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Predictors for the development of haemolytic uraemic syndrome with *Escherichia coli* O157:H7 infections: with focus on the day of illness

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SUMMARY

A large outbreak of *Escherichia coli* O157 infections via school lunches occurred at primary schools in 1996 in Sakai City, Japan. As many as 10000 patients suffered from diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome (HUS). Using data on 288 inpatient school children affected by this outbreak, of whom 36 presented complete HUS and the remaining 252 tested positive for *E. coli* O157 culture, we attempted to identify predictors for the progression to HUS. Within the first 5 days of illness, clinical features associated with inpatients who developed HUS compared with those without HUS included a C reactive protein (CRP) level higher than 1·2 mg/dl (OR 44·26; 95% CI 5·83–336·23), a white blood cell (WBC) count greater than $11{\cdot}0 \times 10^9/1$ (OR 5·03; 95% CI 2·13–11·87) and a temperature higher than 38·0 °C (OR 5·00; 95% CI 2·25–11·08). It can be concluded that these three factors are predictive factors for the development of HUS in patients with *E. coli* O157 infection, and patients who have two or all of these factors should be observed closely.

INTRODUCTION

Escherichia coli O157:H7 was first recognized as a human pathogen in 1982 after outbreaks in Oregon and Michigan, USA [1]. The rate of recognition of *E. coli* O157 infections has increased in recent years, and this bacterium is estimated to cause more than 20000 infections and up to 250 deaths annually in the United States alone [2].

E. coli O157 produces a range of symptoms from a mild watery diarrhoea to a severe haemorrhagic colitis, and complications such as haemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura can cause severe sequelae including renal failure [3–5]. Risk factors for the progression to HUS are reported to be extreme youth or old age [3, 6], female sex [7, 8], bloody diarrhoea [9], fever [10], elevated leucocyte count [10, 11] as well as antimicrobial therapy [9, 10, 12] and use of antimotility agents [7] and antidiarrhoeal agents [13, 14].

Although the association between infection with *E. coli* O157 and HUS has been established, little is known about whether or which early clinical features can identify patients who are more likely to suffer severe complications. A large outbreak of *E. coli* O157 infections via school lunches occurred at primary schools in 1996 in Sakai City, Japan, and as many as 10000 patients suffered from diarrhoea, haemorrhagic colitis and HUS [15]. Using data on inpatients affected by the outbreak in Sakai City, we aimed to identify predictors for the progression to HUS by comparing inpatients who developed HUS with those who did not.

MATERIALS AND METHODS

In 1996, among the 92 primary schools in Sakai City, the outbreak of *E. coli* O157 infection occurred at 47

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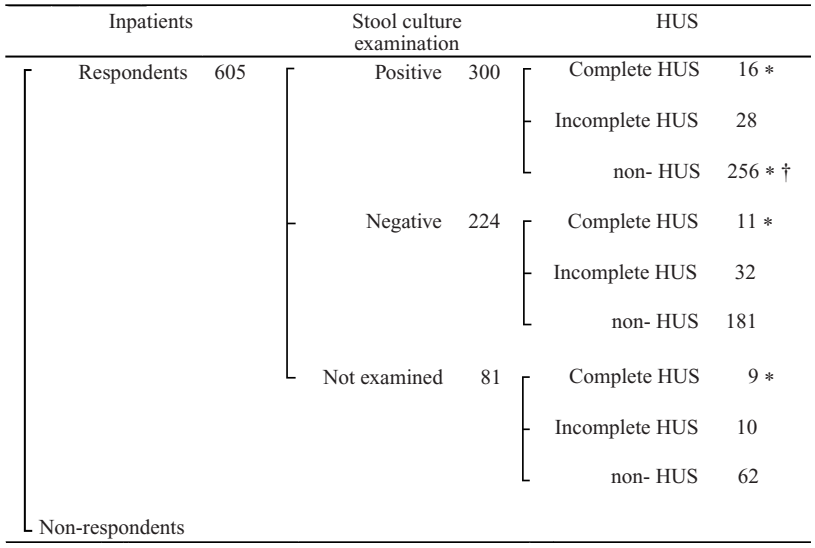

* A total of 288 cases were included in this study, of whom 36 inpatients presented complete HUS and the remaining 252 tested positive for *E coli* O157 by culture.
† Four patients whose onset date of diarrhea was not sure were included.

