Coagulation Abnormalities in Dengue Hemorrhagic Fever: Serial Investigations in 167 Vietnamese Children with Dengue Shock Syndrome

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The pathophysiological basis of hemorrhage in dengue infections remains poorly understood, despite the increasing global importance of these infections. A large prospective study of 167 Vietnamese children with dengue shock syndrome documented only minor prolongations of prothrombin and partial thromboplastin times but moderate to severe depression of plasma fibrinogen concentrations. A detailed study of 48 children revealed low plasma concentrations of the anticoagulant proteins C, S, and antithrombin III, which decreased with increasing severity of shock, probably because of capillary leakage. Concurrent increases in the levels of thrombomodulin, tissue factor, and plasminogen activator inhibitor type 1 (PAI-1) indicated increased production of these proteins. Thrombomodulin levels suggestive of endothelial activation correlated with increasing shock severity, whereas PAI-1 levels correlated with bleeding severity. Dengue virus can directly activate plasminogen in vitro. Rather than causing true disseminated intravascular coagulation, dengue infection may activate fibrinolysis primarily, degrading fibrinogen directly and prompting secondary activation of procoagulant homeostatic mechanisms.

Dengue fever (DF) and dengue hemorrhagic fever (DHF) are 2 apparently distinct clinical manifestations of dengue virus infection, and each may be caused by any of the 4 antigenically related dengue virus serotypes [1, 2]. DF is classically a self-limiting, nonspecific illness characterized by fever, headache, myalgia, and consti-

tutional symptoms. During the 1950s, a more serious clinical entity, termed DHF, began to emerge among children in Southeast Asia [3] and has proved to be a significant cause of pediatric morbidity and mortality in that region. An estimated 100 million dengue infections, of which >250,000 are cases of DHF, occur worldwide each year [4].

Despite its name, the cardinal feature that differentiates DHF from DF is not hemorrhage but increased vascular permeability that results in capillary leakage [2, 5]; the most severely affected children develop dengue shock syndrome (DSS) because of excessive depletion of intravascular volume. Minor bleeding manifestations, most commonly skin petechiae or bruising, are apparent in many children with DHF [6], but major hemorrhage is unusual. If severe bleeding does occur,

Table 1. World Health Organization guidelines for the diagnosis of dengue shock syndrome in areas of endemicity [9, 10].

Criterion	Definition and notes
Fever	Duration of 2–7 days
Hemorrhagic tendency	Any of the following findings: a positive tourniquet test result, spontaneous petechiae or other skin bleeding, or mucosal/gastrointestinal tract bleeding
Thrombocytopenia[a]	Platelet count of ≤100,000 cells/mm³
Evidence of plasma leakage	Any of the following findings: elevation of the admission hematocrit to ≥20% greater than expected mean for age, sex, and population; reduction of the hematocrit to ≥20% of the baseline value after resuscitation; or clinical signs of plasma leakage, such as pleural effusion or ascites
Circulatory compromise	Narrow pulse pressure of ≤20 mm Hg, with tachycardia or hypotension for age

[a] A few patients in our study, although thrombocytopenic, did not have absolute platelet counts ≤100,000 cells/mm³ at the time of admission to the hospital.

it is almost invariably in children with profound or protracted shock who also have evidence of multiple-organ failure. Conversely, mucosal hemorrhage may occur infrequently in children with otherwise uncomplicated DF and can be severe [6].

The underlying mechanisms responsible for bleeding in dengue infections remain poorly understood. Thrombocytopenia is universal in DHF and is one of the criteria stipulated by the World Health Organization (WHO) for the clinical case definition [7, 8], but it has also been noted in up to 50% of cases of DF [9]. Platelet function is abnormal in dengue infections. In the acute phase of disease, platelet aggregation in response to ADP is impaired, and concurrent increases in plasma β-thromboglobulin and platelet factor 4 indicate that platelet secretory activity is increased [10]. The duration of survival of transfused platelets is markedly shortened [11], and immune complexes containing dengue antigen have been found on platelet cell surfaces [12]. Mild prolongation of the prothrombin and partial thromboplastin times and reduced fibrinogen levels have been noted in several studies [13–15], but levels of fibrin degradation products are not elevated to a degree consistent with classical disseminated intravascular coagulation. Variable reductions in the activities of specific coagulation factors, including factors II, V, VII, VIII, IX, X, antithrombin, and α-2–antiplasmin, have been demonstrated during the acute phase of DHF in small numbers of patients [13, 15].

