUS were from West Japan, further assessments are required to reveal the genetic characteristics in Japan. Complement-related genes have also been investigated in the patients with congenital TTP having renal damage by Fan et al [74]. Six complement and complement regulatory genes of aHUS were sequenced, and they have suggested that rare predisposing complement genetic mutations of aHUS do not contribute to renal insufficiency in congenital TTP patients.

In 2013, the use of eculizumab was approved for treating complement-mediated aHUS patients by Ministry of Health, Labour and Welfare in Japan. Several case reports have described the efficacy and safety of this drug for aHUS in Japan. Ito et al. have retrospectively analyzed the clinical course of 10 pediatric aHUS cases treated with eculizumab, and shown that all patients achieved the rapid hematological remission with withdrawal from plasma therapy [75]. Ohta et al. have reported that one infant patient with compound heterozygous *DGKE* mutation, who significantly recovered from severe hypertensions and peritoneal dialysis by eculizumab administration [72, 76]. Of note, this patient showed the severely decreased level of C3 suggesting unregulated complement activation; however, it is unclear whether the mutation in *DGKE* is associated with exhausted C3 or not in this particular patient. In contrast to pediatric cases with aHUS, little has been reported on adult cases in Japan. The study published by Okumi et al. has shown one male patient with *CFH* mutation, who received living-related kidney transplant after first episode of aHUS [77]. This patient developed aHUS again after transplantation, but his hematological parameters and renal function was fully recovered by initiation of eculizumab treatment.

Currently, the diagnostic system of aHUS was moved from Nara Medical University to the Division of Nephrology and Endocrinology, the University of Tokyo Hospital. Protein-based analyses (the hemolytic assay and the screening of anti-CFH autoantibodies) are performed in the University of Tokyo Hospital, and the DNA analyses of six candidate genes (*CFH*, *CFI*, *MCP*, *C3*, *CFB*, and THBD) are in National Cerebral and Cardiovascular Center. Moreover, epidemiologic study of aHUS is ongoing in the University of Tokyo Hospital. Consultation for diagnostic test of aHUS is available by e-mail (ahus-office@umin.ac.jp).

## Conclusions

The recent progresses in the field of aHUS during the last two decade have significantly clarified the underlying pathology of aHUS, which led to new era for complement blockade; eculizumab therapy. Early administration of eculizumab has dramatically prevented the progression to ESRD in the patients with aHUS. In Japan, an established structured diagnostic system of aHUS has gradually revealed both clinical characteristic and genetic background of this disease. On the other hand, the data on the prognosis (risk of ESRD, death, relapse, and recurrence after renal transplantation) or outcome of eculizumab therapy still have not been well documented. Now, our groups are addressing these issues, and we hope that this

ongoing study will lead to early diagnosis and appropriate treatment for patients with aHUS in Japan.

#### Abbreviations

ADAMTS13: A disintegrin-like and metalloprotease with thrombospondin type 1 motif, 13; aHUS: Atypical hemolytic uremic syndrome; CFB: Complement factor B; CFD: complement factor D; CFH: Complement factor H; CFHR: Complement factor H related; CFI: Complement factor I; DGKE: Diacylglycerol kinase epsilon; EDTA: Ethylenediaminetetraacetic acid; ESRD: End-stage renal disease; HELLP: Hemolysis, elevated liver enzyme, low platelet count; MCP: Membrane cofactor protein; RBC: Red blood cell; STEC: Shiga toxin-producing *Escherichia coli*; TMA: Thrombotic microangiopathy; TTP: Thrombotic thrombocytopenic purpura; VWF: von Willebrand factor

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#### Availability of data and materials

Not applicable

#### Authors' contributions

YY and HK made the original form of this manuscript. MN organized the comprehensive study project and edited of this manuscript. All authors read and approved the final manuscript.

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