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Leukocytosis in children with *Escherichia coli* O157:H7 enteritis developing the hemolytic-uremic syndrome

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Background. **Fewer than 10% of children with *Escherichia coli* O157:H7 enteritis develop hemolytic-uremic syndrome (HUS).**

Objective. **To determine whether circulating leukocytes are independent risk markers of developing HUS during *E. coli* O157:H7 enteritis.**

Methods. **We reviewed the charts of all children with culture-proved *E. coli* O157:H7 infections seen at Sainte-Justine Hospital between 1987 and 1997. Epidemiologic data, laboratory indices and circulating leukocytes counts were noted. HUS diagnosis was validated with independent HUS patient lists from the pediatric nephrology services of tertiary care hospitals in the Montreal metropolitan area. The date of onset of enteritis was determined by two independent observers. Leukocyte counts were compared among the following independent groups: (1) uncomplicated O157:H7 enteritis (Group 1); (2) O157:H7 enteritis with the subsequent development of HUS (Group 2); (3) HUS already present at the time of medical consultation (Group 3).**

Results. **There were 369 children with *E. coli* O157:H7 infection. A complete blood count was not performed in 114 (31%) patients. Observers disagreed on the date of onset of gastroenteritis in 34 (9%) children only (kappa 0.92). The study population thus included 221 patients: Group 1, n = 161; Group 2, n = 27; and Group 3, n = 33. Patients developing HUS (Group 2) presented greater total leukocyte ($P < 0.008$), polymorphonuclear ($P < 0.008$) and monocyte ($P < 0.07$) counts than those with an uncomplicated course (Group 1). Logistic regression analysis showed that young age [odds ratio (OR), 0.98; 95% confidence interval (CI), 0.96 to 0.99], duration of enteric prodrome ≤3 days (OR 4.8, 95% CI 1.13 to 20.7) and initial leukocytosis (OR 1.22, 95% CI, 1.11 to 1.35) were independent predictors of HUS.**

Conclusions. **Based on the variables identified above, further studies are needed to determine whether the inflammatory response of the host represents only a marker of the severity of gastrointestinal infection or whether, alternatively, it is a pathophysiologic factor that leads to HUS.**

INTRODUCTION

Classic hemolytic-uremic syndrome (HUS) occurs after a prodrome of hemorrhagic colitis caused by verotoxin-producing *Escherichia coli* (VTEC).[1] In Canada the serotype O157:H7 is the most frequently involved.[2] The pathophysiology of VTEC-associated HUS is unknown,[3] but experimental data suggest that inflammatory response of the host to verotoxin (VT) and/or lipopolysaccharide is involved in the pathophysiology of VTEC infections.[4–7] *In vivo* the measurement of circulating inflammatory mediators is associated with the prognosis of VTEC and *Shigella* infections.[8–16] Characteristically there is a short prodrome between the onset of symptomatic VTEC enteritis and the development of HUS. Young age is a well-recognized risk marker of developing HUS.[17] The severity of the inflammatory response of the host may also vary over time after the onset of enteritis.[12] Once HUS is established, the severity of renal dysfunction is predicted by an increased circulating neutrophil and macrophage count.[18–23]

The prognostic value of the white blood cell count on the risk of developing HUS is unknown. The purpose of this study was to determine whether increased circulating leukocytes are independent risk markers of developing HUS in children with *E. coli* O157:H7 infections.

METHODS

The medical charts of all children <18 years old with culture-proved, sporadic *E. coli* O157:H7 infections,

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treated at Sainte-Justine hospital between June 15, 1987, and December 1st, 1997, were reviewed. Sex, age, date of medical consultation, need for hospitalization, history of diarrhea, bloody stools, abdominal pain, vomiting and results of stool cultures were recorded. Laboratory values obtained at the time of initial medical consultation included hemoglobin, platelet count, presence of schistocytes on peripheral blood smear, serum urea and creatinine and hematuria. The lowest hemoglobin concentration and the lowest platelet count as well as the highest serum urea and creatinine concentrations observed during the course of illness were also recorded. The time of onset of gastroenteritis was defined as the occurrence of either diarrhea (>2 loose stools per day) and/or abdominal pain and/or bloody stools. It was determined by two independent investigators (CB and MC). Disagreements between the observers were noted, and patients for whom a consensus was not reached were excluded.

