management personnel, and statisticians were all masked to the treatment assignment throughout the study.

Procedures

Patients received assigned treatments for 52 weeks, and antihypertensive treatments received at baseline were continued. Other usual care medications were allowed throughout the study. If blood pressure was 130/80 mm Hg or more, the addition of antihypertensive medication (apart from a mineralocorticoid receptor antagonist, angiotensin-converting enzyme inhibitor, or angiotensin receptor blocker) was allowed to reach the treatment goal. Changes to RAS inhibitor drug classes and their doses was not allowed after the stratified randomisation.

Patient follow-up visits were at 4, 8, 16, 28, 40, and 52 weeks after initiation of the study drug. Office standard cuff blood pressure, blood samples, and urine specimens (first morning void urine), and adverse effects were assessed at every visit. Urinary albumin, liver-type fatty acid-binding protein (L-FABP), sodium, and creatinine were measured at the central laboratory (SRL, Tokyo, Japan), and the other measurements measured at each clinic. Data were collected via the University Medical Information Network Internet Data and Information Center for Medical Research (UMIN INDICE) system (Tokyo, Japan).

Outcomes

The primary efficacy measure was percent change from baseline in UACR in the first morning void urine at 52 weeks or last visit in patients who discontinued. Secondary endpoints were absolute values and percent changes from baseline at 4, 8, 28, and 52 weeks in the UACR in first morning void urine, serum creatinine concentrations, eGFR, urinary L-FABP, estimated 24-h urinary sodium excretion, and the incidence of cerebrovascular and cardiovascular events. Changes in plasma and urinary aldosterone were prespecified secondary outcomes, but on-treatment values were variable and meaningless, so we report only baseline values. We also assessed the safety profile of eplerenone treatment with the endpoints of changes in serum potassium concentrations and incidence of adverse effects. We calculated eGFR with the modification in diet in renal disease formula modified by the Japanese Society of Nephrology.[17] We estimated 24-h urinary sodium excretion with a previously reported formula.[18] Cerebrovascular and cardiovascular events included deaths (fatal myocardial infarction, fatal heart failure, sudden death, fatal stroke, and other cardiovascular deaths) and hospital admission (non-fatal myocardial infarction, angina, heart failure, cerebral bleeding, cerebral infarction, and transient ischaemic attack) from these causes.

Statistical analysis

We calculated the sample size on the assumption that the treatment for hypertensive patients with albuminuria who were receiving RAS inhibitors would decrease urinary albumin concentration (primary efficacy measure) by 45%, whereas RAS inhibitor plus eplerenone decreases it by 74%.[19] A sample size of 340 patients (170 patients per group) provided 80% power to detect a 30% reduction in urinary albumin concentration in the eplerenone group compared with that in the placebo group, with a significance level of 0·05 and accounting for a dropout rate of 10%.

Efficacy analyses included all randomly allocated patients who received at least one dose of study drug who had a valid baseline and at least one valid post-baseline assessment. For safety assessments, data from all patients who took the study drug at least once were included in the analyses. We compared differences in the primary efficacy measure (percent change in UACR after 52 weeks [or last visit] from baseline) between the eplerenone and placebo groups with one-way ANOVA. We analysed the secondary outcome measures in the same way. eGFR, 24-h urinary sodium excretion, and serum potassium concentration

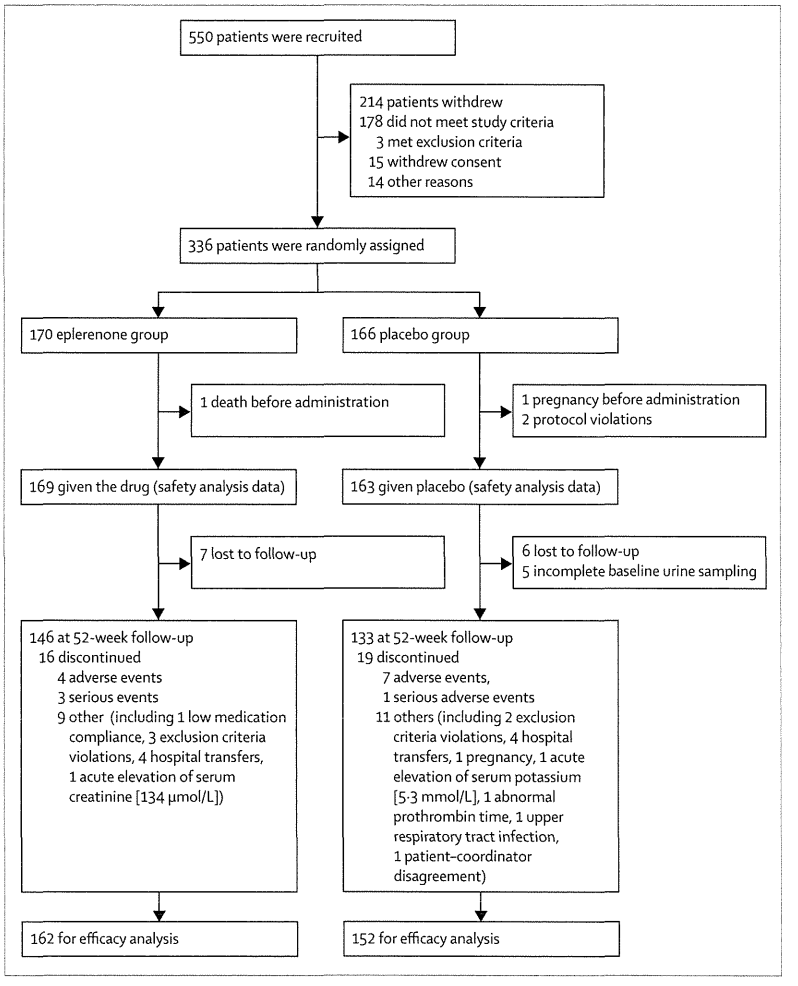

Figure 1: **Trial profile**

were analysed at each visit. Additionally, we assessed both the percent changes in UACR and changes in blood pressure from baseline with the area under the curve technique. In post-hoc analyses, we compared percent change in UACR between groups by dividing patients into two groups according to baseline urinary sodium excretion (≥160 mmol/day and <160 mmol/day). We also assessed the correlation of percent change in UACR with baseline urinary sodium excretion, baseline plasma and urinary aldosterone, percent decrease in systolic blood pressure, and percent decrease in eGFR, with hypothesis tests of correlation. All analyses were two-sided and the significance level was 0·05. Statistical analyses were done by a trial statistician (HO) with the JMP Pro software version 10.0.2 (SAS Institute).

This trial was registered with the clinical trials registry of University Hospital Medical Information Network (UMIN), number UMIN000001803.

	Eplerenone group (n=162)	Placebo group (n=152)
Sex		
Male	114 (70%)	100 (66%)
Female	48 (30%)	52 (34%)
Age (years)	58·6 (13·0)	58·6 (13·8)
Height (cm)	163·8 (9·4)	162·2 (9·3)
Bodyweight (kg)	68·7 (14·5)	68·0 (13·0)
Cause of chronic kidney disease		
Hypertensive nephrosclerosis	91 (56%)	99 (65%)
Primary glomerular disease	63 (39%)	45 (30%)
Other	8 (5%)	8 (5%)
Office blood pressure (mm Hg)		
Systolic	138·6 (11·1)	138·8 (12·6)
Diastolic	82·4 (10·2)	81·9 (10·0)
Pulse rate (beats per min)	72·4 (10·1)	72·1 (11·1)
Laboratory data		
UACR (mg/g)	163·1 (148·0)	156·8 (133·6)
BUN (mg/dL)	15·3 (4·5)	15·5 (4·0)
Serum creatinine (μmol/L)	77·3 (17·2)	74·8 (14·9)
eGFR (mL/min per 1·73 m²)*	67·7 (14·3)	68·6 (13·6)
Blood sugar (mg/dL)	100·1 (11·1)	102·2 (10·9)
Triglycerides (mg/dL)	169·1 (137·8)	155·8 (96·5)
LDL cholesterol (mmol/L)	2·89 (0·709)	2·82 (0·714)
HDL cholesterol (mmol/L)	1·44 (0·422)	1·43 (0·422)
Serum sodium (mmol/L)	141·1 (2·2)	140·8 (2·1)
Serum potassium (mmol/L)	4·15 (0·36)	4·15 (0·40)
Estimated 24 h urinary sodium (mmol/day)	219·5 (66·1)	217·6 (58·1)
Urinary L-FABP/creatinine ratio (μg/g)	0·07 (0·37)	0·04 (0·12)
Plasma renin activity (ng/mL per h)	3·94 (4·97)	4·07 (4·93)
Plasma aldosterone (pg/mL)	89·0 (44·9)	90·1 (51·0)
Urinary aldosterone/creatinine ratio (ng/g)	0·038 (0·029)	0·044 (0·043)

