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GUIDELINE

Clinical guides for atypical hemolytic uremic syndrome in Japan

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Abstract Atypical hemolytic uremic syndrome (aHUS) is a rare disease characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury. In 2013, we developed diagnostic criteria to enable early diagnosis and timely initiation of appropriate treatment for aHUS. Recent clinical and molecular findings have resulted in several proposed classifications and definitions of thrombotic microangiopathy and aHUS. Based on recent advances in this field and the emerging international consensus to exclude secondary TMAs from the definition of aHUS, we have redefined aHUS and proposed diagnostic algorithms, differential diagnosis, and therapeutic strategies for aHUS.

Keywords Atypical hemolytic uremic syndrome · Thrombotic microangiopathy · Eculizumab · Alternative complement pathway

Introduction

Thrombotic microangiopathy (TMA) is a pathophysiological process characterized by the triad of microangiopathic hemolytic anemia (MAHA), consumptive thrombocytopenia, and platelet-mediated microvascular occlusion, leading to organ failure. Classic forms of TMA include hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic

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purpura (TTP). TMAs caused by Shiga toxin-producing *Escherichia coli* (STEC) are termed STEC-HUS, while TMAs caused by severely reduced activity (levels less than 10 % of normal) of a disintegrin-like metalloproteinase with thrombospondin type 1 repeat motifs 13 (ADAMTS13) are termed TTP.

Approximately 90 % of patients presenting with HUS symptoms have STEC infection with bloody diarrhea. The remaining 10 % do not present with diarrhea and their samples are negative for Shiga toxins; such cases were previously classified as diarrhea-negative HUS (D(-)HUS). In 1981, the first case of D(-)HUS accompanied by complement factor H (CFH) deficiency was reported [1]. Warwicker et al. suggested CFH gene mutations as a possible cause of HUS in a linkage analysis study performed in 1998 [2], one of the earliest works to propose genetic involvement in atypical HUS (aHUS). Subsequently, a series of studies indicated that aHUS pathogenesis involved genetic abnormalities of the complements, such as *C3*, complement factor B (*CFB*), complement factor I (*CFI*), membrane cofactor protein (*MCP* or *CD46*), and thrombomodulin (*THBD*). In addition, an acquired form of aHUS with positive anti-CFH antibodies has been identified.

Patients with aHUS have also been reported in Japan [3], and a series of cases prompted the Japanese Society of Nephrology and the Japan Pediatric Society to jointly develop the aHUS diagnostic criteria in 2013 [4, 5]. These criteria broadly defined aHUS as a TMA condition unrelated to STEC-HUS or TTP; thus, the definition included aHUS with complement regulation abnormality and TMA with coexisting diseases (secondary TMA; also called other TMA). However, the international consensus to exclude secondary TMAs from the definition of aHUS [6–8] suggested the need to revise the 2013 edition. This article explains the diagnosis and treatment guides for aHUS, which have incorporated diagnostic algorithms and therapeutic recommendations to assist in clinical practice.

Definitions of TMA and aHUS

Originally, TMA was used to describe pathologic conditions involving systemic microvascular thrombosis and endothelial injury. Currently, TMA also refers to the clinical conditions with the triad of MAHA, consumptive thrombocytopenia, and platelet-mediated microvascular occlusion, leading to organ failures. Common forms of TMA include TTP, STEC-HUS, complement-related aHUS, and secondary TMA. Different types of TMA cause thrombosis in preferential organs, and renal impairment is the most frequent with STEC-HUS and aHUS.

There is currently no international consensus regarding the classification of diseases under TMA. According to the definition of the aHUS diagnostic criteria jointly proposed by the Japanese Society of Nephrology and the Japan Pediatric Society in 2013, aHUS involved the triad of MAHA, thrombocytopenia, and acute kidney failure in the absence of Shiga toxins and TTP [4, 5].

Quoting the diagnostic criteria established by the UK aHUS Rare Disease Group, Scully and Goodship [7] proposed to exclude the following from aHUS: STEC-HUS, TTP, and secondary TMAs resulting from drugs, infection, transplantation, cobalamin deficiency, systemic lupus erythematosus, antiphospholipid syndrome, scleroderma, and other causes.

The definitions of aHUS and TMA have been considerably revised in the current aHUS clinical guides, based on findings reported by several publications [7–9]. Specifically, aHUS associated with congenital and acquired "complement regulation abnormality", as defined in the 2013 version, has been termed "aHUS (complement-mediated HUS)" in the current edition. In addition, TMAs arising from other causes have been defined as "secondary TMAs."

