deficit [9].

5. Cardiovascular sequelae

Myocarditis, cardiac thrombotic microangiopathy, dilated cardiomyopathy, cardiac tamponade and ischemic myocardial involvement were reported as cardiovascular sequelae in HUS patients after the acute phase. However, the long-term outcome of these cardiac complications is not known [a, 10 – 12].

[Secondary evidence]

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3. Suri RS, *et al.* Relationship between *Escherichia coli* O157:H7 and diabetes mellitus. Kidney Int Suppl 2009; 112: S44-S46. (level 4)
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7. Sheth KJ, *et al.* Neurological involvement in hemolytic-uremic syndrome. Ann Neurol 1986; 19: 90-93. (level 4)
8. Schlieper A, *et al.* Sequelae of hemolytic uremic syndrome. Arch Dis Child 1992; 67: 930-934. (level 4)
9. Schlieper A, *et al.* Neuropsychological sequelae of haemolytic uraemic syndrome. Investigators of the HUS Cognitive Study. Arch Dis Child 1999; 80: 214-220. (level 4)
10. Poulton J, *et al.* Dilated cardiomyopathy associated with haemolytic uraemic syndrome. Br Heart J 1987; 57: 181-183. (level 5)
11. Mohammed J, *et al.* Cardiac tamponade in diarrhea-positive haemolytic uraemic syndrome. Nephrol Dial Transplant 2009; 24: 679-681. (level 5)

12. Askiti V, *et al.* Troponin I levels in a hemolytic uremic syndrome patient with severe cardiac failure. Pediatr Nephrol 2004; 19: 345-348. (level 5)

IV. Diagnosis and treatment of HUS in adults

IV.1 Diagnosis of HUS in adults

There are a variety of etiologies in HUS in adults. Possibilities other than STEC-associated HUS should be explored particularly when it occurs in the absence of bloody diarrhea. **[Grade of Recommendation: Not Graded]**

[Comments]

The etiologies of adult HUS differ from those in children. Most HUS are caused by secondary diseases such as thrombotic thrombocytopenic purpura (TTP) associated with ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13) abnormality and various disorders that lead to aHUS [1-4, a-f] (Table 1). Oftentimes, it is difficult to distinguish between HUS and TTP at the onset, and plasmapheresis without delay being considered for most patients. Hence, at the stage of tentative diagnosis, the abbreviation of TTP/HUS (or HUS/TTP) is often used to describe the syndromes. Typical HUS caused by STX, which represents more than 90% of HUS in children, is seen in only 5-10% of the TTP/HUS cases in adults [a, b]. Table 1 shows the incidence of various TTP/HUS causes reported in the Japanese registry that covers mainly secondary causes [f].

STEC-associated HUS is usually considered in adult patients if they present with hemorrhagic diarrhea. Otherwise, other causes of secondary TTP/HUS should be explored. It is noteworthy that non-hemorrhagic diarrhea may be seen in about 30% of non-STEC-associated HUS. On the contrary, hemorrhagic diarrhea can be seen when patients manifest ischemic colitis or peptic ulcers.

As shown in Table 1, the etiologies of TTP and atypical HUS varied and should be investigated according to the patient history and findings (see Chapter V) [g]. DIC, malignant hypertension and scleroderma kidney sometimes resemble HUS, but are usually diagnosed separately [a, d].

Prognosis of HUS in adults depends on its causes but is generally worse in the elderly patients. It was reported previously that the magnitude of renal damages could predict patient survival [4].

Table 1. Etiology and prevalence of HUS in adults [e]

- TTP (ADAMTS13 deficiency and anti- ADAMTS13 antibody) 30-40%
- STEC-HUS 4-10%
- others (atypical HUS) 50-60%
  - Hereditary (abnormality of complement-regulated gene and others) No data available
  - Idiopathic
  - Drugs
    Antiplatelet drugs: ticlopidine, clopidogril
    Anticancer drugs: mitomycin C, gemcitabine
    Calcineurin inhibitors: cyclosporine, tacrolimus
    Quinine
  - Pregnancy (HELLP syndrome, pregnancy-associated hypertension, etc.)
  - Infection (HIV, streptococcus pneumonia, influenza virus, etc.)
  - Autologous hematological stem cell transplantation
  - Connective tissue disease (SLE, anti-phospholipid antibody syndrome, systemic sclerosis, etc.)
  - Malignancy (malignant lymphoma, gastric cancer, etc.)
  - Others

