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MAJOR ARTICLE

Predicting Hemolytic Uremic Syndrome and Renal Replacement Therapy in Shiga Toxin–producing *Escherichia coli*–infected Children

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Background. Shiga toxin–producing *Escherichia coli* (STEC) infections are leading causes of pediatric acute renal failure. Identifying hemolytic uremic syndrome (HUS) risk factors is needed to guide care.

Methods. We conducted a multicenter, historical cohort study to identify features associated with development of HUS (primary outcome) and need for renal replacement therapy (RRT) (secondary outcome) in STEC-infected children without HUS at initial presentation. Children aged <18 years who submitted STEC-positive specimens between January 2011 and December 2015 at a participating study institution were eligible.

Results. Of 927 STEC-infected children, 41 (4.4%) had HUS at presentation; of the remaining 886, 126 (14.2%) developed HUS. Predictors (all shown as odds ratio [OR] with 95% confidence interval [CI]) of HUS included younger age (0.77 [.69–.85] per year), leukocyte count ≥13.0 × 10^3/μL (2.54 [1.42–4.54]), higher hematocrit (1.83 [1.21–2.77] per 5% increase) and serum creatinine (10.82 [1.49–78.69] per 1 mg/dL increase), platelet count <250 × 10^3/μL (1.92 [1.02–3.60]), lower serum sodium (1.12 [1.02–1.23

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per 1 mmol/L decrease), and intravenous fluid administration initiated ≥4 days following diarrhea onset (2.50 [1.14–5.46]). A longer interval from diarrhea onset to index visit was associated with reduced HUS risk (OR, 0.70 [95% CI, .54–.90]). RRT predictors (all shown as OR [95% CI]) included female sex (2.27 [1.14–4.50]), younger age (0.83 [.74–.92] per year), lower serum sodium (1.15 [1.04–1.27] per mmol/L decrease), higher leukocyte count ≥13.0 × 10^3/μL (2.35 [1.17–4.72]) and creatinine (7.75 [1.20–50.16] per 1 mg/dL increase) concentrations, and initial intravenous fluid administration ≥4 days following diarrhea onset (2.71 [1.18–6.21]).

Conclusions. The complex nature of STEC infection renders predicting its course a challenge. Risk factors we identified highlight the importance of avoiding dehydration and performing close clinical and laboratory monitoring.

Keywords. Shiga-toxigenic *Escherichia coli*; hemolytic uremic syndrome; renal replacement therapy; emergency service; child.

Acute bloody diarrhea is a medical emergency that commonly prompts visits to emergency departments (EDs) and is present in up to 15% of children with diarrhea who seek ED care [1]. Shiga toxin–producing *Escherichia coli* (STEC) and, in particular, *E. coli* O157:H7 [2] are the most concerning pathogens in this scenario, because of their ability to cause hemolytic uremic syndrome (HUS), which consists of nonimmune hemolytic anemia, thrombocytopenia, and azotemia [3]. The risk of poor outcomes in STEC infections is not trivial: *E. coli* O157:H7, the most frequently recovered STEC in EDs [4], causes HUS in approximately 15% of infected children <10 years of age [5].

STEC-infected children frequently present to North American EDs [6]. On this first visit, critical decisions might influence disease course, which evolves over several days to either HUS or spontaneous resolution. Individual physicians rarely develop experiential knowledge about these uncommon infections and must rely on published evidence. Some risk factors for the development of HUS [5, 7, 8] and need for renal replacement therapy (RRT) among those who already have HUS [9–11] have been identified. However, the paucity of studies relating early illness characteristics to these outcomes perpetuates the assumption that little can be done to change disease course [12]. Here, we present a multicenter, multinational, ED-based, historical cohort study of STEC-infected children to identify modifiers of disease severity.

METHODS

Study Design and Setting

Thirty-eight tertiary care pediatric EDs in 21 US states and 6 Canadian provinces participated (Supplementary Table 1). The protocol was endorsed by Pediatric Emergency Research Canada (https://perc-canada.ca/) [13] and the Pediatric Emergency Medicine Collaborative Research Committee (PEMCRC; http://pemcrc.pemfellows.com/). All sites obtained institutional review board approval of waiver of informed consent and data-sharing agreements with the coordinating institution and the PEMCRC data center (Baylor School of Medicine).

Study Population

We queried each institution's microbiology databases to identify all children <18 years of age who had STEC detected in their stools between 1 January 2011 and 31 December 2015. Microbiologic testing included screening specimens on chromogenic and selective agar with serologic confirmation of candidate colonies, polymerase chain reaction directly on specimens or after overnight growth, and/or Shiga toxin detection on broth culture of stool by enzyme immunoassay and varied by institution and over time. We analyzed records of STEC-positive patients who visited the study institution ED and reviewed the records of children who returned up to 30 days later. Children admitted directly to inpatient units were excluded because of incomplete prehospital data.

