Correct evaluation of intravascular volume is indispensable for appropriate supportive therapy. Intravascular volume is evaluated according to vital signs, cardiothoracic ratio (by chest roentgenography), the aorta and inferior vena cava ratio (by abdominal ultrasonography), central venous pressure, etc. In many cases, intravascular volume decreases (intravascular dehydration) when the patient's predominant symptoms are gastrointestinal before HUS develops. Initial fluid therapy by isotonic infusion may prevent onset of AKI. However, when gastrointestinal symptom persists, intravascular volume may increase due to reduced urine output after HUS develops In such instances, the infusion volume is required to be adjusted.

In order to maintain intravascular volume appropriately, appropriate fluid therapy and diuretic drugs are required against excessive intravascular volume. The first line agent for diuretics is furosemide: 1-5 mg/kg/dose intravenously. If the effect is insufficient, dialysis is needed. The first line agent of antihypertensives is an oral administration of a calcium channel blocker (nifedipine or amlodipine). Nicardipine infusion is appropriate for urgent cases when oral antihypertensive is not possible or ineffective (Table 3). Since inhibitors of RAS may cause decrease in renal blood flow, they do not qualify as a first line agent. However, when antihypertensive is insufficient, inhibitors of RAS may be used together with a calcium channel blocker. Inhibitors of RAS may be used for the renoprotection over a long-term period when hypertension or proteinuria persists after HUS subsided [1, b].

Table 1. Definition of hypertension [a]

Normal blood pressure (BP)	Both systolic and diastolic BP are less than 90th percentile
Prehypertension	Systolic and/or diastolic blood pressure levels are greater than or equal to the 90th percentile but less than the 95th percentile, or if BP exceeds 120/80 mmHg (even if BP is less than the 90th percentile for gender, age, and height)
Hypertension	Systolic and/or diastolic blood pressure that is greater than or equal to the 95th percentile for gender, age, and height on three or more separate occasions. Stage1 : systolic and/or diastolic BP between the 95th percentile and 5 mmHg above the 99th percentile Stage2 : systolic and/or diastolic BP higher than 5 mmHg above the 99th percentile

Table 2. Blood pressure levels of 50th percentile height based on gender, age of children and adolescents in the USA [a]

Age	boys			girls		
(year)	90th	95th	99th	90th	95th	99th
1	99/52	103/56	110/64	100/54	104/58	111/65
2	102/57	106/61	113/69	101/59	105/63	112/70
3	105/61	109/65	116/73	103/63	107/67	114/74
4	107/65	111/69	118/77	104/66	108/70	115/77
5	108/68	112/72	120/80	106/68	110/72	117/79
6	110/70	114/74	121/82	108/70	111/74	119/81
7	111/72	115/76	122/84	109/71	113/75	120/82
8	112/73	116/78	123/86	111/72	115/76	122/83
9	114/75	118/79	125/87	113/73	117/77	124/84
10	115/75	119/80	127/88	115/74	119/78	126/86
11	117/76	121/80	129/88	117/75	121/79	128/87
12	120/76	123/81	131/89	119/76	123/80	130/88
13	122/77	126/81	133/89	121/77	124/81	132/89
14	125/78	128/82	136/90	122/78	126/82	133/90
15	127/79	131/83	138/91	123/79	127/83	134/91
16	130/80	134/84	141/92	124/80	128/84	135/91
17	132/82	136/87	143/94	125/80	129/84	136/91

systolic/diastolic BP (mmHg)

Table 3. Antihypertensive agents for children

Generic name	Brand name	Dosage and administration
Nifedipine	Sepamit® fine granules 1%	0.25 – 0.5 mg/kg/dose orally every 4 – 6 hours. Maximum: 10 mg/dose or 3 mg/kg/day
Amlodipine besylate	Norvasc® Amlodin® Tablets / OD Tablets (2.5, 5 mg)	2.5 mg once daily in children ≧6 years. Dosage must be titrated according to age, body weight, and patient's response, but does not exceed the dosage for adult.
Nicardipine hydrochloride	Nicardipine® injection (1 mg/ml)	Initial: 0.1 - 1.0 μg/kg/min; titrate dose according to blood pressure: rate of infusion may be increased every 15 – 30 minutes; maximum dose: 4 - 5 μg/kg/min
Enalapril maleate	Renivace® Tablets (2.5, 5, 10 mg)	Initial: 0.08 mg/kg/day every 24 hours in children ≧1 month. Dosage must be titrated according to age, patient's response.
Lisinopril	Longes® Tablets Zestril® Tablets (5, 10 20 mg)	Initial: 0.07 mg/kg/dose once daily in children ≧6 years. Dosage must be titrated according to age, patient's response.
Valsartan	Diovan® Tablets (20, 40, 80, 160 mg)	Initial: dose dependent upon patient weight: < 35 kg: 20mg; ≧35 kg: 40 mg/day every 24 hours orally. Dosage may titrate to age, body weight, and patient's response up to a maximum dose of 40 mg < 35 kg.

