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 C5a–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 2 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, Tables 14 and 15 show the recommended dosages and regimens stated in the packaging insert [s].

**Table 14** 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 × 4 doses | 1200 mg at week 5; then 1200 mg every 2 weeks |
| 30 kg to less than 40 kg | 600 mg weekly × 2 doses | 900 mg at week 3; then 900 mg every 2 weeks |
| 20 kg to less than 30 kg | 600 mg weekly × 2 doses | 600 mg at week 3; then 600 mg every 2 weeks |
| 10 kg to less than 20 kg | 600 mg weekly × 1 dose | 300 mg at week 2; then 300 mg every 2 weeks |
| 5 kg to less than 10 kg | 300 mg weekly × 1 dose | 300 mg at week 2; then 300 mg every 3 weeks |

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.

**Table 15** 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 min 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 min 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 min after each plasma exchange session or at 60 min before fresh frozen plasma infusion should be considered (dosage shown in Table 15). As the supplemental dose of eculizumab is estimated on the basis of simulation results, it is necessary to observe patients carefully post eculizumab supplementation

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 DGKE mutations, because DKGE encodes an intracellular enzyme. Moreover, two patients with DGKE mutations have been reported to show recurrent aHUS while receiving anticomplement therapy including eculizumab and plasma infusion. To date, two allografts have survived for 2 years. In three cases of cadaveric kidney transplantation, the patients survived for 4 years. One allograft failed after 6 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 [t]. Further analysis is necessary to clarify the pathogenesis and clinical course of aHUS in patients with DGKE mutations.

Supplementary articles


a. Kagami S, et al. Diagnostic criteria of atypical hemolytic uremic syndrome. Nihon Jinzo Gakkai Shi. 2013;55:91–93.
b. Besbas N, et al. A classification of hemolytic uremic syndrome and thrombotic thrombocytopenic purpura and related disorders. Kidney Int. 2006;70:423–431.
c. Copelovitch L, et al. Streptococcus pneumonia-associated hemolytic uremic syndrome. Pediatr Nephrol. 2008;23:1951–1956.
d. Ariceta G, et al. Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. Pediatr Nephrol. 2009;24:687–696.
e. Sánchez-Corral P, et al. Functional analysis in serum from atypical hemolytic uremic syndrome patients reveals impaired protection of host cells associated with mutations with factor H. Mol Immunol. 2004;41:81–84.
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 United Kingdom. Br J Haematol. 2009;148:37–47.
i. Loirat C, et al. Plasmatherapy in atypical hemolytic uremic syndrome. Semin Thrombo Hemost. 2010;36:673–681.
j. Noris M, et al. Atypical hemolytic-uremic syndrome. N Eng J Med. 2009;361:1676–1687.
k. Loirat C, et al. Complement and the atypical hemolytic syndrome in children. Pediatr Nephrol. 2008;23:1957–1972.
l. Bresin E, et al. Outcome of renal transplantation in patients with Non-Shiga-Toxin associated hemolytic uremic syndrome: prognostic significance of background. Clin J Am Nephrol. 2006;1:88–89.
m. Loirat C, et al. Hemolytic uremic syndrome recurrence after renal transplantation. Pediatr Transpl. 2008;12:619–629.
n. Noris M, et al. Thrombotic microangiopathy after kidney transplantation. Am J Transpl. 2010;10:1517–1523.
o. Sánchez-Coral P, et al. Advances in understanding the aetiology of atypical haemolytic syndrome. Br J Haematol. 2010;150:529–542.
p. Saland JM, et al. Liver-kidney transplantation to cure atypical hemolytic uremic syndrome. J Am Soc Nephrol. 2009;20:940–949.
q. UpToDate: Atypical hemolytic uremic syndrome in children. (Accessed on April 17, 2012)
r. Schmidtko J, et al. Treatment of atypical hemolytic uremic syndrome and thrombotic microangiopathies: A focus on eculizumab. Am J Kidney Dis. 2013;61:289–299.
s. Soliris® (eculizumab) Concentrated solution for intravenous infusion. Japanese Package Insert.
t. Ozaltin F, et al. DGKE variants cause a glomerular microangiopathy that mimics membranoproliferative GN. J Am Soc Nephrol. 2013;24:377–384.


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## References

### 1 Diagnosis and treatment of Shiga toxin producing *Escherichia coli* infection

#### 1.1 Diagnosis of Shiga toxin producing *Escherichia coli* infection

1. Saito T, et al. Reported cases of hemorrhagic uremic syndrome associated with EHEC infection in 2008-NESID. IASR 2009;30:122–123. [in Japanese] (level 5)

2. Komiya N, et al. Reported cases of hemorrhagic uremic syndrome associated with EHEC infection in 2008-NESID. IASR. 2010;31:170–172. [in Japanese] (level 5)
3. Saito T, et al. Reported cases of hemorrhagic uremic syndrome associated with EHEC infection in 2010-NESID. IASR 2011;32:141–143. [in Japanese] (level 5)
4. Saito T, et al. Reported cases of hemorrhagic uremic syndrome associated with EHEC infection in 2011-NESID. IASR. 2012;33:128–130. [in Japanese] (level 5)
5. Kamioka I, et al. Risk factors for developing severe clinical course in HUS patients: a national survey in Japan. Pediatr Int. 2008;50:441–446. (level 4)

1.2 Treatment of STEC infection

1. Safdar N, et al. Risk of hemolytic uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 enteritis: a meta-analysis. JAMA. 2002;288:996–1001. (level 2)
2. Proulx F, et al. Randomized, controlled trial of antibiotic therapy for *Escherichia coli* O157:H7 enteritis. J Pediatr. 1992;121:299–303. (level 2)
3. Menne J, et al. EHEC-HUS consortium: Validation of treatment strategies for enterohaemorrhagic *Escherichia coli* O104:H4 induced haemolytic uraemic syndrome: case–control study. BMJ. 2012;345:e4565. (level 4)
4. Smith KE, et al. Antibiotic treatment of Escherichia coli O157 infection and the risk of hemolytic uremic syndrome, Minnesota. Pediatr Infect Dis J. 2012;31:37–41. (level 4)
5. Wong CS, et al. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. N Engl J Med. 2000;342:1930–1936. (level 4)
6. Wong CS, et al. Risk factors for the hemolytic uremic syndrome in children infected with *Escherichia coli* O157:H7: a multivariable analysis. Clin Infect Dis. 2012;55:33–41. (level 4)
7. 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)
8. Shiomi M, et al. Effect of early oral fluoroquinolones in hemorrhagic colitis due to *Escherichia coli* O157:H7. Pediatr Int. 1999;41:228–232. (level 4)
9. Ikeda K, et al. Effect of early fosfomycin treatment on prevention of hemolytic uremic syndrome accompanying *Escherichia coli* O157:H7 infection. Clin Nephrol. 1999;52:357–362. (level 4)
10. Bell BP, et al. Predictors of hemolytic uremic syndrome in children during a large outbreak of *Escherichia coli* O157:H7 infections. Pediatrics. 1997;100:E12. (level 4)
11. Cimolai N, et al. A continuing assessment of risk factors for the development of *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. Clin Nephrol. 1994;42:85–89. (level 4)
12. Cimolai N, et al. Risk factors for the central nervous system manifestations of gastroenteritis-associated hemolytic-uremic syndrome. Pediatrics. 1992;90:616–621. (level 4)

## 2 Diagnosis of HUS

2.1 Diagnosis procedure

1. Kawamura N, et al. Risk factors for the development of *Escherichia coli* O157: H7 associated with hemolytic uremic syndrome. Pediatr Int. 1999;41:218–222. (level 4)
2. Kamioka I, et al. Japanese Society for Pediatric Nephrology: Risk factors for developing severe clinical course in HUS patients: a national survey in Japan. Pediatr Int. 2008;50:441–446. (level 4)
3. Oakes RS, et al. Predictors of fatality in postdiarrheal hemolytic uremic syndrome. Pediatrics. 2006;117:1656–1662. (level 4)

2.2 Assessment of acute kidney injury (AKI)

1. Kamioka I, et al. Japanese Society for Pediatric Nephrology. Risk factors for developing severe clinical course in HUS patients: a national survey in Japan. Pediatr Int. 2008;50:441–446. (level 4)
2. Gerber A, et al. Clinical course and the role of shiga toxin-producing *Escherichia coli* infection in the hemolytic-uremic syndrome in pediatric patients, 1997–2000, in Germany and Austria: a prospective study. J Infect Dis. 2002;15:493–500. (level 4)
3. Balestracci A, et al. Dehydration at admission increased the need for dialysis in hemolytic uremic syndrome children. Pediatr Nephrol. 2012;27:1407–1410. (level 4)
4. Hickey CA, et al. Early volume expansion during diarrhea and relative nephroprotection during subsequent hemolytic uremic syndrome. Arch Pediatr Adolesc Med. 2011;165:884–889. (level 4)

2.3 Diagnosis of encephalopathy

1. Gasser C, et al. Haemolytisch-uraemische syndrome: Bilaterale nierenrindennekrosen bei akuten erworbenen haemolytischen anaemien. Schweiz Med Wochenschr. 1955;85:905–909. (level 5)
2. Akashi S, et al. An outbreak of *Escherichia coli* associated colitis in a kindergarten. Committee for epidemiological study of epidemic diarrhea due to pathogenic *E. coli* in a kindergarten, Saitama, Japan. J Jpn Pediatr Soc. 1991;95:2607–2615. [in Japanese] (level 5)
3. Siegler RL. Spectrum of extrarenal involvement in postdiarrheal hemolytic-uremic syndrome. J Pediatr. 1994;125:511–518. (level 5)
4. Furuse A. Clinical analysis of the cases complicated with central nervous system involvement in diarrhea associated hemolytic uremic syndrome. J Jpn Pediatr Soc. 2006;110:919–925. [in Japanese] (level 5)
5. Sheth KJ, et al. Neurologic involvement in hemolytic-uremic syndrome. Ann Neurol. 1986;19:90–93. (level 5)
6. Bale CP, et al. CNS manifestations of the hemolytic-uremic syndrome. Am J Dis Child. 1980;134:869–872. (level 5)
7. Theobald I, et al. Central nervous system involvement in hemolytic uremic syndrome (HUS)–a retrospective analysis of cerebral CT and MRI studies. Clin Nephrol. 2001;56:S3–8. (level 5)
8. Steinborn M, et al. CT and MRI in haemolytic uraemic syndrome with central nervous system involvement: distribution of lesions and prognostic value of imaging findings. Pediatr Radiol. 2004;34:805–810. (level 5)
9. Donnerstag F, et al. Patterns in early diffusion-weighted MRI in children with haemolytic uraemic syndrome and CNS involvement. Eur Radiol. 2012;22:506–513. (level 5).
10. Dhuna A, et al. EEG and seizures in children with hemolytic-uremic syndrome. Epilepsia. 1992;33:482–486. (level 5)
11. Shiraishi M, et al. Soluble tumor necrosis factor receptor 1 and tissue inhibitor of metalloproteinase-1 in hemolytic uremic syndrome with encephalopathy. J Neuroimmunol. 2008;196:147–152. (level 4)
12. Shimizu M, et al. Cytokine profiles of patients with enterohemorrhagic *Escherichia coli* O111-induced hemolytic-uremic syndrome. Cytokine. 2012;60:694–700. (level 4)
13. Crisp DE, et al. Hemorrhagic cerebral infarction in the hemolytic-uremic syndrome. J Pediatr. 1981;99:273–276. (level 5)
14. DiMario FJ, et al. Lacunar infarction of the basal ganglia as a complication of hemolytic-uremic syndrome. Clin Pediatr. 1987;26:586–590. (level 5)