IV.2 Treatment of HUS in adults

We recommend treatment of underlying diseases and systemic supportive care for adult patients with HUS according to the guidelines for children. **[Grade of Recommendation: B]**

We suggest initiating plasmapheresis without delay in adult patients with severe HUS, even if the etiology has not been determined. **[Grade of Recommendation: C1]**

We suggest plasma infusion when plasmapheresis is not immediately available in adult patients with severe HUS. **[Grade of Recommendation: C1]**

[Comments]

The basis of treatment for adult patients with HUS is supportive care with careful systemic management similar to that for children. In addition, treatment of underlying diseases is of particular importance in adult TTP/HUS. Supportive management encompasses fluid infusion, transfusion of blood and its components, nutritional care and management of AKI including dialysis therapy [g]. The prognosis of TTP/HUS used to be very poor decades ago. However, it

has since improved tremendously with the progress in supportive cares and the prevalent use of plasmapheresis [5 - 9, h]. Indication of plasmapheresis includes TTP with ADAMTS13 abnormality and most cases of complement-mediated aHUS (except for those caused by membrane cofactor protein/CD46 mutation) [9, g–i]. HUS secondary to certain drugs (ticlopidine, clopidogril, quinine) and HIV may also be indicated.

In contrast, HUS secondary to disseminated malignancy and most cases of HSCT and STEC are not indicated. Plasmapheresis should be avoided for invasive pneumococcus-derived HUS usually seen in children, as anti-Thomsen-Friedenreich IgM antibody in serum may induce hemolysis that could exacerbate the pathogenesis of HUS (see Chapter V). In patients with HUS secondary to autoimmune diseases, or for refractory or severe cases, immunosuppressive therapy may be combined with plasmapheresis. Unfortunately, it will take some time before the etiology of HUS is clarified. Prognosis in such cases is extremely poor if the initiation of plasmapheresis is delayed even for one or two days. If the diagnosis of TTP and aHUS is highly suspected, we strongly recommend that plasmapheresis be initiated without delay even with no known etiologies. We suggest that patient serum be taken for the purpose of future diagnostic use. Plasmapheresis should be terminated immediately when the etiology has been revealed in which plasmapheresis is not indicated or contraindicated.

Plasmapheresis is to be performed daily at the beginning and continued until the platelet count in the blood has normalized. Thereafter, it should be arranged according to the platelet count in the blood and serum LDH level. Alternatively, plasma infusion may be considered when prompt plasmapheresis is not available [7]. It has been reported that platelet transfusion might induce formation of microvascular thrombosis, but an analysis of the data in Oklahoma TTP-HUS registry revealed no such effect [10]. Therefore, when the risk for bleeding from thrombocytopenia is relatively high, platelet transfusion can be employed after careful consideration.

In autoimmune diseases such as connective tissue disease, treatment with glucocorticoids and immunosuppressive drugs may be considered. The efficacy of rituximab is not established for TTP, but may be considered in refractory or relapsing cases with anti-ADAMTS13 antibody [h]. Antiplatelet agents have not been shown to be effective for TTP and aHUS [h].

[Supplementary articles]

a. UpToDate: Causes, Diagnosis, and Treatment of thrombotic thrombocytopenic purpura-hemolytic uremic syndrome in adults.
b. Noris M, *et al.* Hemolytic-uremic syndrome. J Am Soc Nephrol 2005; 16:1035-1050.
c. Clark WF, *et al.* Attending rounds: microangiopathic hemolytic anemia with renal insufficiency. Clin J Am Soc Nephrol 2012; 7: 343-347.
d. Kagami S, *et al.* Diagnostic criteria of atypical hemolytic uremic syndrome. Nihon Jinzo

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e. Fujimura Y, *et al.* Registry of 919 Patients with Thrombotic Microangiopathies across Japan: Database of Nara Medical University during 1998-2008. Intern Med 2010; 49: 7-15.

f. George JN. How I treat patients with thrombotic thrombocytopenic purpura: 2010. Blood 2010; 116:4060-4069.

g. Taylor CM, *et al.* on behalf of a working party from the Renal Association, the British Committee for Standards in Haematology and the British Transplantation Society: Clinical Practice Guidelines for the management of atypical Haemolytic Uraemic Syndrome in the United Kingdom. Br J Haematol 2012; 148: 37-47.

h. Scully M, *et al.* on behalf of British Committee for Standards in Haematology: Guidelines on the diagnosis and management of thrombotic thrombocytopenic purpura and other thrombotic microangiopathies. Br J Haematol 2012; 158: 323-335.