[Supplementary articles]

a. JCS Joint Working Group 2012. [Guidelines for drug therapy in pediatric patients with cardiovascular diseases]. Circulation Journal. 2012; 76: 167-187, in Japanese.
b. UpToDate: Treatment and prognosis of Shiga toxin associated (typical) hemolytic uremic syndrome in children. Version 18.0

[References]

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)

III.3 Renal Replacement Therapy

1. Timing to initiate renal replacement therapy in AKI arising from HUS

Indications to initiate renal replacement therapy are oliguria in tractable to therapy (urination: 0.5 mL/kg/hr for over 12 hours), uremic manifestations, hyperkalemia (≧6.5 mEq/L), hyponatremia (＜120 mEq/L), acidemia (pH＜7.20), fluid overload, pulmonary edema, cardiac failure, hypertension and renal impairment (in which crystalloid solution, colloid solution, blood products, and drugs cannot used). **[Grade of Recommendation: C1]**

[Comments]

KDIGO Clinical Practice Guideline for AKI shows the standard value of oliguria in which renal replacement therapy is needed [a]. Other published indications for renal replacement therapy in AKI were also consulted [b - d]. Renal replacement therapy is also indicated when serum sodium is under 115 mEq/L. However, this value is too risky for patients with AKI due to HUS. Hence, serum sodium less than 120 mEq/L should be indicative of commencement of renal replacement therapy in HUS patients. Frequent, low-efficient, short hemodialysis (FLESHD) and arrangement of sodium concentration in dialysate will be taken to correct the serum sodium in the patients.

[Supplementary articles]

a. KDIGO clinical practice guideline for acute kidney injury: Timing of renal replacement therapy in AKI. Kidney Int Suppl 2012; 2: 89-92.
b. Gibney N, *et al.* Timing of initiation and discontinuation of renal replacement therapy in AKI: unanswered key questions. Clin J Am Soc Nephrol 2008; 3: 876-880.
c. Bellomo R, *et al.* Indications and criteria for initiating renal replacement therapy in the intensive care unit. Kidney Int Suppl. 1998; 66: S106-109.
d. Palevsky PM: Clinical review: timing and dose of continuous renal replacement therapy in acute kidney injury. Crit Care 2007; 11: 232-237.

2. Modality of renal replacement therapy for patients with AKI arising from HUS

Modalities of renal replacement therapy are peritoneal dialysis (PD), intermittent hemodialysis (HD) and continuous hemo-dia-filtration (CHDF), including continuous hemo-dialysis (CHD) and continuous hemo-filtration (CHF). **[Grade of Recommendation: B]**
We should take CHDF or PD (for 24 hours) for patients with AKI complicated with acute encephalopathy. **[Grade of Recommendation: C1]**

[Comments]

We should take PD, IHD or CRRT (such as CHD, CHF and CHDF) depending on the status of the patients and nature of the institution. Characteristics of PD, IHD and CRRT are shown in Table 1. However, there are no randomized control studies which modality shows most effective.

CHD and CHDF are usually the first line method for AKI due to HUS, because CHF shows lower efficacy than CHD or CHDF. CHD is usually selected when patient manifests AKI with no neurological involvement. On the other hand, when patient with HUS manifests acute encephalopathy, CHDF is chosen to reduce serum inflammatory cytokines. In cases of patients with unstable circulation, CHDF is preferred over CHD.

AKI guideline of KDIGO suggests CHDF or PD (for 24 hours), rather than intermittent renal replacement therapy (IRRT such as IHD), for AKI patients with acute brain injury, increased intracranial pressure or generalized brain edema (Grade 2B: we suggest the Quality of Evidence Moderate) [a]. IHD can worsen neurological status by changing cerebral perfusion pressure. That is why CHDF or 24-hour PD is taken. IHD can induce sudden disequilibrium syndrome or an increase of intracranial pressure (dialysis disequilibrium) [b]. Both types of therapy usually do not produce disequilibrium syndrome, brain edema or hypotension due to the slow removal of fluids and solutes [1]. The characteristics of modalities of renal replacement therapy are shown in Table 2.