Data Sources, Collection, and Extraction

After standardized training, site investigators reviewed and systematically entered medical data into a REDCap (Research Electronic Data Capture) database [14]. The manual of operations detailed all data extraction procedures.

Objectives and Outcome Measures

Our primary and secondary objectives were to identify independent risk factors for development of HUS among STEC-infected children who did not have HUS at the index visit, and for use of RRT if and when HUS developed [15, 16]. We also sought to quantify the frequency of other interventions and complications and to identify clinically relevant cut-points for laboratory variables that might predict HUS: serum sodium, lactate dehydrogenase (LDH) [17] and creatinine concentrations, platelet and peripheral white blood cell (WBC) counts [18], and hematocrit [11].

Definitions

Employing a validated case definition [19], we classified participants as having HUS if each the following was present [5]: hematocrit ≤30%, platelet count ≤150 × 10^3/μL, and serum creatinine concentration above the upper limit of normal for age [20]. We defined RRT as hemodialysis (including continuous veno-veno hemodialysis) or peritoneal dialysis. We recorded as present or absent neurologic (ie, seizures, stroke, coma), respiratory (ie, pulmonary edema, acute respiratory distress syndrome), gastrointestinal (ie, pancreatitis, intussusception, laparotomy, bowel resection), cardiovascular (ie, arrhythmias, myocardial depression), and/or infectious (ie, bacteremia, peritonitis, urinary tract infection, abscess) complications and in-hospital mortality. We also identified use of erythrocyte

and/or platelet transfusions, therapeutic plasma exchange, eculizumab infusion, endotracheal intubation, central line insertion, and intensive care unit (ICU) admission.

We defined the index ED visit as the first visit to the participating site related to the STEC infection. We defined the first days of illness and of HUS as the first calendar day of diarrhea and the calendar day on which all laboratory criteria for HUS were met, respectively [21]. We categorized intravenous fluid (IVF) administration in the ED as yes/no. We also calculated the interval from the first day of diarrhea to the first IVF administration, and based on earlier work, a priori dichotomously evaluated initial IVF administration as occurring before vs on or after day 4 of illness [21, 22]. We considered nondocumentation of antibiotic administration, fever, dark urine, diarrhea, bloody diarrhea, vomiting, swelling/edema, delayed capillary refill, jaundice, bruising or petechiae, crackles on auscultation, and abdominal tenderness to imply "not present" [23, 24]. We employed explicit terminology to classify participants as ill-appearing, dehydrated, or in respiratory distress [25]. We defined anuria as absence of urine output for >12 hours [26].

Statistical Analysis

For the primary outcome, we compared characteristics of those who developed HUS with those who did not. Categorical and continuous variables were compared between groups using χ^2 and Mann-Whitney *U* tests, respectively. Confidence intervals (CIs) around frequencies were determined using the binomial exact method. Median differences of WBC and platelet counts, hematocrit values, and serum creatinine, sodium, and LDH concentrations were computed with the Hodges-Lehmann estimate based on the Wilcoxon rank-sum test of the difference between medians.

For regression models, we used complete case analysis. We initially performed a mixed-effect, multilevel logistic regression to account for clustering by center with study site as the random-effect intercept, but the negligible intracluster correlation coefficient (0.04 for HUS; 0.05 for RRT) implied minimal grouping effect. Therefore, we used logistic regression to estimate independent associations between HUS and a priori hypothesized risk factors: age, sex, presence of vomiting, hematochezia, dehydration, antibiotic administration before HUS diagnosis, index ED visit hematocrit, WBC and platelet count, serum creatinine and sodium, and duration of diarrhea before the first ED visit and to IVF administration. Covariates were tested for co-linearity. If present, the covariate with the greatest significance in the original model was retained. We included WBC and platelet counts as dichotomous covariates employing 13.0 × 10^3/μL and 250 × 10^3/μL as cut-points, respectively. LDH was not included in the model because it was relatively infrequently performed. We used the −2 log likelihood statistic and the Hosmer-Lemeshow test to evaluate the goodness of fit.

RRT use was analyzed as described for the primary outcome. STEC-associated complications were summarized using counts and percentages with 95% CIs. For laboratory parameter cut-points, we plotted receiver operating characteristic curves with HUS outcome status, and we used the Youden index and distance to corner values on the curve to identify cutoffs.

A 2-sided α of .05 was used. To control for false discovery, we corrected *P* values using the Benjamini-Hochberg method within test sets [27]. Data were analyzed using SPSS version 24.0 for Windows (SPSS Inc, Chicago, Illinois) and Stata version 15.0 (StataCorp, College Station, Texas) software.

