

PROTHROMBOTIC COAGULATION ABNORMALITIES PRECEDING THE HEMOLYTIC-UREMIC SYNDROME

WAYNE L. CHANDLER, M.D., SRDJAN JELACIC, B.S., DANIEL R. BOSTER, B.S., MARCIA A. CIOL, PH.D.,
 GLYN D. WILLIAMS, M.B., CH.B., SANDRA L. WATKINS, M.D., TAKASHI IGARASHI, M.D., PH.D.,
 AND PHILLIP I. TARR, M.D.

ABSTRACT

Background The hemolytic-uremic syndrome is a thrombotic complication of *Escherichia coli* O157:H7 infection. It is not known whether the coagulation abnormalities precede, and potentially cause, this disorder.

Methods In 53 children infected with *E. coli* O157:H7, we measured a panel of markers indicating activation of the clotting cascade and renal function within four days after the onset of illness. These markers were measured again in as many as possible of the 16 children in whom the hemolytic-uremic syndrome developed.

Results The children in whom the hemolytic-uremic syndrome subsequently developed had significantly higher median plasma concentrations of prothrombin fragment 1+2, tissue plasminogen activator (t-PA) antigen, t-PA-plasminogen-activator inhibitor type 1 (PAI-1) complex, and D-dimer than children with uncomplicated infection. These abnormalities preceded the development of azotemia and thrombocytopenia. When the hemolytic-uremic syndrome developed, the urinary concentrations of beta₂-microglobulin and *N*-acetyl-β-glucosaminidase rose significantly ($P=0.03$ for both increases); the plasma concentrations of t-PA antigen, t-PA-PAI-1 complex, D-dimer, and plasmin-antiplasmin complex also increased significantly. The concentration of t-PA antigen correlated with that of the t-PA-PAI-1 complex in a linear regression model (squared correlation coefficient, 0.80; $P<0.001$).

Conclusions In the hemolytic-uremic syndrome, thrombin generation (probably due to accelerated thrombogenesis) and inhibition of fibrinolysis precede renal injury and may be the cause of such injury. (N Engl J Med 2002;346:23-32.)

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THE hemolytic-uremic syndrome consists of thrombocytopenia, nonimmune hemolytic anemia, and renal insufficiency. The syndrome develops about one week after the onset of diarrhea in approximately 15 percent of children infected with *Escherichia coli* O157:H7.^{1,2} The development of microvascular thrombi, composed largely of fibrin, and endothelial-cell swelling are common in this disorder.³⁻⁷ *E. coli* O157:H7 produces Shiga toxins, which have diverse effects on eukaryot-

ic cells.⁸ Presumably, these toxins injure host endothelial cells during the early stages of *E. coli* O157:H7 infection and initiate a cascade that leads to the hemolytic-uremic syndrome.

Coagulation abnormalities in the hemolytic-uremic syndrome differ from those observed in classic disseminated intravascular coagulation. In the hemolytic-uremic syndrome, unlike the consumptive coagulopathies, the concentration of fibrinogen is normal or elevated,⁹⁻¹¹ and the prothrombin time and partial-thromboplastin time are normal or only slightly prolonged.⁹ However, the concentration of circulating prothrombin fragment 1+2, the prothrombin-activation peptide that is cleaved from prothrombin when thrombin is generated, increases.^{10,11} Elevated plasma plasminogen-activator inhibitor type 1 (PAI-1) activity and increased concentrations of tissue plasminogen activator (t-PA) antigen and D-dimer¹⁰⁻¹³ further characterize the coagulopathy of the hemolytic-uremic syndrome.

In persons with the hemolytic-uremic syndrome, it is not known whether intravascular thrombin forms before renal insufficiency develops. In addition, the paradox of elevated t-PA concentrations during a thrombotic disorder remains unexplained. We examined a panel of coagulation markers in children with *E. coli* O157:H7 infection to determine the sequence of vascular and renal injury as the hemolytic-uremic syndrome develops and to characterize more completely the associated coagulation abnormalities.

METHODS

Study Subjects and Procedures

The children with *E. coli* O157:H7 infection who participated in this study have been described previously.¹ Microbiology laboratories in Washington, Oregon, Idaho, and Wyoming notified us whenever *E. coli* O157:H7 was identified in the stool culture of a child under the age of 10 years. We immediately contacted the physician of each infected child and asked him or her to seek permission from the child's family for us to approach them about participation in the study. If permission was granted, we explained

From the Departments of Laboratory Medicine (W.L.C.), Anesthesiology (G.D.W.), and Pediatrics (S.L.W., P.I.T.), University of Washington School of Medicine, Seattle; Children's Hospital and Regional Medical Center, Seattle (S.J., D.R.B., M.A.C., G.D.W., S.L.W., P.I.T.); and the Department of Pediatrics, University of Tokyo Graduate School of Medicine, Tokyo, Japan (T.I.). Address reprint requests to Dr. Tarr at the Division of Gastroenterology, CH-24, Children's Hospital and Regional Medical Center, 4800 Sand Point Way NE, Seattle, WA 98105, or at tarr@u.washington.edu.

the purpose of the investigation to the family and obtained written informed consent from the child's parent or guardian. If appropriate, we also obtained assent from the child. In addition, children under the age of 10 years who presented to the Emergency Department of the Children's Hospital and Regional Medical Center with acute bloody diarrhea were enrolled at the time of presentation if the consent of the parent or guardian and, if appropriate, the assent of the child were granted. Data from these children were analyzed as part of this study if a stool culture subsequently yielded *E. coli* O157:H7.