(Table 1 continues in next column)

Role of the funding source

EVALUATE was funded by Pfizer. Pfizer had no role in the design or conduct of the study; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript. The principal investigator (TF) had full access to all data in the study and takes responsibility for the integrity of the data, the accuracy of the data analysis, and the final decision to submit for publication.

Results

Between April 1, 2009, and March 31, 2012, we enrolled 336 hypertensive patients with chronic kidney disease and randomly assigned 170 to the eplerenone group and 166 to the placebo group (figure 1, table 1). Of allocated patients, four were excluded before receipt of any study drug and thus 332 patients were included in the safety assessment (figure 1). During the study period, 13 patients were lost to follow-up, and five did not provide complete baseline urine sampling. Thus, we included 314 patients in efficacy assessments (figure 1). UACR was not measured at 52 weeks in ten of the 314 patients, so the primary endpoint (%UACR) was

	Eplerenone group (n=162)	Placebo group (n=152)
(Continued from previous column)		
Cardiovascular diseases		
Myocardial infarction	0	0
Heart failure or arrhythmia	7 (4%)	8 (5%)
Stroke	0	0
Other atherosclerotic diseases	2 (1%)	2 (1%)
Concomitant treatments at baseline		
Antihypertensive drugs		
ARB†	148 (91%)	136 (89%)
ACE-I	20 (12%)	18 (12%)
Calcium-channel blocker	100 (62%)	95 (62%)
β-blocker or α-blocker and β-blocker	20 (12%)	22 (14%)
Diuretic		
Thiazide	23 (14%)	18 (11%)
Loop	2 (1%)	1 (<1%)
α-blocker	9 (6%)	7 (5%)
Lipid-lowering drugs		
Statins	52 (32%)	48 (32%)
Fibrates	8 (5%)	2 (1%)
Aspirin	10 (6%)	10 (7%)
Others	2 (1%)	1 (<1%)

Data are n (%) or mean (SD), unless otherwise stated. UACR=urinary albumin-to-creatinine ratio. BUN=blood urea nitrogen. eGFR=estimated glomerular filtration rate. L-FABP=liver-type fatty acid-binding protein. ARB=angiotensin receptor blocker. ACE-I=angiotensin-converting enzyme inhibitor. *eGFR was calculated with the modification of diet in renal disease formula modified by the Japanese Society of Nephrology.[16] †Six patients in the eplerenone group and two in the placebo group had ARB and ACE-I combination therapy.

Table 1: **Baseline characteristics of patients included in efficacy analyses**

	Eplerenone group			Placebo group			Maximum dose‡ (mg/day)
	n	Dose* (mg/day)	Dose category (n)†	n	Dose* (mg/day)	Dose category (n)†	
Angiotensin receptor blocker	148	..	41 high, 75 medium, 32 low	136	..	21 high, 76 medium, 39 low	..
Valsartan	41	90·24 (45·85)	11 high, 22 medium, 8 low	35	80 (38·81)	6 high, 18 medium, 11 low	160
Candesartan	32	8·75 (2·77)	11 high, 16 medium, 5 low	33	7·39 (2·42)	3 high, 23 medium, 7 low	12
Olmesartan	29	23·62 (12·02)	9 high, 13 medium, 7 low	31	22·58 (11·25)	8 high, 15 medium, 8 low	40
Telmisartan	24	40 (19·34)	4 high, 14 medium, 6 low	15	36 (21·31)	2 high, 7 medium, 6 low	80
Losartan	18	53·78 (28·30)	4 high, 8 medium, 6 low	16	45·31 (19·3)	1 high, 10 medium, 5 low	100
Irbesartan	4	150 (57·74)	2 high, 2 medium, 0 low	6	100 (54·77)	1 high, 3 medium, 2 low	200
Angiotensin-converting enzyme inhibitor	20	..	12 high, 2 medium, 6 low	18	..	4 high, 9 medium, 5 low	..
Enalapril	6	7·71 (3·74)	4 high, 1 medium, 1 low	4	5 (3·54)	1 high, 1 medium, 2 low	10
Imidapril	6	7·5 (3·87)	4 high, 0 medium, 2 low	5	3·25 (1·68)	0 high, 2 medium, 3 low	10
Perindopril	2	4, 4	0 high, 2 medium, 0 low	3	3·33 (1·15)	2 high, 1 medium, 0 low	8
Trandolapril	2	2, 2	2 high, 0 medium, 0 low	0	..	0 high, 0 medium, 0 low	2
Temocapril	1	2	0 high, 1 medium, 0 low	4	2·5 (1)	1 high, 3 medium, 0 low	4
Cilazapril	1	0·25	0 high, 0 medium, 1 low	0	..	0 high, 0 medium, 0 low	2
Lisinopril	1	2	0 high, 0 medium, 1 low	1	10	0 high, 1 medium, 0 low	20
Captopril	1	56·25	0 high, 0 medium, 1 low	0	..	0 high, 0 medium, 0 low	150
Benazepril	0	..	0 high, 0 medium, 0 low	1	5	0 high, 1 medium, 0 low	10

*Dose is mean (SD), but the dose for individual patients is shown where there are only one or two patients. †Dose categories were divided as high, medium, and low: medium dose is the standard dose shown in the drug package insert in Japan, high dose is higher than standard, and low dose is lower than standard. ‡Maximum dose approved in Japan.

Table 2: **Doses of angiotensin receptor blocker and angiotensin-converting enzyme inhibitor in patients included in the efficacy analyses**

assessed in 304 patients. For the same reason, 24-h urinary sodium excretion and urinary L-FABP were assessed in 307 and 289 patients, respectively.

Baseline demographic, clinical, and biochemical characteristics were well balanced between groups (table 1). Mean urinary sodium excretion was high in both groups (218 mmol/day); higher than mean daily sodium excretion (about 160 mmol/day) recorded in 129 global datasets.[20] Concomitant drug use at baseline and doses administered were also much the same (table 1). In terms of RAS inhibitors, about one quarter of patients in both groups were taking high doses, about one half were taking medium doses, and about one quarter were taking low doses (table 2).

The percent reduction in early morning UACR from baseline was significantly greater in the eplerenone group than in the placebo group after 52 weeks (between-group difference −27·6%; 95% CI −51·15 to 3·96; p=0·0222; table 3). The anti-albuminuric effect of eplerenone was observed early (from week 4) and continued until the end of treatment; percent change in UACR from baseline as assessed by area under the curve was significantly (p<0·0001) lower in the eplerenone group than the placebo group (figure 2).

In post-hoc analyses stratified by urinary sodium excretion, in patients with high urinary sodium excretion (≥160 mmol/day), percent change in UACR from baseline was significantly greater in those in the eplerenone group than in those in the placebo group, but we noted no significant difference in percent change in UACR between patients in the eplerenone group and those in the placebo group with low urinary sodium excretion (<160 mmol/day) (table 3). In the eplerenone group, there was no significant correlation of percent decrease in UACR with baseline urinary excretion (r=0·0400, p=0·619) or baseline concentrations of plasma (r=0·134, p=0·096) or urinary (r=−0·144, p=0·148) aldosterone. No change in urinary sodium excretion or urinary L-FABP in either group was recorded (table 3).