More specifically, aHUS defined in the current version relates to one of the following:

1. Congenital genetic abnormalities (known as of 2015) in seven complement component and complement regulatory genes; i.e., abnormalities in the *CFH*, *CFI*, *CD46* (*MCP*), *C3*, *CFB*, *THBD*, and diacylglycerol kinase ε (*DGKE*) genes. Note that several researchers do not regard DGKE abnormalities as a cause of aHUS because of the absence of compelling evidence of the interplay between the DGKE and complement systems. Further, plasminogen (*PLG*) gene mutations have been suggested to contribute to the etiology of aHUS, but warrant further investigation.
2. Anti-CFH autoantibody positivity (a cause of acquired aHUS).
3. Patients who have none of the genetic mutations mentioned above, but whose clinical manifestations suggest aHUS that cannot be classified as STEC-HUS, TTP, or secondary TMA.

Epidemiology

The exact incidence rates of aHUS are unknown. It is estimated that 2 per million adults and 3.3 per million children develop aHUS each year [10]. Approximately

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40 % of patients who are newly diagnosed with aHUS are under 18 years of age [9, 11]. A one-year prospective study conducted in the United Kingdom reported that the incidence rate was 0.4 patients per million population [12]. In Japan, current estimates suggest that 100 to 200 patients are diagnosed with aHUS.

Etiology and pathophysiology

Complement-mediated HUS is caused by dysregulation of the alternative pathway of the complement system. The genetic causes of aHUS can be divided into loss-of-function and gain-of-function mutations. Loss-of-function mutations relate to the *CFH*, *CFI*, *CD46*, and *THBD* genes. Anti-CFH antibody also results in CFH dysfunction. Gain-of-function mutations relate to the *CFB* and *C3* genes. Loss-of-function and gain-of-function mutations both cause hyperactivation of the alternative complement pathway, which in turn induces aHUS by triggering endothelial damage and platelet aggregation.

Anti-CFH autoantibodies have been detected in approximately 10 % of patients with aHUS [13]. These antibodies bind to the C-terminal domain of CFH and impair CFH-mediated cell surface protection by interfering with the interaction between CFH and its surface ligands.

Recent genetic studies of patients with TMA have identified abnormalities in the *THBD*, *DGKE*, and *PLG* encoding components of the coagulation and fibrinolytic pathways [14, 15]. However, the details of the involvement of these mutations in TMA pathogenesis remain to be clarified. While THBD is a key mediator of anticoagulant response, it also induces C3b inactivation by binding to C3b or CFH. In the current clinical guides, patients with THBD, DGKE, and PLG abnormalities are categorized as having aHUS.

Diagnosis

Clinical manifestations

According to a UK national survey, the onset of many cases of aHUS is either idiopathic or secondary to infection and other disease triggers [16]. Similar to STEC-HUS, aHUS is frequently accompanied by hemolytic anemia, thrombocytopenia, and renal failure. The clinical manifestations may also include central neuropathy, cardiac failure, respiratory disorders, enterocolitis, hypertension, and other conditions affecting multiple organs or systems. Patients with aHUS may present with ischemic enterocolitis and other gastrointestinal problems. In addition, aHUS may be precipitated by microbial or viral infections of the digestive system. Therefore, attention should be paid to the fact that the presence of diarrhea does not exclude the diagnosis of aHUS [16].

Clinical diagnostic criteria

Patients with TMA are clinically diagnosed with aHUS if the following diagnoses can be excluded: STEC-HUS, TTP, TMA secondary to metabolism-related, infection, drug-induced, autoimmune diseases, malignant tumors, hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome, transplantation, or other known causes. TMA typically, but not necessarily, involves the following conditions:

1. **MAHA with hemoglobin levels below 10 g/dL**. In addition to blood hemoglobin levels, elevation of serum lactate dehydrogenase (LDH) level, notable decrease of serum haptoglobin level, and the presence of schistocytes on a peripheral blood smear should be taken into consideration to confirm the diagnosis of MAHA. Detection of schistocytes is not a necessary criterion for diagnosis of MAHA.
2. **Thrombocytopenia with platelet counts less than 150,000/μL** [9].
3. **Acute kidney injury (AKI)**. In pediatric patients, AKI is defined as serum creatinine levels at least 1.5 times the upper limit of the age- and sex-specific pediatric reference range defined by the Japanese Society for Pediatric Nephrology [17]. For adult patients, the diagnosis of AKI should be made according to well-established diagnostic guidelines [18].