A standardized questionnaire was administered to each child's caregiver to determine when the diarrhea began relative to enrollment in the study. The first day on which diarrhea occurred was defined as day 1 of the illness. Blood for research was obtained during the first clinically indicated phlebotomy after enrollment. Urine was obtained as a clean-void or bagged specimen as soon as possible after enrollment. If the hemolytic-uremic syndrome developed, specimens were obtained again, if possible, within 24 hours after the child fulfilled the case definition of the syndrome (as defined below). Phlebotomy and bladder catheterization were not performed solely for research purposes.

In all the children with infection, daily complete blood counts were obtained and renal-function tests performed for clinical purposes until the hemolytic-uremic syndrome developed and resolved or until it became apparent that the infection was resolving without this complication. The period of risk for the development of the hemolytic-uremic syndrome was considered to be 14 days after the onset of diarrhea, according to the results of previous studies in the state of Washington.^{2,14} The hemolytic-uremic syndrome was defined by the presence of the following: hemolytic anemia (a hematocrit below 30 percent, with evidence of fragmented erythrocytes on a peripheral-blood smear), thrombocytopenia (a platelet count of less than 150,000 per cubic millimeter), and renal insufficiency (a serum creatinine concentration that exceeded the upper limit of normal for the child's age). Uncomplicated infection was defined as illness that resolved without progressing to the hemolytic-uremic syndrome. Medical records were reviewed to verify the fulfillment of these classification criteria.

Children 1 to 10 years of age who had no hematologic, renal, inflammatory, or infectious processes and who were undergoing elective operations at the Children's Hospital and Regional Medical Center served as controls. Written informed consent was obtained from each child's parent or guardian, and assent was also obtained from the child, if appropriate. Blood from these children was obtained when an intravenous catheter was inserted at the beginning of anesthesia.

Laboratory Analysis

Blood collected by phlebotomy was added in 1.8-ml quantities to chilled glass tubes containing 0.2 ml of 0.105 M sodium citrate and kept on ice until centrifugation, which was performed within an hour after collection. Plasma was then aspirated and frozen (at -70°C) in aliquots until analysis. Urine specimens were kept on ice and then frozen (at -70°C) in aliquots within four hours after collection. The plasma samples were rapidly thawed, and the concentrations of the following factors were determined with the use of commercially available enzyme immunoassays: D-dimer¹⁵ and t-PA antigen (Asserachrom, Diagnostica Stago, Parsippany, N.J.), PAI-1 activity (Chromolize PAI-1, Biopool, Ventura, Calif.), and plasmin-antiplasmin complexes¹⁶ and prothrombin fragment 1+2 (Enzygnost F1+2, Dade Behring, Marburg, Germany).¹⁷ The concentration of t-PA-PAI-1 complex was measured if sufficient plasma remained after all the other coagulation assays had been performed.¹⁸ The urine samples were thawed on ice. Beta₂-microglobulin concentrations were determined by latex agglutination,¹⁹ and *N*-acetyl- β -glucosaminidase concentrations were determined by a fluorogenic assay with the use of methylumbelliferyl *N*-acetyl- β -glucosaminidase as a substrate.²⁰

The assays of coagulation markers and urinary beta₂-microglobulin and *N*-acetyl- β -glucosaminidase were performed by staff members who were unaware of the enrollment characteristics of the children from whom the samples had been obtained. Information on the identity of the samples was retained in the laboratory of one of the authors. Assays were performed in batches and included simultaneous analysis of samples from all three groups of children.

The samples were assigned to one of three groups for initial analysis: those obtained from the children without infection (controls), those obtained on or before day 4 of illness from children with uncomplicated infection, and those obtained on or before day 4 from children in whom the hemolytic-uremic syndrome subsequently developed. We chose to analyze data from samples collected during the first four days of illness because the earliest point at which the hemolytic-uremic syndrome occurred in any of the children in this study was day 5 of illness, and we wished to avoid analyzing data on samples from children in whom one or more of the criteria for the hemolytic-uremic syndrome had already been fulfilled. In children in whom the hemolytic-uremic syndrome did develop, we obtained informed consent (and, if necessary, assent) to collect a second set of plasma samples, urine samples, or both from subjects after the case definition of the syndrome had been met, in order to identify any significant changes in the results of the laboratory assays.

Statistical Analysis

The Wilcoxon rank-sum test was used to test the significance of differences between the children with uncomplicated infection and those in whom the hemolytic-uremic syndrome subsequently developed; between the children with uncomplicated infection and the controls; and between the children in whom the hemolytic-uremic syndrome subsequently developed and the controls. The significance level was set at 0.05, and all tests to assess *P* values were two-sided. A linear regression model was used to analyze the relation between the concentrations of t-PA antigen and t-PA-PAI-1 complex on or before day 4 of illness in the two groups of infected children. Paired data from the same children, obtained at two different times during the illness, were analyzed with use of the nonparametric Wilcoxon signed-rank test.

RESULTS

Between May 1, 1997, and February 1, 2001, plasma was obtained on or before day 4 of illness from 53 infected children (Table 1); all of the samples except one were obtained after microbiologic diagnosis of *E. coli* O157:H7 infection. Sufficient plasma to measure the concentration of t-PA-PAI-1 complex was available from all except three of the infected children. Urine samples were obtained on or before day 4 of illness from 35 of the infected children. The hemolytic-uremic syndrome developed between days 5 and 13 of illness, inclusive, in 16 of the 53 children. Urine samples had been obtained from 11 of these 16 children, and 14 subsequently contributed a second plasma sample and 6 a second urine sample for research purposes when the hemolytic-uremic syndrome developed. All of the 14 control children contributed plasma, and 6 of them contributed urine.