A small but significant reduction in eGFR was recorded at week 8 in the eplerenone group only, but eGFR did not further decrease in the remaining study period (table 3). Percent decrease in eGFR from baseline at 52 weeks was also greater in the eplerenone group than the placebo group (table 3). The proportion of patients at each chronic kidney disease stage, based on eGFR, before and after treatment, did not differ between the groups (table 4).

We noted differences in systolic blood pressure between the groups throughout the study (figure 3). Systolic blood pressure decreased from baseline by week 4 in the eplerenone group. Systolic blood pressure also decreased in the placebo group, but to a lesser

	Eplerenone group			Placebo group			Between-group difference		p value
	N	Mean	95% CI	N	Mean	95% CI	Mean	95% CI	
Primary endpoint*									
UACR change from baseline (%)	158	-17·3	-33·65 to -0·94	146	10·3	-6·75 to 22·3	-27·6	-51·15 to -3·96	0·0222
Secondary endpoint†									
eGFR change from baseline (%)	156	-4·6	-7·07 to -2·19	151	0·47	-2·00 to 2·96	-5·1	-8·58 to -1·63	0·0041
24 h UNa change from baseline (mmol/day)	159	0·12	-12·21 to 12·44	148	6·4	-6·37 to 19·18	-6·3	-24·05 to 11·46	0·486
Urinary L-FABP change from baseline (μg/L)	150	-0·29	-1·67 to 1·08	139	0·62	-0·814 to 2·045	-0·91	-2·896 to 1·073	0·367
Urinary L-FABP/creatinine change from baseline (%)	150	-2·01	-19·55 to 15·53	139	14·03	-4·19 to 32·26	-16·04	-41·34 to 9·253	0·213
Safety analysis‡									
Serum potassium change from baseline (mmol/L)	158	0·17	0·102 to 0·244	151	0·02	-0·048 to 0·097	0·14	0·057 to 0·250	0·0043
Comparison of groups at each visit									
eGFR (mL/min per 1·73 m²)									
8 weeks	148	64·8	62·27 to 67·33	135	69·0	66·38 to 71·67	-4·2	-7·88 to -0·56	0·0241
28 weeks	147	64·6	62·18 to 66·96	133	68·3	65·77 to 70·80	-3·7	-7·19 to -0·25	0·0358
52 weeks	146	64·1	61·56 to 66·67	138	68·0	65·39 to 70·64	-3·9	-7·56 to -0·23	0·0372
Serum potassium (mmol/L)									
4 weeks	154	4·30	4·24 to 4·37	147	4·15	4·089 to 4·213	0·15	0·240 to 0·067	0·0006
8 weeks	148	4·31	4·25 to 4·37	135	4·20	4·139 to 4·266	0·11	0·022 to 0·198	0·0142
28 weeks	146	4·34	4·28 to 4·40	133	4·19	4·125 to 4·259	0·15	0·054 to 0·240	0·0020
52 weeks	146	4·32	4·25 to 4·340	133	4·16	4·082 to 4·233	0·17	0·062 to 0·270	0·0019
24 h UNa (mmol/day)									
8 weeks	148	209·6	195·90 to 217·31	136	218·0	206·85 to 229·19	-11·4	-26·89 to 4·01	0·148
28 weeks	147	207·2	196·50 to 217·95	133	212·0	200·70 to 223·25	-4·7	-30·31 to 10·81	0·549
52 weeks	146	223·0	211·71 to 234·34	133	218·2	206·33 to 230·04	4·8	-11·55 to 21·22	0·562
Post-hoc subgroup analysis									
UACR change from baseline (%)									
24-h UNa <160 mmol/day	31	-6·0	-30·82 to 18·79	23	6·7	-22·13 to 35·46	-12·7	-50·68 to 25·33	0·506
24-h UNa ≥160 mmol/day	127	-20·0	-39·41 to -0·69	123	10·9	-8·74 to 30·61	-31·0	-58·58 to -3·38	0·0280

UACR=urinary albumin-to-creatinine ratio. eGFR=estimated glomerular filtration rate. UNa=urinary sodium excretion. L-FABP=liver-type fatty acid-binding protein. *At 52 weeks, or last visit for efficacy assessment in patients who discontinued the study. †At 52 weeks, or last visit for efficacy assessment. ‡At 52 weeks, or last visit for safety asssessment.

Table 3: **Changes in variables from baseline values in patients included in efficacy analyses**

extent than in the eplerenone group; similar changes were recorded for diastolic blood pressure. Percent reduction in UACR correlated with percent decrease in systolic blood pressure (r=0·140, p=0·0153), suggesting that the anti-albuminuric effects of eplerenone might be dependent on blood pressure reduction. However, percent reduction in UACR did not correlate with percent decrease in eGFR (r=0·0007, p=0·990).

In safety analyses, seven (4%) of 169 patients in the eplerenone group and eight (5%) of 163 patients in the placebo group discontinued the study drugs because of adverse events (figure 1, table 5). Incidence of serious and non-serious adverse events did not differ between groups (table 5). There was one death in the eplerenone group from unknown causes, cardiovascular and cerebrovascular events were rare, with no difference between groups (table 5). Serum potassium concentrations throughout the study were slightly but significantly higher in the eplerenone group than the placebo group (table 3). Notably, no patient in either group had hyperkalaemia, defined as a potassium concentration greater than 5·5 mmol/L, although 15 patients who received eplerenone and four patients who received placebo had a serum potassium concentration of 5·1–5·5 mmol/L. Two of 15 patients in the eplerenone group had sustained increases in serum potassium concentration, whereas others had transient increases during the treatment period.

Discussion

In our trial, addition of low-dose eplerenone (50 mg/day), a selective aldosterone receptor antagonist, to RAS inhibitor treatment in patients with non-diabetic chronic kidney disease with albuminuria reduced UACR compared with placebo (panel). Notably, residual albuminuria is a strong predictor of adverse renal outcomes in long-term studies of patients with chronic kidney disease treated with RAS inhibitors,[2–5,21,22] and so, reduction of albuminuria might be of benefit. Moreover, early reduction of 24-h urinary protein excretion with a RAS inhibitor reportedly correlated with the long-term effect on creatinine concentrations or end-stage renal

disease.[2] In our study, the anti-albuminuric effect of eplerenone was recorded in the early phase (4 weeks) of treatment and continued in the long term (52 weeks) without attenuation. In view of the hypothesis that albuminuria is sustained during long-term treatment of chronic kidney disease,[23] combination therapy with RAS blockade and a mineralocorticoid receptor blocker to further reduce albuminuria might prevent and slow progressive renal function loss.

With respect to the presence of residual albuminuria in patients with chronic kidney disease treated with RAS inhibitors, the occurrence of aldosterone breakthrough could be connected with resistance to RAS inhibitors. The incidence of aldosterone breakthrough ranges from 10% over 6 months to 53% over 1 year, as shown by a review of eight studies that enrolled patients with congestive heart failure in four, chronic kidney disease in three, and hypertension in one.[24] Treatment with spironolactone added to angiotensin-converting enzyme inhibitor apparently reduced UACR to a greater extent in 40% of patients with diabetes who developed breakthrough than the remaining patients without breakthrough.[11] Thus, addition of a mineralocorticoid receptor antagonist to RAS inhibitors is promising for the treatment of renal injury in patients with chronic kidney disease and aldosterone breakthrough. However, no reliable markers are available to predict the occurrence of breakthrough.