2.4 Acute-phase extrarenal complication (excluding encephalopathy)

1. Spinale JM, et al. Long-term outcomes of Shiga toxin hemolytic uremic syndrome. Pediatr Nephrol 2013 Jan 4. [Epub ahead of print]. (level 4)
2. Habib R, et al. Hemolytic-uremic syndrome in children and arterial hypertension. Arch Mal Coeur Vaiss. 1981;74:37–43. (level 5)
3. Siegler RL, et al. Hemolytic-uremic syndrome in adolescents. Arch Pediatr Adolesc Med. 1997;151:165–169. (level 4)
4. Friedland JA, et al. *Escherichia coli* O157: H7-associated hemolytic-uremic syndrome: Value of colonic color Doppler sonography. Pediatr Radiol. 1995;25:S65–S67. (level 5)
5. Bernard A, et al. Digestive manifestations in hemolytic uremic syndrome in children. Arch Pediatr. 1996;3:533–540. (level 4)
6. Yoden A. Echographic significance in gastrointestinal disease. Jp J Pediatric Medicine. 1999;31:1700–1707. [in Japanese]. (level 5)
7. Crabbe DCG, et al. Gastrointestinal complications of the haemolytic uraemic syndrome. J Roy Soc Med. 1990;83:773–775. (level 5)
8. de Buys Roessingh AS, et al. Gastrointestinal complications of post-diarrheal hemolytic uremic syndrome. Eur J Pediatr Surg. 2007;17:328–334. (level 4)
9. Brandt JR, et al. Cholelithiasis following *Escherichia coli* O157: H7-assosiated hemolytic uremic syndrome. Pediatr Nephrol. 1998;12:222–225. (level 4)
10. Nagita A, et al. Report on nine cases of gallbladder calculus disease. J Jpn Pediatric Society. 1993;97:2140–2144. [in Japanese] (level 5)
11. Yamazaki T, et al. Case Report of Two-year-old Boy with Bile-duct Stones Associated with Hemolytic Uremic Syndrome. J Jpn Pediatric Society. 1996;103:865–868. [in Japanese] (level 5)
12. Spizirri FD, et al. Childhood hemolytic uremic syndrome in Argentina: Long-term follow-up and prognostic features. Pediatr Nephrol. 1997;11:156–160. (level 4)
13. Poulton J, et al. Dilated cardiomyopathy associated with haemolytic uraemic syndrome. Br Heart J. 1987;57:181–183. (level 5)
14. Mohammed J, et al. Cardiac tamponade in diarrhea-positive haemolytic uraemic syndrome. Nephrol Dial Transplant. 2009;24:679–681. (level 5)
15. 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)
16. Abu-Arafeh I, et al. Myocarditis and haemolytic uraemic syndrome. Arch Dis Child. 1995;72:46–47. (level 5)

## 3 Treatment of HUS

3.1 Fluid therapy and blood transfusion

1. Ake JA, et al. Relative nephroprotection during *Escherichia coli* O157:H7 infections: Association with intravenous volume expansion. Pediatrics. 2005;115:e673–80. (level 4)
2. Hickey CA, et al. Early volume expansion during diarrhea and relative nephroprotection during subsequent hemolytic uremic syndrome. JAMA 2012;165:884–889. (level 4)
3. Balestracci A, et al. Dehydration at admission increased the need for dialysis in hemolytic uremic syndrome children. Pediatr Nephrol. 2012;27:1407–1410. (level 4)
4. Pape L, et al. Early erythropoietin reduced the need for red blood cell transfusion in childhood hemolytic uremic syndrome: a randomized prospective pilot trial. Pediatr Nephrol. 2009;24:1061–1064. (level 2)
5. Weil BR, et al. Bleeding risk for surgical dialysis procedures in children with hemolytic uremic syndrome. Pediatr Nephrol. 2010;25:1693–1698. (level 4)

3.2 Antihypertensive therapy

1. Spinale JM, et al. Long-term outcomes of Shiga toxin hemolytic uremic syndrome. Pediatr Nephrol. 2013 Jan 4. [Epub ahead of print]. (level 4)

3.3 Renal replacement therapy

1. Bagshaw SM, et al. Dialysis Disequilibrium Syndrome: brain death following hemodialysis for metabolic acidosis and acute renal failure–a case report. BMC Nephrol. 2004;9:5–9. (level 5)

3.4 Plasma exchange therapy

1. Dundas S, et al. Effectiveness of therapeutic plasma exchange in the 1996 Lanarkshire *Escherichia coli* O157:H7 outbreak. Lancet. 1999;354:1327–1330. (level 5)
2. Menne J, et al. Validation of treatment strategies for enterohaemorrhagic *Escherichia coli* O104:H4 induced haemolytic uraemic syndrome: case–control study. BMJ. 2012;345:e4565. (level 5)
3. Yagi K et al. Clinical experience of E. coli O-157-related hemolytic uremic syndrome. J Jpn Soc Peiatr Nephrol. 1997;10:209–213. [in Japanese] (level 5)

3.5 Antithrombotic therapy for HUS

1. Diekmann L: Treatment of the hemolytic-uremic syndrome with streptokinase and heparin (author's transl). Klin padiatr. 1980;192:430–435. (level 4)
2. Loirat C, et al. Treatment of childhood hemolytic-uremic syndrome with urokinase. Cooperative controlled trial. Arch Fr Pediatr. 1984;41:15–19. (level 4)
3. Van Damme-Lombaarts R, et al. Heparin plus dipyridamole in childhood hemolytic-uremic syndrome: a prospective, randomized study. J Pediatr. 1988;113:913–918. (level 2)
4. O'Regan S, et al. Aspirin and dipyridamole therapy in the hemolytic-uremic syndrome. J Pediatr. 1980;97:473–476. (level 4)
5. Asaga T, et al: A case of thrombotic thrombocytopenic purpura/hemolytic uremic syndrome coincident with disseminated intravascular coagulation caused by abdominal hysterectomy. J Jpn Soc Intensive Care Med. 2008;15:339–340. [in Japanese] (level 5)
6. Kaneda M, et al. Treatment of hemolytic uremic syndrome with recombinant thrombomodulin. Thrombosis Medicine. 2012;2:198–202. [in Japanese] (level 5)

3.6 Treatment of encephalopathy associated with STEC infection

1. Robson WL, et al. Causes of death in hemolytic uremic syndrome. Child Nephrol Urol. 1991;11:228–233. (level 5)
2. Kahn SI, et al. Spontaneous recovery of the hemolytic uremic syndrome with prolonged renal and neurological manifestations. Nephron. 1982;32:188–191. (level 5)
3. Steel BT, et al. Recovery from prolonged coma in hemolytic uremic syndrome. J Pediatr. 1983;102:402–404. (level 5)
4. Siegler RL. Spectrum of extrarenal involvement in postdiarrheal hemolytic-uremic syndrome. J Pediatr. 1994;125:511–518. (level 5)
5. Perez N, et al. Steroids in the hemolytic uremic syndrome. Pediatr Nephrol. 1998;12:101–104. (level 4)
6. Shimizu M, et al. Cytokine profiles of patients with enterohemorrhagic *Escherichia coli* O111-induced hemolytic-uremic syndrome. Cytokine. 2012;60:694–700. (level 4)
7. Shiraishi M, et al. Soluble tumor necrosis factor receptor 1 and tissue inhibitor of metalloproteinase-1 in hemolytic uremic syndrome with encephalopathy. J Neuroimmunol. 2008;196:147–152. (level 4)

8. Yanagisawa A, et al. [Hemolytic uremic syndrome complicated by acute childhood necrotizing encephalopathy]. J Jpn Soc Peiatr Nephrol. 2009;22:161–165., in Japanese. (level 5)
9. Dundas S, et al. Effectiveness of therapeutic plasma exchange in the 1996 Lanarkshire *Escherichia coli* O157:H7 outbreak. Lancet. 1999;354:1327–1330. (level 5)
10. Nathanson S, et al. Acute neurological involvement in diarrhea-associated hemolytic uremic syndrome. Clin J Am Soc Nephrol. 2010;5:1218–1228. (level 4)
11. 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 4)
12. Lapeyraque AL, et al. Eculizumab in severe Shiga-toxin-associated HUS. N Engl J Med. 2011;364:2561–2563. (level 5)
13. Menne J, et al. STEC-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)
14. Honda T, et al. A novel strategy for hemolytic uremic syndrome: successful treatment with thrombomodulin α. Pediatrics. 2013;131:e928–33. (level 5)