RESULTS

Population, Testing, and STEC Serotype

Stool from 704 of 927 (75.9%) cases (Figure 1) underwent Shiga toxin testing and culture; 216 (23.3%) and 7 (0.8%) underwent only culture and Shiga toxin testing, respectively. Among the cultured stools, 79.9% (735/920) contained an STEC isolate, and 18.8% (173/920) and 1.3% (12/920) were culture negative or had no results available, respectively. Forty-one of 927 children (4.4% [95% CI, 3.2%–6.0%]) presented with established HUS and were excluded from primary and secondary outcome analyses. *Escherichia coli* O157:H7 accounted for 79.0% (132/167) of HUS cases (Supplementary Figure 1).

Hemolytic Uremic Syndrome

Hemolytic uremic syndrome developed in 126 of the 866 (14.2% [95% CI, 12.0%–16.7%]) children who did not have HUS at the index visit, after a median of 3 days (interquartile range [IQR], 2–5 days); of these, 35 (27.8% [95% CI, 20.2%–36.5%]) returned with HUS after initial discharge. STEC-infected children who subsequently developed HUS were more likely to report fever, reduced oral intake and urine output, hematochezia, vomiting, and abdominal pain and were younger, more likely to appear ill, considered to be dehydrated, and to have received antibiotics than those who did not (Table 1). Patients who developed HUS after the index ED visit also had higher first-visit median WBC counts (14.8 vs 10.6 × 10^3/μL; difference, 4.4 [95% CI, 3.4–5.5]) and serum LDH concentrations (802 vs 242 U/L; difference, 525 [95% CI, 109–2306]) compared with those who did not (Table 2; Figure 2).

Covariates independently associated with HUS in regression analysis (Table 3) included younger age (odds ratio [OR], 0.77 [95% CI, .69–.85] per year increase), higher hematocrit (OR, 1.83 [95% CI, 1.21–2.77] per 5% increase), WBC count ≥13.0 × 10^3/μL (OR, 2.54 [95% CI, 1.42–4.54]), and platelet count <250 × 10^3/μL (OR, 1.92 [95% CI, 1.02–3.60]). IVF started on or after day 4 of diarrhea compared to those who received no IVF or for whom it was administered before day 4 of diarrhea was associated with an increased risk of HUS (OR, 2.50 [95% CI, 1.14–5.46]). The later a child presented for ED care after

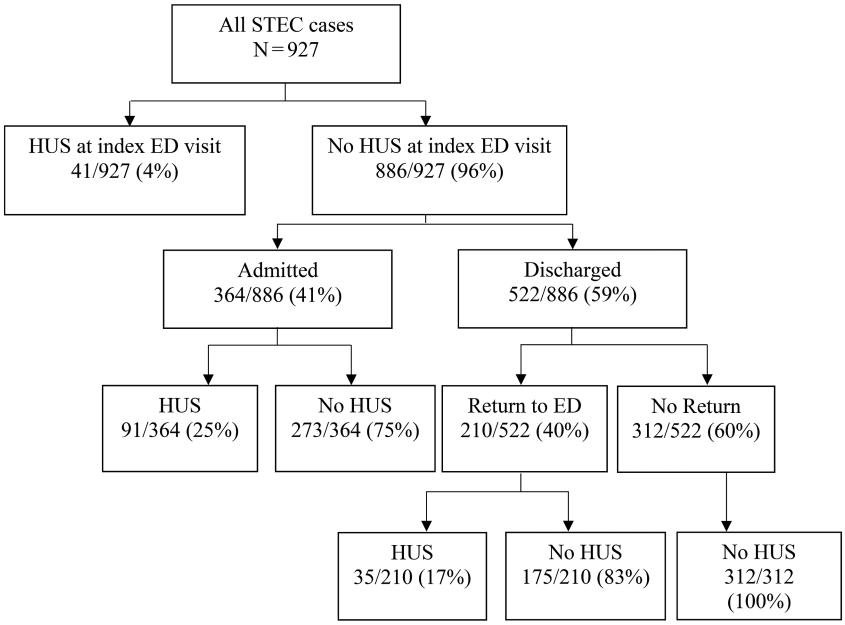

Figure 1. Flow diagram of study participants, including development of hemolytic uremic syndrome and emergency department disposition. Abbreviations: ED, emergency department; HUS, hemolytic uremic syndrome; STEC, Shiga toxin–producing *Escherichia coli*.

diarrhea onset, the less likely they were to develop HUS (OR, 0.70 [95% CI, .54–.90]). Other covariates independently associated with increased HUS risk included higher serum creatinine and lower serum sodium values.