Regarding another possible cause of resistance to RAS inhibitors, urinary sodium excretion in patients recruited in this study (218 mmol/day) was higher than the mean of about 160 mmol/day recorded in 129 global datasets.[20] Moreover, anti-albuminuric effects of eplerenone were evident in patients with high concentrations of the estimated 24-h urinary sodium excretion (≥160 mmol/day) but not in those with low urinary sodium excretion (<160 mmol/day). Daily salt (NaCl) intake of greater than 11·7 g (equivalent to 200 mmol/g creatinine) seems to blunt the antiproteinuric effect of angiotensin-converting enzyme inhibitor therapy and increase the risk of end-stage renal disease, independent of blood pressure control.[25] Conversely, sodium depletion with a low-sodium diet or diuretic treatment is beneficial for individuals with proteinuria that are resistant to RAS blockade when previously on a high salt diet,[26] suggesting that knowledge of the salt intake of patients with chronic kidney disease is important in assessment of the potential antiproteinuric and renoprotective effects of RAS inhibitors. Regarding the putative mechanisms of salt-induced resistance to RAS inhibitors, high salt intake increased renal mineralocorticoid receptor activity despite decreased plasma aldosterone in salt-sensitive hypertensive rats,[27] possibly through an aldosterone-independent mineralocorticoid receptor-mediated pathway.[28] Because we noted no significant correlation of percent change in UACR with baseline plasma or urinary aldosterone in this study, it is possible that high salt-induced enhancement of mineralocorticoid receptor signalling at the level of the kidney is closely associated with the progression of renal injury in the setting of inadequate suppression of plasma aldosterone via aldosterone breakthrough, even if adequate suppression is provided by RAS inhibitors.

In our study, mean systolic blood pressure was lower in patients who received eplerenone than in those who received placebo after 4 weeks of eplerenone treatment, an effect observed until study end at 52 weeks. Notably,

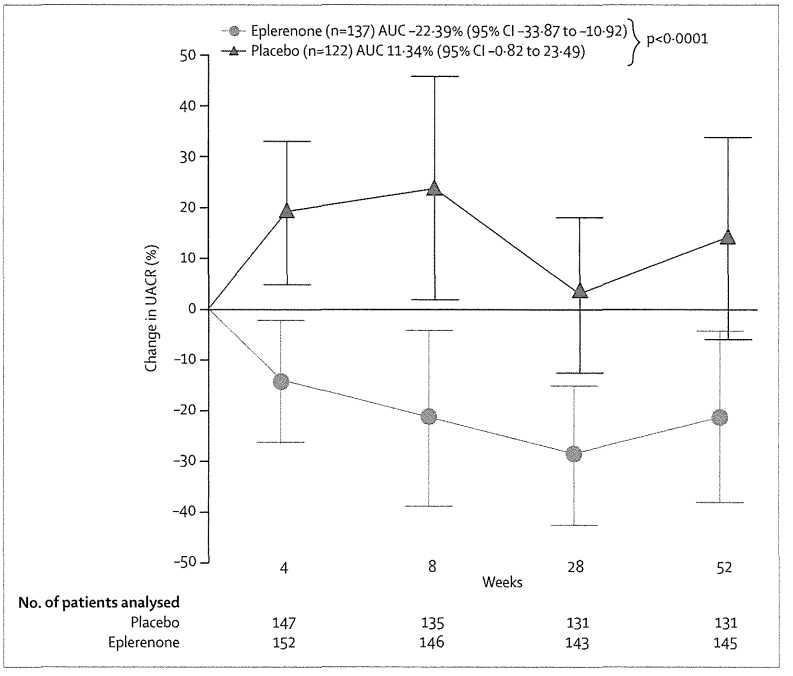

Figure 2: **Time course of percent change from baseline in urinary albumin-to-creatinine ratio (UACR) during the study period**

Comparisons between eplerenone and placebo groups: p=0·0005 (4 weeks), p=0·0015 (8 weeks), p=0·0022 (28 weeks), p=0·0079 (52 weeks). Data are means and 95% CIs. Time course during the study quantified as the area under the curve (AUC) of serial assessments from baseline to week 52.

	Baseline		Study completion	
	Eplerenone group (n=159)	Placebo group (n=151)	Eplerenone group (n=158)	Placebo group (n=152)
1	12 (8%)	13 (9%)	8 (5%)	16 (11%)
2	86 (54%)	88 (58%)	77 (49%)	82 (54%)
3a	59 (37%)	45 (30%)	61 (39%)	45 (30%)
3b	2 (1%)	5 (3%)	11 (7%)	9 (6%)
4	0	0	1 (1%)	0
Missing	3 (2%)	1 (<1%)	4 (3%)	0

Data are n (%). Stage 1: estimated glomerular filtration rate (eGFR) ≥90 mL/min per 1·73 m²; stage 2: 60 mL/min per 1·73 m² ≤ eGFR < 90 mL/min per 1·73 m²; stage 3a: 45 mL/min per 1·73 m² ≤ eGFR < 60 mL/min per 1·73 m²; Stage 3b: 30 mL/min per 1·73 m² ≤ eGFR <45 mL/min per 1·73 m²; stage 4: 15 mL/min per 1·73 m² ≤ eGFR < 30 mL/min per 1·73 m². CKD=chronic kidney disease.

Table 4: **Chronic kidney disease stages at baseline and study completion**

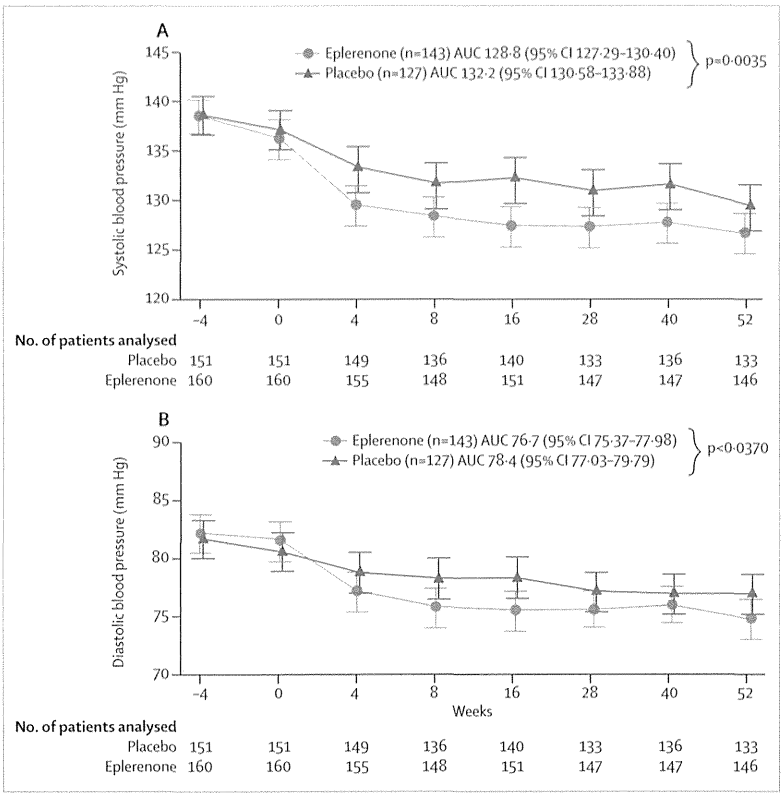

Figure 3: **Time course of office blood pressure during the study period**
Office (A) systolic and (B) diastolic blood pressures. Comparisons between eplerenone and placebo groups: (A) p=0·0149 (4 weeks), p=0·0289 (8 weeks), p=0·0018 (16 weeks), p=0·0227 (28 weeks), p=0·0137 (40 weeks), p=0·036 (52 weeks), and (B) p=0·0180 (16 weeks). Data are means and 95% CIs. Time course during the study quantified as the area under the curve (AUC) of serial assessments from baseline to week 52.

	Eplerenone group (n=169)	Placebo group (n=163)
Patients with adverse events	53 (31%)	49 (30%)
Serious adverse events	5 (3%)*	7 (4%)†
Non-serious adverse events	103 (61%)	104 (64%)
Dizziness	2 (1%)	6 (4%)
Tiredness	3 (2%)	3 (2%)
Digestive trouble	3 (2%)	1 (1%)
Headache	3 (2%)	1 (1%)
Increased liver enzymes	2 (1%)	1 (1%)
Hyperuricaemia	1 (1%)	1 (1%)
Others	89 (53%)	91 (56%)

Data are n (%). No patients were affected by hyperkalaemia, which was defined as a serum potassium level greater than 5·5 mmol/L. *Serious adverse events in the eplerenone group included sudden death (unknown causes), atrial fibrillation, renal dysfunction, gastric cancer, and liver dysfunction. †Serious adverse events in the placebo group included cerebrovascular infarction, colon cancer, bile duct cancer, uterine cervix cancer, colon polyp, bone fractures, and pyelonephritis.