We set out to study the coagulopathy associated with DSS, focusing on factors important in the 3 major pathways—prothrombotic, antithrombotic, and fibrinolytic—that, together, determine the overall balance of the coagulation cascade.

PATIENTS AND METHODS

Patients and clinical methods

The clinical work took place on the pediatric intensive care unit at the Centre for Tropical Diseases of Ho Chi Minh City, Vietnam. All children aged 1–15 years who presented directly to the hospital with clinical DSS were enrolled in this study, provided that a parent or guardian gave informed consent. The children were also enrolled in an intervention trial of fluid resuscitation. WHO guidelines (table 1) were used for diagnosis of DSS, with a minor modification relating to the platelet count. Ethical approval of the study was obtained from the Scientific and Ethical Committee of the Centre for Tropical Diseases of Ho Chi Minh City.

As part of the fluid study, the children were randomized to receive 1 of 3 resuscitation fluids (dextran 70, 6% hydroxyethyl starch, or Ringers' lactate), depending on the initial severity of disease. All children received 25 mL/kg of the specified fluid over 2 h, followed by a standardized reducing schedule of Ringers' lactate. Patients whose condition failed to improve or deteriorated subsequently received further boluses of colloid solution, as judged necessary on clinical grounds; patients with the most severe cases received inotropic support in addition to fluid resuscitation. Careful note was made of all parenteral fluid therapy administered.

For each patient, basic demographic details, history, examination findings, and subsequent progress were recorded on standard proforma. Pulse and blood pressure were measured every hour until they were stable for at least 24 h and then every 4 h until the patient was discharged from the hospital. Patients were examined daily by a member of the study team, with particular attention paid to the occurrence and severity of any bleeding manifestations. At discharge, all patients were asked to return for a follow-up visit after 1 month.

Sample collection and handling

Serum and citrated plasma samples were obtained at presentation (i.e., on day 1) prior to fluid resuscitation. Additional samples were obtained at 8 A.M. on days 2 and 4 of the study and at the 1-month follow up visit. The samples were separated as quickly as possible and stored at −70°C. Many coagulation tests are affected by delays in separation, so only test results

for samples that were processed within 6 h after being obtained and in which there was no visible hemolysis or clot formation were considered suitable for inclusion in this report.

Severity scoring

After discharge, each child was classified as having had "mild," "moderate," or "severe" shock according to the following system. Those who achieved cardiovascular stability in ⩽6 h after admission to the hospital, without the need for further administration of colloid solution, were considered to have had mild DSS. Those children who recovered from shock within 48 h and required ⩽50 mL/kg of additional colloid solution were classified as having had moderately severe shock. Those children who recovered from shock in >48 h, required >50 mL/kg of extra colloid solution, or died from intractable shock were classified as having had severe DSS.

For each child, bleeding was assigned to 1 of 4 categories, as follows. Patients with only spontaneous petechiae or bruising at venipuncture sites were considered to have mild bleeding; those with mucosal bleeding that did not affect the hematocrit were considered to have moderate bleeding; and those with epistaxis or gastrointestinal bleeding sufficient to cause a detectable decrease in the hematocrit or sufficient to warrant transfusion were considered to have severe bleeding. A number of children had no bleeding manifestations at all throughout their hospital stay.

Laboratory methods

United Kingdom. Stored samples obtained from a random selection of patients with shock of mild and moderate severity, together with obtained samples from all those with severe shock, were transported to the United Kingdom for detailed study. Laboratory work in Vietnam was carried out in accordance with US regulations, which classify dengue as a category 2 pathogen. In the United Kingdom, dengue is regarded as a category 3 pathogen; therefore, all specimens analyzed in the United Kingdom were first inactivated with Triton X100 (final concentration, 0.5%) in a category 3 facility. Triton is a nonionic detergent with recognized virucidal activity against dengue virus, but it is known to interfere with certain laboratory assays [16]. Preliminary studies in volunteers showed that, at a final concentration of 0.5%, it had no effect on the specific antigen–based coagulation factor ELISAs of interest. However, coagulation screening tests, particularly the prothrombin time, were compromised.

Levels of the following proteins were measured in the treated plasma samples by use of commercial ELISA kits (Imubind; American Diagnostica): plasminogen activator inhibitor type 1 (PAI-1), thrombomodulin, tissue factor, and total and truncated tissue factor pathway inhibitor (TFPI). The laboratory methods used and the interpretation of results for all kits were as specified in the manufacturer's instructions. Levels of proteins C, S, and antithrombin III were measured by ELISA as described elsewhere [17–19], in each case with use of the appropriate rabbit antihuman antibody (Dako).