The samples were obtained after similar numbers of days of illness in the two groups of children with infection: those with uncomplicated infection and those in whom the hemolytic-uremic syndrome subsequently developed. The hematocrit, platelet count,

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TABLE 1. CHARACTERISTICS OF THE CHILDREN.*

CHARACTERISTIC	NO INFECTION (CONTROLS) (N=14)	UNCOMPLICATED INFECTION (N=37)	BEFORE HEMOLYTIC- UREMIC SYNDROME (N=16)	AFTER ONSET OF HEMOLYTIC- UREMIC SYNDROME (N=14)
Female sex — no. (%)	2 (14)	13 (35)	5 (31)	5 (36)
Age — yr	4.3±3.2	3.9±2.4	3.4±1.8	3.5±1.9
Race or ethnic group — no. (%)				
White	12 (86)	30 (81)	13 (81)	12 (86)
Hispanic	0	5 (14)	2 (12)	1 (7)
Black	1 (7)	2 (5)	0	0
Asian or Pacific Islander	0	0	1 (6)	1 (7)
Native American	1 (7)	0	0	0
Bloody diarrhea — no. (%)	—	30 (81)	14 (88)	12 (86)
Plasma sample obtained — no. (%)				
Day 2 of illness	—	4 (11)	2 (12)	—
Day 3 of illness	—	10 (27)	5 (31)	—
Day 4 of illness	—	23 (62)	9 (56)	—
Day of illness plasma sample obtained after hemolytic-uremic syndrome case definition fulfilled (in 14 children)	—	—	—	
Median				8
Range				6–13
Day of illness urine sample obtained after hemolytic-uremic syndrome case definition fulfilled (in 6 children)	—	—	—	
Median				8
Range				6–13
Hematocrit — %	35.6±3.0	37.4±2.6	38.2±5.0	22.0±3.3
Platelet count — ×10 ⁻³ per mm ³	321±70	317±74	322±97	43±30
Creatinine — mg/dl†	0.4±0.1	0.4±0.1	0.4±0.2	2.0±1.2
Prothrombin fragment 1+2 — nmol/liter				
Median	0.73	1.44‡	2.45§	3.94¶
Range	0.30–1.37	0.48–5.40	0.70–11.0	1.75–20.6
PAI-1 activity — IU/ml				
Median	1.4	1.7	6.1§	11.5
Range	0.4–7.0	0.1–17.6	0.5–48.7	2.0–28.6
t-PA-PAI-1 complex — ng/ml				
Median	2.0	2.6	4.5§	9.2¶
Range	0.9–4.2	0.5–9.9	1.4–18.0	3.4–18.7
t-PA antigen — ng/ml				
Median	3.1	3.3	4.3§	11.4¶
Range	1.8–9.1	0.5–8.7	1.5–18.7	4.1–15.4
D-Dimer — log ₁₀ ng/ml				
Median	5.88	7.20‡	7.80§	9.17¶
Range	5.03–6.43	5.96–8.27	6.59–8.87	7.26–10.85
Plasmin-antiplasmin complex — ng/ml				
Median	405	691‡	847§	2159¶
Range	140–683	321–1673	300–3286	1250–5874
Beta ₂ -microglobulin — mg/ml				
Median	≤70	89	326‡	19,398¶
Range	≤70–220	≤70–5655	≤70–4159	714–44,658
N-acetyl-β-glucosaminidase — U/liter				
Median	2.3	2.7	3.4	17.0¶
Range	1.4–3.9	1.0–9.0	0.5–11.0	8.4–74.6

*Plus-minus values are means ±SD. Day 1 of illness was defined as the first day of diarrhea. PAI-1 denotes plasminogen-activator inhibitor, and t-PA tissue plasminogen activator. Sufficient plasma to measure the concentration of t-PA-PAI-1 complex was available from all except three of the infected children. Urine samples were obtained on or before day 4 of illness from 35 of the infected children. Dashes indicate that the variable is not applicable. Because of rounding, not all percentages total 100.

†To convert values for serum creatinine to micromoles per liter, multiply by 88.4.

‡P<0.05 by the Wilcoxon rank-sum test for the comparison with the median value in the controls.

§P<0.05 by the Wilcoxon rank-sum test for the comparison with the median value in the children with uncomplicated infection and with the median value in the controls.

¶P<0.05 by the Wilcoxon signed-rank test for the comparison with the median value obtained on or before day 4 of illness, before the onset of the hemolytic-uremic syndrome in children from whom paired samples were available.

and serum creatinine concentration were normal and similar in these two groups, as well as in the control group (Table 1). Thus, hemolysis, quantitative platelet abnormalities, and renal insufficiency had yet to evolve in the children in whom the hemolytic-uremic syndrome subsequently developed.