Differential diagnosis

Patients with TMA should be clinically diagnosed with aHUS after confirming that they do not meet the criteria for the following: first, STEC-HUS or TTP, and then, TMA secondary to known causative underlying conditions [7, 19]. It should be noted that some cases of secondary TMA have been reported to have complement gene mutations and anti-CFH antibodies. Future research should investigate the extent of the involvement of abnormal complement activation in the etiology of secondary TMA, the proportion of patients with complement gene mutations among the population with secondary TMA, and the effectiveness of eculizumab for treating secondary TMA.

Clinicians should strongly suspect aHUS if the patient's family history includes individuals with the following diagnoses: aHUS; HUS, TTP, or TMA in the era when aHUS was not well recognized; or renal failure of unknown cause.

1. Differentiation between TMA and similar conditions
   - **Diagnosis of hemolytic anemia and differentiation of MAHA from other forms of hemolytic anemia**. Elevated LDH level, schistocytes in blood smears, and marked decreases in haptoglobin levels are consistent with the diagnosis of hemolytic anemia. The Coombs test is helpful in diagnosing autoimmune hemolytic anemia.
   - **Differentiation between TMA and other disorders causing AKI**.
   - **Differentiation of disseminated intravascular coagulation (DIC)**. Physicians should use well-established diagnostic criteria for DIC. For this purpose, appropriate parameters should be evaluated, such as prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrin degradation product (FDP), D-dimer, and fibrinogen levels. In general, DIC occurs secondary to sepsis, malignant tumors, hematologic disorders, trauma, and other underlying causes.
   - **Differentiation of pernicious anemia**. Pernicious anemia has been reported to present with clinical manifestations similar to those of TMA [20]. Measurements of vitamin B12 and folic acid levels are helpful for its identification. Patients with pernicious anemia frequently have low reticulocyte counts.
   - **Differentiation of heparin-induced thrombocytopenia (HIT)**.
2. **Differentiation of STEC-HUS**
   Results of stool culture assays, direct detection of Shiga toxins in feces, and anti-lipopolysaccharide (LPS) immunoglobulin (Ig) M antibody measurements assist the diagnosis of STEC infection. Approximately 80 % of patients with STEC-HUS have bloody diarrhea, which is often severe. Ultrasound scans typically show extreme wall thickening of the ascending colon with elevated echogenicity. In pediatric patients, STEC-HUS accounts for approximately 90 % of all TMA cases. Therefore, STEC-HUS should be primarily suspected in children aged 6 months or older presenting with severe bloody diarrhea and other common gastrointestinal complications.
3. **Differentiation of TTP**
   Patients who have less than 10 % of normal ADAMTS13 activity and are positive for anti-ADAMTS13 neutralizing antibodies (inhibitors) are diagnosed with acquired TTP. Congenital TTP is suspected if ADAMTS13 activity is less than 10 % and anti-ADAMTS13 inhibitors are not present [21]. To confirm the diagnosis of congenital TTP, *ADAMST13* gene analysis is necessary. TMAs other than TTP, such as aHUS, HUS, and secondary TMA, are occasionally associated with decreased ADAMTS13 activity; however, in most such cases, ADAMTS13 activity does not decrease below 20 % of normal [22].
4. **Differentiation of Secondary TMA**
   - **Cobalamin C deficiency** (particularly in infants). Disorders of cobalamin metabolism are frequently detected in infants less than 12 months of age presenting with feeding problems, vomiting, poor growth, enervation, hypotonia, and convulsions. Cobalamin C deficiency has also been reported in adults in recent years. This disease presents with hyperhomocysteinemia, decreased plasma methionine levels, and methylmalonic aciduria [23].
   - **Autoimmune diseases and connective tissue diseases**, in particular, systemic lupus erythematosus, scleroderma renal crisis, antiphospholipid syndrome, multiple myositis/dermatomyositis, and vasculitis. These disorders often present with signs and symptoms similar to TMA. The following assessments should be conducted, as appropriate: antinuclear antibodies, antiphospholipid antibodies, anti-DNA antibodies, anti-centromere antibodies, anti-Scl-70 antibodies, C3, C4, CH50, immunoglobulin (Ig)G, IgA, IgM, and anti-neutrophil cytoplasmic antibodies (ANCA).
   - **Accelerated or malignant hypertension**. Patients with accelerated or malignant hypertension often present with TMA. Patients with aHUS sometimes present with accelerated or malignant hypertension; thus, when TMA persists after treatment of hypertension, efforts should be made to differentiate aHUS from these disorders.
   - **Malignant tumors**. Advanced malignant tumors often cause TMA. In a review of cancer-related TMA cases reported in the literature, more than 90 % had advanced cancers, including tumors of the gastrointestinal tract, breast, prostate, and lung [24].
   - **Infections**. Pneumococcal infections, particularly invasive pneumococcal infections, cause TMA mostly in children. Therapeutic plasma exchange may aggravate the condition. Approximately 90 % of patients with pneumococcus-associated HUS have positive direct Coombs test results [25]. In addition to pneumococcal infection, infections with human immunodeficiency virus (HIV), influenza A H1N1 virus, hepatitis C virus, and cytomegalovirus, as well as pertussis, varicella, and severe streptococcal infection, have been reported to cause TMA [16, 26, 27]. Attention should be paid to the