E. coli O157:H7 was identified by the presence of sorbitol-negative colonies growing on MacConkey-sorbitol agar. Those were subcultured onto blood agar and screened for serotype O157 by slide agglutination (Difco, Detroit, MI). Colonies agglutinating with the antiserum were identified as *E. coli* by standard biochemical reactions. Confirmation of the lipopolysaccharide O157 antigen and the flagellar H7 antigen was obtained from the Laboratory Center for Disease Control (Ottawa). HUS was defined as a prodrome of enteritis with all the following criteria: (1) thrombocytopenia (<150 000 units/l); (2) hemolytic anemia (hemoglobin below the third percentile for age and sex; and (3) acute renal failure (serum creatinine >50 μmol/l if <5 years old, >60 μmol/l if between 5 and 9 years old, >90 μmol/l if between 10 and 13 years old; and >110 μmol/l if older than 13 years old).[23] Uncomplicated O157:H7 infection (Group 1) was defined as all of the following criteria: hemoglobin level >110 g/l; no schistocyte on peripheral blood smear; platelet count >150 000 units/l, no red blood cells on urine analysis and normal serum urea and creatinine values. Children were considered to have developed HUS (Group 2) if they presented criteria for uncomplicated O157:H7 infection at initial medical consultation and subsequently met any criterion for HUS. Both the diagnosis of HUS and the ascertainment of patients to the three independent groups were validated among our study population using the pediatric nephrology services patients list of Sainte-Justine Hospital and that of the Montreal Children's Hospital. These are the only two tertiary care hospitals with pediatric nephrology services in the greater Montreal metropolitan area. Children who already presented to us with clinical evidence of HUS (Group 3) were distinguished from those previously described (Group 2).

Statistical analysis. Descriptive statistics are presented as mean ± SD. For categoric data comparative analyses between the three groups were performed using a 2 × 3 contingency table. An analysis of variance for nonrepeated measurements was used to compare the continuous variables between the three groups; all orthogonal comparisons (Group 1 *vs.* 2, Group 2 *vs.* 3, Group 1 *vs.* 3) were subsequently determined by the test of Dunn. All statistical tests were two-sided and P values <0.05 were considered significant; $P < 0.0167$ was considered significant for orthogonal comparisons.

A logistic regression model was then constructed with development of HUS as the dependent variable. Every potential risk marker studied was tested by a forward step method with a score test inclusion criterion of $P < 0.1$ and a −2 log likelihood ratio improvement exclusion criterion of $P > 0.1$. Both age and the duration of enteric prodrome were forced into the model. There was no interaction between the tested variables. The linearity of each variable was also evaluated according to its distribution. Both age and leukocyte counts were treated as continuous variables, whereas the duration of enteric prodrome was dichotomized (≤3 days *vs.* 4 days or more). The Hosmer-Lemeshow goodness of fit test was performed. The sensitivity and specificity of the model were determined with $P > 0.5$ of developing HUS as a cutoff point. Because predictors of HUS included continuous and dichotomous variables, we determined their relative contribution to the assessment of risk as a percentage of global variance. Statistics were performed on SAS release 6.12 (1996; SAS, Cary, NC).

Ethics. The study was approved by the Ethics Committee of Sainte-Justine Hospital.

RESULTS

From June 15, 1987, to December 1, 1997, there were 369 children (49% male *vs.* 51% female; P not significant) with *E. coli* O157:H7 infection proved by stool culture. Of all patients with *E. coli* O157:H7 enteritis, 72 children had HUS (19%) and 2 of the 72 patients (3%) died. There were (1) 297 (81%) children who remained with an uncomplicated infection, (2) 30 (8%) patients who were seen at the stage of enteritis and then subsequently developed HUS and (3) 42 (11%) who already had HUS on initial presentation. These groups were comparable for sex and history of diarrhea. However, patients with uncomplicated O157:H7 enteritis less frequently were seen with vomiting (39% *vs.* 77% *vs.* 83%, respectively; $P < 0.0001$) and were less often hospitalized (58% *vs.* 100% *vs.* 100%, respectively; $P < 0.0001$) than children with HUS. Patients in whom HUS subsequently developed were younger (59 ± 47 *vs.* 38 ± 27 *vs.* 47 ± 42 months, respectively; $P < 0.04$) and more frequently reported abdominal pain

(65% *vs.* 80% *vs.* 59%, respectively; $P < 0.005$) than other groups. However, the presence of bloody stool was similar between groups (86% *vs.* 85% *vs.* 90%, respectively; *P* not significant).