***Table 5:* Adverse events**

the reduction of albuminuria was also recorded during the early period of eplerenone treatment, concomitant with blood pressure lowering. Consistent with changes in blood pressure, a significant reduction in eGFR also occurred early during eplerenone treatment, but this decline did not progress throughout the study, a result also shown by a previous study.[12] Thus, the early reduction of eGFR implies the functional effects of mineralocorticoid receptor antagonism. Mineralocorticoid receptor antagonists reduce the intraglomerular pressure via impaired tubuloglomerular feedback.[29] Functional reduction of eGFR induced by the decreased intraglomerular pressure might be renoprotective in the long term in most patients with chronic kidney disease, possibly through the inhibition of albuminuria.[23]

Serum potassium concentration was significantly increased during treatment with eplerenone, but no patients in either group had serum potassium concentrations greater than 5·5 mmol/L, suggesting that combination therapy with 50 mg/day eplerenone and standard doses of RAS inhibitors is safe for patients with chronic kidney disease, albuminuria, and eGFR of 50 mL/min per 1·73 m² or more. However, combination therapy with mineralocorticoid receptor antagonists and RAS inhibitors in patients with renal insufficiency increases the risk of serious, life-threatening hyperkalaemia. Thus, frequent measurement of serum potassium concentrations in all patients receiving combination treatment is mandatory.[30,31]

Our study has several limitations. The main limitation was the short treatment duration of 52 weeks. Despite 2·2 years of follow-up, a 2013 study did not show the renoprotective effects of dual therapy with an angiotensin receptor blocker and an angiotensin-converting enzyme inhibitor for the treatment of diabetic nephropathy despite the presence of antiproteinuric effects.[32] Notably, after 4 years of the STENO trial, in patients with diabetic nephropathy, intensive risk factor control did not decrease the risk of mortality despite reduced albuminuria, but after a 10-year follow-up, the intensive treatment reduced the risk of mortality and end-stage renal failure events.[33] Therefore, longer observation periods might be needed to assess the effects of study drugs on the prognosis of patients with renal dysfunction, and measurements of eGFR and the incidence of end-stage renal disease are necessary. In addition, background doses of angiotensin receptor blockers and angiotensin-converting enzyme inhibitors in our study were smaller compared with doses used in other trials that examined the effect of these drugs on urinary albumin excretion. Theoretically, maximum dose RAS inhibitors should be used whenever resistance to RAS inhibitors is assessed. However, full RAS system blockade may not be seen even with maximal doses of RAS inhibitors: the full antiproteinuric potential of the angiotensin receptor blocker irbesartan was not reached at the approved maximum dose of 300 mg once daily, and further increase of the dose up to 900 mg once daily resulted in a more complete RAS blockade and additional reduction in urinary albumin excretion.[34] Finally, we used a formula to estimate sodium excretion in a single sample

Panel: Research in context

Systematic review

We searched PubMed for randomised controlled trials published in English before January, 2009, and identified four articles that provided prospective data to assess the anti-albuminuric effects of mineralocorticoid receptor antagonists in patients with non-diabetic chronic kidney disease who received renin-angiotensin system (RAS) inhibitors. Addition of spironolactone to RAS inhibitors for the treatment of non-diabetic chronic kidney disease significantly reduced proteinuria by 37–70%. However, these studies enrolled few patients and none of the studies used the selective mineralocortcoid receptor antagonist, eplerenone. When preparing the report, our updated literature search identified an additional open-label trial, which showed that eplerenone, a mineralocorticoid receptor antagonist, reduced albuminuria in non-diabetic chronic kidney disease.

In patients with diabetic nephropathy, eight double-blind, placebo-controlled trials, most of which were small in size, showed that mineralocorticoid receptor antagonists reduced albuminuria in patients with diabetic nephropathy. We cited the most relevant articles. Systematic search of publications on "double-blind, placebo-controlled trial" with "eplerenone" or "spironolactone" in non-diabetic chronic kidney disease retrieved no articles.

Interpretation

In this double-blind, randomised, placebo-controlled trial, we compared eplerenone 50 mg with placebo in 314 hypertensive patients with albuminuria who were treated with standard doses of RAS inhibitors. Eplerenone reduced albuminuria to a greater extent than placebo in the early phase and the effect was continued to 52 weeks. Anti-albuminuric effects of eplerenone were recorded in patients with higher sodium excretion (≥160 mmol/day) but not in those with lower sodium excretion (<160 mmol/day), suggesting that high dietary sodium intake causes resistance to RAS inhibitors through mineralocorticoid receptor-mediated activation. Our results are based on surrogate endpoints; whether the observed effects will translate into improved clinical outcomes needs prospective testing in an appropriately sized outcomes study.

of morning fasting urine because this method is considered an adequate substitute for 24-h urinary sodium excretion.[35] Salt intake can be assessed by measuring urinary sodium excretion collected during 24 h, but this method is impractical in clinical trials.

We conclude that low-dose eplerenone might be safe and efficacious as add-on treatment to RAS inhibitors for hypertensive patients with non-diabetic chronic kidney disease.

Contributors

KA was responsible for the protocol, protocol review, study coordination, and management, including ethical and regulatory approvals, drug sourcing and management, data review, and drafting and review of the manuscript. HO did the protocol review, statistical analysis, and drafted and reviewed the manuscript. SU and SK reviewed the protocol, recruited patients, followed the protocol, and reviewed the manuscript. YA did the protocol review and masking. TF did the executive coordination, protocol and document drafting, protocol review, study coordination and management, data review, and drafted and reviewed the manuscript.

Declaration of interests

KA has received research grants from Daiichi-Sankyo, Kyowa Hakko Kirin, Boehringer Ingelheim, and Shionogi Pharm. HO has received honoraria from Statocom, EPS Corporation, Ministry of Health, Labour and Welfare, Daiichi-Sankyo, Chugai, Astellas, and Mitsubishi-Tanabe Pharm. SK has received research grants from Chugai, Genzyme Japan, Takeda, and Otsuka Pharm. YA has received a research grant from Toray. TF has received research grants and honoraria from Astellas, Toray, Boehringer Ingelheim, Chugai, Fukuda Denshi, Kyowa Hakko Kirin, Mitsubishi Tanabe, Mochida, Omron, Novartis, Pfizer, and Takeda Pharm. SU declares no competing interests.

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Guideline

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 approximately 25%, and with approximately 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.

Key words ADAMTS13, alternative complement pathway, atypical hemolytic uremic syndrome, complement dysregulation, thrombotic microangiopathy.

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 approximately 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 approximately 25%, and with approximately 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

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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 1.5-fold 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, 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

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 Presence confirmed based on: – increased serum LDH levels – marked decreases in serum haptoglobin levels – 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. Pediatric cases: Serum creatinine should be increased to a level that is 1.5-fold higher than the serum creatinine reference values based on age and gender issued by the Japanese Society for Pediatric Nephrology [7]. 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.

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 100,000–150,000/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, sclerodermatous 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*.[12] 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.

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	
(i) Congenital	
Genetic mutations of complement proteins, factor H, factor I, membrane cofactor protein, C3, factor B, and thrombomodulin	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
(ii) Acquired	Detection of anti-factor H antibody by ELISA, western blot, etc
Production of autoantibodies, including anti-factor H antibody	
(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.

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 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 approximately 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 hours 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 United States. 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.