Fig. 1. Cases analysed in this study.

(51·1%). From the beginning of the outbreak, information on symptoms and diagnosis of each food poisoning case was provided by physicians as required by the Food Safety Act and the Infectious Disease Prevention Act. School staff visited all the schoolchildren enrolled at the 47 schools and gathered detailed information about their medical consultations. Following up these two surveys, public health nurses also visited the symptomatic schoolchildren at home and verified information on the medical consultation and symptoms of each patient. In addition, the city office requested regular information on inpatients from each hospital. Detailed information on all schoolchildren at the 47 schools could thus be gathered and it was verified that there were 684 inpatients with *E. coli* O157.

In February 1997, we conducted a questionnaire survey of physicians who treated these inpatients and asked for all inpatients' clinical records related to this *E. coli* O157 outbreak as well as outpatients' records. The survey asked about date of onset, result of stool culture examination, initial symptoms and results of urine and blood tests. Of the 684 inpatients school children, 605 valid replies were received (88·5% recovery). There were no significant differences according to age and gender between the 605 respondents and the 79 non-respondents.

We diagnosed HUS on the basis of the criteria by the Japanese Society for Pediatric Nephrology [16]. A complete HUS case was defined as a patient who satisfied all the following laboratory criteria: (1) haemoglobin $\leqslant 10$ mg/dl, (2) platelets $\leqslant 100000/\mu$l and (3) 50% increase in serum creatinine over the upper limit of the reference range for age. Those satisfying one or two of these criteria were classified as a case of incomplete HUS. There were 106 HUS cases (17·5%), of which 36 inpatients represented complete cases (34·0%) and 70 inpatients incomplete (66·0%); 499 cases with *E. coli* O157 infection were diagnosed as non-HUS (82·5%). The day of HUS onset was defined as the day when all of the three criteria for HUS were met.

Of the 605 inpatients, 524 (86·6%) had their stools examined, and 300 (57·3%) tested positive for *E. coli* O157 culture (Fig. 1). In order to enhance the validity of our study, incomplete HUS cases were excluded from the analysis, and both inpatients with complete HUS and those non-HUS from whose gut *E. coli* O157 was cultured were selected as study subjects. There were 36 complete HUS patients and 256 non-HUS patients who tested positive for *E. coli* O157 culture.

We defined the first day of illness as the day when diarrhoea first occurred. Of those 256 patients, there were 4 patients whose onset date of diarrhoea was not sure. Thus, the population of this study consisted of 288 inpatients, 36 of whom represented complete HUS and 252 tested positive for *E. coli* O157 culture. There were no statistical differences in terms of age, gender and symptoms between the 288 analysed cases and the 317 non-analysed cases.

To identify risk factors for the development of HUS

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among the inpatients, inpatients who had developed HUS were compared with those who had not, and clinical characteristics present at any time before HUS developed were examined. We also analysed clinical symptoms, which consisted of fever, diarrhoea, bloody diarrhoea, abdominal cramps and vomiting, as well as laboratory data comprising C reactive protein (CRP) level, white blood cell (WBC) count, lactate dehydrogenase (LDH) activity, platelet count and proteinuria content. Urinary protein excretion was assessed with reagent strips.

Pearson's χ^2 test was used to test for differences in frequency distributions and proportions, and Fisher's exact test was used when the expected value was less than 5. All continuous data were compared by means of the two-tailed t test. Sensitivity and specificity for each test were each calculated over a range of cut-off points (CRP value, every 0·2 mg/dl from 0·2 to 3·0 mg/dl; WBC count, every $1{\cdot}0 \times 10^9/1$ from 5·0 to $20{\cdot}0 \times 10^9/1$; temperature, every 0·5 °C from 36·5 to 40·0 °C). The accuracy of the tests was compared by means of receiver operating characteristic (ROC) curves. If the test results are based on chance alone, the proportion of true-positive and false-positive results will be the same and the curve will be a 45° straight line. Comparison is based on the measurement of the area under the curve (AUC) of ROC. Tests with greater AUCs are considered superior when ROC curves of tests do not overlap [17–19]. The AUC and standard error for the tests were measured with the method of Hanley and McNeil [17–19], and any significant difference among AUCs was examined further.