cases where infections with influenza virus and other infections often trigger the onset of aHUS [28].

- **Pregnancy-induced HELLP syndrome and eclampsia**. HELLP syndrome and eclampsia usually resolve quickly after delivery. However, cases of TTP and aHUS triggered by pregnancy have been reported in the literature. In a cohort study, patients with aHUS developed HELLP syndrome primarily postpartum [29]; however, the incidence of aHUS among patients with HELLP syndrome or postpartum HELLP syndrome is unknown.
- **Drug-induced TMA**. Anti-tumor agents, antiplatelet drugs, immunosuppressive agents, and other medications may cause TMA (Table 1) [30]. Agents suspected of causing TMA should be tapered or discontinued wherever possible.
- **Acute pancreatitis**. Acute pancreatitis is a possible or probable precipitating event for TMA episodes [31]. In a review of seven cases of TMA precipitated by acute pancreatitis, patients responded well to therapeutic plasma exchange [32].
- **Post-transplant TMA subsequent to hematopoietic stem cell or organ transplantation**. Post-transplant TMA following hematopoietic stem cell transplantation has been widely documented. In patients with post-transplant TMA, ADAMTS13 activities usually do not fall below 10 % of normal, and plasma exchange is not very effective. Typical interventions include discontinuation or dose reduction of immunosuppressive calcineurin inhibitors [33]. Recent research revealed a high prevalence of anti-CFH autoantibodies in pediatric patients with hematopoietic stem cell transplant-associated TMA [34]; however, the involvement of complement dysregulation in the pathogenesis of TMA following hematopoietic stem cell transplant requires further investigation.

  Patients with end-stage renal disease due to aHUS who are undergoing kidney transplant are at high risk of TMA recurrence and graft loss. It is therefore advisable to conduct genetic testing preoperatively in prospective kidney recipients suspected of having aHUS. TMAs occurring subsequent to kidney transplant (*de novo*) involve new onset of aHUS, with complement abnormalities [35], as well as transplant-induced TMA [30]. The clinical approaches for patients with aHUS undergoing kidney transplantation and TMA after kidney transplantation are beyond the scope of this guide; please see the current consensus [8]. The occurrence of TMA has been documented not only in patients with kidney transplants, but in those receiving liver, heart, lung, and small intestine transplants [36].

Considerations concerning pediatric diagnosis

STEC-HUS should primarily be suspected in children with TMA aged 6 months or older who manifest severe bloody diarrhea, because STEC-HUS accounts for approximately 90 % of all pediatric TMA cases. Conditions that predispose pediatric patients to TMA, not accompanied by diarrhea or bloody stool include pneumococcal and other infections in infants, and systemic lupus erythematosus and antiphospholipid syndrome. When a pediatric patient is diagnosed with TMA, the physician should immediately examine the possibility of TTP and determine whether existing medical conditions or oral medications are causing TMA. If these possibilities are ruled out, the physician should initiate eculizumab therapy while continuing to investigate whether rarer etiologies are responsible for TMA.