Commercially available dialysates for patients with AKI contained low potassium (2 mEq/L), low magnesium (1 or 1.5 mEq/L) and no phosphorus. As CRRT is usually maintained for over 24 hours, potassium, magnesium and phosphorus can be added to dialysates to maintain the appropriate serum levels in patients [c].

Table 1. Characteristics of PD, IHD, and CRRT

	PD	IHD	CRRT
Duration	Continuously for 24 hours	Intermittent	Continuously for 24 hours
Simplicity	Technically simple	Technically complex	Technically more complex
Influence on hemodynamics	Small	Large	Small
Control of the removed fluids	Not accurately	Accurately	Accurately and easily
Anticoagulation	Not necessary	Necessary	Necessary
Disequilibrium syndrome	No	Yes	No
Catheter related trouble	Obstruction, fluid leak, and peritonitis	Hemorrhage, thrombosis, and sepsis	Hemorrhage, thrombosis, and sepsis
Availability to infant	Available, good indication	Available	Available but depend on the institute activity
Restraint to the patients	Not necessary	Necessary	Necessary

PD: Peritoneal Dialysis, IHD: Intermittent Hemodialysis, CRRT: Continuous Renal Replacement Therapy

Table 2. Aplications of renal replacement therapy in various complications

Complications	Modality	Reasons of indication
Unstable circulation	CRRT・PD	Both can prevent hypotension.
Hyperkalemia	IHD	IHD can reduce plasma potassium concentration rapidly.
	CRRT	CRRT can not rapidly normalize hyperlkalemia compared to IHD. Iit is recommended to infants.
Acute encephalopathy /increased intracranial pressure	CRRT・PD	Both are preferred to IHD. Both can keep the intracranial pressure stable.
Bleeding tendency	PD	Anticoagulation is not necessary. PD will not accelerate bleeding tendency.
	IHD	Treatment time of IHD is shorter than that of CRRT, resulting in less use of anticoagulants.
Severe fluid overload	CRRT	CRRT can enable precise and continuous removal of fluid.
During mechanical ventilation	CRRT	CRRT easily and accurately can control fluid balance. CRRT is also helpful to restore from mechanical ventilation.
Intestinal perforation	CRRT	PD can not be used in patients with intestinal perforation.

PD; Peritoneal Dialysis, IHD; Intermittent Hemodialysis, CRRT; Continuous Renal Replacement Therapy

[Supplementary articles]

a. KDIGO clinical practice guideline for acute kidney injury: Modality of renal replacement therapy for patients with AKI. Kidney Int suppl 2012: 107-110.
b. Davenport A: Continuous renal replacement therapies in patients with liver disease. Semin Dial 2009; 22:169-172.
c. Sawada M, *et al.* Necessity of adding phosphorus and magnesium to dialysate in CHDF. J Jpn Soc Pediatr Dialysis Transplantation 2007; 27: 95-97. [in Japanese]

[References]

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)

III.4 Plasma exchange therapy

Plasma exchange therapy has no beneficial effect to reduce nephropathy in HUS. **[Grade of Recommendation: C2]**

When plasma exchange therapy is performed, we must prevent fluid overload by using renal replacement therapy in the patients with HUS. **[Grade of Recommendation: Not Graded]**

[Comments]

For aHUS, plasma exchange therapy is the first line treatment. Conversely, plasma exchange shows no beneficial effect for patients with HUS due to STEC infection [1, 2, a] (See Chapter III. 6).

Plasma exchange therapy can increase plasma osmolality, as it promotes the migration of fluids from the third space to the intravascular space. Precise management of fluid, electrolytes and acid-base balance is mandatory. Rapid increase of intravascular volume leads to severe complications such as hypertension, cardiac failure, pulmonary edema and brain edema. When plasma exchange therapy is performed, fluid overload must be avoided by using renal replacement therapy in the patients with HUS [3]. We suggest plasma exchange therapy for infants with HUS in hospitals with adequate clinical experience.