3.7 Renal sequelae of HUS

1. Gerber A, et al. Clinical course and the role of Shiga toxin-producing *Escherichia coli* infection in the hemolytic-uremic syndrome in pediatric patients, 1997–2000, in Germany and Austria: a prospective study. J Infect Dis. 2002;186:493–500. (level 4)
2. Kamioka I, et al. Japanese Society for Pediatric Nephrology: Risk factors for developing severe clinical course in HUS patients: a national survey in Japan. Pediatr Int. 2008;50:441–446 (level 4)
3. Oakes RS, et al. Predictors of fatality in postdiarrheal hemolytic uremic syndrome. Pediatrics. 2006;117:1656–1662. (level 4)
4. Spinale JM, et al. Long-term outcomes of Shiga toxin hemolytic uremic syndrome. Pediatr Nephrol. 2013 Jan 4. [Epub ahead of print]
5. Garg AX, et al. Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome: A systematic review, meta-analysis, and meta-regression. JAMA. 2003;290:1360–1370. (level 4)
6. Garg AX, et al. Microalbuminuria three years after recovery from *Escherichia coli* O157 hemolytic uremic syndrome due to municipal water contamination. Kidney Int. 2005;67:1476–1482. (level 4)
7. Sharma AP, et al. Chronic renal disease is more prevalent in patients with hemolytic uremic syndrome who had a positive history of diarrhea. Kidney Int. 2010;78:598–604. (level 4)
8. Siegler RL, et al. Long-term outcome and prognostic indicators in the hemolytic-uremic syndrome. J Pediatr. 1991;118:195–200. (level 4)
9. Fitzpatrick MM, et al. Long term renal outcome of childhood haemolytic uraemic syndrome. BMJ. 1991;303:489–492. (level 4)
10. Small G, et al. Hemolytic uremic syndrome: defining the need for long-term follow-up. Clin Nephrol. 1999;52:352–356. (level 4)
11. Kelles A, et al. Childhood haemolytic uraemic syndrome: long-term outcome and prognostic features. Eur J Pediatr. 1994;153:38–42. (level 4)
12. Spizzirri FD, et al. Childhood hemolytic uremic syndrome in Argentina: long-term follow-up and prognostic features. Pediatr Nephrol. 1997;11:156–160. (level 4)
13. Gagnadoux MF, et al. Long-term (15–25 years) outcome of childhood hemolytic-uremic syndrome. Clin Nephrol. 1996;46:39–41. (level 4)
14. Hüseman D, et al. Long-term prognosis of hemolytic uremic syndrome and effective renal plasma flow. Pediatr Nephrol. 1999;13:672–677. (level 4)
15. Oakes RS, et al. Duration of oliguria and anuria as predictors of chronic renal-related sequelae in post-diarrheal hemolytic uremic syndrome. Pediatr Nephrol. 2008;23:1303–1308. (level 4)
16. Cobeñas CJ, et al. Long-term follow-up of Argentinean patients with hemolytic uremic syndrome who had not undergone dialysis. Pediatr Nephrol. 2007;22:1343–1347. (level 4)
17. Garg AX, et al. Absence of renal sequelae after childhood *Escherichia coli* O157:H7 gastroenteritis. Kidney Int. 2006;70:807–812. (level 4)

3.8 Extra-renal sequelae in patients with HUS

1. Crabbe DCG, et al. Gastrointestinal complications of the haemolytic uraemic syndrome. J Roy Soc Med. 1990;83:773–775. (level 5)
2. Brandt JR, et al. Cholelithiasis following *Escherichia coli* O157:H7-associated hemolytic uremic syndrome. Pediatr Nephrol. 1988;12:222–225. (level 5)
3. Suri RS, et al. Relationship between *Escherichia coli* O157:H7 and diabetes mellitus. Kidney Int Suppl. 2009;112:S44–S46. (level 4)
4. Suri RS, et al. Diabetes during diarrhea-associated hemolytic uremic syndrome: a systematic review and meta-analysis. Diabetes Care. 2005;28:2556–2562. (level 4)
5. Nathanson S, et al. Acute neurological involvement in diarrhea-associated hemolytic uremic syndrome. Clin J Am Soc Nephrol. 2010;5:1218–1228. (level 5)
6. Brasher C, et al. The hemolytic-uremic syndrome. West J Med. 1981;134:193–197. (level 5)
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)

## 4 Diagnosis and treatment of HUS in adults

4.1 Diagnosis and treatment of HUS in adults

1. Melnyk AMS, et al. Adult hemolytic-uremic syndrome: a review of 37 cases. Arch Intern Med. 1995;155:2077–2084. (level 4)
2. Tostivint I, et al. Adult haemolytic and uraemic syndrome: causes and prognostic factors in the last decade. Nephrol Dial Transplant. 2002;17:1228–1234. (level 4)
3. George JN: The thrombotic thromobytopenic purpura and hemolytic uremic syndromes: overview of pathogenesis (Experience of the Oklahoma TTP-HUS Registry, 1989–2007). Kidney Int. 2009;75:S8–S10. (level 4)
4. Schieppati A, et al. Renal function at hospital admission as a prognosis factor in adult hemolytic uremic syndrome. J Am Soc Nephrol. 1992;2:1640–1644. (level 4)
5. Bell WR, et al. Improved survival in thrombotic thrombocytopenic purpura-hemolytic uremic syndrome. Clinical experience in 108 patients. N Engl J Med. 1991;325:398–403. (level 4)

6. von Baeyer H: Plasmapheresis in thrombotic microangiopathy-associated syndromes: review of outcome data derived from clinical trials and open studies. Ther Apher. 2002;6:320–328. (level 4)
7. Brunskill SJ, et al. A systematic review of randomized controlled trials for plasma exchange in the treatment of thrombotic thrombocytopenic purpura. Transfus Med. 2007;17:17–35. (level 1)
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)

4.2 Diagnosis and treatment of STEC-associated HUS in adults

1. Karpac CA, et al. Sporadic bloody diarrhoea-associated thrombotic thrombocytopenic purpura-haemolytic uraemic syndrome: an adult and paediatric comparison. Br J Haematol. 2008;141:696–707. (level 4)
2. Frank C, et al. HUS Investigation Team. Epidemic profile of Shiga-toxin-producing *Escherichia Coli* O104:H4 outbreak in Germany. N Eng J Med. 2011;365:1771–1780. (level 4)
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)

## 5 Diagnosis and treatment of atypical hemolytic uremic syndrome (aHUS)

5.2 Treatment of aHUS

1. Krysan DJ, et al. Renal transplantation after streptococcus pneumonia-associated hemolytic uremic syndrome. Am J Kidney Dis. 2001;37:e15. (level 5)
2. McGraw ME, et al. Haemolytic uremic syndrome and the Thomsen-Friedenreich antigen. Pediatr Nephrol. 1989;3:135–139. (level 5)
3. Gilbert RD, et al. Streptococcus pneumonia-associated hemolytic uremic syndrome. Pediatr Infect Dis J. 1998;17:530–532. (level 5)
4. Noris M, et al. Relative role of genetic complement abnormalities in sporadic and familiar aHUS and their impact on clinical phenotype. Clin J Am Soc Nephrol. 2010;5:1844–1859. (level 4)
5. Remuzzi G, et al. Combined kidney and liver transplantation for familial haemolytic uremic syndrome. Lancet. 2002;359: 1671–1672. (level 5)
6. Noris M, et al. Relative role of genetic complement abnormalities in sporadic and familiar aHUS and their impact on clinical phenotype. Clin J Am Soc Nephrol. 2010;5:1844–1859. (level 4)
7. Remuzzi G, et al. Hemolytic uremic syndrome: a fatal outcome after kidney and liver transplantation performed to correct factor H gene mutation. Am J Transplant. 2005;5:1146–1150. (level 5)
8. Remuzzi G, et al. Hemolytic uremic syndrome: a fatal outcome after kidney and liver transplantation performed to correct factor H gene mutation. Am J Transplant. 2005;5:1146–1150. (level 5)
9. Cheong HI, et al. Attempted treatment of factor H deficiency by liver transplantation. Pediatr Nephrol. 2004;19:454–458. (level 5)
10. Saland JM, et al. Favorable long-term outcome after liver-kidney transplant for recurrent hemolytic uremic syndrome associated with a factor H mutation. Am J Transplant. 2006;6:1948–1952. (level 5)
11. Jalanko H, et al. Successful liver-kidney transplantation in two children with aHUS caused by a mutation in complement factor H. Am J Transplant. 2008;8:8216–221. (level 5)
12. Saland JM, et al. Successful split liver-kidney transplant for factor H associated hemolytic uremic syndrome. Clin J Am Soc Nephrol. 2009;4:201–206. (level 5)
13. Gruppo RA, et al. Eculizumab for congenital atypical hemolytic-uremic syndrome. N Eng J Med. 2009;360:544–546. (level 5)
14. Nürnberger J, et al. Eculizumab for atypical hemolytic-uremic syndrome. N Eng J Med. 2009;360:542–544 (level 5)
15. Ohanian M, et al. Eculizumab safety reverses neurologic impairment and eliminates need for dialysis in severe atypical hemolytic uremic syndrome. Clin Pharmacol. 2011;3:5–12. (level 5)
16. Dorresteijn EM, et al. Eculizumab as rescue therapy for atypical hemolytic uremic syndrome with normal platelet count. Pediatr Nephrol. 2012;27:1193–1195. (level 5)
17. Zimmerhackl LBHofer J, et al. Prophylactic eculizumab after renal transplantation in atypical hemolytic uremic syndrome. N Eng J Med. 2010;362:1746–1748. (level 5)
18 Weitz M, et al. Prophyractic eculizumab prior to kidney transplantation for atypical hemolytic uremic syndrome. Pediatr Nephrol. 2011;26:1325–1329. (level 5)
19. Al-Akash SI, et al. Eculizumab induces long-term remission in recurrent post-transplant HUS associated with C3 gene mutation. Pediatr Nephrol. 2011;26:613–619. (level 5)
20. Zuber J, et al. for the French Study Group for atypical HUS: Eculizumab for atypical haemolytic uremic syndrome recurrence in renal transplantation. Am J Transplant. 2012;12:3337–3354. (level 5)

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SPECIAL ARTICLE

# Diagnostic criteria for atypical hemolytic uremic syndrome proposed by the joint committee of the Japanese society of nephrology and the Japan pediatric society

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**Abstract** Atypical hemolytic uremic syndrome (aHUS) is rare and comprises the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury. Recently, abnormalities in the mechanisms underlying complement regulation have been focused upon as causes of aHUS. The prognosis for patients who present with aHUS is very poor, with the first aHUS attack being associated with a mortality rate of ~25 %, and with ~50 % of cases resulting in end-stage renal disease requiring dialysis. If treatment is delayed, there is a high risk of this syndrome progressing to renal failure. Therefore, we have developed diagnostic criteria for aHUS to enable its early diagnosis and to facilitate the timely initiation of appropriate treatment. We hope these diagnostic criteria will be disseminated to as many clinicians as possible and that they will be used widely.

**Keywords** Atypical hemolytic uremic syndrome · Thrombotic microangiopathy · Complement dysregulation · Alternative complement pathway · ADAMTS13

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## Introduction

Hemolytic uremic syndrome (HUS) is characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute kidney injury (AKI) [1]. Approximately 90 % of pediatric patients develop this syndrome after infection with *Shigella dysenteriae*, which produces true Shiga toxins, or *Escherichia coli*, some strains of which produce Shiga-like toxins. Shiga toxin was originally called verotoxin because Vero cells derived from the kidney epithelial cells of the African green monkey are hypersensitive to this toxin [2]. Subsequently, other toxins were called Shiga-like toxin because of their similarities to Shiga toxin in terms of their antigenicity and structure. Shiga-like toxin-1 differs from Shiga toxin by only 1 amino acid, whereas Shiga-like toxin-2 shares 56 % sequence homology with Shiga-like toxin-1. Although Shiga-like toxin-producing *E. coli*-HUS (STEC-HUS) strains most often trigger HUS, certain Shiga toxin-secreting strains of *S. dysenteriae* can also cause HUS. They are currently known as the Shiga toxin family, and the terms are often used interchangeably. HUS occurring from infection with STEC-HUS was formerly called diarrhea + HUS (D + HUS) or typical HUS.