Conflicts of interest

Advisory role: Yoshihiro Fujimura (Baxter Bioscience and Alexion Pharmaceuticals). Honoraria: Masaomi Nangaku (Kyowa Hakko Kirin Co. Ltd and Daiichi Sankyo Co. Ltd). Subsidies: Masaomi Nangaku (Kyowa Hakko Kirin Co. Ltd, Daiichi Sankyo Co. Ltd, Astellas Pharma Inc., Mitsubishi Tanabe Pharma Corporation, Chugai Pharmaceutical Co. Ltd and Takeda Pharmaceutical Co. Ltd). The other authors have no conflicts of interest.

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GUIDELINE

Guidelines for the management and investigation of hemolytic uremic syndrome

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Outline of the Guidelines

1. Necessity to provide comprehensive guidelines for hemolytic uremic syndrome (HUS)

The first guidelines for the diagnosis and treatment of HUS following the Shiga toxin producing *Escherichia coli* (STEC) infection was published by The Japanese Society of Pediatric Nephrology (JSPN) in 2000. Since then, there has been considerable advancement in the understanding and treatment of acute encephalopathy - one of the most serious complications in HUS. Furthermore, the etiology, conditions and treatments of atypical HUS have been elucidated. Therefore, a set of comprehensive guidelines for HUS that reflects recent clinical evidence is necessary.

The aim of this set of guidelines is to provide a support, tool for daily medical practice and to contribute to the standardization and accessibility of HUS-related medical care, as well as to improve level of safety for HUS patients.

2. Preparation of guidelines

The present guidelines are produced according to the procedures proposed by the Medical Information Network Distribution Service (Minds) of the Japan Council for Quality Health Care.

The guideline writing committee (GWC) consists of members from these societies: JSPN, The Japanese Society of Nephrology (JSN), The Japanese Society of Child Neurology (JSCN), Japanese Society for Pediatric Infectious Diseases (JSPID) and The Japanese Association for Infectious Diseases (JAID).

The GWC members set the keywords in conjunction with the clinical question and critically reviewed relevant

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This is the English version of Guidelines for the Management and Investigation of Hemolytic Uremic Syndrome, which was published in 2013 (in Japanese). (http://www.jspn.jp/kaiin/guideline.shtml)

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literatures published between January 1, 1992 and August 31, 2012, through the use of major databases (e.g., PubMed and the Japana Centra Revuo Medicina [Ichushi]) in cooperation with The Japan Medical Library Association. As there is a lack of high quality publications on HUS currently, publications with low quality evidences or without retrieval target period were still carefully reviewed.

All documents used are supported by evidence. A grade of recommendation was assigned to the statements. The grades were determined based on the level of evidence, as well as on the quality and clinical significance of the evidence. The levels of evidence and grades of recommendation are shown in Tables 1 and 2.

3. Independent assessment

The present guidelines were reviewed by the assessment committee members derived from three JSPN and one Child Support Whole Country Network of Intractable Disease representatives. The final draft of the guidelines, together with a request for public comments, was published on the websites of JPS, JSN and JSPN. The GWC then took on board the comments and suggestions by the public to revise and finalize the present set of guidelines accordingly.

4. Cautionary notes on the use of the present guidelines

Users should be aware that the guidelines do not always equate to evidence-based medicine (EBM). The guidelines are not meant to overrule a physician's experience. Users should bear in mind that the guidelines are developed in accordance with evidence at the time of preparation and that the quantity and level of evidence may subsequently change. The guidelines serve to assist physicians and patients in making decisions about treatment. This set of guidelines does not provide any legal basis in the event of medical lawsuits.

Table 1 Level of evidence

Level I	Data obtained from a systematic review or a meta-analysis of randomized clinical trials
Level II	Data obtained from at least one randomized comparative clinical trial
Level III	Data obtained from non-randomized comparative clinical trials
Level IV	Cohort studies, case–control studies, or cross-sectional studies
Level V	Case reports, or case series
Level VI	Opinions of special committees or specialists with no basis of patient data

Table 2 Grade of recommendation

Grade A	A given treatment or procedure was recommended based on robust scientific evidence
Grade B	A given treatment or procedure was suggested based on scientific evidence
Grade C1	A given treatment or procedure may be considered although scientific evidence is not available
Grade C2	A given treatment or procedure may not be considered due to missing scientific evidence
Grade D	A given treatment or procedure is not recommended as scientific evidence indicated inefficacy or harm

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5. Conflict of interest

The expense for GWS meetings were provided by the Health Labor Sciences Research Grant (for the study on standardization of the pathogenic factor and the medical treatment for severe enterohemorrhagic *Escherichia coli* infection) supported by the Ministry of Health, Labor and Welfare (MHLW). Dr. Makoto Ohnishi chairs this research project. All committee members confirmed their conflict of interest (COI) declaration based on the Acts of COI established by JPS, JSN and JSPN.

Contents

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

1.1 Diagnosis of Shiga toxin producing *Escherichia coli* infection

Methods for Shiga toxin producing *Escherichia coli* (STEC) infection diagnosis defined by the Ministry of Health, Labor and Welfare, Japan. [Grade of Recommendation: Not Graded]

STEC infection is diagnosed when a patient manifests clinical symptoms and signs suggestive of STEC infection and meets criterion 1, 2 or 3 below.

1. *E. coli* isolated from stool is confirmed to have the ability to produce Shiga toxin (STX) by one of the following criteria:
   a. Confirmation of STX being produced.
   b. Isolation of STX-producing genes by PCR or other methods.
2. Isolation of STX from stool of a patient with HUS.
3. Isolation of serum anti-O antigen of *E. coli* antibody or anti-STX antibody from a patient with HUS.

Comments

1. What is enterohemorrhagic *Escherichia coli* infection?

According to the definition established by the MHLW under the Law concerning the Prevention of Infections and Medical Care for Patients of Infections (Infectious Diseases Control Law), enterohemorrhagic *Escherichia coli* (EHEC) is an infection caused by diarrheagenic *E. coli* that produces STX [a]. STX is also known as Verotoxin (VT). EHEC infects human intestine, where it produces STX and induces diarrhea. EHEC may also be referred to as Verotoxin producing *Escherichia coli* (VTEC).

2. Causative food

Humans usually contract STEC infection by ingesting food such as raw or inadequately cooked beef, sprout, vegetables, pickles or water contaminated with the organism. In many cases, however, specific causative food cannot be identified. Hence, the route of infection remains unconfirmed.

3. Symptoms and signs

Abdominal pain and watery diarrhea develop 3–7 days after oral ingestion of STEC, and likely to be followed by bloody stool, which has similar consistency with blood in severe cases (hemorrhagic colitis, Fig. 1). The wall of the large intestine shows edematous change (Figs. 2, 3), accompanied by the erosion and bleeding. In more severe cases, the patient experiences diarrhea more than ten times per day and suffers serious abdominal pain. According to the MHLW, abdominal pain, watery diarrhea and bloody stool are the main symptoms of STEC infection. A high body temperature of over 38 °C and nausea are observed in some STEC patients [b]. High fever over 39 °C is a rare complication.