[Supplementary articles]

a. Michael M, Elliott EJ, Ridley GF, Hodson EM, Craig JC, Editorial Group: Cochrane Renal Group: Interventions for haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura. 21 JAN 2009

[References]

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)

III.5 Antithrombotic therapy for HUS

We do not recommend administration of antithrombotics including heparin, dipyridamole, and urokinase for HUS patients, especially those without complications of disseminated intravascular coagulation (DIC). To date, there is no clinical evidence that it is beneficial. **[Grade of Recommendation: D]**

For patients with HUS complicated by DIC, it is reasonable to use agents including nafamostat mesilate, gabexate mesilate, recombinant human thrombomodulin alpha, or antithrombin (AT, formerly known as antithrombin III). **[Grade of Recommendation: C1]**

[Comments]

Differential diagnosis between HUS and DIC is usually difficult, and can only be made on the basis of results of examinations and careful observation of symptoms. DIC is a thrombotic microangiopathy resulting from activation of the coagulation system, accompanied by deposition of fibrin clots in the lumina of blood vessels, consumption of coagulation factors, and microangiopathic hemolysis. These in turn lead to thrombocytopenia, a decreased plasma fibrinogen level, prolongation of prothrombin time (PT) and activated partial thromboplastin time (aPTT). HUS, on the other hand, is a thrombotic microangiopathy resulting from primary platelet activation due to primary endothelial injury, with the levels of coagulation factors, PT and aPTT all within the normal range. Therefore, the differences in coagulation abnormalities between these two disorders usually permit them to be distinguished.

Four prospective trials have compared combination of supportive care with antithrombotic agents (e.g. either urokinase and heparin, or dipyridamole and heparin) or supportive care alone [1 – 4]. However, thrombocytopenia, microangiopathic hemolytic anemia and the duration of renal failure were similar in both the control and treated groups. Accordingly, these studies did not demonstrate any advantages of these treatments, nor the comparison between streptokinase therapy and heparin therapy with supportive care alone. Hemorrhagic complications were more common in the group treated with streptokinase. Therefore, antithrombotic therapy has not been deemed suitable for use in HUS patients.

On the other hand, HUS patients complicated by DIC are often reported [5, 6]. To date, the published data are insufficient to allow any conclusions to be drawn about the efficacy or safety of treatment for HUS complicated by DIC. Therefore, we suggest administration of agents such as nafamostat mesilate, gabexate mesilate, recombinant human thrombomodulin alpha and antithrombin, in accordance with the diagnostic and therapeutic guidelines for HUS caused by enterohemorrhagic *Escherichia coli* infection (established by the Japanese Society for Pediatric Nephrology), as well as expert consensus based on evidence for the treatment of DIC due to

infection (established by the Japanese Society for Thrombosis and Hemostasis). However, it is important to note that hyperkalemia may occur during treatment with nafamostat mesilate, and that any bleeding tendency can be exacerbated by treatment with antithrombin. Further, it is noteworthy that the established data are insufficient to allow any conclusions to be drawn about the efficacy of recombinant human thrombomodulin alpha for treatment of symptoms of HUS, including encephalopathy, although this treatment may be effective for DIC.

[Supplementary articles]

a. UpToDate: Treatment and prognosis of Shiga toxin associated (typical) hemolytic uremic syndrome in children.
b. The Japanese Society for Pediatric Nephrology [Diagnostic and therapeutic guideline for hemolytic uremic syndrome associated with enterohemorrhagic *Escherichia coli* infection (revised version)], in Japanese. http://www.jspn.jp/gakujyutsu.html
c. Maruyama I, *et al.* [Scientific Standardization Committee of the Japanese Society on Thrombosis and Hemostasis: Expert consensus based on the evidence for the treatment of disseminated intravascular coagulation arising from intravascular infection]. Jap J Thrombo Hemost 2009; 20: 77-113, in Japanese.
d. Aoki N, *et al.* [Revision of examination data and findings for diagnosis of disseminated intravascular coagulation]. Research Report 1988: 37-41. Grant-in-Aid for Research of Special Disease of Blood Coagulation Abnormalities from the Ministry of Health and Welfare of Japan, in Japanese.

[References]

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] (level5)
6. Kaneda M, *et al*. Treatment of hemolytic uremic syndrome with recombinant thrombomodulin. Thrombosis Medicine 2012; 2: 198-202. [in Japanese] (level 5)

III.6 Treatment of encephalopathy associated with STEC infection

1. Supportive therapy for encephalopathy associated with STEC infection

Supportive therapy includes the basic treatment of encephalopathy associated with STEC infection. To suppress brain edema and seizures (convulsions), management of systemic organs and treatment of the central nervous system (CNS) signs are critical. The former aims to stabilize circulation and respiration; while the latter, to treat seizures (convulsions) and to lower the intracranial pressure. **[Grade of Recommendation: C1]**

[Comments]

(1) Factors to be considered in the treatment of encephalopathy associated with STEC infection.