In contrast, HUS that is not related to Shiga toxins and accounts for ~10 % of all HUS cases, is called atypical HUS (aHUS). Although STEC-HUS is relatively common in children, aHUS occurs in individuals of all ages and is often familial. The prognosis is very poor, with the first aHUS attack being associated with a mortality rate of ~25 %, and with ~50 % of cases resulting in end-stage renal disease requiring dialysis [3].

In recent years, abnormalities in the mechanisms underlying complement regulation have been focused on as causes of aHUS. Various genetic abnormalities in complement regulatory factors, including complement factor H, have been noted in 50–60 % of patients. The analysis of the pathology underlying this condition is currently progressing rapidly [4].

The differential diagnosis of aHUS from STEC-HUS or thrombotic thrombocytopenic purpura (TTP), another form of thrombotic microangiopathy (TMA) caused by a deficiency of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13), is not necessarily easy at the early stages of disease onset. However, if treatment is delayed, there is a high risk of this syndrome progressing to renal failure. Therefore, the Joint Committee of the Japanese Society of Nephrology and the Japan Pediatric Society (JSN/JPS) has developed diagnostic criteria for aHUS to enable its early diagnosis and to facilitate the timely initiation of appropriate treatment [5, 6]. We hope that the diagnostic criteria presented in this report will become familiar to as many clinicians as possible and that they will be used widely.

### Definition of aHUS

aHUS is a type of TMA that differs from STEC-HUS and TTP, with the latter being caused by markedly reduced ADAMTS13 activity. aHUS is a syndrome characterized by the triad of microangiopathic hemolytic anemia, thrombocytopenia, and AKI, which is similar to STEC-HUS.

## Guidelines for the diagnosis of aHUS

### Definitive diagnosis

A definitive diagnosis of aHUS is made when the triad of microangiopathic hemolytic anemia, thrombocytopenia, and AKI is present. The disease should not be associated with Shiga toxins, and TTP should also be excluded.

The Joint Committee of the JSN/JPS defined microangiopathic hemolytic anemia based on a hemoglobin (Hb) level of <10 g/dL. The presence of microangiopathic hemolytic anemia should be confirmed based on increased serum lactate dehydrogenase levels, a marked decrease in serum haptoglobin levels, and the presence of red blood cell fragments in a peripheral blood smear.

Thrombocytopenia is defined as a platelet (PLT) count of <150,000/μL.

The definition of AKI has been updated, with the most recent definition given by the international guidelines group, the Kidney Disease: Improving Global Outcomes that integrates both the Risk, Injury, Failure, Loss, End-stage kidney disease and the Acute Kidney Injury Network classifications to facilitate identification. Thus, we recommend diagnosis based on the most recent guidelines, along with the following definitions. For pediatric cases, the serum creatinine should be increased to a level that is 1.5fold higher than the serum creatinine reference values based on age and gender issued by the Japanese Society for Pediatric Nephrology [7]. For adult cases, the diagnostic criteria for AKI should be used.

## Guidelines for the diagnosis of aHUS

### Definitive diagnosis

A definitive diagnosis of aHUS is made when the triad of microangiopathic hemolytic anemia, thrombocytopenia,

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**Table 1** Definitions of microangiopathic hemolytic anemia, thrombocytopenia, and AKI that have been established by the joint committee of the JSN/JPS

| Microangiopathic hemolytic anemia | Thrombocytopenia | Acute kidney injury |
|---|---|---|
| Defined as an Hb level <10 g/dL<br>Presence confirmed based on:<br>Increased serum LDH levels<br>Marked decreases in serum haptoglobin levels<br>The presence of red blood cell fragments in a peripheral blood smear | Defined as a PLT count <150,000/μL | The most recent AKI definition is provided by the international guideline group, the KDIGO, integrating the RIFLE and AKIN classifications to facilitate identification. Thus, diagnosis should be based on the most recent guidelines, and the following definitions should be used.<br>Pediatric cases: Serum creatinine should be increased to a level that is 1.5fold higher than the serum creatinine reference values based on age and gender issued by the Japanese Society for Pediatric Nephrology [7].<br>Adult cases: Diagnostic criteria for AKI should be used |

*Hb* hemoglobin, *LDH* lactate dehydrogenase, *PLT* platelet, *AKI* acute kidney injury, *KDIGO* kidney disease: improving global outcomes, *RIFLE* risk, injury, failure, loss, end-stage kidney disease, *AKIN* acute kidney injury network

and AKI is present. The disease should have no association with Shiga toxins, and TTP should also be excluded. Table 1 presents the definitions of microangiopathic hemolytic anemia, thrombocytopenia, and AKI that are established by the Joint Committee of the JSN/JPS.

Probable diagnosis

A probable diagnosis of aHUS is made when 2 of the following 3 conditions are found: microangiopathic hemolytic anemia, thrombocytopenia, and AKI. The disease should have no association with Shiga toxins and TTP should be excluded.

## Applicability of these diagnostic criteria

When we applied these diagnostic criteria to the Nara Medical University (NMU) TMA cohort, 15 out of 37 individuals who had all the data required for the assessment were diagnosed as having definitive aHUS. Since the data were recorded at one time point only, we speculate that the sensitivity of the diagnostic criteria would increase if we could assess data from multiple time points. The cut-off value for anemia, defined as an Hb level of <10 g/dL, and the cut-off value for thrombocytopenia, defined as a PLT count of <150,000/μL, are equivalent to those employed by the International Registry of Recurrent and Familial HUS/TTP [8]. We had considered using a cut-off value of a PLT count <100,000/μL for thrombocytopenia to reflect that used in the diagnostic criteria for STEC-HUS by the Japanese Society for Pediatric Nephrology (2000), but we only found 1 patient with a PLT count between 100,000 and 150,000/μL in the NMU cohort. Therefore, it is likely that this difference will not have a large impact on the sensitivity or specificity of our diagnostic criteria. Our diagnostic criteria include the category of "Probable" aHUS because we believe that this tentative diagnosis will help in the early diagnosis of aHUS and avoid delays in developing appropriate therapeutic approaches for patients with aHUS.

## Evaluation of inappropriate complement activation

Abnormalities in complement regulation are among the main causes of aHUS. The diagnosis of aHUS that is caused by inappropriate complement activation has become more critical because eculizumab, a humanized anti-C5 monoclonal antibody, has been shown to be an effective therapeutic modality [9] that has been approved for the treatment of aHUS patients in Europe and the United States. Recently, Fan and colleagues evaluated genotype–phenotype relationships in 10 Japanese patients with aHUS and identified potentially causative mutations in complement factor H, C3, membrane cofactor protein, and thrombomodulin in 8 of the patients [10]. However, the definitive diagnosis of inappropriate complement activation in aHUS patients is difficult because some patients show normal serum levels of complement components [11] and there are a number of complement regulatory proteins, making it difficult to decide which complement regulatory protein is responsible for a particular patient developing aHUS.

## Excluding Shiga toxin-producing *E. coli* infection

STEC-HUS is characterized by diarrhea accompanied by bloody stools. However, diarrhea may also be present in some aHUS cases. Diarrhea in aHUS can be a manifestation of ischemic colitis. In addition, enteritis that is not caused by STEC can trigger aHUS. Therefore, a diagnosis of STEC-HUS cannot be made based on symptoms alone, and the earlier nomenclature that used "D + HUS" to correspond with STEC-HUS and "D-HUS" to correspond

with aHUS is not used at present [11]. The involvement of Shiga toxins should be confirmed by stool culture, the direct detection of Shiga toxins, or the detection of anti-lipopolysaccharide-IgM antibodies.

### Excluding TTP

Conventionally, TTP has been diagnosed based on the classic pentad (microangiopathic hemolytic anemia, thrombocytopenia, labile psychoneurotic disorder, fever, and renal failure). However, the discovery of ADAMTS13 led to the finding that 60–90 % of patients with TTP have a marked reduction in the activity of ADAMTS13, to a level of <5 %, regardless of race. Therefore, when diagnosing aHUS, patients who have markedly reduced levels of ADAMTS13 activity (<5 %) should be diagnosed as having TTP, thereby ruling out a diagnosis of aHUS. However, some patients may show the classic TTP pentad and have normal or slightly reduced levels of ADAMTS activity. Therefore, if a patient has levels of ADAMTS13 activity ≥5 %, a differential diagnosis of aHUS or TTP may be necessary to account for other clinical symptoms.

### Excluding TMA caused by other distinct factors

Diseases that evidently cause a clinical state of TMA, including disseminated intravascular coagulation, scleroderma kidney, and malignant hypertension, should be excluded when diagnosing aHUS.

### When a probable case of aHUS is suspected

When a probable case of aHUS is suspected, samples that are necessary to determine the appropriate diagnosis should be collected, and the therapeutic strategy should be established after consultation with an institution that has extensive experience of managing aHUS cases.

### Cases where aHUS should be strongly suspected

If there are features that are characteristic of HUS, aHUS should be strongly suspected if the following criteria are fulfilled, regardless of the presence of diarrhea: the patient is younger than 6 months of age; time of onset is unclear (latent onset); the patient has a history of HUS (recurrent case); the patient has a history of anemia of unknown cause; recurrent HUS after kidney transplantation; the patient has a family history of HUS (excluding cases of food poisoning); and, the patient has no diarrhea or bloody stools.

### Classification of aHUS causes, excluding TTP caused by the ADAMTS13 defect

Table 2 classifies the causes of aHUS and presents methods to determine the causes.

## Discussion

Nineteen years after Gasser et al. [1] reported HUS, an interesting report was published in the *Lancet* [10]. This report indicated that although C3-predominant activity is initiated in the blood vessels in TMA patients, this is not observed in typical cases of HUS, suggesting that complement activation is involved in aHUS onset [12]. Subsequently, numerous researchers have elucidated further information on the pathology of aHUS. At present, the reported causes of aHUS include, complement regulation abnormalities, cobalamin metabolism disorder, infection with *Streptococcus pneumoniae* and other microorganisms, drugs, pregnancy, and autoimmune diseases.