Renal Replacement Therapy

Ninety-four of 927 (10.1% [95% CI, 8.3%–12.3%]) children underwent RRT, including 74 of 886 (8.4% [95% CI, 6.7% 10.4%]) without HUS at initial presentation. Of the latter group of 74 children, 15 (20.3% [95% CI, 12.2%–31.5%]) were discharged to home following the index ED visit. RRT was independently associated with female sex (OR, 2.27 [95% CI, 1.14–4.50]), younger age (OR, 0.83 [95% CI, .74–.92] per year increase), and noninitiation of IVFs before day 4 of diarrhea (OR, 2.71 [95% CI, 1.18–6.21]) compared with those who did not receive IVF or for whom it was administered before this point in illness (Table 3). Other covariates independently associated with RRT use included higher WBC count and serum creatinine and lower serum sodium concentrations.

Complications

Complications are shown in Supplementary Table 2. Of the 167 participants with HUS, 32 (19.2%) were admitted to the ICU from the ED including 26 (15.6%) who required mechanical ventilation. In addition, 144 (86.2%) children received erythrocyte transfusions and 50 (29.9%) received platelet transfusions. Two children (1.2%) with HUS died during hospital admission. Eight of the 9 children who received eculizumab required RRT, and 1 of these died. Complications were uncommon among children without HUS. There was no difference between sites in the provision rates of antibiotics or IVFs in participants with STEC infection, or in the provision rates of RRT in participants who developed HUS (Supplementary Tables 1, 3, and 4).

Laboratory Parameters

In the bivariate analyses, only WBC and LDH concentration predicted HUS development. The optimal index ED visit cut-points to predict development of HUS were 13.0 × 10^3/μL and 275 U/L for WBC count and LDH concentration, respectively. These values had the following sensitivities and specificities: 63.9% (95% CI, 54.0%–72.8%) and 71.9% (95% CI, 67.6%–75.8%), respectively, for WBC count, and 77.8% (95% CI, 51.9%–92.6%) and 63.4% (95% CI, 46.9%–77.4%), respectively, for LDH (Supplementary Figure 2).

DISCUSSION

Our findings reinforce the notion that EDs are crucial venues for care of children at risk of developing HUS. Specifically, 1 in 7 STEC-infected children without HUS at the index visit developed HUS within the next week. It is concerning that nearly 30% of those who developed HUS did so after being discharged following their index ED evaluation, possibly because the laboratory features of HUS (ie, anemia, thrombocytopenia, and azotemia) do not clearly identify those who will develop HUS. Notably, a disproportionate number of children in this returning group required RRT when HUS ensued.

Our venue-based study confirmed several risk factors for development of HUS, including younger age [28, 29] and higher WBC count [5, 30]. Although prior work suggests that

Table 1. Clinical Characteristics of Subjects Without Hemolytic Uremic Syndrome at the Index Emergency Department Visit