Main symptoms of encephalopathy associated with STEC infection are seizures (convulsions) and impaired consciousness. Many of the severe cases show diffuse brain edema and/or bilateral deep gray matter lesions (basal ganglia or thalamus) on cranial imaging studies (CT or MRI) (See Chapter Ⅱ.3). To correct the pathology and pathogenesis for such cases, supportive therapy is performed. Since neither randomized nor case-control studies with regard to the treatment of encephalopathy associated with STEC has been done to date, there is no report of any therapy that has a high degree of evidence. It should be reasonable, however, to apply a therapeutic strategy similar to that against acute encephalopathy associated with influenza and other viral infections [a].

It should be kept in mind that, in cases of encephalopathy associated with STEC infection, most patients have acute kidney injury associated with HUS, prompting the consideration for water overload, electrolytes imbalance, as well as changes in blood concentration of drugs due to hemodialysis. Secondary injuries to other organs, such as the liver and heart, may also occur, although they are less serious compared to the some severe cases of influenza-associated encephalopathy. Taken together, encephalopathy associated with STEC infection differs in several aspects from other encephalopathies.

While encephalopathy associated with STEC is the main cause of death in HUS [1], there have been several case reports that described patients recovering after several weeks of coma [2, 3]. Thus, active and continuous therapy should be kept in mind.

(2) Supportive therapy of encephalopathy associated with STEC infection.

In the acute period of encephalopathy, principle of treatment consists of several aspects. First, management of systemic organs should be done vigorously. Circulation and respiration should be continuously monitored and stabilized by hydration, drug therapy, dialysis and mechanical ventilation. Carbon bicarbonate concentration in arterial blood should be maintained at the

normal range, and the volume of body fluid managed adequately to avoid both overhydration and dehydration. Abnormalities of the body fluid components, such as serum electrolytes and glucose, if any, should be corrected.

Second, CNS signs and symptoms should be treated. The level of consciousness and seizures (convulsions) should be monitored continuously. Seizures are to be halted primarily with intravenous antiepileptic drugs. Many patients respond to benzodiazepines (diazepam, midazolam and others), whereas some intractable cases have clusters of seizures or status epilepticus, necessitating intravenous injection of a large amount of barbiturates (thiopental and others). To prevent the recurrence of seizures, antiepileptic drugs (midazolam, phenobarbital, fosphenytoin and others) are given with monitoring of their blood concentration. Attention should be paid to seizures due to hyponatremia and other abnormalities of electrolytes, as well as hypoglycemia. Treatment of increased intracranial pressure includes sedation and hyperosmolar therapy (glycerol and fructose). Mannitol is not recommended for encephalopathy associated with HUS, since this is excreted via kidneys and may aggravate renal failure. Monitoring of intracranial pressure should be considered in severe cases. Cooling must be introduced to the patients with hyperthermia [a, 4].

(3) Follow-up during convalescence and after discharge

During convalescence, patients should undergo cranial imaging studies, electroencephalography and, if necessary, developmental tests to check for residual abnormalities, immediately before or after discharge. If a patient is left with disabilities in intellect, higher cortical and motor function, or epilepsy, treatment and rehabilitation should be started for each condition. Even if no apparent sequelae were noted at discharge, learning disabilities or behavioral problems may manifest later in life. Thus, a long-term follow-up on the mental development is required.

[Supplementary articles]

a. Morishima T, *et al.* Guideline for influenza encephalopathy: Revised edition. Jpn J Pediatr 2009; 62:2483-2528. [in Japanese]

[References]

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)

2. Specific therapy of encephalopathy associated with STEC infection.

Many cases of encephalopathy associated with STEC have an unfavorable prognosis. To date, no specific therapy has been established for this condition. **[Grade of Recommendation: Not Graded]**

Methylprednisolone (mPSL) pulse therapy may be considered in patients with severe STEC-associated encephalopathy whereby poor outcome is predicted with regard to neurologic function and/or survival. However, its efficacy has not established. **[Grade of Recommendation: Not Graded]**

Plasma exchange may be considered in patients with STEC-associated encephalopathy (when its safety is verified) although its efficacy has not been established. This therapy should be performed in a medical facility with adequate experience. **[Grade of Recommendation: Not Graded]**

[Comments]

Encephalopathy (CNS involvement) associated with STEC infection is recognized globally to be a predictor of poor outcome. The management of encephalopathy is primarily based on systemic supportive care. Although no specific interventions have been shown to be efficacious, there may be a role for mPSL pulse therapy, plasma exchange and other treatments. The efficacy of these agents is still unclear due to the small number of clinical experience reports.