The complement system plays an important role as part of the immune systems of living organisms. It is activated via 3 pathways, the classical, alternative, and lectin pathways. As a result of the activation of the host's alternative and classical pathways, C5b-9, a membrane attack complex, is generated and destroys cells by forming transmembrane pores. The alternative pathway is involved in the onset of aHUS. Unlike the classical and lectin pathways, activation of the alternative pathway does not require initiators; it is continuously activated by the spontaneous hydrolysis of C3.

When complement proteins are inappropriately activated, there is a risk of inducing cell dysfunction within the host itself. Thus, humoral factors in the circulating plasma and several plasma membrane-bound factors are involved in the regulation of complement activation and act at various stages, such as the inactivation of C3b or C4b, and the inhibition of the generation of membrane attack complexes. The regulators involved in the alternative pathway include complement factors H and I, which are humoral factors, and membrane cofactor protein and thrombomodulin, which are membrane-bound factors. If these factors are abnormal, the subsequent failure of regulation will hyperactivate the complement proteins, leading to the onset of aHUS. Some cases of aHUS develop after trigger events, for example, infections of the respiratory tract and the gastrointestinal tract, and it is likely that activation of the complement cascade by these trigger events and the

**Table 2** Classification and determination of the causes of aHUS, excluding TTP caused by the ADAMTS13 defect

| Cause of aHUS | Method to determine the cause |
|---|---|
| Complement regulation abnormality | Hemolysis test, quantification of complement proteins and complement regulatory proteins, and gene analysis. Even if the amounts of complement proteins and complement regulatory proteins are within the normal ranges, it does not serve as a basis for excluding complement-related aHUS |
| (i) Congenital | |
| Genetic mutations of complement proteins, factor H, factor I, membrane cofactor protein, C3, factor B, and thrombomodulin | |
| (ii) Acquired | |
| Production of autoantibodies, including anti-factor H antibody | Detection of anti-factor H antibody by ELISA, western blot, etc. |
| (2) Cobalamin metabolism disorder | Age at onset should be considered (<6 months old), and hypomethioninemia or hyperhomocysteinemia is detected on plasma amino acid analysis |
| (3) Infection | Definitive diagnosis by identification of pathogenic microorganisms and serological examination |
| (i) Pneumococcus | |
| (ii) Human immunodeficiency virus | |
| (iii) Pertussis | |
| (iv) Influenza | |
| (v) Varicella | |
| (4) Drug-induced | Identification of the drug |
| (i) Anticancer drugs | |
| (ii) Immunomodulatory drugs | |
| (iii) Antiplatelet drugs | |
| (5) Pregnancy-related | |
| (i) Hemolysis, elevated liver enzymes, low platelet counts (HELLP) syndrome | |
| (ii) Eclampsia | |
| (6) Autoimmune disease, collagen disease | Definitive diagnosis by autoantibody test, antiphospholipid antibody test, and serological examination |
| (i) Systemic lupus erythematosus | |
| (7) Bone-marrow transplant, organ transplant-related | |
| (8) Others | |

*aHUS* atypical hemolytic uremic syndrome, *ELISA* enzyme-linked immunosorbent assay

subsequent amplification of complement activation by the alternative pathway cannot be regulated in patients with deficiencies in complement regulation. Gain-of-function mutations in C3 and complement factor B, which are complement-activating factors, also cause hyperactivation of complement proteins and, ultimately, aHUS.

It has been reported that ~50 % of aHUS patients have genetic abnormalities in complement regulatory factors, including complement factor H. The frequency of the presence of certain mutations among aHUS cases, responsiveness to plasma therapy, prognosis of kidney function, and the recurrence rate after kidney transplantation, vary depending on the type of genetic abnormalities present [13]. Although plasmapheresis within 24 h of confirmation of the diagnosis has been recommended as the initial treatment for aHUS [14], its effects are not always satisfactory. The mortality or incidence of end-stage renal disease is considered to be between 70 and 80 %, and the recurrence rate after kidney transplantation may be as high as 80–90 %, particularly in patients with abnormal complement factor H, which is the most frequent abnormality [15].

In 2011, eculizumab (Soliris®, Alexion Pharmaceuticals), a terminal complement inhibitor, was approved as a new drug for the treatment of aHUS in Europe and the US. Eculizumab is a humanized recombinant immunoglobulin G2/4 monoclonal antibody directed against the complement component C5, which was developed as a treatment for paroxysmal nocturnal hemoglobinuria. By binding to complement component C5, the drug inhibits the generation of C5a and C5b-9, and thus subsequently inhibits the complement system.

There are a number of reports stating that only HUS that is associated with complement regulation abnormalities is defined as aHUS. On the basis of the current diagnostic criteria, we have defined aHUS to include all types of HUS that are not related to Shiga toxins or other distinct causes. In cases where aHUS is associated with complement

dysregulation, the introduction of eculizumab may markedly change therapeutic strategies. It should be noted, however, that recommendations of specific therapeutic modalities are beyond the scope of the current diagnostic criteria. However, in cases where complement dysregulation is confirmed as the cause, treatment with eculizumab is established. Thus, it may be desirable to assign HUS associated with complement dysregulation a separate disease name rather than it being classified as "aHUS", as in the case of definitive "complement-mediated TMA".

As described in previous reports, aHUS is a disease that may frequently cause renal failure and be fatal if it is not appropriately diagnosed and treated at the early stages of disease onset. In Japan, aHUS may be misdiagnosed as HUS caused by Shiga toxins because clinicians are not sufficiently aware of aHUS, and consequently, treatment may be delayed. Thus, our diagnostic criteria include the category of "Probable" aHUS to ensure that the clinicians consider aHUS during diagnosis. Many issues should be addressed in the future, including the development of diagnostic strategies to diagnose cases of suspected aHUS, the establishment of insurance coverage for ADAMTS13 activity measurement testing that is necessary to differentiate aHUS from TTP, and the development of treatment guidelines. We hope that our diagnostic criteria will be used widely and will contribute to the diagnosis and treatment of aHUS patients.

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## References

1. Gasser C, Gautier E, Steck A, Siebenmann R, Oechslin R. Hemolytic-uremic syndrome: bilateral necrosis of the renal cortex in acute acquired hemolytic anemia. Schweizerische medizinische Wochenschrift. 1955;85(38–39):905–9.
2. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun. 1977;18:775–9.
3. Noris M, Remuzzi G. Atypical hemolytic-uremic syndrome. N Engl J Med. 2009;361(17):1676–87. doi:10.1056/NEJMra0902814.
4. Kavanagh D, Goodship T. Genetics and complement in atypical HUS. Pediatric Nephrol (Berlin, Germany). 2010;25(12):2431–42. doi:10.1007/s00467-010-1555-5.
5. Kagami S, Okada H, Kaname S, Sato W, Nangaku M, Yasuda T, et al. Diagnostic criteria of atypical hemolytic uremic syndrome. Jpn J Nephrol. 2013;55(2):91–3 (in Japanese).
6. Kagami S, Okada H, Kaname S, Sato W, Nangaku M, Yasuda T et al. Diagnostic criteria of atypical hemolytic uremic syndrome. J Jpn Pediatr Soc. 2013; http://www.jpeds.or.jp/uploads/files/saisin_130201.pdf (in Japanese).
7. 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.
8. 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 Amer Soc Nephrol. 2010;5(10):1844–59. doi:10.2215/CJN.02210310.
9. Legendre C, Licht C, Muus P, Greenbaum L, 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.
10. 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.
11. Loirat C, Frémeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis. 2011;6:60. doi:10.1186/1750-1172-6-60.
12. Stühlinger W, Kourilsky O, Kanfer A, Sraer J. Letter: haemolytic-uraemic syndrome: evidence for intravascular C3 activation. Lancet. 1974;2(7883):788–9.
13. Nester C, Thomas C. Atypical hemolytic uremic syndrome: what is it, how is it diagnosed, and how is it treated? Hematology. 2012;2012:617–25. doi:10.1182/asheducation-2012.1.617.
14. Ariceta G, Besbas N, Johnson S, Karpman D, Landau D, Licht C, et al. Guideline for the investigation and initial therapy of diarrhea-negative hemolytic uremic syndrome. Pediatric Nephrol (Berlin, Germany). 2009;24(4):687–96. doi:10.1007/s00467-008-0964-1.
15. Waters A, Licht C. aHUS caused by complement dysregulation: new therapies on the horizon. Pediatric Nephrol (Berlin, Germany). 2011;26(1):41–57. doi:10.1007/s00467-010-1556-4.

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ORIGINAL ARTICLE

# Relationship between dietary protein intake and the changes in creatinine clearance and glomerular cross-sectional area in patients with IgA nephropathy

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**Abstract**
*Background* Dietary protein intake (PI) induces glomerular hyperfiltration and reduced dietary PI can be effective in preserving kidney function. However, there is limited information regarding the relationship between dietary PI and glomerular histological changes in chronic kidney disease. We investigated the relationship between changes in dietary PI and both the changes in creatinine clearance and glomerular histomorphometry in adult patients with IgA nephropathy (IgAN).
*Methods* A total of 24 consecutive adult patients with biopsy-confirmed IgAN were enrolled and glomerular histomorphometric variables and clinical variables were investigated. The main clinical variables were differences in creatinine clearance (Ccr) (dCcr) and in PI (dPI) which were calculated by subtracting PI and Ccr values in patients on a controlled diet during hospitalization for kidney biopsy from the respective values in patients on daily diets as outpatients. These values of PI were estimated from urinary urea excretion measured by 24-h urine collection. The main renal histomorphometric variable was glomerular tuft area (GTA) ($\mu m^2$).
*Results* dCcr positively correlated with dPI ($r = 0.726$, $P < 0.001$). GTA correlated positively with dPI ($r = 0.556$, $P = 0.013$). Multiple regression analysis showed that dPI was independently associated with both dCcr and GTA. Additionally, GTA positively correlated with dietary PI as outpatients ($r = 0.457$, $P = 0.043$).
*Conclusion* Changes in dietary PI were associated with the changes in glomerular filtration rate. Furthermore, histomorphometric findings suggested that a greater dietary PI can affect the glomerular size at the time of the initial diagnostic biopsy for IgAN.

**Keywords** Dietary protein intake · Chronic kidney disease · Glomerular hypertrophy · Glomerular hyperfiltration · Glomerular filtration rate

## Introduction

Chronic kidney disease (CKD) is recognized as a worldwide public health problem [1]. IgA nephropathy (IgAN) is the most common form of glomerulonephritis and is one of the major causes of CKD in Japan, leading to end-stage renal disease in about 40 % of patients 20 years after onset [2, 3].