Despite the absence of microangiopathic changes or renal insufficiency, the children in whom the hemolytic-uremic syndrome subsequently developed had higher median values for each of the coagulation factors and urinary proteins than the children with uncomplicated infection, who, in turn, had higher median values for each of these variables than the controls. These differences were significant with respect to the plasma concentrations of fragment 1+2 ($P=0.003$ for the comparison between the children who later had the hemolytic-uremic syndrome and those with uncomplicated infection and $P<0.001$ for the comparison between those with uncomplicated infection and the controls) and D-dimer ($P=0.002$ and $P<0.001$, respectively), according to the Wilcoxon rank-sum test (Fig. 1 and Table 1). Children in whom the hemolytic-uremic syndrome subsequently developed had significantly higher median plasma concentrations of t-PA antigen than those with uncomplicated infection and the controls ($P=0.006$ and $P=0.01$, respectively); the same was true with respect to the median concentrations of PAI-1 ($P=0.02$ and $P=0.005$, respectively) and of t-PA-PAI-1 complex ($P=0.006$ and $P=0.001$, respectively). The median plasma concentrations of plasmin-antiplasmin complex and median urinary concentrations of beta₂-microglobulin were significantly greater in the children in whom the hemolytic-uremic syndrome developed than in the controls ($P<0.001$ and $P=0.04$, respectively) but were not significantly greater than those in the children with uncomplicated infection (Fig. 2 and Table 1). The overlap between the infected groups in plasma and urinary values was usually considerable, as shown for selected variables in Figures 1 and 2.

In the children in whom the hemolytic-uremic syndrome eventually developed, the median changes between the initial concentrations of coagulation fac-

tors measured after enrollment and the concentrations measured after the onset of this complication (among the 14 children from whom paired plasma samples were available) were statistically significant with respect to the following variables: t-PA antigen (median increase, 4.90 ng per milliliter; $P=0.01$), t-PA-PAI-1 complex (median increase, 4.0 ng per milliliter; $P=0.002$), D-dimer (median increase, 0.56 log_e ng per milliliter; $P=0.02$), and plasmin-antiplasmin complex (median increase, 982 mg per milliliter; $P<0.001$) (Fig. 3 and Table 1). The changes in urinary indexes between these two time points among the children from whom paired urine samples were available were also significant: the urinary beta₂-microglobulin concentration increased by a median of 20,899 mg per milliliter ($P=0.03$), and the *N*-acetyl-β-glucosaminidase concentration increased by a median of 20.75 U per liter ($P=0.03$) (Fig. 2 and Table 1). In contrast, the median changes in the concentration of prothrombin fragment 1+2 and PAI-1 activity were 0.915 nmol per liter ($P=0.17$) and 4.45 IU per milliliter ($P=0.24$), respectively (Fig. 3 and Table 1).

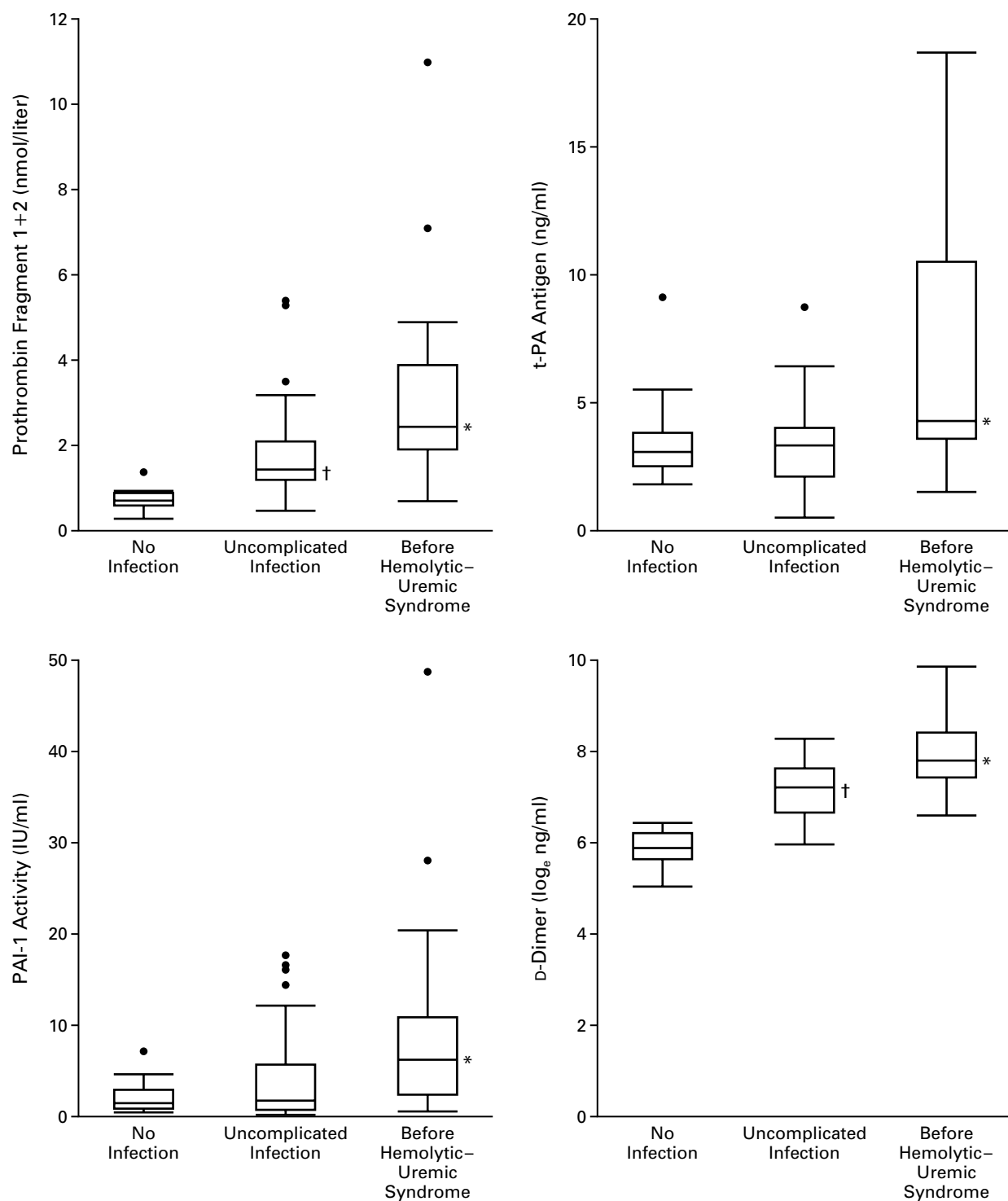
During the first four days of illness the concentrations of t-PA-PAI-1 complex were strongly correlated with the concentration of circulating t-PA antigen (squared correlation coefficient, 0.80; $P<0.001$) among the children with infection (Fig. 4). The median percentages of total t-PA antigen in complexes with PAI-1 were 81 percent in the children with uncomplicated infection, 95 percent in those in whom the hemolytic-uremic syndrome subsequently developed, and 86 percent in those with the hemolytic-uremic syndrome.