Vietnam. Basic coagulation screening tests, including determination of prothrombin and partial thromboplastin times and fibrinogen levels, were performed with use of commercial kits (Neoplastine CI, CK Prest, and Fibri-Prest, respectively; Diagnostica Stago) on the serial (untreated) samples from the remaining children (i.e., all patients excluding those whose samples were sent to the United Kingdom for study). For confirmation of dengue infection, paired (untreated) serum samples were examined by use of Dengue Duo IgM and IgG Capture ELISA kits (Panbio). When possible, the specific dengue serotype was identified by reverse transcriptase–PCR, according to the method of Lanciotti et al. [20].

Statistical analysis

Data on patients characteristics and laboratory results were compared between different groups by use of the nonparametric Kruskal-Wallis test for continuous variables; the Cuzick nonparametric test for trend, where appropriate; and the χ^2 test or Fisher's exact test for categorical variables. Comparisons between results obtained at different time points were performed by use of the Wilcoxon signed-rank test. All statistical computations were carried out by use of Stata software (Stata).

RESULTS

From July 1998 through February 1999, 170 children with DSS were admitted to the pediatric intensive care unit at the Centre for Tropical Diseases in Ho Chi Minh City, Vietnam, and recruited to the study. Serology confirmed acute dengue infection in 163 of 170 patients. For 4 patients, all of whom had classical DSS, serology results were indeterminate, because no convalescent-phase samples were available. Of the 98 serum samples tested by PCR, 33 were positive for a dengue virus: serotype 2 was detected in 19 samples, serotype 3 was detected in 11 samples, and serotype 4 was detected in 1 sample; >1 serotype was detected in 2. Three patients remained seronegative throughout the study and were excluded from the subsequent analyses.

Samples studied in Vietnam and the United Kingdom were from patients with generally similar characteristics (table 2), except that samples from all the children with severe shock were included in the UK group of samples, which therefore biased this group toward the more severe end of the disease spectrum. Four children died, all of whom had fulminant shock unresponsive to aggressive management and had severe gas-

Table 2. Characteristics of 167 Vietnamese children with dengue shock syndrome at the time of admission to the hospital, comparing the group whose plasma samples were chosen for detailed study in the United Kingdom with the overall group.

Characteristic	United Kingdom ($n = 48$)	Vietnam ($n = 119$)	P[a]
Age, median years (range)	8 (3–14)	9 (1–14)	.26
Male sex	21 (44)	61 (51)	.38
Days of illness at presentation[b]			
3–4	18 (38)	50 (42)	
5–7	28 (58)	69 (58)	
>7	2 (4)	0 (0)	.13 (F)
Temperature, median °C (range)	37 (36.5–38.5)	37 (37–39.8)	.61
Pulse			
Undetectable	4 (8)	5 (4)	.28 (F)
Rate, median beats/min (range)[c]	120 (88–150)	116 (80–160)	.40
Blood pressure			
Unrecordable	5 (10)	6 (5)	.30 (F)
Systolic, median mm Hg (range)[c]	90 (85–125)	100 (70–120)	.02
Pulse pressure			
Unrecordable	5 (10)	6 (5)	
≤10 mm Hg	8 (17)	13 (11)	
>10 and ≤20 mm Hg	35 (73)	100 (84)	.23
Spontaneous bleeding	39 (81)	89 (75)	.37
Hematocrit, median % (range)	49 (40–58)	48 (40–63)	.11
Platelet count, median cells/mm³ (range)	93,000 (9000–180,000)	100,000 (12,000–250,000)	.30

NOTE. Data are no. (%) of patients, unless otherwise specified.

[a] For categorical variables, the χ^2 or Fisher's exact test (F) was used. For continuous variables, the nonparametric Kruskal-Wallis test was used.

[b] Days the patient had been ill at the time of presentation to the hospital with shock.

[c] For patients with recordable values.

trointestinal bleeding in the terminal stages of their illness. Suitable samples were obtained from only 2 of these children; therefore, for the purposes of analysis, the data for these patients have been combined with the data for the group with moderately severe shock. The clinical course was complicated by severe bleeding in 2 other children, both of whom had mild shock. The remaining patients had shock of mild or moderate severity, in general without any spontaneous bleeding or with only mild hemorrhagic manifestations, and all recovered fully. Of the survivors, 110 (66%) returned for a follow-up visit after 1 month. All were well at that visit, and we assume that the follow-up results represent the normal values for these children.