Immunological mechanisms, such as an aberrant IgA immune response to a variety of different antigens, contribute to the development of IgAN. In addition, non-immunological mechanisms, such as glomerular hypertension (considered to be a common factor in the progression of glomerular diseases), may also play a role in IgAN progression.

Dietary protein intake (PI) can modulate renal function [4–6], but its role in kidney disease provokes ongoing debate, in particular, the concern that excessive dietary PI promotes or aggravates chronic kidney disease (CKD) [6–8]. The habitual excessive consumption of dietary protein was demonstrated to negatively affect kidney function by inducing a sustained increase in glomerular pressure [6, 7]. High glomerular pressure induces glomerular hyperfiltration that mediates injury to the glomeruli,

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which subsequently progress to interstitial fibrosis and consequently loss of renal function [7, 9]. Dietary protein restriction limits the adaptive increase in the single-nephron glomerular filtration rate and glomerular capillary hydraulic pressure that is observed in response to a reduction in functional nephron number, as demonstrated in various models of experimental renal disease [10–12]. In micropuncture studies in the rat, the increased glomerular filtration rate in response to amino acid infusion was associated with a reduction in afferent arteriolar resistance and a subsequent increase in single-nephron plasma flow [8].

Dietary PI can also induce phosphorus load and acid load, and the reduction of dietary PI together with these loads preserves kidney function, prevents the development of uremic symptoms, and delays the need for dialysis therapy, particularly in the later stages of CKD [13–15]. A meta-analysis of randomized controlled clinical trials on the effects of low-protein diets yielded favorable results in inhibiting the progression of renal function decline in patients with non-diabetic CKD [16].

The results of quantitative morphometric analysis in IgAN patients indicate an increase in the overall glomerular area compared with the respective glomerular area in normal patients [17]. Previous studies of the quantitative analysis of renal histology demonstrated that a lower glomerular density and larger glomeruli are prognostic indicators for IgAN [18–21].

However, there is limited information regarding the relationship between dietary PI and glomerular histomorphometry in CKD [22]. The objectives of this study were (1) to confirm whether the glomerular filtration rate varies with changes in protein intake, and (2) to quantitatively assess the glomerular tuft area (GTA) in patients with IgAN and investigate the relationship between glomerular histomorphometry and dietary PI. The present study is an observational study based on the changes between food intake of patients during hospitalization and as outpatients undergoing routine medical care.

## Materials and methods

### Subjects

A total of 24 consecutive patients (14 men and 10 women) who had undergone renal biopsy and were confirmed to have IgAN between March 2005 and February 2008 in our department were enrolled. All patients provided written informed consent to use their clinical data, and this study was approved by the Institutional Review Board of Tokyo Medical University (No. 1987). Patients whose total number of obtained glomeruli was less than 4 and who had previously been given diagnoses of IgAN by renal biopsy or who had complications of diabetes mellitus, liver cirrhosis, or purpura nephritis were excluded from the analysis. No patients had received corticosteroid therapy or dietary instruction from a dietitian before receiving a diagnosis of IgAN.

### Diets

All patients had unrestricted diets during the period of outpatient department attendance before receiving a diagnosis of IgAN. The hospital stay was 4–5 days for diagnostic kidney biopsy. During their hospital stay, the daily diet which was provided to patients as part of their care before diagnosis included 120 mEq of sodium, 65 g of protein, and 1800 kcal of energy, and their dietary intake was at least 80 % and substantially 80–90 % of the diet based on reviewed medical records.

### Dietary protein and sodium intake

Dietary PI was determined according to the formula of Maroni et al. on the basis of 24-h urine collection [23]. Dietary sodium intake (SI) was determined on the basis of 24-h urinary sodium excretion.

### Clinical data

Creatinine clearance (Ccr) was determined by the standard clearance technique on the basis of 24-h urine collection, and 24-h urinary protein (UP) excretion was measured. The mean arterial pressure (MAP) was defined as diastolic blood pressure plus pulse pressure/3. Routine biochemical measurements, which included measurements of the levels of serum low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and hemoglobin A1c, were performed in the morning after overnight fasting on the day following admission for renal biopsy. The estimated glomerular filtration rate (mL/min/1.73 $m^2$) was calculated from the following formula for Japanese patients: $194 \times Cr^{-1.094} \times age^{-0.287}$ ($0.739\times$ for women) [24].

### Data analysis

Two values each of PI, SI, Ccr, MAP, and Serum creatinine (Scr) (measured at the outpatient department in the most recent days before treatment for IgA nephropathy which was diagnosed by kidney biopsy) were averaged, and these average values were applied as the outpatient department value. The mean elapsed time between the hospitalization measurements and the most recent outpatient

measurements was 2.0 months [interquartile range (IQR), 1.0–4.0]. The hospitalization period for renal biopsy was 4–5 days and renal biopsy was performed on the intermediate day of the hospital stay. The values of PI, SI, Ccr, MAP, and Scr (obtained once on the third or fourth day of admission for kidney biopsy with conceivable reduced effect of diet on an outpatient basis) were applied as the values during hospitalization. Differences in protein intake (dPI), sodium intake (dSI), creatinine clearance (dCcr), mean arterial pressure (dMAP), and serum creatinine (dScr) values between those obtained at the outpatient department and those obtained during hospitalization were investigated. Then, dPI, dSI, dCcr, dMAP, and dScr values were calculated by subtracting the PI, SI, Ccr, MAP, and Scr values of patients obtained during hospitalization from the respective values of the same patients when measured at the outpatient department.

### Quantitative morphology of kidney tissue sections

The diagnosis of IgAN was made by a pathologist. All patients had both kidneys. Kidney tissue specimens were obtained by standard percutaneous kidney biopsy.

Biopsies were divided into three portions including the glomeruli, which were identified for light, immunofluorescence, and electron microscopies. The tissue was embedded in paraffin, cut into 1-μm-thick sections for light microscopy, and stained with hematoxylin–eosin, periodic acid–Schiff (PAS), periodic-acid methenamine silver, and Masson trichrome (MT).

Quantitative morphometry of the renal histological specimens was performed using an automated image analyzing system (Image-Pro Plus ver. 6.1, Media Cybernetics Inc., Silver Spring, MD, USA) which provided digital image capture and computer-assisted image analysis. Measurements were performed on the routinely processed kidney tissue slide samples for pathological diagnosis. The glomeruli were stained with PAS for morphometric analysis. For each biopsy specimen, the top quartile of the large glomeruli was selected based on the morphometry of each GTA, and the mean tuft area of the glomeruli and the capillary area were measured by a nephrologist (T.W.). The nephrologist performing the morphometric assessments was blinded to the clinical data. To measure a cross-section of the maximal planar area to reflect the area inside a circular line on the surface of a sphere, the glomerulus was intersected with a plane passing through as nearly as possible to the center of the glomerulus. We selected a larger area of glomeruli in a tissue section slide in which the plane passed through as nearly as possible to the center of the glomerulus as a sphere.

The tuft area of the glomerulus was defined as the inner area indicated by the outer capillary loops of the tuft. The capillary area was defined as the area expressed as a blank space of the inner glomerulus on an obtained image. The averaged values of each morphometric variable on the selected glomeruli were calculated, and the following morphometric indices were investigated:

1. glomerular tuft area (GTA) ($\mu m^2$) = mean tuft area of the glomeruli;
2. capillary area ratio (CapR) (%) = mean percentage of capillary area/tuft area of the glomeruli × 100.

### Statistical analysis

The data were expressed as mean (standard deviation, SD) for parametric data, or median (interquartile range, IQR) for non-parametric data. A $P$ value of less than 0.05 was considered to indicate a statistically significant difference. The Student unpaired $t$ test for parametric data or the Mann–Whitney $U$ test for non-parametric data was used to compare the differences between variables. The Student paired $t$ test for parametric data or the Wilcoxon signed rank test was used to compare values both at the outpatient department and during hospitalization. Simple correlations between two values were analyzed by Pearson correlation (for parametric data) or Spearman correlation (for non-parametric data). Multiple regression analysis was conducted to evaluate the independent determinant factors for dCcr and GTA. Data analysis was performed using PASW Statistics 18 (IBM, Chicago, IL, USA).

**Table 1** Clinical characteristics of the patients at renal biopsy ($N = 24$)

| | |
|---|---|
| Age (years) | 27 (22–36) |
| Men:women | 14:10 |
| Duration of presence of urine protein (years) | 3.5 (0.6–6.0) |
| Body mass index (kg/m$^2$) | 21.1 (2.2) |
| Mean arterial pressure (mmHg) | 81.9 (8.4) |
| HDL-C (mg/dL) | 66 (19) |
| LDL-C (mg/dL) | 107 (32) |
| HbA1c (%) | 4.8 (0.3) |
| UP[a] (g/day) | 0.43 (0.16–0.58) |
| eGFR (mL/min/1.73 m$^2$) | 95.2 (29.1) |

The ratio of men: women is expressed as a number; other values are expressed as mean (*SD* standard deviation) or median (*IQR* interquartile range)

eGFR was calculated from the following formula for Japanese patients: $194 \times Cr^{-1.094} \times age^{-0.287}$ (0.739× for women) 24

*HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HbA1c* hemoglobin A1c, *UP* urinary protein excretion, *eGFR* estimated glomerular filtration rate

[a] $N = 22$

**Table 2** Clinical values at the outpatient department, during hospitalization, and the differences between them ($N = 24$)

| | Outpatient | During hospitalization | $P$ value | Difference ($d$) |
|---|---|---|---|---|
| PI[a] (g/kg/day) | 1.13 (0.26) | 0.84 (0.65–1.18) | 0.064 | 0.15 (0.34) |
| SI[a] (mEq/day) | 192 (44) | 111 (31) | <0.001 | 84 (51) |
| Ccr[a] (mL/min) | 121.0 (35.5) | 93.2 (83.0–120.1) | 0.049 | 12.8 (25.0) |
| MAP (mmHg) | 86.0 (12.1) | 81.9 (8.4) | 0.044 | 4.1 (9.5) |
| Scr[b] (mg/dL) | 0.72 (0.19) | 0.77 (0.25) | 0.320 | −0.01 (−0.06 −0.03) |

Values are expressed as mean (SD) or median (IQR). Differences ($d$) in the values of dPI, dSI, dCcr, dMAP, and dScr were determined by subtracting the values obtained during hospitalization from those obtained at the outpatient department

*PI* protein intake, *SI* sodium intake, *Ccr* creatinine clearance, *Scr* serum creatinine, *MAP* mean arterial pressure

[a] $N = 20$ at the outpatient department, $N = 22$ during hospitalization, $d$ values were valid for $N = 19$

[b] $N = 20$ at the outpatient department, $N = 24$ during hospitalization, $d$ values were valid for $N = 20$

## Results

### Baseline characteristics

The baseline values of the patients at the point of renal biopsy are listed in Table 1. The average body mass index was 21.1 kg/m$^2$ which was less than 22 kg/m$^2$ defined as the standard (Japan Society for the Study of Obesity 1999). A total of 3 patients received angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Of these, 1 patient received both a calcium channel blocker and a beta blocker.