All calculated P-values are shown as two-tailed. A P-value less than 0·05 was considered to be significant. We analysed all data with SPSS 7.5.1 J for Windows.

RESULTS

Of the 288 inpatients, 36 represented complete HUS and 252 tested positive for *E. coli* O157 culture (Fig. 1). Females accounted for 150 (52·1%) and the mean age (±S.D.) of the inpatients was 8·28 (±1·66) years (range 6–11). Of the 36 inpatients with HUS, 23 (63·9%) were female and the mean age was 7·89 (±1·79) years. Inpatients with HUS did not differ significantly from those without HUS with regard to gender ($P = 0{\cdot}130$) or age ($P = 0{\cdot}161$).

The onset of HUS occurred between the 5th and the 13th day of illness. The number of inpatients with HUS increased relatively slowly until the 6th day of illness but from the 7th day on it increased rapidly. In fact, most of the HUS inpatients (94·4%) developed the complication between the 7th and 13th day of illness. To determine early predictors of HUS, therefore, we evaluated the risk of HUS on the basis of clinical symptoms and laboratory findings observed and measured within the first 5 days of illness.

Inpatients with HUS were significantly more likely than those without HUS to report having had fever and vomiting (Table 1). Within the first 5 days of illness, fever within 3 days of the onset was the only symptom significantly associated with the risk of HUS. Over 90% of all the inpatients had bloody diarrhoea and abdominal cramps within the first 5 days of illness.

The mean CRP level was significantly higher for inpatients with HUS than without from the second day on, and kept at a high level (approx. $2{\cdot}0 \sim 3{\cdot}0$ mg/dl), but remained at a lower level ($< 1{\cdot}3$ mg/dl) among those without HUS (Fig. 2). The mean WBC count continued to rise within the first 4 days of illness and kept at a high level (approx. $13{\cdot}0 \times 10^9/1$) in inpatients with HUS, but decreased and remained at a lower level (less than $10{\cdot}0 \times 10^9/1$) among those without HUS. On the fourth day of illness and afterwards, the mean WBC count was significantly higher in HUS patients. The mean temperature on the first day of illness was also significantly higher for those with HUS than those without HUS.

Figure 3 shows ROC curves for the highest CRP level, WBC count and temperature within the first 5 days of illness. The AUC±S.E. for the CRP level ($0{\cdot}823 \pm 0{\cdot}136$) was larger than those for WBC count ($0{\cdot}734 \pm 0{\cdot}072$) and for temperature ($0{\cdot}679 \pm 0{\cdot}108$), although the differences did not achieve statistical significance. Compared with inpatients without HUS, those with HUS had a higher maximum CRP level (mean±S.D.: $3{\cdot}3 \pm 2{\cdot}3$ mg/dl *vs.* $1{\cdot}4 \pm 2{\cdot}0$ mg/dl; $P < 0{\cdot}01$), a higher maximum WBC count (mean±S.D.: $13{\cdot}4 \pm 6{\cdot}2 \times 10^9/1$ *vs.* $9{\cdot}7 \pm 3{\cdot}2 \times 10^9/1$; $P < 0{\cdot}01$) and a higher maximum temperature (mean±S.D.: $38{\cdot}0 \pm 0{\cdot}7$ °C *vs.* $37{\cdot}6 \pm 0{\cdot}6$ °C; $P < 0{\cdot}01$), within the first 5 days of illness.