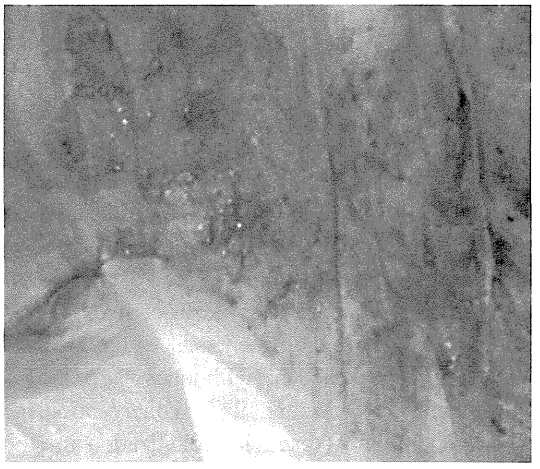

Fig. 1 Bloody stool from a patient with STEC infection

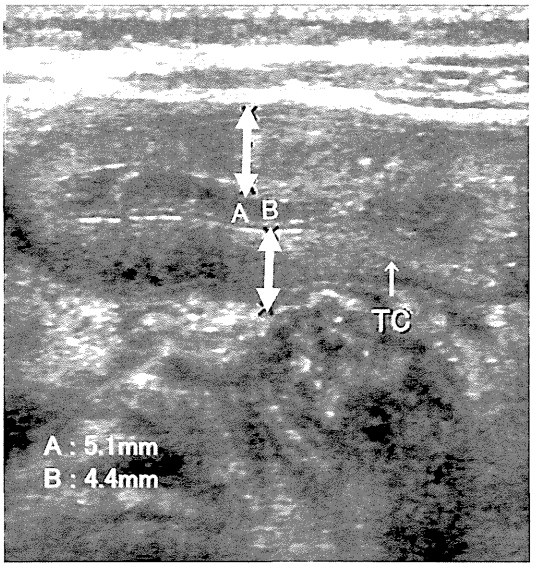

Fig. 2 Edematous change of the transverse colon of a patient with STEC infection (abdominal ultrasonography)

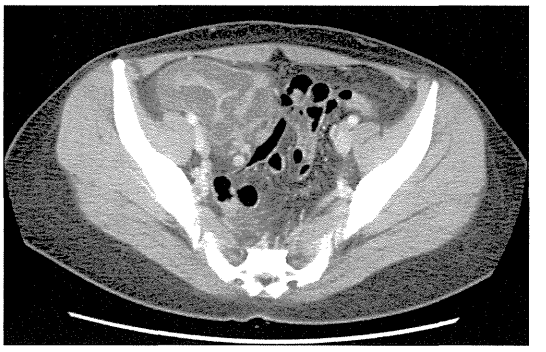

Fig. 3 Edematous change of the cecum and ascending colon of a patient with STEC infection (abdominal CT scan)

Table 3 Number of patients with HUS and STEC infection in Japan (2008–2011)

No. of patients with HUS	371
No. of HUS patients with detected STEC	242
No. of HUS patients with detected STEC O157	203
No. of HUS patients with detected STEC O157(producing both STX1 & STX2)	117
No. of HUS patients with detected STEC O157(producing STX2)	76
No. of HUS patients with detected STEC O157(producing unclassifiable STX)	10
No. of HUS patients with detected STEC excluding O157	39

Figures in this table are based on data from IASR 2009, 2010, 2011 and 2012

Some patients with STEC infection develop HUS several days from the onset of diarrhea. A triad of symptoms typically appears in HUS, including hemolytic anemia, thrombocytopenia and acute kidney injury (AKI).

4. STEC as causative agent of HUS

Table 3 shows the reported cases of HUS in Japan from 2008 to 2011 (retrieved from the records of the Infectious Disease Surveillance Center under the National Institute of Infectious Diseases of Japan). The most common serotype of STEC isolated from HUS patients in Japan was O157. For patients who are O157 negative, O121, O111, O26, and O145 are identified [1–4].

5. Diagnosis

Final diagnosis requires the identification of STEC in stool. Therefore, stool sample should be obtained and cultured before antibiotics are administered to patients. According to the guidelines for the examination of intestinal infections by the Japanese Society of Clinical

Table 4 Specimen, subject, measurement principle, and reaction time of commercially available rapid diagnostic methods

Specimen	Subject	Measurement principle	Reaction (required) time
Stool	Antigen of STEC O157[a]	Immunochromatography	10–15 min
		Latex agglutination	2 min
Stool	Shiga toxin	ELISA	~3 h
Serum	Antibody against STEC O157 LPS	Latex agglutination	3 min

[a] Diagnosis of STEC infection should not be based on STEC antigen detected in the stool from the patient solely

Microbiology, the presence of STX is the most reliable marker of STEC [c]. The guidelines from the Center for Disease Control and Prevention in the USA recommends the use of a culture that could identify STEC O157 and other serotypes in stool samples in addition to the confirmation of STX in the stool [d]. It remains difficult to diagnose STEC infection as other bacteria besides STEC can produce STX. It is also challenging to diagnose STEC infection based solely on the presence of STX in stool. The MHLW reported that the presence of STX in stool, serum antibody against *E. coli* O antigen or anti STX antibody in serum would be enough for the diagnosis of STEC infection only in cases with HUS. The MHLW arrived at this decision due to the fact that STEC is the leading cause of HUS in Japan [5], and that it is difficult to detect STEC in stool when antibiotics were administered to patients before examination of stool sample. Specimen, subjects, measurement principles and reaction time of commercially available rapid diagnostic methods are shown in Table 4.

Supplementary articles

a. Ministry of Health, Labour and Welfare: Report of three cases of enterohemorrhagic *Escherichia coli* infection by doctors and veterinarians (http://www.mhlw.go.jp/bunya/kenkou/kekkaku-kansenshou11/01-03-03.html).
b. Legal act on the medical care, prevention and treatment of infectious diseases (Law 114th, October 2, 1998. Revision: Law 122nd, December 14, 2011).
c. Japanese Society of Clinical Microbiology. Guidelines for examination of infectious enteritis. J Jpn Soc Clin Microbiol. 2010;20:1–138.
d. Gould LH, et al. Recommendations for diagnosis of Shiga toxin-producing *Escherichia coli* infections by clinical laboratories. MMWR Recomm Rep. 2009;58(RR-12):1–14.

1.2 Treatment of STEC infection

1. Antibiotics

> No conclusion has been made regarding the association between the use of antibiotics for STEC infection and the onset of HUS. [Grade of Recommendation: Not Graded]
>
> The use of antibiotics is considered for carrier of STEC (such as patient's family members) to prevent further transmission of the disease.

Comments

Treatment for children with STEC infection is primarily by supportive care. In the set of guidelines in the USA, the use of antibiotics is not recommended for the treatment of STEC infection as it is a risk factor for HUS. Antibiotics kill bacteria and provoke the release of toxin resulting in HUS [a, b]. However, a global meta-analysis performed between January 1981 and February 2001 demonstrated that the use of antibiotics did not influence the incidence of HUS. This indicated the need of the appropriate randomized controlled study (RCT) [1]. One RCT comparing the incidence of HUS between antibiotics-use group and antibiotics non-use group in STEC infected patients demonstrated no differences [2]. Another case–control study evaluating patients with STEC infection outbreak in Europe showed that antibiotics-use group ($n = 52$) had lower incidence of seizure, surgical intervention, mortality and shorter duration of bacterial colonization in stool than antibiotics non-use group $n = 246$) [3].

In contrast, several cohort studies evaluating STEC O157 patients demonstrated that antibiotics -use group had higher incidence of HUS than antibiotics non-use group, and concluded that the use of antibiotics is indeed a risk factor for HUS [4–7]. In the studies, antibiotics such as β-lactams (penicillins and cephalosporins), fluoroquinolones, and sulfamethoxazole/trimethoprim were used. Furthermore, recent in vivo data revealed that fluoroquinolones facilitated STX production while azithromycin did not induce STX production [c, d]. Hence, in cases where antibiotics are administered, it is crucial to consider the type being used.

During an outbreak of STEC in Japan, antibiotics—particularly fosfomycin—was used [8]. A retrospective analysis demonstrated that patients who used fosfomycin in

the early onset of diarrhea (within 2 days) had lower incidence of HUS than those who did not [9].

As the indication of antibiotics differs between Japan and other countries, it is difficult to draw comparisons. To date, there has been no conclusion on whether the use of antibiotics is effective in preventing HUS. A recommendation grade is not provided as further investigation is necessary for this treatment option.

For carriers of STEC (such as patient's family members), the use antibiotics should be considered to prevent further transmission of the disease.

2. Anti-diarrheal drug

> We do not recommend the use of anti-diarrheal drug for pediatric patients with STEC as it is a risk factor for HUS. [Grade of Recommendation: D]

Comments

It was previously reported that anti-diarrheal drug is a risk factor for HUS in patients with STEC infection [10–12]. Current foreign guidelines do not recommend the use of anti-diarrheal drugs [a, b]. The use of such drugs should therefore be avoided.