(1) Methylprednisolone (mPSL) pulse therapy

To date, there are no studies that have evaluated the efficacy of mPSL pulse therapy for STEC-associated encephalopathy. There are only a few case reports in Japan. To determine whether steroids could be of clinical benefit in the treatment of HUS, Perez *et al.* conducted trials of mPSL (5 mg/kg/day over seven days). They reported that there was no significant difference between the treatment groups in terms of the number of convulsive episodes or transfusion requirements [1].

In April 2011, an outbreak of STEC O111 infection occurred principally in Toyama prefecture, Japan. During this outbreak, mPSL pulse therapy was introduced for encephalopathy. 20 children with STEC O111 infection were eventually identified with eight of them developed encephalopathy. Three children without mPSL pulse therapy died, while all five children treated

with mPSL therapy survived without neurological sequelae [a]. However, there is still insufficient evidence to establish the effectiveness of mPSL pulse therapy for STEC-associated encephalopathy (as other interventions, such as plasma exchange, were used concurrently).

STEC-HUS is systemic disorder characterized by thrombotic microangiopathy (TMA). However, postmortem examination of brain tissue has shown little evidence of TMA. Affected patients often have generalized cerebral edema and enlarged spaces around blood vessels that indicate increasing permeability [a]. Previous studies showed that the pathogenesis of STEC-associated encephalopathy involves inflammatory cytokines such as TNF-α and IL-6 [2, 3]. Moreover, two cases of HUS complicated by acute necrotizing encephalopathy (ANE) were reported [4]. The efficacy of mPSL pulse therapy has been established for ANE. Although there were no obvious side effects of mPSL pulse therapy in the STEC O111 outbreak, this therapy should only be administered for STEC-associated encephalopathy when close attention is paid to the potential side effects, such as infections, thrombus formation and hypertension.

In spite of the treatment of STEC-associated encephalopathy with mPSL pulse therapy not being established, we suggest that it can be considered in patients with severe STEC-associated encephalopathy (with the safety of the patients ensured as a prerequisite).

(2) Plasma exchange therapy

Plasma exchange therapy is sometimes used to treat severe STEC-HUS (especially when there is CNS involvement) based upon the reported benefits of plasma exchange in adults with TTP. Dundas *et al.* reported that five of 16 adult cases (31%) treated with plasma exchange died, while five of the six cases without plasma exchange (83%) died [5]. Nathanson *et al.* recently investigated 52 patients with severe initial neurological involvement associated with D+HUS (HUS associated with diarrhea). Eleven patients were treated with plasma exchange within 24 hours after the first presentation of neurological signs. However, the outcome of this group was not significantly different from that of the others who were not treated with plasma exchange [6]. Colic *et al.* reported that an earlier start of plasma exchange for five patients with STEC O104:H4 associated HUS reduced the lactate dehydrogenase concentrations more effectively than later treatment, possibly indicating that early therapy ameliorates the course of severe HUS [7].

The efficacy and mechanism(s) of plasma exchange for severe HUS and CNS involvement are currently unknown. Moreover, this therapy is associated with problems such as pulmonary edema, infection and the high cost of treatment.

While the treatment of STEC-associated encephalopathy with plasma exchange is not established, we suggest that it can be considered in patients with severe STEC-associated encephalopathy (with the safety of the patients ensured as a prerequisite).

(3) Other treatments

In a German outbreak of STEC O104 in 2011, it was reported that eculizmab, a monoclonal antibody against complement factor C5, was beneficial in patients with STEC-HUS and CNS involvement [8]. In contrast, eculizmab did not show any efficacy in a cohort study by Menne *et al* [9]. Recombinant human soluble thrombomodulin (rTM) has effects on the complement control and has anti-inflammatory properties. There was one report of a small number of patients that evaluated the efficacy of rTM in children with STEC-HUS [10]. More evidence is needed to establish a new therapeutic strategy for STEC-associated encephalopathy.