	No HUS Following the Index ED Visit (n = 760)		Developed HUS Following the Index ED Visit (n = 126)		
Characteristic	No. With Data Available	No. (%)	No. With Data Available	No. (%)	*P* Value[a]
Categorical variables					
Sex, female	760	349 (45.9)	126	70 (55.6)	.05
Month of index visit	760		126		
January–March		83 (10.9)		14 (11.1)	.69
April–June		183 (24.1)		29 (23.0)	
July–September		364 (47.9)		56 (44.4)	
October–December		130 (17.1)		27 (21.4)	
Travel outside US/Canada past 30 d (yes)	527	37 (7.0)	87	4 (4.6)	.49
NSAID use past 2 wk (yes)	228	52 (22.8)	42	9 (21.4)	>.99
Fever (yes)	760	259 (34.1)	125	63 (50.4)	<.001
Decreased urine output (yes)	464	119 (25.6)	90	45 (50.0)	<.001
Anuria (yes)[b]	412	7 (1.7)	73	9 (12.3)	<.001
Diarrhea (yes)	760	739 (97.2)	125	123 (98.4)	.56
Hematochezia (yes)	759	576 (75.9)	125	107 (85.6)	.02
Vomiting (yes)	759	323 (42.6)	125	82 (65.6)	<.001
Abdominal pain (yes)	691	585 (84.7)	110	101 (91.8)	.06
Decreased oral fluid intake (yes)	553	317 (57.3)	105	78 (74.3)	.002
Ill appearance (yes)[c]	710	84 (11.8)	120	46 (38.3)	<.001
Dehydration (yes)[d]	682	164 (24.0)	117	49 (41.9)	<.001
Capillary refill time increased (yes)	760	44 (5.8)	125	17 (13.6)	.003
Abdominal tenderness (yes)	760	361 (47.5)	125	69 (55.2)	.12
Antibiotics used in past 2 wk (includes ED)	760	51 (6.7)	124	12 (9.7)	.26
IVF started before HUS diagnosed	747		125		<.001
On day 1 of diarrhea		30 (4.0)		11 (8.8)	
On day 2 of diarrhea		84 (11.2)		23 (18.4)	
On day 3 of diarrhea		128 (17.1)		28 (22.4)	
On or after day 4 of diarrhea		223 (29.9)		63 (50.4)	
No IVF		282 (37.8)		0 (0)	
Continuous variables	No. in Analysis	Median (IQR)	No. in Analysis	Median (IQR)	
Age, mo	760	79.5 (35.6–141.3)	126	56.8 (33.0–91.0)	<.001
Weight, kg	751	21.8 (14.2–41.0)	126	17.8 (13.4–25.1)	<.001
Days of illness at presentation	741	2.9 (1.9–4.4)	125	2.8 (1.6–3.6)	.02
Days of bloody diarrhea at presentation	565	1.2 (0.6–1.9)	113	0.9 (0.5–1.8)	.14
Days of diarrhea at presentation	738	2.9 (1.9–4.4)	125	2.8 (1.6–3.6)	.01
Maximum temperature in ED, °C	722	37.0 (36.7–37.3)	121	37.1 (36.8–37.5)	.03
Systolic blood pressure, mm Hg	672	113 (103–123)	116	112 (103–122)	.63
Diastolic blood pressure, mm Hg	668	70 (62–78)	115	73 (65–80)	.02
Heart rate, beats/minute, age group					
<1.0 y	40	132 (120–140)	2	131 (127–131)	.89
1.0 to <2.0 y	81	130 (113–140)	20	132 (120–150)	.13
2.0 to <5.0 y	183	115 (101–125)	46	124 (112–140)	.002
5.0 to <11.0 y	228	97 (86–112)	47	112 (96–125)	<.001
11.0 to <18.0 y	220	88 (78–100)	11	99 (72–110)	.67
Respiratory rate, breaths/min, age group					
<1.0 y	40	32 (28–38)	2	33 (28–33)	.88
1.0 to <2.0 y	79	28 (24–32)	20	29 (24–32)	.60
2.0 to <5.0 y	178	24 (22–26)	45	24 (23–28)	.12
5.0 to <11.0 y	227	20 (20–24)	47	22 (20–24)	.007
11.0 to <18.0 y	219	20 (18–20)	11	20 (18–20)	.99

Abbreviations: ED, emergency department; HUS, hemolytic uremic syndrome; IQR, interquartile range; IVF, intravenous fluids; NSAID, nonsteroidal anti-inflammatory drug; US, United States.

[a] $P < .03$ was considered statistically significant with *P* value adjustment via Benjamini-Hochberg procedure for multiple comparisons (n = 36) [27].

[b] No urine output for >12 hours.

[c] Sick, toxic, shocky, decreased mental status, lethargic, unresponsive, irritable, fussy, inconsolable, not looking well, poor or decreased pulses, decreased pulses, or other similar terms.

[d] Dehydrated, dry-appearing, dry mucous membranes, tented skin, sunken eyes, decreased perfusion, or other similar terms.

Table 2. Laboratory Characteristics of Subjects Without Hemolytic Uremic Syndrome at the Index Emergency Department Visit[a]

	No HUS Following the Index ED Visit (n = 760)		Developed HUS Following the Index ED Visit (n = 126)		
Characteristic	No. Performed	Median (IQR)	No. Performed	Median (IQR)	*P* Value[b]
WBC count, $\times 10^3/\mu L$	484	10.6 (8.7–13.2)	108	14.8 (11.8–21.0)	<.001
Hemoglobin, g/dL	484	13.7 (12.6–14.7)	109	14.0 (12.6–15.0)	.28
Hematocrit, %	483	39.7 (37.0–43.0)	109	40.7 (37.2–43.0)	.25
Platelet count, $\times 10^3/\mu L$	481	270 (230–329)	106	270 (207–332)	.45
Lactate dehydrogenase, U/L	41	242 (190–293)	18	802 (282–4103)	<.001
Serum sodium, mEq/L	454	138 (136–140)	103	136 (133–138)	<.001
Serum bicarbonate, mEq/L	422	22 (20–24)	101	20.0 (18–23)	<.001
Serum creatinine, mg/dL	455	0.4 (0.3–0.6)	102	0.4 (0.3–0.6)	.71
Blood urea nitrogen, mg/dL	440	9.0 (7.0–12.0)	101	11.0 (8.0–14.3)	<.001
Erythrocyte sedimentation rate, mm/h	124	10 (6–15)	21	8 (6–13)	.22
C-reactive protein, mg/dL	174	2.0 (1.0–5.3)	35	5.6 (1.6–33.3)	.002
Urine protein positive,[c] No. (%)	242	78 (32.2)	40	19 (47.5)	.07
Urine protein ≥3+ (ie, large), No. (%)	76	8 (10.5)	17	3 (17.6)	.68
Urine blood positive[d], No. (%)	231	83 (35.5)	40	16 (40.0)	.60
Urine blood ≥3+ (ie, large), No. (%)	80	9 (11.3)	15	5 (33.3)	.04

Data are presented as median (IQR) unless otherwise indicated.