Within the first 5 days of illness, clinical features associated with inpatients who developed HUS included a CRP level higher than 1·2 mg/dl (OR 44·26; 95% CI, 5·83–336·23), a WBC count greater than $11{\cdot}0 \times 10^9/1$ (OR 5·03; 95% CI 2·13–11·87) and a temperature higher than 38·0 °C (OR 5·00; 95% CI 2·25–11·08).

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Table 1. *Cumulative frequency of symptoms during* E. coli *O157 infection according to day of illness* (%)

			Day of illness								
Symptom	HUS	*n*	1	2	3	4	5	6	7	8+	Total†
Fever	Yes	36	77·8**	83·3**	83·3*	83·3	86·1	94·4*	94·4*	94·4	94·4
	No	244	38·1	53·7	65·2	74·6	77·9	78·3	79·1	81·1	82·0
Bloody	Yes	36	41·7	58·3	75·0	91·7	94·4	97·2	100·0	100·0	100·0
diarrhoea	No	248	29·0	59·7	81·5	90·3	91·5	91·9	92·3	93·1	93·1
Abdominal	Yes	35	65·7	82·9	94·3	100·0	100·0	100·0	100·0	100·0	100·0
cramps	No	251	73·7	87·6	94·4	97·6	98·0	98·0	98·0	98·8	98·8
Vomiting	Yes	35	11·4	25·7	37·1	48·6	51·4	65·7*	68·6**	82·9**	85·7**
	No	229	15·7	27·5	38·9	43·2	43·7	44·5	45·0	45·9	47·2

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$ by χ^2 test or Fisher's exact test.

† Cases for which onset day was not clear were included in total.

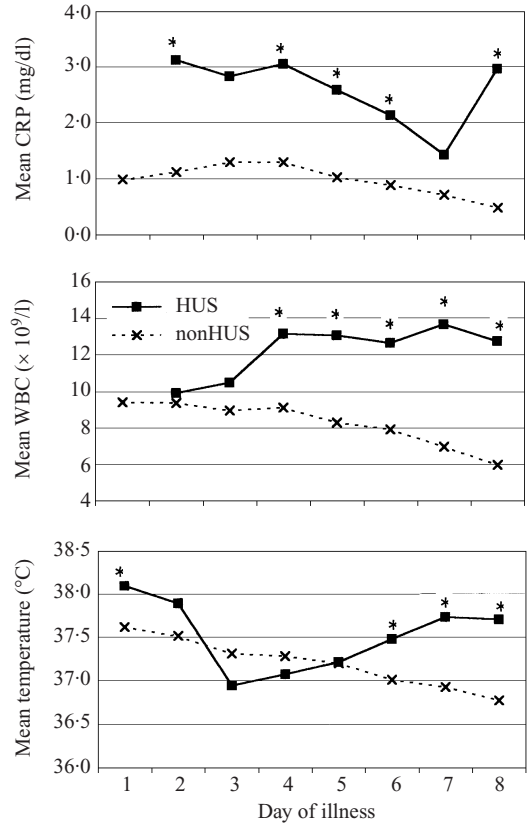

Fig. 2. Clinical characteristics by day of illness among inpatients with HUS (boxes) and among those without complications (crosses). * $P < 0{\cdot}05$ by the two-tailed t test.

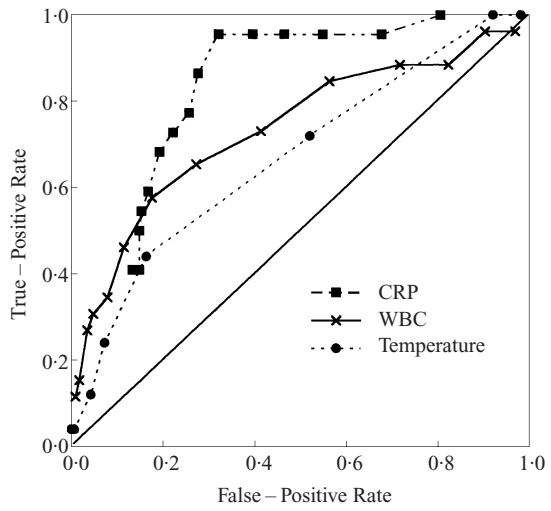

Fig. 3. Receiver operating characteristic curves for the highest CRP, WBC and temperature within the first 5 days of illness. The diagonal line indicates the equality of false–positive rate and true–positive rate.