Basic coagulation screening tests. Prothrombin and partial thromboplastin times were marginally longer on day 2 than day 1 and were significantly longer than the values at follow-up (table 3). However, the median values still fell

Table 3. Results of serial coagulation tests for 119 children with dengue shock syndrome, according to day of sampling.

Test	Day of sampling[a]			P[b]	
	Day 1 ($n = 28$)	Day 2 ($n = 84$)	1 Month ($n = 50$)	Day 1 vs. day 2 ($n = 23$)	Day 2 vs. 1 month ($n = 35$)
Prothrombin time, median s (range)	13.4 (11.3–15.9)	13.4 (10.9–19.1)	12.7 (10.9–17.3)	NS	.01
Partial thromboplastin time, median s (range)	37.7 (18.8–58.1)	39.2 (22.8–63.1)	31.4 (24.7–50.9)	NS	<.01
Fibrinogen level, median g/L (range)	1.59 (0.7–5.63)	1.22 (0.61–4.33)	2.74 (0.87–7.25)	.02	<.01

NOTE. Only results from samples that were without visible hemolysis or clots and that were separated ≤6 h after being obtained are included.

[a] Day 1 was the day of admission to the hospital with shock.

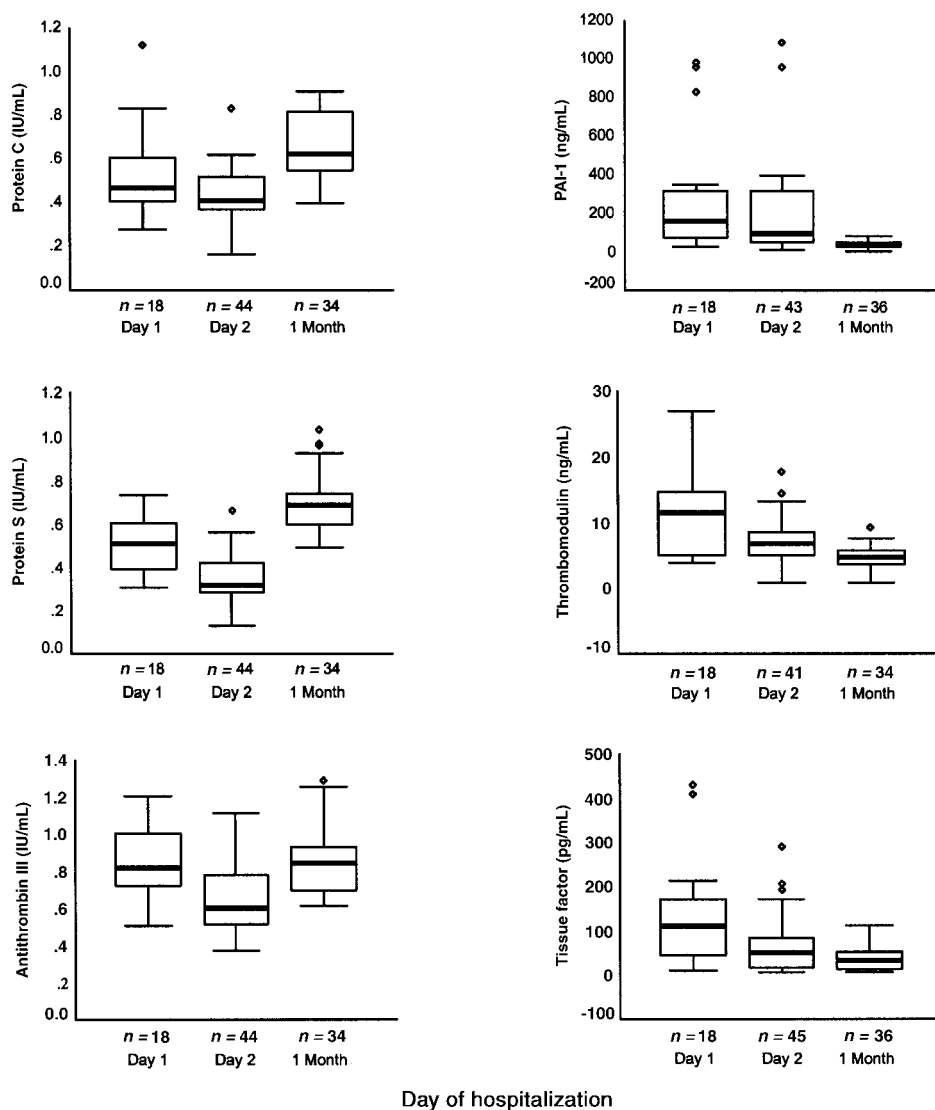[b] The Wilcoxon signed-rank test was used for paired data.