Abbreviations: ED, emergency department; HUS, hemolytic uremic syndrome; IQR, interquartile range; WBC, white blood cell.

[a]Table excludes the 41 children who had HUS at the time of the index ED visit.

[b]$P < .04$ was considered statistically significant after *P* value adjustment via Benjamini-Hochberg procedure for multiple comparisons (n = 15) [27].

[c]≥1+ protein.

[d]≥1+ blood.

intravascular volume expansion early in illness is associated with better outcomes if HUS ensues [21, 22], we identified for the first time that intravenous volume expansion on or before day 4 of illness was associated with a lower risk of HUS. The value of volume expansion may be understated because children who present early in illness have higher rates of HUS, reflecting a severe, fulminant course. This early-presenting group therefore presents a paradox: They are the patients most likely to experience HUS and require RRT, and simultaneously, their early presentation offers an opportunity to improve outcomes. Our work also identified several novel pre-HUS variables relevant to HUS severity. Notably, lower serum sodium concentration and elevated serum creatinine at presentation and failure to administer IVF before day 4 of illness were associated with an increased risk of requiring RRT in those who develop HUS. These findings are important because dehydration and relative hyponatremia are potentially correctable or avoidable pre-HUS risk factors, and there is precedent for vigorous intravascular volume expansion among children with established HUS [31].

We note that initial presentation analyses such as ours should be interpreted in light of the kinetics of progression of STEC infections to HUS. Though a highly patterned sequence of events occurs in STEC-infected individuals, culminating in HUS vs uncomplicated resolution, trajectories of deterioration are quite variable. In prospective studies, the median day of meeting the criteria for HUS is 6.5–7.0. Although diagnostic criteria are rarely met before day 5 of illness [5, 32], a diagnosis of HUS can occur as late as day 13 [5, 21]. Our finding that the risk of HUS is lower in children with a greater interval between diarrhea onset and ED presentation, an association noted previously [33, 34], suggests that a rapidly progressive early in illness course is associated with worse outcome. In infected children, fecal and circulating Shiga toxin concentrations diminish rapidly and often disappear before HUS ensues [30, 35]. Hence, it is possible that the shorter the duration to presentation, the greater the vascular injury, and that later-presenting children may be experiencing a less accelerated course. The association between later-in-illness administration of IVF and HUS development and need for RRT likely reflects the deteriorating clinical condition and the lack of IVF administration early in illness. These issues highlight the need for a prospective trial to evaluate the role of IVF early in illness.

Literature is sparse on the ability of serum LDH to predict HUS. As LDH is an enzyme expressed in erythrocytes, early intravascular hemolysis [36] might offer a pathophysiologic explanation for this finding. However, this variable was available for only 59 of the children, thereby precluding its inclusion in the regression model. Urinalyses are too insensitive to use as a screening test. Interestingly, the bivariate association between occult blood in the urine and subsequent development of HUS may reflect early hemolysis, which could also explain the elevated LDH concentrations seen at the index visit. Further studies should explore the utility of microhematuria