To date, there is no available data on the efficacy or risk of probiotics in patients with STEC infection.

3. Infection control for patients with STEC infection

> In addition to standard precaution, we recommend adopting contact precaution for hospitalized patients with acute diarrhea caused by STEC until two consecutive negative stool cultures. [Grade of Recommendation: B]

Comments

In addition to standard precaution, the wearing of apron and gloves is recommended when coming into contact with patients with acute diarrhea caused by STEC [e]. Contact precaution can be lifted when two consecutive stool cultures proved negative [e].

Supplementary articles

a. Guerrant RL, et al. Infectious Diseases Society of America: Practice guidelines for the management of infectious diarrhea. Clin Infect Dis 2001; 32:331–351.
b. Thielman NM, et al. Clinical practice: Acute infectious diarrhea. N Engl J Med. 2004;350:38–47.
c. Zhang X, et al. Quinolone antibiotics induce Shiga toxin-encoding bacteriophages, toxin production, and death in mice. J Infect Dis. 2000;181:664–670.
d. Zhang Q, et al. Gnotobiotic piglet infection model for evaluating the safe use of antibiotics against Escherichia coli O157:H7 infection. J Infect Dis 2009;199:486–493.
e. American Academy of Pediatrics. Committee on Infectious Diseases. Report of the Committee on Infectious Diseases. In. Evanston, Ill.: American Academy of Pediatrics; 2011.

2 Diagnosis of HUS

2.1 Diagnosis procedure

> STEC causes HUS characterized by thrombotic microangiopathy. Definitive diagnosis of STEC-HUS should be based on the following tests. [Grade of Recommendation: Not Graded]
>
> A. Diagnostic tests
>
> 1. Hemolytic anemia (Hb <10 g/dL, positive for schistocytes, Fig. 4)
> 2. Thrombocytopenia (platelet count $<15 \times 10^4/\mu L$)
> 3. Acute kidney injury (AKI; serum creatinine 1.5 times that of age- and gender-matched standard values, according to the Japanese Pediatric Nephrology Society; Table 5)
>
> B. Concomitant symptoms
>
> 1. Central nervous system (CNS) involvement: conscious disturbance, seizure, headache, and hemorrhagic infarction
> 2. Gastrointestinal involvement: diarrhea, bloody stool, abdominal pain, intestinal perforation, intestinal stenosis, rectal prolapse and intussusceptions
> 3. Cardiac involvement: cardiac infarction and cardiac failure due to myocardial injury
> 4. Pancreatic involvement: pancreatitis
> 5. Disseminated intravascular coagulation (DIC)
>
> Notes
>
> 1. The following markers in serum may support diagnosis: marked elevation of lactate dehydrogenase (LDH), decreased haptoglobin and negative Coombs test despite hyperbilirubinemia.
> 2. Serum O157 lipopolysaccharide (LPS) antibody, rapid diagnostic test for stool O157 antigen or Shiga toxin, and isolation of STEC by stool culture help definitive diagnosis.

Table 5 Standard serum creatinine values by age and gender in Japanese children [f]

Age	2.50 %	50 %	97.5 %
3–5 months	0.14	0.2	0.26
6–8 months	0.14	0.22	0.31
9–11 months	0.14	0.22	0.34
1 year	0.16	0.23	0.32
2 years	0.21	0.24	0.37
3 years	0.21	0.27	0.37
4 years	0.2	0.3	0.4
5 years	0.25	0.34	0.45
6 years	0.25	0.34	0.48
7 years	0.28	0.37	0.49
8 years	0.29	0.4	0.53
9 years	0.34	0.41	0.51
10 years	0.3	0.41	0.57
11 years	0.35	0.45	0.58
12 years boy	0.4	0.53	0.61
13 years boy	0.42	0.59	0.8
14 years boy	0.54	0.65	0.96
15 years boy	0.48	0.68	0.93
16 years boy	0.62	0.73	0.96
12 years girl	0.4	0.52	0.66
13 years girl	0.41	0.53	0.69
14 years girl	0.46	0.58	0.71
15 years girl	0.47	0.59	0.72
16 years girl	0.51	0.59	0.74

Comments

1. Clinical manifestations and diagnosis of HUS

Up to 10 % of patients infected with STEC developed HUS 4–10 days after the onset of diarrhea. Patients who developed HUS within 3 days after the onset of diarrhea may take a rapid and severe clinical course. 20–60 % of patients with HUS need dialysis due to AKI, and between 25 and 33 % of patients have CNS involvement. Mortality in the acute phase may reach to 2–5 %, mainly caused by CNS involvement or intestinal perforation [a–d].

Diagnosis of HUS should be based on the summary described above. Age- and gender-matched standard values should be referred to in order to monitor the increase of serum creatinine in children (Table 5) [e].

Isolation of STEC from stool culture, positivity of stool O157 antigen or STX test, and positivity of serum anti O157 LPS antibody, can help definitive diagnosis. However, some patients do not show gastrointestinal involvement, together with negative STEC results.

Decreased level of platelets in the blood and elevated serum LDH are initial abnormal findings observed in patients with HUS. In particular, a marked increase in LDH of more than 1000 IU/mL is characteristic of HUS, and is helpful for diagnosis. Subsequently, hemolytic anemia and elevated serum creatinine (leading to AKI) occurs. Additionally, marked thickening of the large intestinal wall on abdominal CT and increased echogenicity of the kidney on abdominal ultrasound are characteristic findings that are detectable even in the early phase of HUS (Fig. 5. See also Sect. 2.4, Concomitant Symptoms: Gastrointestinal involvement).

STEC-HUS accounts for 90 % of HUS. Non-STEC HUS is defined as atypical HUS (aHUS). To confirm STEC-HUS, both aHUS and von Willebrand factor-cleaving protease (ADAMTS13)-related thrombotic thrombocytopenic purpura (TTP) should be excluded. Plasma therapy or plasma exchange is the first line treatment against aHUS and TTP, and differs from the treatment for STEC-HUS. It is noteworthy that patients with aHUS are also frequently complicated by gastrointestinal manifestations.

2. Risk factors for developing HUS from STEC infection

According to the survey of the largest outbreak of STEC in 1996 in Sakai city, Japan, risk factors for developing HUS are increased white blood cell (WBC) count in blood (HUS group vs non HUS group: WBC 13,900 vs. 8,300/μL, $p < 0.001$) and increased serum C-reactive protein (CRP; HUS group vs non HUS group: CRP 1.3 vs 0.5 mg/dL, $p < 0.001$) [1].

3. Risk factors for progression to severe HUS

According to a Japanese nationwide survey of childhood STEC–HUS conducted between January 2001 and December 2002, the risk factors for AKI requiring dialysis are low serum sodium (≤130 mEq/L, odds ratio 8.1) and increased serum aspartate transaminase (AST; ≥70 mg/dL, odds ratio 8.9) at the onset of HUS. In total, 64 % of patients with serum sodium ≤130 mEq/L and 73 % of those with AST ≥70 IU/dL received dialysis [2]. The risk factors for CNS involvement are the need for dialysis (odds ratio 6.6) or CRP ≥5.0 mg/dL (odds ratio 6.3). In total, 75 % of patients with CRP ≥5.0 mg/dL and 51 % of patients requiring dialysis have CNS involvement [2].

A registry of 352 children with post-diarrheal HUS in the USA showed that risk factors for death are increased blood WBC count (>20,000/μL, $p = 0.025$) and hematocrit >23 % ($p = 0.00045$). A hematocrit of >23 % seems paradoxical, but the authors provided an argument that the patients took a very rapid and progressive course, and expired before the emergence of decreased hematocrit [3]. CNS involvement was the highest cause of death ($n = 8$).

HUS patients with a serum creatinine level double that of the age- and gender-matched standard value have a higher chance of requiring dialysis. Such patients should be promptly transferred to a hospital for renal replacement therapy [f].