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8. Forzley BR, *et al.* Treating TTP/HUS with plasma exchange: a single centre's 25-year experience. Br J Haematol 2008; 143: 100-106. (level 5)
9. Clark WF: Thrombotic microangiopathy: current knowledge and outcomes with plasma exchange. Semin Dial 2012; 25: 214-219. (level 4)

10. Swisher KK, *et al.* Clinical outcomes after platelet transfusions in patients with thrombotic thrombocytopenic purpura. Transfusion 2009; 49:873-887. (level 4)

IV.3 Diagnosis and treatment of STEC-associated HUS in adults

1. Clinical features of STEC-associated HUS in adults

STEC-associated HUS may occur in an outbreak or sporadically in adults, although the incidence is lower compared to in children. **[Grade of Recommendation: Not Graded]**
Elderly people with STEC infection are likely to develop HUS and prognosis is usually poor for such cases. **[Grade of Recommendation: Not Graded]**

[Comments]

STEC infection is seen in 5-10% of adult TTP/HUS [a]. Sporadic and community-based infection may occur, with outbreaks being reported in elderly nursing homes. Currently, it is still not known why sporadic infection is seen more frequently in females together with slightly higher incidence of outbreak [1, 2]. O157 is the most common specie that causes HUS in adult, and the same is observed in children. However, in Germany and Japan, O104 accounted for the majority of adult cases. Other species such as O111, O145, O26 and O121 have been reported before [a]. The Oklahoma TTP/HUS registry showed that in comparison with children, 21 adult cases (21-89 years with a median of 59 years) showed more severe manifestations in the CNS, anemia, thrombocytopenia and poor prognosis, although the degree of AKI was similar between adults and children [1].

In the outbreak of O104 in Germany in 2011, there were almost no differences in clinical features between the cases with and without HUS. The incidence of HUS was lower in adults (average 37 years) than in childhood cases, although hemorrhagic diarrhea was seen more frequently [2]. The reason has not been elucidated; the differential expression of Gb3 receptor for STX in the intestines, which was suggested in animal studies, has not been examined in human cases of HUS [c]. For adults with HUS, it was seen mainly in females.

Outbreak of STEC infection may occur among elderly people in facilities like nursing home, and ages older than 65 years are reported as a risk factor for development of HUS in patients infected with STEC [3]. Therefore, elderly patients with STEC infection should be managed more carefully from the onset. If they present with HUS, systemic treatment should be initiated without delay. Possible explanations as to why elderly people with infection show poor outcome include decreased antibody titers against STX [4] and reduced defense mechanisms against infection in the stomach. The latter is most likely to be caused by reduced gastric juice secretion,

gastrectomy and the use of antacid agents [3].

2. Treatment of adult HUS caused by STEC infection

We recommend treatment of underlying diseases and systemic supportive care for adult patients with STEC-associated HUS according to guidelines for childhood cases. **[Grade of Recommendation: B]**
We suggest plasmapheresis or combined therapy of immunoadsorption with IgG infusion in adult patients with severe STEC-associated HUS presented with CNS involvement. **[Grade of Recommendation: C1]**
For STEC-associated HUS in adults, no evidence is present for the use of antibiotics and eculizumab. **[Grade of Recommendation: Not Graded]**

[Comments]

Basic management for adult patients with STEC-associated HUS is similar to that for childhood cases. It includes systemic supportive care such as fluid infusion, transfusion of blood and its components, nutritional care and management of AKI. In severe cases, intensive care with respiratory and circulatory management is mandatory. In some reports, plasmapheresis was shown to be beneficial for the improvement of patient survival [5, 6]. In the outbreak of O104 in Denmark in 2011, plasmapheresis was reported to be effective for patients with neurological disturbances showing consciousness loss or convulsion [6]. In contrast, plasmapheresis did not show any efficacy in the 2011 German outbreak of O104 [7]. These are contradicting reports with regard to the efficacy of plasmapheresis. Randomized controlled trials are therefore necessary to determine the efficacy and indication for plasmapheresis. Overall, we suggest performing plasmapheresis for severe patients with poor prognosis who have no other suitable treatment options.

It was reported recently that a combined therapy of immunoadsorption and IgG infusion was effective for 12 HUS patients with severe neurological disturbances [8], and that efficacy was observed even in the patients who were refractory to treatment with plasmapheresis. Although the study has a limited patient number, such combined therapy may be considered for adult patients with refractory cases of severe HUS with neurological disturbances. While it remains unknown which treatment of immunoabsorption and IgG infusion is more important, there was a report that IgG infusion alone was not effective in childhood cases [d].