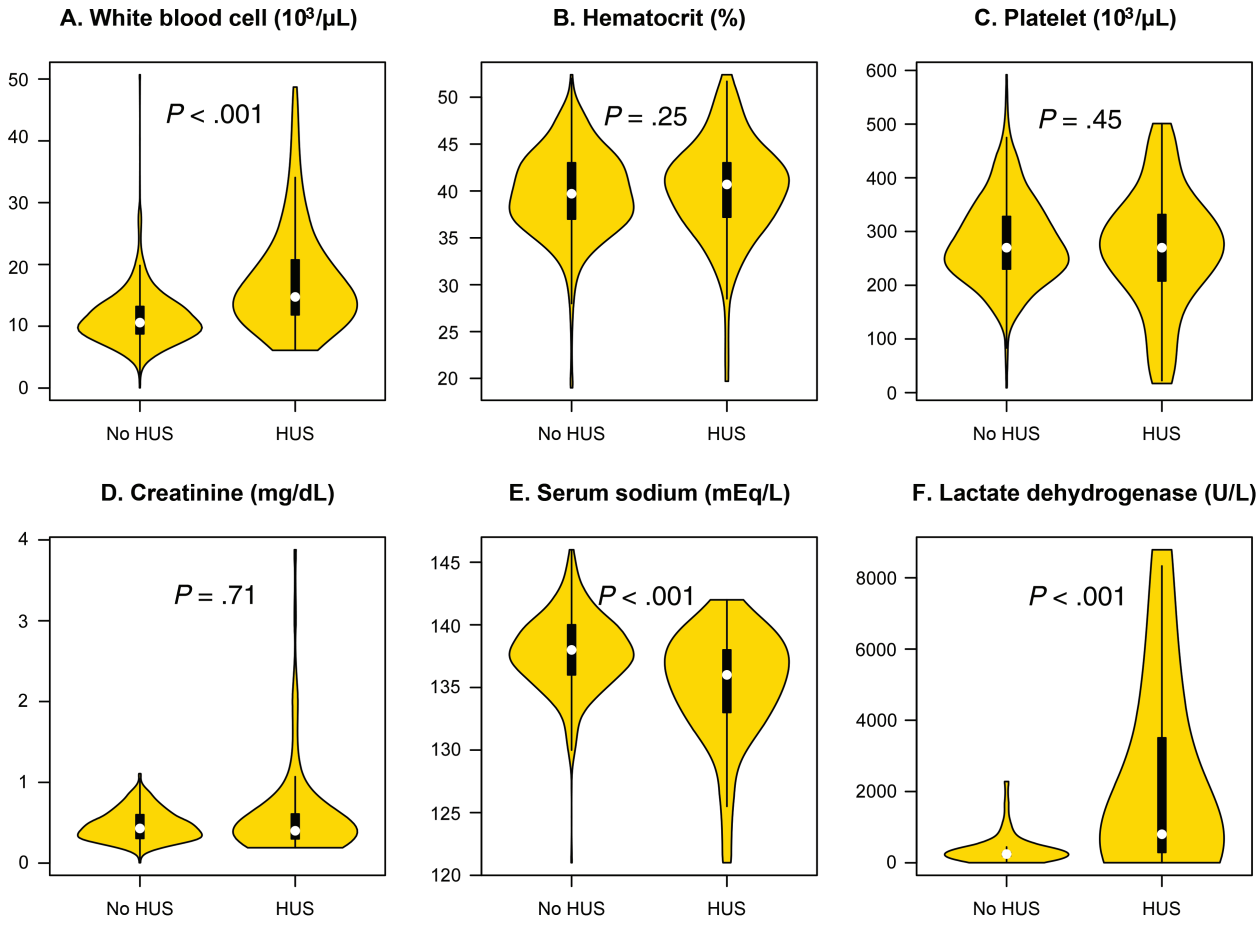

Figure 2. Violin plots of white blood cell count, hematocrit, platelet, serum creatinine, serum sodium, and lactate dehydrogenase of children who did and those who did not develop HUS after the index emergency department visit. The white dot represents the median, the black vertical bar the interquartile range, the thin vertical line the 95% confidence interval, and the yellow shape the kernel density estimation (ie, distribution of data – wider sections = higher probability). Abbreviation: HUS, hemolytic uremic syndrome.

and elevated LDH as early markers of hemolysis in patients infected with STEC. However, elevations should be interpreted cautiously, as many children who did not develop HUS had serum LDH concentrations above the upper limit of normal for age, and the day-to-day trajectories of the abnormalities we present have not been established.

Our findings also highlight the possibility that the strict definition of HUS, particularly the criteria of anemia, serves as an obstacle to the early diagnosis and management of HUS. During the pre-HUS phase, children suffering from diarrhea and vomiting are often dehydrated; consequently, the serum hemoglobin is disproportionately elevated relative to the intravascular red cell mass, yielding a value that prevents the establishment of a diagnosis of HUS. This discordance is ominous, because it reflects severe relative hemoconcentration and a poor prognosis [37]. Serum LDH concentrations may serve to identify hemolysis prior to the development of anemia, and clinicians should consider such children as having HUS [37], thereby triggering more aggressive monitoring and potentially volume expansion [21, 22, 31].

We did not include precise microbial etiology as a risk factor, even though postdiarrheal HUS is almost exclusively caused by STEC that contain a gene encoding Shiga toxin 2, a genotype that includes almost all *E. coli* O157:H7 [38]. Increasingly, clinicians have rapid access to the pathogen's genotype [39], and the presence of Shiga toxin 2 will be factored into HUS risk-assessment paradigms. The availability of such etiologic clarity at the index ED visit will obviate concerns that our study limited risk assessment only to patients who were subsequently determined to be infected with STEC. This is important because we did not include in our analyses the larger population of children with bloody diarrhea caused by pathogens that have no risk of causing HUS, such as *Campylobacter*, salmonellae, or non-*dysenteriae* shigellae.