Observers disagreed on the date of onset of enteric symptoms in 34 patients (9%) by 2 days ($n = 10$), 3 days ($n = 6$), 4 days or more ($n = 18$); (kappa 0.92; a value >0.75 indicates an appropriate level of consensus). These children were thus excluded from other comparative analysis. A complete blood count was not performed in 114 patients (31%) who were also excluded. These children were younger than the study population (43 ± 40 *vs.* 60 ± 46 months, respectively; $P < 0.001$). Most of them were, however, immediately discharged from the emergency room (95% *vs.* 25%, respectively; $P < 0.0001$). They presented a similar rate of diarrhea and bloody stools but showed significantly less frequent episodes of vomiting (30% *vs.* 46%, respectively; $P < 0.01$) and abdominal pain (56% *vs.* 82%, respectively; $P < 0.0001$) than children with an uncomplicated infection. On the basis of the pediatric nephrology services patient lists, none of the excluded patients had developed HUS.

Our study population thus included 221 children with evidence of O157:H7 enteritis in whom a complete blood count was performed at the initial time of medical consultation and a clearly identifiable date of onset of gastrointestinal symptoms was available. Among this cohort 161 continued an uncomplicated course (Group 1), 27 initially presented an enteritis followed by the development of HUS (Group 2) and 33 already had HUS on arrival (Group 3). The initial circulating leukocyte counts among the three groups are shown in Figure 1, which shows that increased neutrophil and monocyte responses were noted in patients developing HUS (Group 2), whereas a higher lymphocyte count was observed in children admitted with established HUS (Group 3). The time interval between the onset of enteritis and white blood cell count measurements is shown in Figure 2. A trend for a shorter interval before leukocyte measurements at admission was noted in Group 2, although statistical significance was not reached (3.5 ± 3.5 *vs.* 2.5 ± 1.5 *vs.* 3.9 ± 3.0 days, respectively; $P < 0.2$). Serial measurements of the white blood cell count during the development of HUS (Group 2) are given in Figure 3. The predictive value of the white blood cell count for development of HUS during *E. coli* O157:H7 enteritis is presented in Table 1. We noted that children in Group 2 were significantly younger than those remaining with an uncomplicated course (Group 1); their age was, however, comparable with those with HUS diagnosed on admission (59 ± 47 *vs.* 38 ± 27 *vs.* 47 ± 42 months, respectively; $P < 0.03$). A logistic regression model was built. The probability of developing HUS during *E. coli* O157:H7 enteritis was

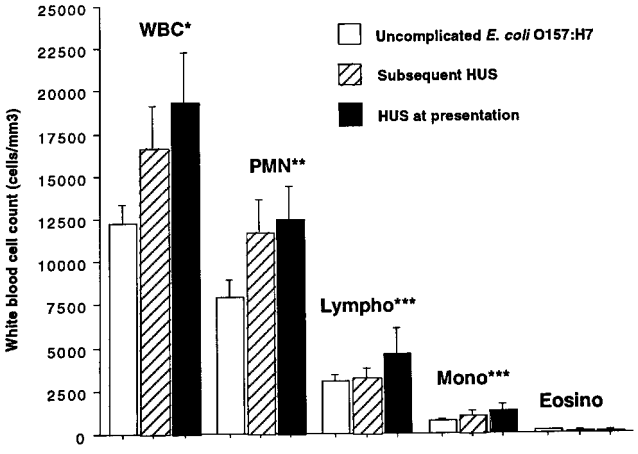

FIG. 1. Circulating white blood cells among 161 children with uncomplicated *E. coli* O157:H7 enteritis (□), 27 children with *E. coli* O157:H7 enteritis who subsequently developed HUS (▨) and 33 children with established HUS on medical consultation (■). Total white blood cell (*WBC*) (*, $P < 0.0001$), absolute polymorphonuclear (*PMN*) (**, $P < 0.0003$) and lymphocyte (*lympho*) and monocyte (*mono*) (***, $P < 0.001$) counts were significantly different among the three groups. Children in whom HUS subsequently developed presented increased total leukocyte and neutrophil ($P < 0.008$) counts compared with those with an uncomplicated course; a trend was noted for the monocyte count ($P < 0.07$). Children with established HUS also showed increased lymphocyte ($P < 0.002$) and monocyte ($P < 0.0001$) counts compared with both of the other groups.