There are several reports showing the use of antibiotics may worsen the prognosis of HUS patients. On the other hand, it has recently been reported that azithromycin may shorten the

duration of bacteremia in adult patients with O104-associated HUS [9], although it did not affect renal and patient survival [9]. In the 2011 German outbreak of 104, it was reported that involvement of the CNS was less as a result of antibiotics use. HUS patients treated with a multiple regimen of antibiotics has better prognosis than those who were not [7]. However, there are no other reports suggesting the usefulness of antibiotic therapy. As such, the efficacy of antibiotics remains to be clarified.

Eculizumab has been used for patients with STEC-associated HUS to suppress activated complement system, and showed good efficacy in childhood cases [10]. However, no efficacy was observed in the cohort study of adult cases (average age 47.7) in the O104 outbreak shown above [7]. We have decided not to show recommendation grade for antibiotics and eculizumab. More evidence is clearly required to determine its efficacy.

[Supplementary articles]

a. Noris M, *et al.* Hemolytic-uremic syndrome. J Am Soc Nephrol 2005; 16: 1035-1050.
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c. Mobassaleh M, *et al.* Developmentally regulated Gb3 galactosyltransferase and a-galactosidase determine Shiga toxin receptors in intestine. Am J Physiol 1994; 267: G618-G624.
d. Remuzzi G, *et al.* The hemolytic uremic syndrome. Kidney Int 1995; 48:2-19.

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3. Dundas S, *et al.* The Central Scotland *Escherichia coli* O157:H7 outbreak: risk factors for the hemolytic uremic syndrome and death among hospitalized patients. Clin Infect Dis 2001; 33: 923-931. (level 4)
4. Karmali MA, *et al.* Age-specific frequencies of antibodies to *Escherichia coli* verocytotoxins (Shiga toxins) 1 and 2 among urban and rural populations in southern Ontario. J Infect Dis 2003; 188: 1724-1729. (level 4)
5. Dundas S, *et al.* Effectiveness of therapeutic plasma exchange in the 1996 Lanarkshire

Escherichia coli O157:H7 outbreak. Lancet 1999; 354:1327-1330. (level 4)

6. Colic E, *et al.* Management of an acute outbreak of diarrhoea-associated haemolytic uraemic syndrome with early plasma exchange in adults from southern Denmark: an observational study. Lancet 2011; 378: 1089-1093. (level 5)
7. Menne J, *et al.* EHEC-HUS consortium. Validation of treatment strategies for enterohaemorrhagic Escherichia coli O104:H4 induced haemolytic uraemic syndrome: case-control study. Br Med J 2012; 345: e4598. (level 4)
8. Greinacher A, *et al.* Treatment of severe neurological deficits with IgG depletion through immunoadsorption in patients with *Escherichia coli* O104:H4-associated haemolytic uraemic syndrome: a prospective trial. Lancet. 2011; 378: 1166-1173. (level 4)
9. Nitschke M, *et al.* Association between azithromycin therapy and duration of bacterial shedding among patients with Shiga toxin-producing enteroaggregative *Escherichia coli* O104:H4. JAMA 2012; 307: 1046-1052. (level 4)
10. LapeyraqueAL, *et al.* Eculizumab in severe Shiga-toxin–associated HUS. N Engl J Med 2011; 364: 2561-2563. (level 5)

V. Diagnosis and treatment of atypical hemolytic uremic syndrome (aHUS)

V. 1 The diagnosis of aHUS

aHUS is a type of HUS characterized by a triad of microangiopathic hemolytic anemia, thrombocytopenia and AKI; and excludes STEC-associated HUS and TTP caused by markedly decreased ADAMTS13. **[Grade of Recommendation: Not Graded]**

Diagnostic criteria

Definite:

Definitive diagnosis of aHUS based on the presence of the complete triad, but an absence of STEC infection and TTP caused by a marked decrease of ADAMTS13.

1. Microangiopathic hemolytic anemia: the level of hemoglobin (Hb) is less than 10 g/dL (We defined microangiopathic hemolytic anemia as an Hb level of less than 10 g/dL. At diagnosis, the presence of microangiopathic hemolysis should be confirmed on the basis of reference data including elevation of LDH level, a markedly decreased serum haptoglobin level, and the presence of schistocytes in blood smears.)
2. Thrombocytopenia: a platelet count of less than 150,000 /μL
3. AKI in pediatric case: a serum creatinine level exceeding 1.5-fold the reference value by age and gender issued by the Japanese Society for Pediatric Nephrology.