DISCUSSION

Our data shed light on the pathophysiology of the progression of *E. coli* O157:H7 infection to the hemolytic-uremic syndrome. The concentrations of prothrombin fragment 1+2 during the first four days of illness were as high as those in patients with disseminated intravascular coagulation caused by abdominal sepsis or cancer²¹ and were similar to the concentrations of fragment 1+2 observed in children with

Figure 1 (facing page). Box Plot of Coagulation Factors in Children without *Escherichia coli* O157:H7 Infection and within Four Days after the Onset of Diarrhea in Children with Infection.

Shown for each coagulation factor is the distribution of values from the 14 children without infection (controls), the 37 children whose *E. coli* O157:H7 infection resolved without development of the hemolytic-uremic syndrome, and the 16 children in whom the hemolytic-uremic syndrome subsequently developed. The horizontal line within each box represents the median, the lower and upper borders of each box represent the 25th and the 75th percentiles, respectively, and the T bars represent the differences between the lower and upper borders multiplied by 1.5. Outliers (values that exceed these boundaries) are depicted as single points. The asterisks indicate $P<0.05$ by the Wilcoxon rank-sum test for the comparison between the median value in the group of children in whom the hemolytic-uremic syndrome subsequently developed and the median value in each of the other two groups of children, and the dagger indicates $P<0.05$ by the Wilcoxon rank-sum test for the comparison between the median value in the children with uncomplicated infection and the median value in the controls.



the hemolytic–uremic syndrome in European studies.^{10,11} These elevations demonstrate that in *E. coli* O157:H7 infection, prothrombin is being converted to thrombin at an early stage of illness, when the hematocrit, platelet count, and serum creatinine concentration are normal. The thrombin generation at this stage of illness most likely represents thrombogenesis. The formation of fibrin can be inferred from the finding of disseminated thrombi in patients with the hemolytic–uremic syndrome, usually in specimens obtained at autopsy,³⁻⁷ and from the elevated concentration of fibrinopeptide A²² in children with the hemolytic–uremic syndrome, which was presumably caused by *E. coli* O157:H7.²³

The elevation of prothrombin fragment 1+2 before the development of azotemia suggests that thrombogenesis precedes, and may lead to, renal insufficiency in the hemolytic–uremic syndrome caused by *E. coli* O157:H7. Because the serum creatinine concentration can be a delayed indicator of renal injury, we analyzed urinary concentrations of beta₂-microglobulin and *N*-acetyl-β-glucosaminidase (a proximal renal tubular enzyme), which are early markers of injury to the kidneys after exposure to nephrotoxic agents.^{24,25} Despite several outlying elevated values, most of the infected children in our study had normal concentrations of *N*-acetyl-β-glucosaminidase. There was evidence of impaired renal absorption of beta₂-microglobulin, but the concentration of neither of these urinary proteins became markedly elevated until the hemolytic–uremic syndrome developed. Our data probably underestimate the renal tubular injury at the onset of the hemolytic–uremic syndrome, because some of the children became anuric and thus were unable to provide a second urine sample for analysis, which presumably would have had an even higher concentration of these proteins.

The delay in the elevation of these urinary proteins and the delay in the onset of azotemia contrast with the relative stability of the plasma concentrations of prothrombin fragment 1+2 in most of the children in whom paired samples were available for analysis. This sequence of events suggests that pro-