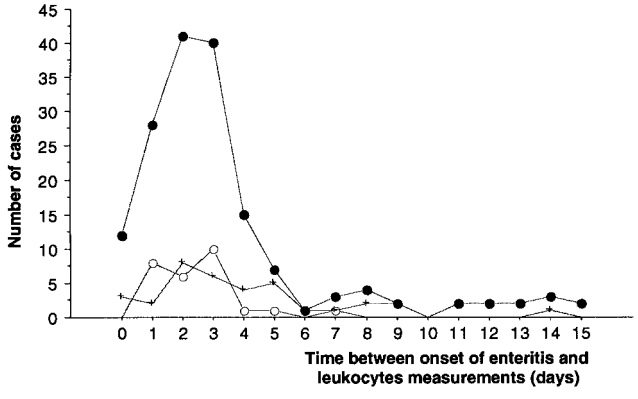

FIG. 2. Time interval between the onset of enteritis and measurements of the white blood cell count on admission among 161 children with uncomplicated *E. coli* O157:H7 enteritis (○), 27 children with *E. coli* O157:H7 enteritis who subsequently developed hemolytic uremic syndrome (HUS) (●) and 33 children with already established HUS (+). Results show a comparable distribution between the three groups (3.5 ± 3.5 *vs.* 2.5 ± 1.5 *vs.* 3.9 ± 3.0 days, respectively; $P < 0.2$).

the following: Logit (Prob) = −4.7 to 0.02 (age in months) + 1.6 (days of prodrome duration) + 0.2 (total leukocyte count) (goodness of fit: chi square, 9.05; $P = 0.34$). Odds ratio and 95% confidence interval of the independent predictors of HUS are shown in Table 2.

DISCUSSION

In North America diarrhea-associated HUS is a prominent cause of acute renal failure in children.[2]

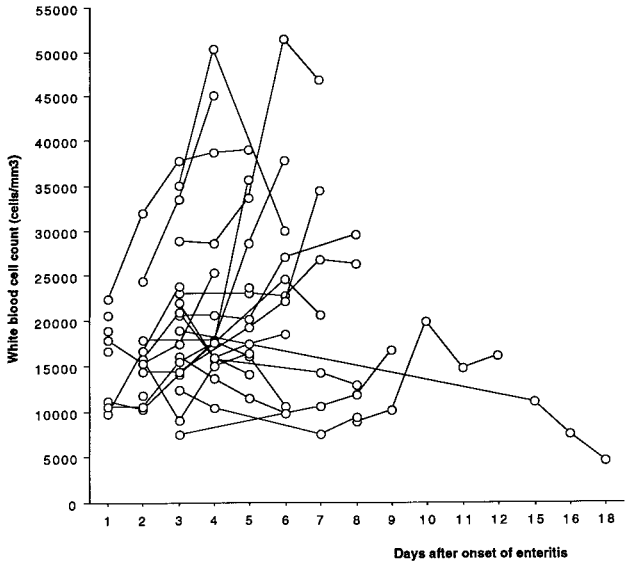

FIG. 3. Serial measurements of the total white blood cell count among children included in Group 2. Results show that most children presented increasing values of total leukocytes before (first point) and after the development of HUS (all other points).

Although the prevalence of O157:H7 infections in the Montreal metropolitan area is high, Rowe et al.[24] reported that only 12% of Canadian children <5 years of age with O157:H7 enteritis subsequently develop HUS. This low incidence rate complicates the assessment of prognostic markers of sporadic cases of O157:H7 enteritis, on a prospective basis. During the past decade 369 children with evidence of *E. coli* O157:H7 infection were evaluated at our institution. HUS diagnostic criteria were already met on arrival in the majority of cases (42 of 72, 58%). This may reflect the referral pattern to our tertiary care hospital.[24] A white blood cell count was not performed in 31% of children with O157:H7 infections. There is a low probability that this caused a significant detection bias of HUS diagnosis for the following reasons. Although patients without any blood testing on initial consultation were young and were theoretically at a significant risk of developing thrombotic microangiopathy, they were less symptomatic than children with uncomplicated O157:H7 infection, and 95% were immediately discharged by emergency room physicians. The lack of development of significant renal failure requiring supportive therapy was also validated by independent data sources. Nevertheless we recognize that some children with either isolated thrombocytopenia or hemolytic anemia may have been missed.