Probable:

Probable diagnosis is based on the presence of two components of the triad with the exclusion of STEC infection and TTP caused by a marked decrease of ADAMTS13.

[Comments]

aHUS has been traditionally regarded as a disease concept that excludes STX-associated HUS, the most common form of HUS in children. aHUS is a heterogeneous disorder responsible for only 10% of cases in children. An increased number of cases of aHUS have been reported to develop from the pathogenesis of HUS. In the present guidelines, we followed and adopted the diagnostic criteria established by the Joint Committee of the Japanese Society of Nephrology and the Japanese Society of Pediatrics [a]. In view of the unreliability of diarrhea as a distinguishing feature, aHUS should be suspected if the following characteristics are present, irrespective of whether diarrhea is present: 1) Patient is less than six months of age, 2) disease recurrence, 3) latent onset, 4) familial history of the disease with food poisoning excluded. Classification of aHUS is shown in Table 1 [b]. For differential diagnosis of aHUS, examinations should be planned with a sound understanding of the characteristics of the causative disease for HUS.

(1) Invasive pneumococcal infection

Invasive pneumococcal infection is defined as severe pneumococcal disease manifested as severe pneumonia, meningitis, bacteremia, sepsis, empyema, and other conditions. The pathogenesis of pneumococci-associated HUS has been suggested to involve the release of N-acetylneuraminidase, which cleaves N-acetylneuraminic acid in the glycocalyx, resulting in the exposure of the Thomsen-Friedenreich antigen on red blood cells, platelets, and glomeruli. Thomsen-Friedenreich antigen is recognized by a natural IgM antibody normally present in plasma leading to polyagglutination of the patient's red cells and hemolysis [c]. For diagnosis of pneumococci-associated HUS, identification of *Streptococcus pneumoniae* is necessary by culture, as well as detection of Thomsen-Friedenreich antigen on red cells [d].

(2) Disorder of regulatory components of the complement system

Dysregulatory changes in complement system components should be estimated through measurement of homolytic complement activity (CH50), assay of complement protein and complement regulatory protein, detection of auto-antibody against complement factor H (CFH), and measurement of membrane cofactor protein (MCP, CD46) expression level on monocytes [e]. Thereafter, genetic complement-associated HUS can be definitively diagnosed through gene analysis of complement proteins and complement regulatory proteins. However, missense mutations of complement proteins typically result in functional impairment without affecting serum complement protein levels [f]. Therefore, analysis of known candidate genes is recommended, if possible.

(3) Deficiency of ADAMTS13

ADAMTS13 deficiency comprised of two types, congenital type (Upshaw-Schulman syndrome) and acquired type due to its inhibitor, anti-ADAMTS13 antibody. A marked decrease of ADAMTS13 activity to a level of less than 5% has been demonstrated in 60-90% of patients with TTP. Therefore, patients with congenital or acquired TTP should be diagnosed and ruled by measuring the activity of ADAMTS13 and its inhibitor.

(4) Cobalamine metabolism abnormality

Inborn error of cobalamine C metabolism is a rare cause of HUS, especially in young infants (less than six months of age). The diagnosis is suggested by a marked increase of homocysteine and a decrease of methionine demonstrated by plasma amino acid chromatography.

(5) Recessive mutation in diacylglycerol kinase ε (DGKE) gene

Mutations in diacylglycerol kinase ε (DGKE) gene were identified using exome sequencing in

four patients with aHUS [g]. Most patients with DGKE gene mutation presented with aHUS in the first year of life show episodes of relapse before five years of age. It was reported that 13 (27%) of 49 patients with aHUS in the first year of life had DGKE gene mutations and that three of six familial disease kindreds had these mutations. Affected individuals present with aHUS in the first year of life have persistent hypertension, hematuria and proteinuria (sometimes in the nephrotic range), and commonly show progression to CKD stage 4 and 5 by the second decade of life. Therefore, DGKE gene mutations should be suspected if characteristic symptoms such as hypertension, hematuria and proteinuria occur after recovery from aHUS attacks, and that there are no pathogenic mutations in known aHUS-related genes or antibodies against CFH.

(6) HIV infection

Definitive diagnosis is performed by serological test for anti-HIV antibody.

(7) Others

Definitive diagnosis is performed with various examinations including serological examinations for anti-nuclear antibody and anti-phospholipid antibody.