### Clinical data

Adequate 24-h urine collection samples were provided by 22 out of 24 patients during hospitalization for kidney biopsy, and from 20 out of 24 patients at the outpatient department. Thus, the comparisons of clinical variables on the basis of 24-h urine collection samples during hospitalization and at the outpatient department were valid for 19 patients. MAP values both at the outpatient department and during hospitalization were obtained from all the 24 patients. The clinical variables are listed in Table 2.

### Quantitative histomorphometric measurements

The total number of glomeruli examined in each biopsy specimen ranged from 4 to 35 with an average of 18.1 ± 7.9, and quantitative morphometry was performed for all of the glomeruli with a GTA. The top quartile of the large glomeruli, the average number of which was 4.2 ± 1.9, was selected based on the morphometry of each GTA and used for histological variable. Representative images of renal histomorphometry are shown in Fig. 1. Summaries of the renal histomorphometric measurements performed in this study are listed in Table 3. There were no statistically significant differences in morphometry variables between men and women. GTA correlated positively with CapR (Fig. 2).

### Relationship between dCcr and dPI

dCcr positively correlated with dPI (Fig. 3), but not with dSI or dMAP. Multiple regression analysis was performed to adjust for the effects of age, gender, BMI, dPI, dSI, and dMAP on dCcr. dPI was independently associated with dCcr (Table 4).

### Relationship between GTA and dPI

GTA correlated positively with dPI (Fig. 4a). There were no statistically significant differences between GTA and dSI, dCcr, dMAP. Multiple regression analysis was performed to adjust for the effects of age, gender, BMI, dPI, dSI, and dMAP on GTA. dPI was independently associated with GTA (Table 4).

Additionally, GTA also correlated positively with dietary PI, but not with SI and MAP at the outpatient department (Fig. 4b).

## Discussion

Our results demonstrated that dCcr, which represents changes in glomerular filtration rate, correlated with changes in protein intake. Moreover, glomerular tuft size correlated with dPI, which represents dietary PI as outpatients when compared with hospitalization on a controlled diet. These findings suggest that greater daily PI may induce glomerular hyperfiltration and increase glomerular size.

The result that dCcr positively correlated with dPI apparently supports previous reports with regard to the increased glomerular filtration rate in response to PI [6–8, 10, 12].

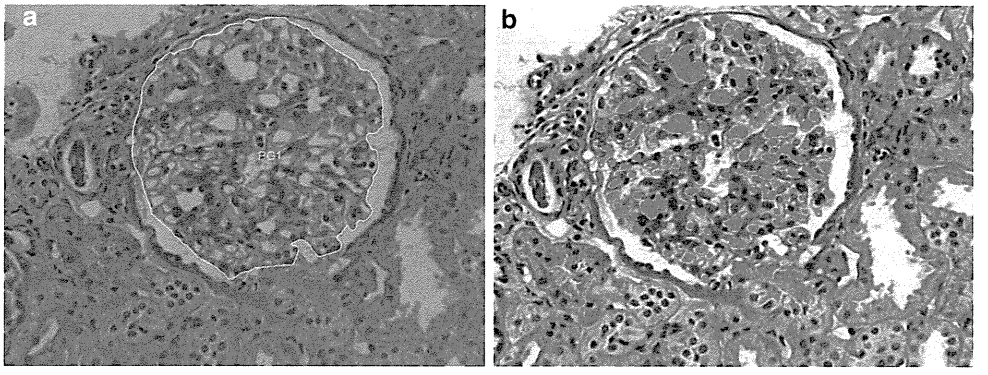


**Fig. 1** Representative images of renal histomorphometry. **a** Tuft area of the glomerulus (inner area of *yellow outline*), **b** capillary area (*red areas*) (PAS stain ×400)

**Table 3** Morphometry of renal histology ($N = 24$)

| Renal histological variable | Mean (SD) or median (IQR) |
|---|---|
| 1) Glomerular tuft area (GTA) ($\mu m^2$) | 21583 (20316–26156) |
| 2) Capillary area ratio (CapR) (%) | 12.3 (3.7) |

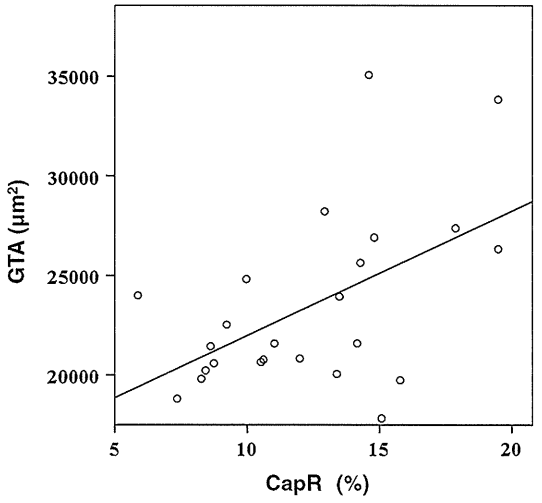


**Fig. 2** Relationship between GTA and CapR. GTA ($\mu m^2$) positively correlated with CapR (%) ($N = 24$; Spearman correlation coefficient, $r = 0.424$; $P = 0.039$)

We simultaneously measured GTA, which represents glomerular size, and the area of the components of the glomerulus such as the glomerular capillaries. We performed a simple histomorphometric technique to measure the plane area using routinely processed kidney tissue slide samples for pathological diagnosis in clinical care. The top quartile of the large glomeruli in a tissue section slide was selected for analysis, in which the plane passed through as nearly as possible to the center of the glomerulus as a sphere to avoid the selection of the edge portion of the glomerulus into the measurements. A larger

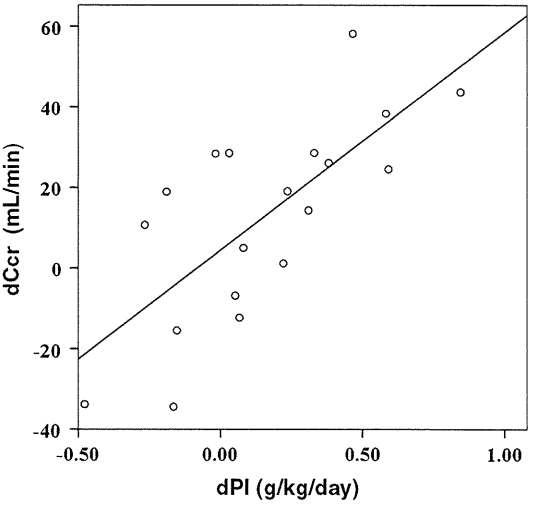


**Fig. 3** Relationship between dCcr and dPI. dCcr (mL/min) positively correlated with dPI (g/kg/day) ($N = 19$; Pearson correlation coefficient, $r = 0.726$; $P < 0.001$). dCcr and dPI were determined by subtracting the values obtained during hospitalization from those obtained at the outpatient department

glomerulus tends to become clearer, making its presence more visible.

In the current results, GTA correlated positively with CapR, which is the ratio of the glomerular capillary area in the glomerulus. An increase in CapR might be due to capillary loop dilatation which contributes to the development of large glomeruli. It is possible that such capillary loop dilation is caused by a high inner glomerular capillary pressure. Previous studies have shown that large glomeruli are frequently observed in the early stages of various kidney diseases, and such findings may indicate a high risk of renal failure [9, 18–21, 25].

In the present study, the patients were provided a diet containing the recommended dietary allowance for healthy Japanese adults during their hospital stay for kidney biopsy [26]. There were no differences in the PI between

**Table 4** Multiple regression analysis of dCcr and GTA

| Variable | dCcr (mL/min) | | GTA ($\mu m^2$) | |
|---|---|---|---|---|
| | Beta | *P* value | Beta | *P* value |
| Age (years) | 0.097 | 0.687 | 0.067 | 0.806 |
| Gender | 0.005 | 0.981 | 0.322 | 0.220 |
| BMI ($kg/m^2$) | 0.156 | 0.503 | −0.033 | 0.901 |
| dPI (g/kg/day) | 0.722 | 0.002 | 0.507 | 0.031 |
| dSI (mEq/day) | −0.170 | 0.389 | 0.222 | 0.327 |
| dMAP (mmHg) | −0.293 | 0.203 | −0.177 | 0.488 |
| $R^2$ | 0.621 | | 0.510 | |

Gender: women were assigned a value of 0 and men were assigned a value of 1. Differences (*d*) in the values of dCcr, dPI, dSI, and dMAP were determined by subtracting the values obtained during hospitalization from those obtained at the outpatient department. GTA ($\mu m^2$) and age (years) were transformed ($\log_{10}$) prior to the analysis

*Ccr* creatinine clearance, *BMI* body mass index, *PI* protein intake, *SI* sodium intake, *MAP* mean arterial pressure

outpatient and during hospitalization because several types of outpatients existed, those who had a large daily PI and those who had a small daily PI, making the outpatient PI as a whole not significantly different from the PI during hospitalization wherein patients were on a controlled diet. With all these considered, higher levels of dPI suggest a greater daily PI; in contrast, low levels of dPI suggest a less daily PI than during hospitalization for patients on a controlled diet. GTA correlated positively with dPI (Fig. 3a), and also with PI at the outpatient department (Fig. 3b). These findings suggest that daily PI affects glomerular size.

Glomerular hyperfiltration is considered to be a normal adaptation mechanism in the remnant glomerulus which compensates for the glomerular filtration loss caused by the insults from various glomerular diseases, and thus the remnant glomerulus of the kidney can become hypertrophic. Moreover, high PI causes high glomerular pressure and hyperfiltration in kidney diseases, and a subsequent increase in glomerular size [6, 7]. Jia et al. and Wakefield et al. performed studies on the effects of long-term dietary PI in healthy pigs or rat and demonstrated that a high-protein diet resulted in renal glomerular hypertrophy and frequent renal glomerulosclerosis compared with a normal-protein diet [27, 28]. Parts of these results are consistent with those of the present study.