Laboratory confirmation of aHUS diagnosis

Besides the laboratory data supporting the diagnosis of TMA mentioned above, low C3 and normal C4 levels strongly suggest activation of the alternative pathway, and hence aHUS. However, previous data show that low C3 levels are detected in approximately half of patients with aHUS, and normal C3 levels do not necessarily rule out its diagnosis. To establish a diagnosis of aHUS, several studies recommend analyses of CFH, CFI, and CFB levels, and leukocyte expression levels of CD46 in addition to routine blood C3 and C4 measurements. However, the levels of these alternative complement molecules do not necessarily lead to the diagnosis of aHUS [6]. Quantitative hemolytic assay protocols using sheep erythrocytes are highly sensitive methods for detecting patients with genetic CFH abnormalities and anti-CFH antibodies [37, 38]. However, these protocols are still not practical for use in routine clinical settings. Urological examination in many patients with aHUS shows hematuria and proteinuria.

Confirmatory diagnosis of aHUS requires genetic testing for known causative genes and analysis of anti-CFH antibodies. However, the absence of causative genetic mutations does not always exclude the diagnosis of aHUS, because approximately 40 % of patients show no known genetic abnormalities.

Physicians caring for patients with suspected aHUS in Japan are advised to contact the Division of Nephrology and Endocrinology, University of Tokyo Hospital (ahus-office@umin.ac.jp), which will conduct hemolytic assay, anti-CFH antibody screening and genetic assays in

Table 1 Examples of medications that may cause TMA (adopted from References [9, 30])

Antiplatelets	Ticlopidine, clopidogrel
Antibacterials	Quinine
Antivirals	Valacyclovir
Interferons	
Antitumor agents	Mitomycin C, gemcitabine, cisplatin, vascular endothelial growth factor (VEGF) inhibitors, tyrosine kinase inhibitors
Immunosuppressants	Cyclosporin, tacrolimus, sirolimus
Oral contraceptives	

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Treatments

Therapeutic considerations

Since the 1980s, plasma exchange therapy has been the mainstay method for management of aHUS. This therapy aims to eliminate abnormal complement regulatory proteins and anti-CFH antibodies, while supplementing normal complement regulatory proteins. Eculizumab is a humanized monoclonal antibody that binds to C5 complement protein. Eculizumab suppresses C5 cleavage to C5a and C5b and thereby prevents the production of the membrane attack complement complex (MAC).

In practical terms, when a patient presents with TMA and is negative for STEC-HUS and invasive pneumococcal infection (the latter of which is not indicated for plasma exchange), the treating physician should start the empirical treatments described below, while continuing diagnostic efforts. Physicians should also pay attention to systemic management such as fluid and electrolyte control, blood pressure control, and supportive therapies for AKI.

If the physician considers plasma exchange appropriate, it should be started immediately. Daily sessions followed by gradual tapering of the plasma therapy are recommended. Plasma infusion may be implemented in pediatric patients in whom plasma exchange is technically difficult to perform, as well as in situations where plasma exchange cannot be performed. The tapering of the plasma therapy will generally be based on improvements in platelet count, LDH and hemoglobin levels [39]. Although plasma infusion and plasma exchange can achieve hematological remission in approximately 70 % of patients with aHUS, long-term outcomes include high incidences of TMA recurrence, progression to end-stage renal failure, and death [40].

If the patient is clinically diagnosed with aHUS after STEC-HUS, and if TTP and secondary TMA are ruled out, the physician should consider eculizumab therapy [7]. Eculizumab is recommended in the early stages of treatment of pediatric patients with clinically diagnosed aHUS because pediatric patients have a lower incidence of secondary TMA than adults and a higher rate of complications related to catheterization for plasma exchange and plasma infusion [8].

Decreased platelet counts observed in patients with aHUS usually resolve after 1 to 2 weeks of eculizumab therapy [41–43].

In anti-CFH antibody-positive patients, plasma exchange combined with immunosuppressants or steroids, as compared to plasma exchange alone, yielded better outcomes with reduced antibody titers [11]. Eculizumab may be considered for treating aHUS accompanied by extra-renal organ injury [8].

Warnings and precautions for eculizumab use

Eculizumab has been shown to elevate the risk of meningococcal infection, and patients should be immunized with meningococcal vaccine at least two weeks prior to receiving eculizumab. If situations require immediate eculizumab administration in a patient who has not been immunized with meningococcal vaccine, the physician must administer appropriate prophylactic antibiotics.

Discontinuation of eculizumab

No expert consensus has been reached regarding the timing of eculizumab withdrawal after achievement of remission.