[Supplementary articles]

a. Sata T (ed.): 2011 Annual Report of the Research Committee on Epidemiologic, Bacteriologic and Clinical Studies of Cases of Food Poisoning due to STEC/O111.
b. UpToDate: Treatment and prognosis of Shiga toxin associated (typical) hemolytic uremic syndrome in children. Version 18.0

[References]

1. Perez N, *et al.* Steroids in the hemolytic uremic syndrome. Pediatr Nephrol 1998; 12: 101-104. (level 4)
2. Shimizu M, *et al.* Cytokine profiles of patients with enterohemorrhagic *Escherichia coli* O111-induced hemolytic-uremic syndrome. Cytokine 2012; 60: 694-700. (level 4)
3. 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)
4. 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)
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 5)
6. Nathanson S, *et al.* Acute neurological involvement in diarrhea-associated hemolytic uremic syndrome. Clin J Am Soc Nephrol 2010; 5: 1218-1228. (level 4)
7. 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)
8. Lapeyraque AL, *et al.* Eculizumab in severe Shiga-toxin–associated HUS. N Engl J Med 2011; 364: 2561-2563. (level 5)

9. 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)
10. Honda T, *et al.* A novel strategy for hemolytic uremic syndrome: successful treatment with thrombomodulin α. Pediatrics. 2013; 131:e928-33. (level 5)

III.7 Renal sequelae of HUS

Renal sequelae of HUS are albuminuria, proteinuria, decreased renal function and hypertension.

About 20-40% of HUS patients developed chronic kidney disease (CKD), a risk factor of end-stage kidney disease and cardiovascular complications. We recommend patient monitoring by examining albuminuria, proteinuria and measurement of blood pressure according to the severity in the acute phase:

1) At least for fifteen years in patients who needed dialysis in acute phase, or anuria for more than six days.
2) At least for fifteen years in patients under two years old at the onset, whose peak serum creatinine was higher than or equal to 1.5 mg/dL.
3) Throughout the life in HUS patients who are positive for albuminuria, proteinuria, decreased renal function, or hypertension during follow-up.
4) For five years for patients without any of the above mentioned conditions or renal sequelae.

[Grade of Recommendation: B]

Renal biopsy in the acute phase for HUS patients is not recommended, because the bleeding risk is high and pathological findings of the acute stage do not correlate to the renal prognosis.
[Grade of Recommendation: C2]

[Comments]

1. Renal sequelae

The mortality rate in the acute phase of HUS is 2-6% in western countries [1] and 1.6% in Japan [2]. Of the fatal cases, 88% occurred in the acute phase [3]. The mortality rate has improved markedly by advances in acute renal replacement therapy and pediatric intensive care compared to the 1980s [a]. About 40% of the HUS patients develop anuria [1] and approximately 40% [b]-60% [1, c] of the HUS patients required dialysis in the acute phase. A national survey

between 2000 and 2001 in Japan revealed that oliguria or anuria was seen in 47% and dialysis in 27% of the patients [2]. Most patients who needed dialysis in the acute phase recovered their renal functions. About 20-40% of the HUS patients, however, developed chronic kidney disease (CKD) for prolonged periods. Since CKD is a risk factor of end-stage kidney disease and cardiovascular complications, continuous management is needed.

Mortality rate was 9% and rate of end-stage kidney disease was 3% based on a meta-analysis of 49 articles that covered 3,476 HUS patients between 1950 and 2001 [5]. Of the 2,372 survivors who were monitored for more than a year, 25% were complicated by renal sequelae. The symptoms and frequency are as follows: decreased renal function 15.8% (GFR; 60-80 mL/min/1.73m^2: 8 %, 30-59 mL/min/1.73m^2: 6 %, 5-29 mL/min/1.73m^2: 1.8 %), proteinuria 15 % and hypertension 10 % (multiple answer) [5].

Albuminuria, a more sensitive indicator of renal damage than proteinuria, is useful for early detection of CKD in HUS patients. The frequency of albuminuria at three years [6] and mild renal dysfunction at six years [7] after the onset of HUS was high compared to normal control.

Hypertension is the most prominent renal sequela in HUS patients [8]. About 25% manifested hypertension in the acute phase, while about 10% did so in chronic periods [5]. Hypertension can also manifest without other complications; hypertension usually develops in patients with proteinuria and renal dysfunction [9]. Monitoring the ambulatory blood pressure measurement (ABPM) for 24 hours can reveal occult hypertension [10].

The frequency of renal sequelae is high in HUS patients and long-term renal prognosis is not always good. Therefore, follow-up of patients according to their condition is necessary.

2. Predictive factor of prognosis for renal function and follow-up

The risk factors of renal sequelae include oliguric or anuric period and dialysis period in acute phase [11]. When anuric period is over 7-10 days, renal sequelae such as proteinuria, renal dysfunction and hypertension increase [12 - 15]. In addition, renal sequela correlates to the period of dialysis [5, 10]. The renal function can be decreased in patients who needed dialysis for more than five weeks [5].