We used the presenting factors observed within the first 5 days of illness to develop a multifactorial assessment of the development of HUS. A score of 0 or 1 was assigned to each selected factor, depending on its value as specified in Table 2, and the scores were added to give a predictor index of 0–3. The predictor index (mean $\pm$ S.D.) was $2{\cdot}1 \pm 0{\cdot}8$ for inpatients with HUS and $0{\cdot}8 \pm 0{\cdot}9$ for those without HUS ($P < 0{\cdot}01$). Furthermore, inpatients with predictor index scores of 2 points or more significantly were more likely to developed HUS than those with scores of less than 2 points (OR 26·07; 95% CI 5·60–121·27; sensitivity 0·88; specificity 0·79).

Tabel 2. *Predictor Index Scoring System**

Factor	Value for score of 0	Value for score of 1
CRP value (mg/dl)	$< 1{\cdot}2$	$\geqslant 1{\cdot}2$
WBC count ($\times 10^9$/l)	$< 11{\cdot}0$	$\geqslant 11{\cdot}0$
Temperature (°C)	$< 38{\cdot}0$	$\geqslant 38{\cdot}0$

* Score 0 or 1 for each factor as listed and add to give a total index of 0–3. The factor values used are those noted at presentation.

The difference between the day when one of the three criteria for HUS was met and the day of HUS onset (mean $\pm$ S.D.) was $2{\cdot}5 \pm 2{\cdot}0$ days. The increase in LDH activity and the proportion of those with proteinuria of 2+ or more were both relatively small before the day when one of the three criteria for HUS was met, but on that day they markedly increased from less than 520 U/l to more than 2000 U/l in LDH activity, and from less than 15% to more than 70% in proteinuria of 2+ or more (Fig. 4). Conversely, the decrease in platelet count was relatively small before the day when one of the three criteria for HUS was met, but on that day it markedly decreased from more than $22{\cdot}0 \times 10^4$/ml to less than $7{\cdot}0 \times 10^4$/ml. On the other hand, LDH activity of those without HUS kept at a low level (less than 450 U/l), the proportion of those with proteinuria of 2+ or more also remained at a low level (less than 10%) and platelet count remained at a high level ($> 25{\cdot}0 \times 10^4$/ml) within the first 10 days of illness.

DISCUSSION

Kawamura and Colleagues [20] reported that there was no significant difference between patients with and without HUS with respect to fever. From the point of the day of illness, in this study, fever within the first 3 days of illness was the only symptom which was identified as having predictive value for the development of HUS. Bell and Colleagues [11] and

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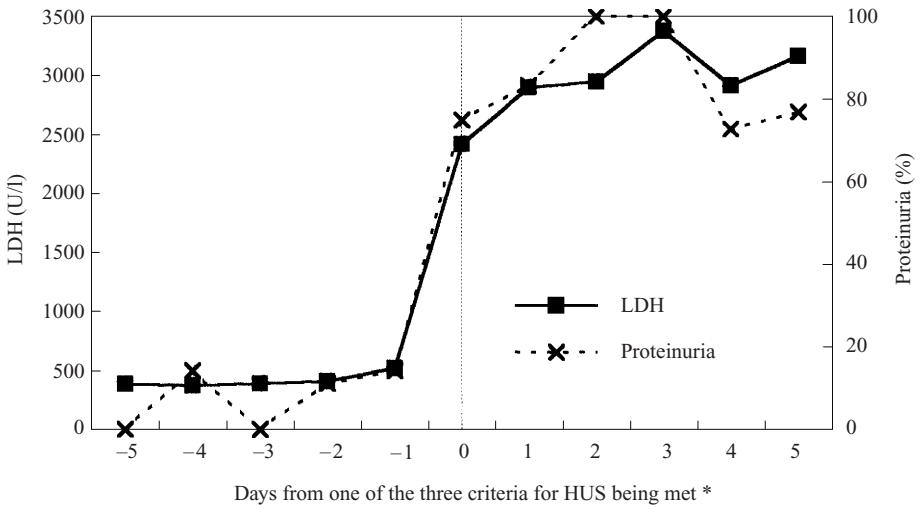

Fig. 4. Lactate dehydrogenase (LDH) activity and the proportion of those with proteinuria 2+ or more among inpatients with HUS by days from one of the three criteria for HUS being met. * Day 0 represents the day when one of the three criteria for HUS was met.