Table 1 Classification of aHUS (excluding TTP due to ADAMTS13 deficiency) [1]

1. Advanced Etiology
   - i) Infection induced
     - *Streptococcus pneumoniae* infection
   - ii) Disorders of complement regulation
     - Genetic disorders of complement regulation: complement factor H (CFH), complement factor I (CFI), membrane cofactor protein (MCP, CD46), C3, complement factor B (CFB),thrombomodulin
     - Acquired disorders of complement regulation: auto-antibody
   - iii) Defective cobalamine metabolism
   - iv) DGKE mutation
   - v) Quinine induced

2. Clinical associations
   - i) HIV
   - ii) Malignancy, cancer chemotherapy, ionizing radiation
   - iii) Transplantation, Immunosuppressant use
   - iv) Pregnancy : HELLP syndrome
   - v) Autoimmune disease, collagen disease
   - vi) Others

V.2 Treatment of aHUS

Treatment of aHUS includes supportive therapy for control of overall body conditions and specific therapy for the causative disease. **[Grade of Recommendation: B]**

1) Pneumococcal-associated aHUS

Plasma therapy, including plasma exchange and plasma infusion with fresh frozen plasma, should be avoided in therapy for pneumococcal-associated HUS as plasma (which contains natural IgM-class antibodies against Thomsen-Friedenreich antigen) may aggravate hemolysis. It is preferable to transfuse washed RBC or platelets. **[Grade of Recommendation: D]**

2) aHUS associated with complement dysregulation and other abnormalities

The guideline indicates that plasma therapy, including plasma exchange and plasma infusion, should be started as soon as possible at diagnosis of aHUS (excluding cobalamine metabolism disorder and pneumococcal-associated HUS). **[Grade of Recommendation: C1]**

Patients diagnosed with aHUS (based on the diagnostic criteria proposed by the Joint Committee of the Japanese Society of Nephrology and the Japanese Society of Pediatrics) should be treated with eclizumab. **[Grade of Recommendation: C1]**

Living-related donor transplantation should not be performed in patients with end-stage renal disease (ESRD) due to aHUS. **[Grade of Recommendation: C2]**

Preventive plasma therapy should be performed in the perioperative period for patients with ESRD due to aHUS undergoing cadaveric unrelated renal transplantation. **[Grade of Recommendation: C1]**

Prophylactic eclizumab administration in the perioperative period is acceptable for patients with ESRD due to aHUS and undergoing cadaveric unrelated renal transplantation. **[Grade of Recommendation: C1]**

[Comments]

As described in Chapter V. 1, aHUS has several etiologies that can affect presentation, management and outcome. Supportive care including dialysis and various type of intensive care to control patient's general conditions is important, as is the case for STEC-associated HUS. Specific therapy is needed for various etiologies. Therefore, we have described the importance of supportive care for treatment of patients with aHUS in the opening statement of this

guideline.

(1) Pneumococci-associated aHUS

Children with pneumococci-associated HUS are usually younger at presentation and show a more severe course than those with STEC-associated HUS. The mortality rate of pneumococci-associated HUS in the acute phase has been reported to be 12.5% [c], and 26% [l]. It has been reported that 10.1% [c], with 8% of patients develop end-stage renal disease [l]. These rates are between two to three times higher than those for STEC-associated HUS. As for the pathophysiology of pneumococci-associated HUS, it has been proposed that neuraminidase, produced by pneumococci, cleaves N-acetyl neuraminic acid from the cell surface of erythrocytes, platelets, and glomerular endothelial cells, exposing the Thomsen-Friedenreich antigen. The latter is identified by a natural IgM antibody as a normal plasma constituent that initiates the cascade of events leading to HUS. Transfusion of plasma products containing anti-Thomsen-Friedenreich IgM antibodies further accelerates hemolysis, and such cases have been documented [2, 3]. The reported morbidity rate of CKD or end-stage renal disease is significantly lower in patients treated with washed blood products than in those treated with unwashed products [1]. These circumstances dictate that plasma therapy, including plasma infusion and plasma exchange with fresh frozen plasma, should not be performed in patients with pneumococci-associated HUS. Washed blood products should be used for blood transfusion and filler in the dialysis circuit for infant dialysis.