In this study we have noted that risk of developing HUS increased with the severity of leukocytosis, except for the last two strata with a very small sample size. We observed that during *E. coli* O157:H7 enteritis, children with ≥13 000 total leukocytes on initial presentation had a 3.8-fold increased risk to develop HUS. We have also shown that children destined to develop HUS sought medical consultation slightly more rapidly after the onset of enteritis than those with an uncomplicated course. Similarly Bell et al.[25] reported a 7-fold increased risk to develop HUS in patients presenting with ≥13 000 leukocytes during the first 3 days of *E. coli* O157:H7 enteritis. We have shown that leukocytosis was caused by both increased polymorphonuclear and monocyte responses. Using multivariate analysis we observed that initial leukocytosis, duration of enteric prodrome ≤3 days before initial medical consultation and young age were independent predictors of developing HUS. Although our model displayed a good specificity, it could account for only 27% of the global variance. This may be caused by the small number of HUS patients or it may signify that other important variables remain unknown. With regard to this we recognize that we have not studied environmental risk factors such as the use of antibiotic therapy or antimotility agents, although these may be important.[17, 25, 26] Recent data indicate that risk may either increase or decrease depending on which antibiotic is given as well as when it is administered after the onset of illness.[17, 25, 26] It might thus have been difficult to account for all these agents considering the number of patients developing HUS in this study. It could also be expected that an increased exposure to VT or lipopolysaccharide induced by pharmaceutic agents would have also modify the circulating leukocyte levels.

There is now an increasing amount of data suggesting that the inflammatory response of the host to VTEC infections may lead to HUS. *In vitro* there is a synergistic cytotoxicity on endothelial cells among verotoxin, lipopolysaccharide and cytokines.[4–7] *In vivo* circulating cytokines levels are associated with the development of HUS,[12] extrarenal manifestations[10] and the severity of acute and long term renal dysfunction.[13] Abnormally increased monocyte and neutrophil infiltration has been noted within the kidney of HUS patients.[15] Finally circulating neutrophils of patients with diarrhea associated thrombotic microangiopathy have been shown to be activated.[27] It remains to be determined whether leukocyte activation precedes the development of renal endothelial cell dysfunction. On one hand increased circulating cytokines[8, 12, 13] and leukocytes in patients developing HUS may only reflect the severity of gastrointestinal infection. Alternatively Tesh[28] has proposed that endothelial cells may be involved with inflammatory cells in a molecular crosstalk between VT and sensitizing cytokines for the development of HUS. In this regard we have recently observed that circulating levels of lipopolysaccharide-binding protein are abnormally increased during O157:H7 infections, the highest concentrations being found among HUS patients.[29] Lipopolysaccharide is a

TABLE 1. Prognostic value of the first white blood cell count in children with *Escherichia coli* O157:H7 enteritis developing hemolytic-uremic syndrome*

WBC (Cells/mm^3)	Developing HUS* (n = 28)	No HUS* (n = 161)	RR	P
≥9000	25	119	2.6 (0.8–10.2)†	0.1
≥11 000	23	80	3.8 (1.5–9.7)	0.002
≥13 000	19	49	3.8 (1.8–7.8)	0.0002
≥15 000	17	30	4.7 (2.4–9.2)	0.0001
≥17 000	12	15	4.5 (2.4–8.4)	0.0001
≥19 000	10	10	7.4 (3.6–15.1)	0.0001
≥21 000	7	7	8.6 (2.6–28.1)	0.0007
≥23 000	4	4	3.8 (1.7–8.2)	0.02
≥25 000	2	2	6.1 (0.8–45.4)	0.1

* No HUS and developing HUS refer to patients included in Groups 1 and 2, respectively.
† Numbers in parentheses, 95% confidence interval.
WBC, white blood cell count; RR, relative risk.

TABLE 2. Multivariate analysis of the risk markers for developing hemolytic-uremic syndrome during *Escherichia coli* O157:H7 enteritis*

Risk Markers	Odds Ratio
Age (mo)	0.98 (0.96–0.99)†
Prodrome duration (≤3 days)	4.84 (1.13–20.7)
Total leukocytes	1.22 (1.11–1.35)

* With a probability of developing HUS of >0.5 as a cutoff point, sensitivity and specificity were 26 and 98%, respectively. We estimated that this model could account for 27% of variance in the risk of developing HUS, 21% being attributed to the combination of age and total leukocyte count and 6% to prodrome duration.
† Numbers in parentheses, 95% confidence interval.

well-recognized proinflammatory initiating agent that may lead to endothelial cell, macrophage or neutrophil activation. An augmented production of granulocyte colony-stimulating factor may also be involved in increasing the neutrophil response during HUS development. We do not have any clear hypothesis to account for the higher circulating lymphocyte count noted once HUS has occurred.

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Shanghai, China: No Chance of Diaper Rash

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