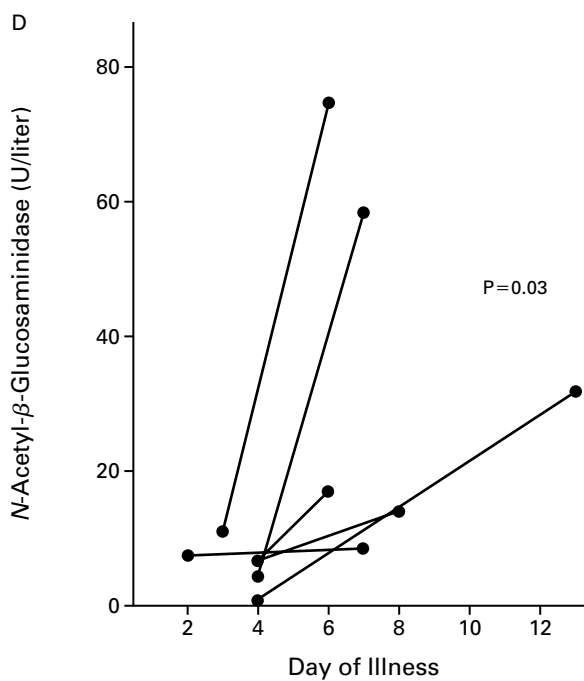
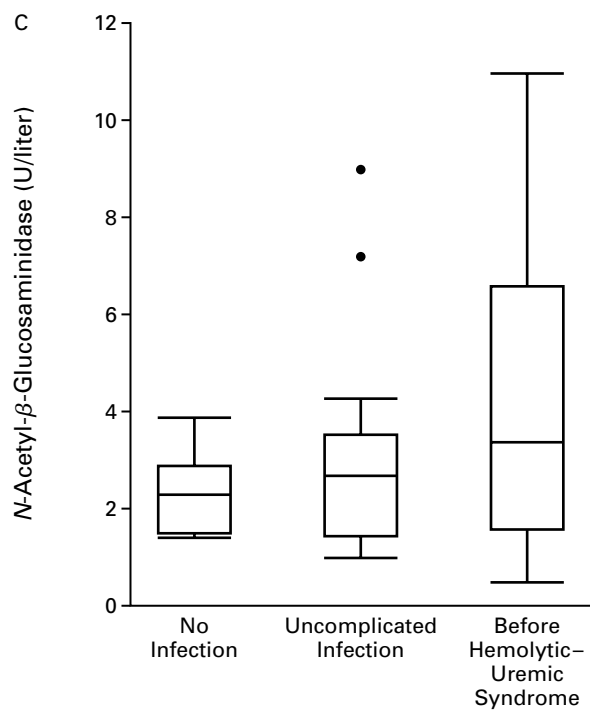
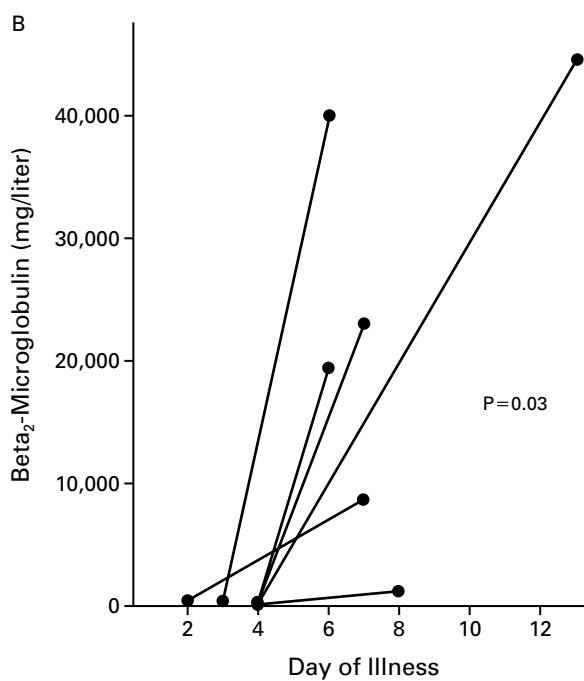
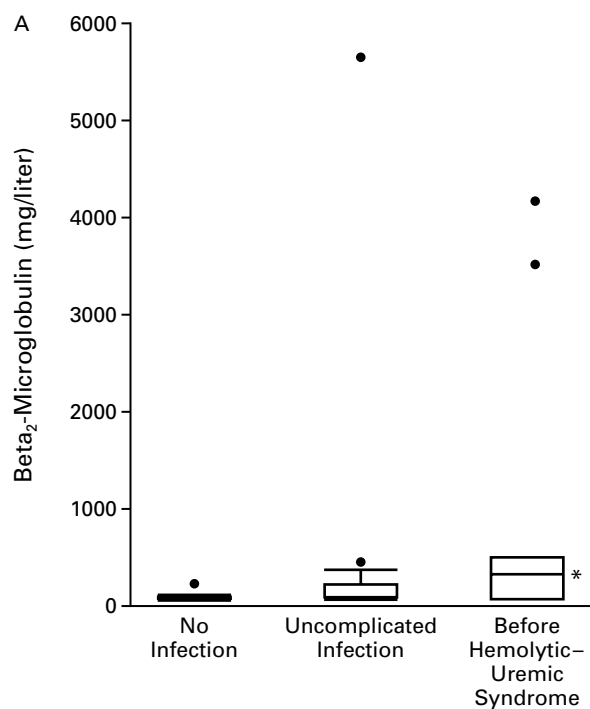
thrombotic abnormalities might underlie the initial pathophysiologic events leading to renal injury in the hemolytic–uremic syndrome and that tubular injury and renal insufficiency are secondary to the formation of fibrin thrombi. An alternative and, in our opinion, less likely explanation is that circulating bacterial products, probably Shiga toxins, directly injure renal cells before or at the same time as they injure vascular cells, but that the renal manifestations of this injury appear later.

The correlation of the concentrations of t-PA–PAI-1 complex with those of the t-PA antigen resolves the seemingly contradictory observation that a putatively profibrinolytic molecule, t-PA, increases in concentration after the onset of the hemolytic–uremic syndrome, which is a thrombotic disorder.^{10,12} Specifically, the half-life of t-PA antigen in the circulation is 2.4 minutes when it is in its free form and 5.0 minutes when it is in t-PA–PAI-1 complexes.²⁶ Therefore, the formation of complexes of t-PA and PAI-1 elevates the concentration of circulating t-PA antigen at the same time that it inhibits the ability of t-PA to activate plasminogen. This mechanism probably underlies the diminished fibrinolytic potential of the plasma of children with the hemolytic–uremic syndrome.¹⁰ The diminished capacity of t-PA to activate plasminogen in infected children before the hemolytic–uremic syndrome develops also extends the findings of Bergstein et al.,¹³ who observed that elevated PAI-1 activity and, presumably, augmented t-PA inhibition are associated with prolongation of the hemolytic–uremic syndrome.