One study reviewed 20 cases of aHUS involving eculizumab therapy discontinuation [8]. Patients with *CFH* mutations and anti-CFH antibody-positive patients had a higher rate of recurrence. However, among patients with *CD46* or *CFI* mutations and those without known causative genes, no recurrence was observed during the study period. In a similar review of 24 patients who terminated eculizumab therapy [44], the incidence rate was 25 %, and

recurrences were noted more frequently in patients with *CFH* mutation and those positive for anti-CFH antibodies.

Available vaccines are insufficient for completely preventing meningococcal and other types of infections in patients receiving eculizumab. Eculizumab therapy requires patients to visit the hospital once every two weeks, a requirement that considerably affects their quality of life. Long-term repeated intravenous administration often leads to compromised vascular access. In addition, cost-benefit analyses should be considered for eculizumab, one of the most expensive drugs on the market. Future research on the relationship between genetic mutations and treatment outcomes, and markers for early detection of recurrence, will shed light on ways of overcoming these problems [8, 44].

Outcome

The literature has reported gene-specific differences in response to therapeutic plasma exchange and in graft survival after kidney transplant [11]. While eculizumab therapy has been shown to improve treatment outcomes, its gene-specific outcomes are not well known.

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Compliance with ethical standards

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References

1. Thompson RA, Winterborn MH. Hypocomplementaemia due to a genetic deficiency of beta 1H globulin. Clin Exp Immunol. 1981;46(1):110–9.
2. Warwicker P, Goodship TH, Donne RL, Pirson Y, Nicholls A, Ward RM, et al. Genetic studies into inherited and sporadic hemolytic uremic syndrome. Kidney Int. 1998;53(4):836–44. doi:10.1111/j.1523-1755.1998.00824.x.
3. Mukai S, Hidaka Y, Hirota-Kawadobora M, Matsuda K, Fujihara N, Takezawa Y, et al. Factor H gene variants in Japanese: its relation to atypical hemolytic uremic syndrome. Mol Immunol. 2011;49(1–2):48–55. doi:10.1016/j.molimm.2011.07.017.
4. Sawai T, Nangaku M, Ashida A, Fujimaru R, Hataya H, Hidaka Y, et al. Diagnostic criteria for atypical hemolytic uremic syndrome proposed by the Joint Committee of the Japanese Society of Nephrology and the Japan Pediatric Society. Clin Exp Nephrol. 2014;18(1):4–9. doi:10.1007/s10157-013-0911-8.
5. Sawai T, Nangaku M, Ashida A, Fujimaru R, Hataya H, Hidaka Y, et al. Diagnostic criteria for atypical hemolytic uremic syndrome proposed by the Joint Committee of the Japanese Society of Nephrology and the Japan Pediatric Society. Pediatr Int. 2014;56(1):1–5. doi:10.1111/ped.12274.
6. Loirat C, Fremeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis. 2011;6:60. doi:10.1186/1750-1172-6-60.
7. Scully M, Goodship T. How I treat thrombotic thrombocytopenic purpura and atypical haemolytic uraemic syndrome. Br J Haematol. 2014;164(6):759–66. doi:10.1111/bjh.12718.
8. Loirat C, Fakhouri F, Ariceta G, Besbas N, Bitzan M, Bjerre A, et al. An international consensus approach to the management of atypical hemolytic uremic syndrome in children. Pediatr Nephrol. 2016;31(1):15–39. doi:10.1007/s00467-015-3076-8.
9. Campistol JM, Arias M, Ariceta G, Blasco M, Espinosa M, Grinyo JM, et al. An update for atypical haemolytic uraemic syndrome: diagnosis and treatment. A consensus document. Nefrologia. 2013;33(1):27–45. doi:10.3265/Nefrologia.pre2012.Nov.11781.
10. Mele C, Remuzzi G, Noris M. Hemolytic uremic syndrome. Semin Immunopathol. 2014;36(4):399–420. doi:10.1007/s00281-014-0416-x.
11. Fremeaux-Bacchi V, Fakhouri F, Garnier A, Bienaime F, Dragon-Durey MA, Ngo S, et al. Genetics and outcome of atypical hemolytic uremic syndrome: a nationwide French series comparing children and adults. Clin J Am Soc Nephrol. 2013;8(4):554–62. doi:10.2215/CJN.04760512.
12. Sheerin NS, Kavanagh D, Goodship TH, Johnson S. A national specialized service in England for atypical haemolytic uraemic syndrome-the first year's experience. QJM. 2015;109(1):27–33. doi:10.1093/qjmed/hcv082.
13. Hofer J, Janecke AR, Zimmerhackl LB, Riedl M, Rosales A, Giner T, et al. Complement factor H-related protein 1 deficiency and factor H antibodies in pediatric patients with atypical hemolytic uremic syndrome. Clin J Am Soc Nephrol. 2013;8(3):407–15. doi:10.2215/CJN.01260212.
14. Lemaire M, Fremeaux-Bacchi V, Schaefer F, Choi M, Tang WH, Le Quintrec M, et al. Recessive mutations in DGKE cause atypical hemolytic-uremic syndrome. Nat Genet. 2013;45(5):531–6. doi:10.1038/ng.2590.
15. Bu F, Maga T, Meyer NC, Wang K, Thomas CP, Nester CM, et al. Comprehensive genetic analysis of complement and