Oshima [21] reported that vomiting was the sign associated with HUS risk, but our results indicated that vomiting within the first 5 days of illness did not show any such significance. The reasons that bloody diarrhoea, abdominal cramps and vomiting are not significantly associated with the risk of HUS at this stage are that most patients, with and without HUS, had bloody diarrhoea and abdominal cramps, and vomiting occurred at a later stage in patients with HUS.

As for laboratory findings, Pavia and Colleagues [10] found that patients with HUS had markedly higher leucocyte counts than those without HUS from the third day of illness and that the leucocyte count peaked on the fourth day of illness and more than 24 h before signs of HUS appeared. We found similar results. On and after the fourth day of illness, the WBC count for those with HUS was significantly higher than for those without HUS. Kawamura and Colleagues [20] and Oshima [21] reported that CRP value was significantly higher in the patients with HUS. We also demonstrated that an elevated CRP value was strongly associated with the development of HUS, and showed the CRP value by day of illness which has not been previously reported.

These factors such as fever, WBC count and CRP level should help clinicians to identify patients with *E. coli* O157 infection who are at high risk of developing HUS. We tried to compare the value of these three factors using the ROC analysis, but the differences did not achieve statistical significance. Furthermore, we suggested the threshold of these factors using ROC curve: CRP $\geqslant$ 1·2 mg/dl, WBC $\geqslant 1{\cdot}1 \times 10^9/1$ and temperature $\geqslant$ 38·0 °C.

A predictive index using a combination of presenting factors was developed. The fact that the predictor index is based on presenting factors means that it could be a useful clinical tool that would be able to facilitate prediction of the development of HUS. A predictor index of 2 points or more was found to be a moderately sensitive predictor for the development of HUS.

In our study, 14 (32·6%) of 43 inpatients with predictor index scores of 2 points or more within the first 5 days of illness subsequently developed HUS compared with 2 (1·8%) of 110 inpatients with predictor index scores of less than 2 points. Assessment of inpatients with *E. coli* O157 infection by means of this multifactorial approach should allow the physician to identify rapidly those patients who are most at risk of developing HUS.

A marked increase in LDH activity and proteinuria of 2+ or more, and a sudden decrease in platelet count occurred on the day when one of the three criteria for HUS was met. These laboratory findings therefore may not represent predictive factors but diagnostic values, because LDH activity and proteinuria changed at the same time as platelet count (one of the criteria of HUS) a few days before the HUS onset.

Our study has several limitations. First, all primary cases in this study were hospitalized school children; this meant that we could not evaluate predictors for the development of HUS for outpatients or other age

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groups. Second, our data pertain only to this outbreak in Sakai City. Perhaps other risk factors apply to patients who are ill after ingestion of other infecting strains Third, some patients were admitted to the hospital on a holiday, as a result their stools were not tested during the early period of illness. Furthermore, antimicrobial agents were prescribed during the same period. These factors might have resulted in a low positive rate for *E. coli* O157 culture. The positive rate for *E. coli* O157 culture was 57·3 %, which appears to be a rather low rate and may have skewed our result.

In conclusion, a CRP level higher than 1·2 mg/dl, a WBC count higher than $11{\cdot}0 \times 10^9/1$ and a temperature higher than 38·0 °C within the first 5 days of illness might be predictive factors for the development of HUS in patients with *E. coli* O157 infection. Patients who have two or all of these factors should be observed closely for 5–13 days after onset of illness.

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