Our data showed no correlation between GTA and dCcr. We may also consider other effects of PI on the glomerulus, not only the effect of PI on GFR by hemodynamic change, the impact of which can be reduced during hospitalization when patients take controlled diet at which time biopsy is performed, but also the nonhemodynamic effect of PI on GTA. A previous study indicated that higher dietary PI produces nonhemodynamic glomerular injury by enhancing mesangial cell responses which contribute to expansion of the glomerular area and mesangial area [22].

To the best of our knowledge, the relationship between PI and renal histomorphometry remains unexplored in IgAN. We believe that the findings of the present study can provide insight regarding the relationship between dietary PI and histomorphometric findings in patients with IgAN.

One of the strengths of the current study is the simultaneous measurement of GTA and CapR, which may clarify whether glomerular capillary loop dilation, possibly caused by a high glomerular pressure, contributes to the increased glomerular size. There are few previous studies investigating glomerular hypertrophy in relation to glomerular capillary loop dilation. Furthermore, in the present study, we evaluated the effects of the dietary PI according to observed differences in PI as estimated from urinary

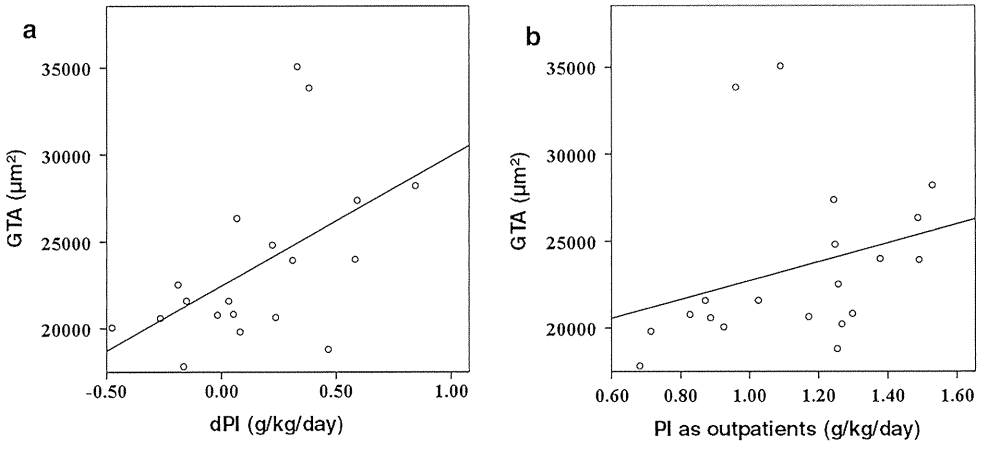


**Fig. 4** Relationship between GTA and dietary PI. **a** Correlation between dPI (g/kg/day) and GTA ($\mu m^2$) ($N = 19$; Spearman correlation coefficient, $r = 0.556$; $P = 0.013$). **b** Correlation between dietary PI at the outpatient department (g/kg/day) and GTA ($\mu m^2$) ($N = 20$; Spearman correlation coefficient, $r = 0.457$; $P = 0.043$). dPI was determined by subtracting the values obtained during hospitalization from those obtained at the outpatient department

urea excretion measured by 24-h urine collection at the outpatient department and during hospitalization. To the best of our knowledge, no previous studies have investigated the correlation between dietary PI and the histomorphometric changes in IgAN.

However, this study has some limitations. First, this study was made without consideration of the disease activity of IgAN to put priority on quantification of glomerular tissue as an objective method. Second, we could not apply multiple measurements for better assessment of PI. As there was almost no chance in many cases, kidney biopsy was performed in the early stages after the first visit as an outpatient in view of obtaining early diagnosis. Third, the sample size of this preliminary study may have been small. Fourth, healthy control subjects were not recruited in this study as ethical issues prevented us from obtaining normal healthy kidney tissue.

In conclusion, our clinical observational study, data based on 24-h urine collection, and glomerular histomorphometry have indicated that a greater dietary PI may induce glomerular hyperfiltration, which is one of the factors that possibly increase glomerular size at the time of the initial diagnostic biopsy for IgAN. The focus of this study as CKD patients was patients with IgA nephropathy which is the most common form of glomerulonephropathy in Japan with a large number of cases. However, the relationship between dietary PI and the changes in Ccr and GTA in other types of glomerulonephritis that cause CKD should be further investigated in future studies on CKD patients. The relationship between PI as a dietary factor and changes in the human kidneys must be furthered clarified and given attention.

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**Conflict of interests** The authors declare that they have no competing interests associated with this study.

## References

1. Stenvinkel P. Chronic kidney disease: a public health priority and harbinger of premature cardiovascular disease. J Intern Med. 2010;268(5):456–67. doi:10.1111/j.1365-2796.2010.02269.x.
2. Koyama A, Igarashi M, Kobayashi M. Natural history and risk factors for immunoglobulin A nephropathy in Japan. Research Group on Progressive Renal Diseases. Am J Kidney Dis. 1997;29(4):526–32 (pii S0272638697000656).
3. Sugiyama H, Yokoyama H, Sato H, Saito T, Kohda Y, Nishi S, et al. Japan Renal Biopsy Registry and Japan Kidney Disease Registry: committee report for 2009 and 2010. Clin Exp Nephrol. 2013;17(2):155–73. doi:10.1007/s10157-012-0746-8.
4. King AJ, Levey AS. Dietary protein and renal function. J Am Soc Nephrol. 1993;3(11):1723–37.
5. Garibotto G, Sofia A, Saffioti S, Bonanni A, Mannucci I, Verzola D. Amino acid and protein metabolism in the human kidney and in patients with chronic kidney disease. Clin Nutr. 2010;29(4):424–33. doi:10.1016/j.clnu.2010.02.005.
6. Martin WF, Armstrong LE, Rodriguez NR. Dietary protein intake and renal function. Nutr Metab (Lond). 2005;2:25. doi:10.1186/1743-7075-2-25.
7. Brenner BM, Meyer TW, Hostetter TH. Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. N Engl J Med. 1982;307(11):652–9. doi:10.1056/NEJM198209093071104.
8. Meyer TW, Ichikawa I, Zatz R, Brenner BM. The renal hemodynamic response to amino acid infusion in the rat. Trans Assoc Am Physicians. 1983;96:76–83.
9. Hill GS. Hypertensive nephrosclerosis. Curr Opin Nephrol Hypertens. 2008;17(3):266–70. doi:10.1097/MNH.0b013e3282f88a1f.
10. Hostetter TH, Olson JL, Rennke HG, Venkatachalam MA, Brenner BM. Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation. Am J Physiol. 1981;241(1):F85–93.
11. Dworkin LD, Feiner HD. Glomerular injury in uninephrectomized spontaneously hypertensive rats. A consequence of glomerular capillary hypertension. J Clin Invest. 1986;77(3):797–809. doi:10.1172/JCI112377.
12. Hostetter TH, Meyer TW, Rennke HG, Brenner BM. Chronic effects of dietary protein in the rat with intact and reduced renal mass. Kidney Int. 1986;30(4):509–17.
13. Mitch WE. Dietary therapy in uremia: the impact on nutrition and progressive renal failure. Kidney Int Suppl. 2000;75:S38–43 (pii kid7510).
14. Kusano K, Segawa H, Ohnishi R, Fukushima N, Miyamoto K. Role of low protein and low phosphorus diet in the progression of chronic kidney disease in uremic rats. J Nutr Sci Vitaminol (Tokyo). 2008;54(3):237–43 (pii JST.JSTAGE/jnsv/54.237).
15. Kanda E, Ai M, Kuriyama R, Yoshida M, Shiigai T. Dietary acid intake and kidney disease progression in the elderly. Am J Nephrol. 2014;39(2):145–52. doi:10.1159/000358262.
16. Fouque D, Laville M. Low protein diets for chronic kidney disease in non diabetic adults. Cochrane Database Syst Rev. 2009;3:CD001892. doi:10.1002/14651858.CD001892.pub3.
17. Vleming LJ, de Fijter JW, Westendorp RG, Daha MR, Bruijn JA, van Es LA. Histomorphometric correlates of renal failure in IgA nephropathy. Clin Nephrol. 1998;49(6):337–44.
18. Kataoka H, Ohara M, Honda K, Mochizuki T, Nitta K. Maximal glomerular diameter as a 10-year prognostic indicator for IgA nephropathy. Nephrol Dial Transpl. 2011;26(12):3937–43. doi:10.1093/ndt/gfr139.
19. Tsuboi N, Kawamura T, Ishii T, Utsunomiya Y, Hosoya T. Changes in the glomerular density and size in serial renal biopsies during the progression of IgA nephropathy. Nephrol Dial Transpl. 2009;24(3):892–9. doi:10.1093/ndt/gfn572.
20. Tsuboi N, Kawamura T, Koike K, Okonogi H, Hirano K, Hamaguchi A, et al. Glomerular density in renal biopsy specimens predicts the long-term prognosis of IgA nephropathy. Clin J Am Soc Nephrol. 2010;5(1):39–44. doi:10.2215/CJN.04680709.
21. Kataoka H, Ohara M, Shibui K, Sato M, Suzuki T, Amemiya N, et al. Overweight and obesity accelerate the progression of IgA nephropathy: prognostic utility of a combination of BMI and histopathological parameters. Clin Exp Nephrol. 2012;16(5):706–12. doi:10.1007/s10157-012-0613-7.
22. Ohkawa S, Yanagida M, Uchikawa T, Yoshida T, Ikegaya N, Kumagai H. Attenuation of the activated mammalian target of rapamycin pathway might be associated with renal function

reserve by a low-protein diet in the rat remnant kidney model. Nutr Res. 2013;33(9):761–71. doi:10.1016/j.nutres.2013.06.003.
23. Maroni BJ, Steinman TI, Mitch WE. A method for estimating nitrogen intake of patients with chronic renal failure. Kidney Int. 1985;27(1):58–65.
24. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis. 2009;53(6):982–92. doi:10.1053/j.ajkd.2008.12.034.
25. Kramer H. Obesity and chronic kidney disease. Contrib Nephrol. 2006;151:1–18. doi:10.1159/000095315.
26. Kido Y, Shizuka F, Shimomura Y, Sugiyama T. Dietary reference intakes for Japanese 2010: protein. J Nutr Sci Vitam. 2012;59(Supplement):S36–43.
27. Jia Y, Hwang SY, House JD, Ogborn MR, Weiler HA, K O, et al. Long-term high intake of whole proteins results in renal damage in pigs. J Nutr. 2010;140(9):1646–52. doi:10.3945/jn.110.123034.
28. Wakefield AP, House JD, Ogborn MR, Weiler HA, Aukema HM. A diet with 35 % of energy from protein leads to kidney damage in female Sprague–Dawley rats. Br J Nutr. 2011;106(5):656–63. doi:10.1017/S0007114511000730.