11 - 16 % of the patients with HUS manifested renal dysfunction (<80 mL/min/1.73m^2) during the follow-up period [5]. Furthermore, proteinuria and renal dysfunction manifested after five years in patients whose serum creatinine level was higher than 1.5 mg/mL in the acute phase [16].

As 951 children with gastrointestinal STEC O157 infection with no HUS did not manifest hypertension or microalbuminuria, long-term follow-up was not needed for these patients [17].

On the basis of the evidence above, we recommend follow-up of patients with HUS by examining albuminuria, proteinuria, and measurement of blood pressure according to the

severity of the disease in the acute phase.

1) At least for fifteen years in patients who needed dialysis in acute phase, or anuria for more than six days.
2) At least for fifteen years in patients under two years old at the onset, whose peak serum creatinine was higher than or equal to 1.5 mg/dL.
3) Throughout the life in HUS patients who are positive for albuminuria, proteinuria, decreased renal function, or hypertension during follow-up.
4) For five years for patients without any of the above mentioned conditions or renal sequelae.

Renal pathological findings in the acute phase cannot predict long-term renal prognosis. However, examination of the kidney during the sub-acute phase revealed that patients with cortical necrosis and glomerular microangiopathy (covering more than 50% of liver area) showed poor long-term renal prognosis (average 18 years) [13]. The indication for renal biopsy in the acute phase is to assist diagnosis [d], and surgical renal biopsy must be considered in the patients with high-risk bleedings. For patients with severe renal dysfunction and persistent proteinuria after the acute phase, renal pathological findings can produce important information for proper treatment. Renal biopsy in the acute phase for HUS patients is not usually recommended.

[Supplementary articles]

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c. Bakkaloglu SA, *et al.* Diseases of the Kidney and Urinary Tract in Children. Taal MW, Chertow GM, Marsden PA, Skorecki K, Yu ASL, Brenner BM ed, Brenner and Rector's The Kidney 9th ed. Pp.2622-2679, Elsevier, Philadelphia, 2012
d. UpToDate: Treatment and prognosis of Shiga toxin associated (typical) hemolytic uremic syndrome in children. Version 18.0

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severe clinical course in HUS patients: a national survey in Japan. Pediatr Int 2008; 50:441-446 (level 4)

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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)
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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)
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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)
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III.8 Extra-renal sequelae in patients with HUS

Patients with HUS can have extra-renal sequelae: sequelae of digestive system, diabetes mellitus, neurological complications, behavioral and cognitive sequelae or cardiovascular sequelae. It is important to conduct follow-up for HUS patients for at least five years after the acute illness. Long-term special care and treatment should be directed to patients with specific sequelae after the acute phase of HUS. **[Grade of Recommendation: B]**

[Comments]

1. Sequelae of digestive system

Cholelithiasis, persistent pancreatitis and colon stricture were reported in HUS patients as extra-renal sequelae [1]. Cholelithiasis is related to hemolysis or the use of parenteral nutrition in the acute illness. Pancreatic microthrombi can cause exocrine cell death resulting in persistent pancreatitis. Hemorrhagic colitis can cause severe inflammation of bowels leading to bowel stricture or obstruction. The transverse and ascending colon are most frequently affected. Bowel resection was indicated in patients with bowel stricture or obstruction who manifested persistent abdominal pain and severe constipation intractable to medication [2].

2. Diabetes mellitus

Pancreatic microthrombi can cause islet cell death resulting in diabetes mellitus. The incidence of diabetes mellitus during the acute phase of HUS was 1.7-3.2% [3, 4]. HUS patients with severe disease (including the need for dialysis and CNS symptoms) were more likely to develop diabetes mellitus. For those who survived, one third had permanent diabetes mellitus. Relapse of diabetes mellitus can occur years after the acute illness. Patients with STEC infections who do not manifest HUS will not have diabetes mellitus.

3. Neurological sequelae

Convulsion and impaired consciousness in the acute phase of HUS are associated with severe renal damage [5]. In contrast, HUS patients with seizure or impaired consciousness in acute phase can recover without permanent neurological complications. Neurological outcomes including epilepsy, hemiplegia, cortical blindness and psychomotor disturbance were reported [6, 7].

4. Behavioral and cognitive sequelae

Patients who recovered from the acute phase of HUS can manifest mild behavioral and cognitive sequelae [8]. In contrast, patients who did not manifest neurological complications in the acute phase of HUS did not manifest learning disability, behavioral disturbance and attention