(2) aHUS associated with complement dysregulation and other abnormalities

The guideline recommends that daily plasma therapy, including plasma exchange and plasma infusion, should be started at the point of diagnosis of aHUS (excluding cobalamine metabolism disorder for which vitamin B12 supplementation is the established therapy) [d, h]. Plasma exchange is commonly undertaken daily using 1.5 to 2 plasma volume per session, employing fresh frozen plasma. Plasma exchange is more efficient than plasma infusion, as the former supplies a large amount of normal complement regulatory protein, avoids any risk of volume overload, and removes fluid-phase causative agents (such as abnormal complement regulatory proteins, anti-CFH antibodies, inflammatory cytokines, and other triggers of platelet hyperaggregability) [f, h]. The results of a case series study suggested that the response to short-term plasma therapy varies according to genotype [j, k, 4, 5]. Patients with CFH mutations have the poorest prognosis. On the other hand, patients with MCP mutations have the best short-term prognosis, with 90% of such patients reported to survive and remain dialysis-free in the long term [4]. Therefore, in patients with MCP mutations, plasma therapy does not affect outcome. This is consistent with the fact that MCP is not a circulating complement regulatory

protein.

In the patients with mutations in genes for complement proteins and their regulators, the outcome of kidney transplantation is poor; overall risk of aHUS recurrence after kidney transplantation is about 50%, and the risk of graft loss occurs in 80-90% of patients with recurrence [l - n, 6]. The outcomes of kidney transplantation vary according to the type of mutated gene, being poor inpatients with CFH, complement factor I (CFI), C3 mutations. In contrast, kidney graft outcome is reportedly favorable, and disease recurrence rates are low in patients with MCP mutations, due to the fact that MCP is a transmembrane protein and that kidney grafts show normal expression of MCP [6]. The efficacies and benefits of plasma therapy in the perioperative period have been reported in some case series, with the purpose of preventing aHUS recurrence after kidney transplantation [l - n]. Therefore, preventive perioperative plasma therapy is recommended when performing kidney transplantation for patients with complement-associated HUS. These data, together with the higher rates of disease recurrence, suggest that living kidney transplantation is not recommended for patients with mutations of CFH, CFI, complement factor B (CFB) and C3. In particular, living-related kidney transplantation is contraindicated, as a living-related donor may be a carrier of mutations and may be at risk of developing *de novo* aHUS after kidney donation.

As CFH, CFI, CFB and C3 are synthesized in the liver or liver-kidney, combined transplantation has been proposed as a logical curative intervention for severe complement-associated HUS in patients harboring mutations of those complement proteins. There have been over 10 combined liver-kidney transplants [o, p, 7 - 12], and a few successful cases have been reported [10 - 12]. However, as data on patient outcome are limited, it is not possible to draw reliable conclusions on this type of transplantation.

Mutations of complement protein components of the alternative complement pathway, including CFH, CFI and MCP, have been reported in many cases of aHUS [f] The proposed pathological mechanism for the development of HUS is uninhibited continuous activation of the alternative pathway, resulting in the formation of membrane attack comples (MAC, C5-9). Eculizumab, a recombinant monoclonal humanized IgG antibody that targets C5, blocks the cleavage of C5 to C5b, ultimately preventing generation of the proinflammatory peptide C5b, and the cytotoxic MAC. Therefore, eculizmab blocks the complement terminal pathway. Two prospective single-arm studies involving adult patients, and one retrospective study involving pediatric patients, have been performed to investigate the efficacy of eculizumab for aHUS [q]. In the autumn of 2011, the use of eculizumab for treatment of aHUS was approved in the USA and Europe based on the results of these studies [q]. Many reports have described the efficacy of eculizumab for patients with plasma therapy-resistant aHUS [13 - 15], and its long-term preventive effect against aHUS recurrence after kidney transplantation [16 - 20]. These reports

suggest that eculizumab may be highly beneficial for patients with aHUS and also for prevention of aHUS recurrence after kidney transplantation. However, blockade of the complement terminal pathway by eculizumab increases the risk of infection by encapsulated bacteria, including *Neisseria meningitidis, Haemophilus influenza* type B, and *Streptococcus pneumoniae.* In particular, *Neisseria meningitidis* infection is life-threatening. Patients must be vaccinated against it at least two weeks before being treated with eculizumab. If this is not possible, adequate antibiotics, including ciproxan, should be administered prophylactically [r]. Moreover, in children, it should be ascertained if they have been vaccinated against *Streptococcus pneumoniae* and *Haemophilus influenza* type B. If not, such vaccination ought to be considered [s]. In Japan, the use of eculizumab for treatment of thrombotic microangiopathy due to aHUS was approved in September 2013. Accurate diagnosis of aHUS is important before initiating treatment with eculizumab, as stated in the packaging insert for the agent: "Examine carefully the appropriateness of eculizumab administration and start the medication based on sufficient understanding of its efficacy and safety" and "Appropriate diagnosis based on diagnostic criteria established by the Joint Committee of the Japanese Society of Nephrology and the Japanese Society of Pediatrics is necessary for use of eculizumab" [s]. With regard to these guidelines, Table 2 and 3 show the recommended dosages and regimens stated in the packaging insert [s].