The elevated concentrations of circulating D-dimer, a fibrin-degradation product, can be misinterpreted as representing the rate of plasminogen activation and thus the concentration of functional plasminogen activators. However, the concentration of D-dimer is elevated when there is excess fibrin in the vascular system, as in patients with deep venous thrombosis or disseminated intravascular coagulation. The D-dimer concentration may increase further when the concentration of circulating t-PA increases in the presence of increased concentrations of fibrin,^{27,28} but

Figure 2 (facing page). Urinary Markers of Renal Injury Associated with *Escherichia coli* O157:H7 Infection.

Panels A and C show box plots of the urinary concentrations of beta₂-microglobulin and *N*-acetyl-β-glucosaminidase, respectively, in children without infection (controls) and within four days after the onset of diarrhea in children with *E. coli* O157:H7 infection. The horizontal line within each box represents the median, the lower and upper borders of each box represent the 25th and the 75th percentiles, respectively, and the T bars represent the differences between the lower and upper borders multiplied by 1.5. Outliers (values that exceed these boundaries) are depicted as single points. The asterisk indicates P<0.05 by the Wilcoxon rank-sum test for the comparison between the median value in the group of children in whom the hemolytic–uremic syndrome subsequently developed and the median value in the controls. Panels B and D show changes in the concentrations of urinary beta₂-microglobulin and *N*-acetyl-β-glucosaminidase, respectively, between the values measured after enrollment and the values measured after the onset of the hemolytic–uremic syndrome in the six children from whom paired urine samples were available for analysis. P values are for the comparison between the pairs of values, by the Wilcoxon signed-rank test.



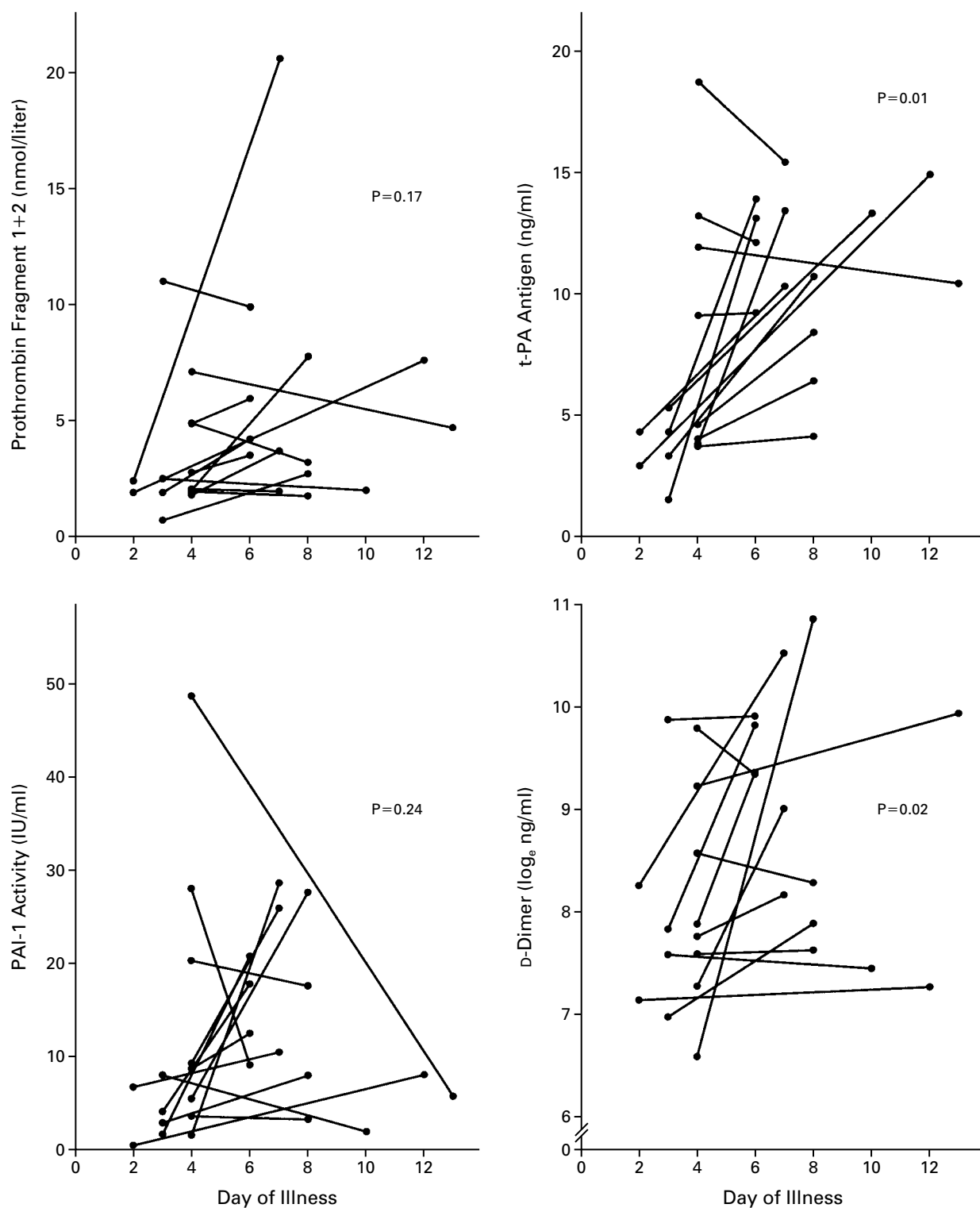


Figure 3. Changes in the Concentrations of Coagulation Factors at the Onset of the Hemolytic–Uremic Syndrome. Changes between the values measured after enrollment and the values measured after the onset of the hemolytic–uremic syndrome are shown for the 14 children from whom paired plasma samples were available for analysis. P values are for the comparison between the pairs of values, by the Wilcoxon signed-rank test.