coagulation genes in atypical hemolytic uremic syndrome. J Am Soc Nephrol. 2014;25(1):55–64. doi:10.1681/ASN.2013050453.
16. Johnson S, Stojanovic J, Ariceta G, Bitzan M, Besbas N, Frieling M, et al. An audit analysis of a guideline for the investigation and initial therapy of diarrhea negative (atypical) hemolytic uremic syndrome. Pediatr Nephrol. 2014;29(10):1967–78. doi:10.1007/s00467-014-2817-4.
17. Uemura O, Honda M, Matsuyama T, Ishikura K, Hataya H, Yata N, et al. Age, gender, and body length effects on reference serum creatinine levels determined by an enzymatic method in Japanese children: a multicenter study. Clin Exp Nephrol. 2011;15(5):694–9. doi:10.1007/s10157-011-0452-y.
18. KDIGO. Clinical practice guideline for acute kidney injury. Kidney Int Suppl. 2012;2:1–138.
19. Kato H, Yoshida Y, Nangaku M. Pathogenesis of complement-mediated and coagulation-mediated atypical hemolytic uremic syndrome. Nihon Jinzo Gakkai Shi. 2014;56(7):1058–66.
20. Tadakamalla AK, Talluri SK, Besur S. Pseudo-thrombotic thrombocytopenic purpura: a rare presentation of pernicious anemia. N Am J Med Sci. 2011;3(10):472–4. doi:10.4297/najms.2011.3472.
21. Fujimura Y, Isonishi A. Pathophysiology of thrombotic thrombocytopenic purpura. Nihon Jinzo Gakkai Shi. 2014;56(7):1043–51.
22. Shah N, Rutherford C, Matevosyan K, Shen YM, Sarode R. Role of ADAMTS13 in the management of thrombotic microangiopathies including thrombotic thrombocytopenic purpura (TTP). Br J Haematol. 2013;163(4):514–9. doi:10.1111/bjh.12569.
23. Hattori M. Pathogenesis and clinical features of HUS and aHUS. Nihon Jinzo Gakkai Shi. 2014;56(7):1052–7.
24. Lechner K, Obermeier HL. Cancer-related microangiopathic hemolytic anemia: clinical and laboratory features in 168 reported cases. Medicine (Baltimore). 2012;91(4):195–205. doi:10.1097/MD.0b013e3182603598.
25. von Vigier RO, Seibel K, Bianchetti MG. Positive Coombs test in pneumococcus-associated hemolytic uremic syndrome. A review of the literature. Nephron. 1999;82(2):183–4.
26. Allen U, Licht C. Pandemic H1N1 influenza A infection and (atypical) HUS–more than just another trigger? Pediatr Nephrol. 2011;26(1):3–5. doi:10.1007/s00467-010-1690-z.
27. Shimizu M, Yokoyama T, Sakashita N, Sato A, Ueno K, Akita C, et al. Thomsen-Friedenreich antigen exposure as a cause of Streptococcus pyogenes-associated hemolytic-uremic syndrome. Clin Nephrol. 2012;78(4):328–31.
28. Fan X, Yoshida Y, Honda S, Matsumoto M, Sawada Y, Hattori M, et al. Analysis of genetic and predisposing factors in Japanese patients with atypical hemolytic uremic syndrome. Mol Immunol. 2013;54(2):238–46. doi:10.1016/j.molimm.2012.12.006.
29. Fakhouri F, Roumenina L, Provot F, Sallee M, Caillard S, Couzi L, et al. Pregnancy-associated hemolytic uremic syndrome revisited in the era of complement gene mutations. J Am Soc Nephrol. 2010;21(5):859–67. doi:10.1681/ASN.2009070706.
30. Matsui K, Yasuda T. Drug/transplant-induced atypical hemolytic uremic syndrome. Nihon Jinzo Gakkai Shi. 2014;56(7):1067–74.