Treatment with eculizumab is highly effective for patients who depend on or resist to plasma exchange, as well as for those whose risks of plasma exchange outweigh the benefits (e.g. allergic reaction to plasma products, technical difficulties in achieving vascular access). For these patients, treatment with eculizumab may become a first line strategy in Japan, just as it has been reported in the USA and Europe [t]. So far, however, only three cases have been examined in a clinical trial and only a handful of cases have been treated with eculizumab through private importation in Japan. Since the efficacy and safety of treatment with eculizumab for Japanese aHUS patients is still unclear, we have decided on a recommendation grade of C1 for treatment with eculizumab. The treatment protocol for aHUS and preventive therapy protocol for disease recurrence after kidney transplantation may change once treatment experience with eculizumab has been accumulated.

In 2013, mutations in the gene coding for DGKE were reported as a cause of aHUS [f]. It is not obvious whether complement activation has a role in patients with DKGE mutations, because DKGE encodes an intracellular enzyme. Moreover, two patients with DKGE mutations have been reported to show recurrent aHUS while receiving anticomplement therapy including eculizumab and plasma infusion. To date, two allografts have survived for two years. In three cases of cadaveric kidney transplantation, the patients survived for four years. One allograft failed after six years due to chronic rejection. It is notable that there were no cases of aHUS

recurrence. Additionally, DKGE mutations have been reported to cause membrane proliferative glomerulonephritis with thrombotic microangiopathy [u]. Further analysis is necessary to clarify the pathogenesis and clinical course of aHUS in patients with DGKE mutations.

Table 2. Dosing recommendation of eclizumab for the patients with aHUS

Patient age and body weight	Induction	Maintenance
18 years and older	900 mg weekly for the first 4 weeks	1200 mg at week 5; then 1200 mg every 2 weeks
Less than 18 years		
40 kg and over	900 mg weekly x 4 doses	1200 mg at week 5; then 1200 mg every 2 weeks
30 kg to less than 40 kg	600 mg weekly x 2 doses	900 mg at week 3; then 900 mg every 2 weeks
20 kg to less than 30 kg	600 mg weekly x 2 doses	600 mg at week 3; then 600 mg every 2 weeks
10 kg to less than 20 kg	600 mg weekly x 1 doses	300 mg at week 2; then 300 mg every 2 weeks
5 kg to less than 10 kg	300 mg weekly x 1 doses	300 mg at week 2; then 300 mg every 3 weeks

Table 3. Supplemental dose of eculizumab after plasma exchange/plasma infusion

	Most recent eculizumab dose	Supplemental eculizumab dose	Timing of supplemental eculizumab dose
Plasma exchange	300 mg	300 mg per plasma exchange session	Within 60 minutes after each plasma exchange
	600 mg and over	600 mg per plasma exchange session	
Fresh frozen Plasma infusion	300 mg and over	300 mg per fresh fozen plasma infusion session	60 minutes prior to fresh frozen plasma infusion session

Eculizumab may be partially lost from plasma due to plasma exchange, and fresh frozen plasma includes complement factor 5 (C5). Therefore, eculizumab supplementations within 60 minutes after each plasma exchange session or at 60 minutes before fresh frozen plasma infusion should be considered (dosage shown in Table 3). As the supplemental dose of eculizumab is estimated on the basis of simulation results, it is necessary to observe patients carefully post eculizumab supplementation.

[Supplementary articles]

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f. Loirat C, *et al.* Atypical hemolytic uremic syndrome. Orphanet J Rare Dis 2011, 6: 60-89.
g. Lemaire M, F *et al.* Recessive mutations in *DKGE* cause atypical hemolytic uremic syndrome. Nat. Genet. 2013; 45: 531-536.
h. Taylor CM, *et al.* on behalf of a working party from the Renal Association, the British Committee for Standards in Haematology and the British Transplantation Society: Clinical Practice Guidelines for the management of atypical haemolytic uraemic syndrome in the