A limitation to our study is its retrospective design, which resulted in missing data, especially day-by-day volume and content of IVF administered. Because data were likely not missing at random, we did not employ multiple imputation to overcome potential biases introduced by incomplete data, as its use would have been inappropriate [40]. Additionally, not all sites produced

Table 3. Regression Analysis: Risk Factors Associated With Development of Hemolytic Uremic Syndrome and Need for Renal Replacement Therapy

	Hemolytic Uremic Syndrome (n = 464)		Renal Replacement Therapy (n = 464)	
Risk Factor	Adjusted OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
Sex, female	1.64 (.93–2.90)	.09	2.27 (1.14–4.50)	.02
Age (per year increase)	0.77 (.69–.85)	<.001	0.83 (.74–.92)	<.001
Received antibiotics (yes)[a]	1.11 (.41–2.97)	.84	1.52 (.51–4.51)	.45
Vomiting (yes)	1.59 (.90–2.81)	.11	1.46 (.74–2.88)	.27
Time from start of diarrhea to first ED visit (per day)	0.70 (.54–.90)	.005	0.83 (.67–1.02)	.07
Hematochezia (yes)	1.03 (.47–2.25)	.94	0.68 (.29–1.57)	.37
Signs of dehydration[b] (yes)	1.20 (.67–2.16)	.55	1.00 (.50–2.00)	.99
WBC count ≥13 × 10^3/μL	2.54 (1.42–4.54)	.002	2.35 (1.17–4.72)	.02
Hematocrit (per 5% increase)	1.83 (1.21–2.77)	.004	1.26 (.81–1.97)	.30
Platelet count <250 × 10^3/μL	1.92 (1.02–3.60)	.04	1.49 (.72–3.07)	.28
Serum creatinine (per 1 mg/dL increase)	10.82 (1.49–78.69)	.02	7.75 (1.2–50.16)	.03
Serum sodium (per 1 mmol/L decrease)	1.12 (1.02–1.23)	.01	1.15 (1.04–1.27)	.007
Initiation of IVF administration[c]				
None or IVF before day 4 of diarrhea	1 (Reference)		1 (Reference)	
On or after day 4 of diarrhea	2.50 (1.14–5.46)	.02	2.71 (1.18–6.21)	.02

Complete data available for 464 participants without HUS at index visit. Model diagnostic statistics for HUS: –2 log likelihood = 336.882, χ^2 = 128.045, degrees of freedom (*df*) = 13, *P* < .001; Nagelkerke R^2 = 0.381; classification percentage, 84.9%; Hosmer-Lemeshow test *P* = .674; largest variance inflation factor (VIF) = 1.862. Model diagnostic statistics for renal replacement therapy: –2 log likelihood = 263.654, χ^2 = 89.861, *df* = 13, *P* < .001; Nagelkerke R^2 = 0.330; classification percentage, 89.2%; Hosmer-Lemeshow test *P* = .260; largest VIF = 1.862.

Abbreviations: CI, confidence interval; ED, emergency department; IVF, intravenous fluid; OR, odds ratio; WBC, white blood cell.

[a]For children with HUS, variable includes antibiotics received before HUS criteria were met.

[b]Signs of dehydration defined by: dehydrated, dry-appearing, dry mucous membranes, tented skin, sunken eyes, decreased perfusion, or other similar terms.

[c]For children with HUS, variable includes IVF before HUS criteria were met.

useful data throughout the 5-year study period. Almost all sites were large tertiary care centers, so our data might not be generalizable to the larger community [41]. However, participating sites were from diverse geographic regions, and many serve rural and urban populations. Also, we have incomplete data on discharged patients who did not return to the index hospital within 30 days, but it is unlikely they would have gone to another institution if HUS developed, as participating study centers have pediatric nephrology expertise. Nonetheless, we cannot exclude the small possibility that we missed some cases of HUS, thereby underestimating its frequency.

In summary, HUS occurred in nearly 1 in 5 STEC-infected children, and 1 in 7 infected children without HUS at the index ED visit. In regression analysis, development of HUS was associated with higher serum creatinine and hematocrit values and lower platelet counts at presentation, and independent predictors included shorter symptom duration, younger age, higher WBC count, lower serum sodium, and IVF administration ≥4 days following diarrhea onset. Female sex, younger age, higher WBC count and serum creatinine, lower serum sodium, and administration of IVF ≥4 days following the onset of diarrhea predicted RRT. Large prospective studies are needed to assess if early intravascular volume expansion and correction of dysnatremia mitigate disease progression.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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