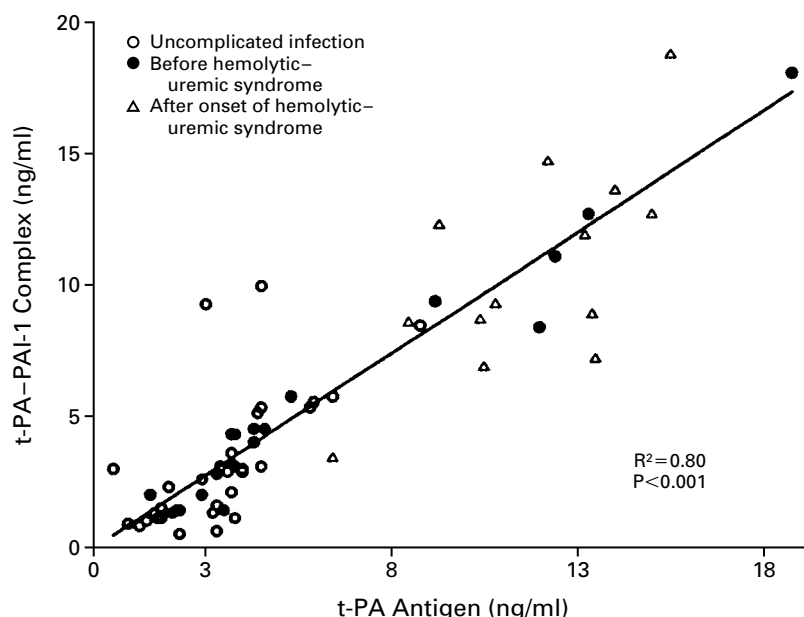


Figure 4. Correlation between Plasma Concentrations of t-PA and t-PA-PAI-1 Complex in Children with *Escherichia coli* O157:H7 Infection.

The values from samples obtained after the onset of the hemolytic-uremic syndrome were not included in the regression analysis but are plotted here to show the persistent correlation between the concentrations of t-PA antigen and t-PA-PAI-1 complex after the onset of the hemolytic-uremic syndrome. R^2 denotes the squared correlation coefficient.

increased amounts of active t-PA alone will not raise the D-dimer concentration. For example, when t-PA is infused into normal subjects, circulating D-dimers^{26,29} are not detected; the appearance of D-dimers in the circulation depends on the presence of intravascular fibrin. Therefore, it is more likely that elevated concentrations of circulating D-dimer in persons with *E. coli* O157:H7 infection reflect the mass of intravascular fibrin present than it is that they signify an increased rate of plasminogen activation or elevated concentrations of functional plasminogen activators. The increasing concentrations of these fibrin-degradation products as the hemolytic-uremic syndrome evolves most likely indicate accelerating accretion of intravascular fibrin.

In our study, children with uncomplicated *E. coli* O157:H7 infection also had higher median values for coagulation factors than did children without infection. Thus, as with the degradation of von Willebrand factor,³ at least some children infected with *E. coli* O157:H7 have vascular injury without evidence of hematologic or renal injury. It is not known whether this vascular injury plays a part in the pathophysiol-

ogy of the gastrointestinal manifestations of *E. coli* O157:H7 infection.

The greater severity of prothrombotic abnormalities in the children in whom the hemolytic-uremic syndrome subsequently developed than in those whose illness resolved spontaneously has several clinical implications. First, the blood count and serum creatinine concentration are normal, and thus these values do not indicate the severity of vascular injury early in the course of illness; considerable amounts of thrombin are generated in infected children, despite normal hematocrits, platelet counts, and serum creatinine concentrations. Second, although our findings are useful in delineating the pathophysiology of the sequelae of *E. coli* O157:H7 infection, we must caution against the use of these data to predict the development of the hemolytic-uremic syndrome. There was extensive overlap between the values in the children with uncomplicated infection and those in the children in whom the hemolytic-uremic syndrome subsequently developed. Third, the coagulation abnormalities that are observed before azotemia develops might present an opportunity for altering the pro-

thrombotic cascade early in the course of the illness, possibly by thrombin inhibition, and thus halting the progression to renal insufficiency. However, the formation of thrombin and the inhibition of fibrinolysis are well under way by the fourth day of illness in children in whom the hemolytic-uremic syndrome subsequently develops; the vascular disorder may already be irreversible by the time of presentation. Clearly, the best way to prevent the hemolytic-uremic syndrome is to prevent primary infection with *E. coli* O157:H7.

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CORRECTION

**Prothrombotic Coagulation Abnormalities Preceding
the Hemolytic-Uremic Syndrome**

Prothrombotic Coagulation Abnormalities Preceding the Hemolytic-Uremic Syndrome . On page 32, the grant support was omitted. It should have read, "Supported by National Institutes of Diabetes and Kidney Diseases grant 1RO1DK52081 and the Ministry of Health and Welfare of Japan." We regret the error.