31. Swisher KK, Doan JT, Vesely SK, Kwaan HC, Kim B, Lammle B, et al. Pancreatitis preceding acute episodes of thrombotic thrombocytopenic purpura-hemolytic uremic syndrome: report of five patients with a systematic review of published reports. Haematologica. 2007;92(7):936–43.
32. McDonald V, Laffan M, Benjamin S, Bevan D, Machin S, Scully MA. Thrombotic thrombocytopenic purpura precipitated by acute pancreatitis: a report of seven cases from a regional UK TTP registry. Br J Haematol. 2009;144(3):430–3. doi:10.1111/j.1365-2141.2008.07458.x.
33. Matsumoto M. Pathophysiology and management of transplantation associated TMA. Rinsho Ketsueki. 2013;54(10):1958–65.
34. Jodele S, Licht C, Goebel J, Dixon BP, Zhang K, Sivakumaran TA, et al. Abnormalities in the alternative pathway of complement in children with hematopoietic stem cell transplant-associated thrombotic microangiopathy. Blood. 2013;122(12):2003–7. doi:10.1182/blood-2013-05-501445.
35. Le Quintrec M, Lionet A, Kamar N, Karras A, Barbier S, Buchler M, et al. Complement mutation-associated de novo thrombotic microangiopathy following kidney transplantation. Am J Transplant. 2008;8(8):1694–701. doi:10.1111/j.1600-6143.2008.02297.x.
36. Verbiest A, Pirenne J, Dierickx D. De novo thrombotic microangiopathy after non-renal solid organ transplantation. Blood Rev. 2014;28(6):269–79. doi:10.1016/j.blre.2014.09.001.
37. Roumenina LT, Roquigny R, Blanc C, Poulain N, Ngo S, Dragon-Durey MA, et al. Functional evaluation of factor H genetic and acquired abnormalities: application for atypical hemolytic uremic syndrome (aHUS). Methods Mol Biol. 2014;1100:237–47. doi:10.1007/978-1-62703-724-2_19.
38. Yoshida Y, Miyata T, Matsumoto M, Shirotani-Ikejima H, Uchida Y, Ohyama Y, et al. A novel quantitative hemolytic assay coupled with restriction fragment length polymorphisms analysis enabled early diagnosis of atypical hemolytic uremic syndrome and identified unique predisposing mutations in Japan. PLoS One. 2015;10(5):e0124655. doi:10.1371/journal.pone.0124655.
39. Sakai N, Wada T. Therapeutic strategy and thrombotic microangiopathy. Nihon Jinzo Gakkai Shi. 2014;56(7):1082–9.
40. Noris M, Caprioli J, Bresin E, Mossali C, Pianetti G, Gamba S, et al. Relative role of genetic complement abnormalities in sporadic and familial aHUS and their impact on clinical phenotype. Clin J Am Soc Nephrol. 2010;5(10):1844–59. doi:10.2215/CJN.02210310.
41. Legendre CM, Licht C, Muus P, Greenbaum LA, Babu S, Bedrosian C, et al. Terminal complement inhibitor eculizumab in atypical hemolytic-uremic syndrome. N Engl J Med. 2013;368(23):2169–81. doi:10.1056/NEJMoa1208981.
42. Licht C, Greenbaum LA, Muus P, Babu S, Bedrosian CL, Cohen DJ, et al. Efficacy and safety of eculizumab in atypical hemolytic uremic syndrome from 2-year extensions of phase 2 studies. Kidney Int. 2015;87(5):1061–73. doi:10.1038/ki.2014.423.
43. Ito N, Hataya H, Saida K, Amano Y, Hidaka Y, Motoyoshi Y, et al. Efficacy and safety of eculizumab in childhood atypical hemolytic uremic syndrome in Japan. Clin Exp Nephrol. 2015;20(2):265–72. doi:10.1007/s10157-015-1142-y.
44. Nester CM. Managing atypical hemolytic uremic syndrome: chapter 2. Kidney Int. 2015;87(5):882–4. doi:10.1038/ki.2015.60.