Figure 1. Box plots showing levels of specific coagulation proteins among 167 Vietnamese children with dengue shock syndrome, according to the day of sampling, where day 1 is the day of admission to the hospital with dengue shock syndrome. *Boxes,* median and interquartile ranges; *whiskers,* overall range, excluding outliers (◇). Comparisons of results for day 1 versus those for 1 month are significant ($P < .02$) for all proteins except antithrombin III (NS). Comparisons of results for day 2 versus those for 1 month are significant ($P < .01$) for all proteins except tissue factor ($P = .05$). The Wilcoxon signed-rank test was used for paired data. PAI-1, plasminogen activator inhibitor type 1.

within the acceptable normal range. Fibrinogen levels were moderately low at admission to the hospital, decreased significantly on day 2, and had returned to normal by 1 month. There was no association with either the bleeding category or the fluid used for initial resuscitation. There was a slight but significant increase in the prothrombin time in children with moderate shock, compared with that in children with mild shock ($P = .03$). The median prothrombin time was 13.2 s (range, 10.9–17.9 s) in patients with mild shock and 13.7 s (range, 12.6–19.1 s) in those with moderately severe shock.

Specific coagulation protein results. For most of the individual proteins measured, concentrations demonstrated

highly significant changes over time and in comparison with the follow-up results (figure 1). Plasma levels of the naturally occurring anticoagulant proteins C and S were significantly reduced at presentation, compared with levels at follow-up, whereas levels of antithrombin III were normal; the levels of all 3 proteins had decreased on day 2. The severity of hemoconcentration reflects the volume of fluid lost from the intravascular compartment. For each protein, absolute results were corrected for the concurrent hematocrit, to a value of 37% (which is the mean hematocrit for local Vietnamese children age 5–10 years [C. X. T. Phuong, personal communication]), and this correction suggested that at each time point the true

Table 4. Levels of specific coagulation proteins for 48 children with dengue shock syndrome, according to day of sampling, corrected for the degree of hemoconcentration at the time of sampling.

Protein	Median value (range), by day of sampling[a]			P[b]		
	Day 1 (n = 18)	Day 2 (n = 41–45)	1 Month (n = 34–36)	Day 1 vs. day 2 (n = 16–18)	Day 1 vs. 1 month (n = 13–15)	Day 2 vs. 1 month (n = 32–36)
Tissue factor, pg/mL	81.0 (7.7–302.4)	39.4 (3.1–295.6)	32.4 (5.7–109)	<.01	<.01	NS
Thrombomodulin, ng/mL	9.0 (2.8–17.2)	5.23 (0.8–28.4)	4.7 (0.8–9.4)	.03	.01	<.01
PAI, ng/mL	114.6 (19.8–674.4)	72.2 (6.1–1715.2)	38 (1.4–217.7)	NS	<.01	<.01
Total TFPI, pg/mL	55.4 (16.8–126.5)	55.9 (25.9–128.3)	47 (27–141)	NS	NS	NS
Free TFPI, pg/mL	4.6 (0–25.4)	3.7 (0–35.5)	7 (0–45)	NS	NS	NS
Protein C, IU/mL	0.36 (0.18–0.90)	0.35 (0.14–0.73)	0.62 (0.40–1.32)	NS	<.01	<.01
Protein S, IU/mL	0.40 (0.23–0.59)	0.27 (0.10–0.63)	0.68 (0.48–1.03)	<.01	<.01	<.01
Antithrombin III, IU/mL	0.62 (0.42–1.25)	0.56 (0.30–1.00)	0.84 (0.62–1.29)	.02	<.01	<.01

NOTE. Only results from samples that were without visible hemolysis or clots and that were separated ≤6 h after being obtained are included. Correction for hemoconcentration: (absolute value/concurrent hematocrit) × 37%. PAI-1, plasminogen activator inhibitor type 1; TFPI, tissue factor pathway inhibitor.

[a] Day 1 was the day of admission to the hospital with shock.
[b] Wilcoxon signed-rank test for paired data.

values were probably considerably lower (table 4). In contrast, levels of tissue factor, thrombomodulin, and PAI-1 at admission were all significantly elevated, and they remained so after correction for hemoconcentration. On day 2 all these levels, although lower, remained significantly elevated, but, after correction, only the elevations in the levels of thrombomodulin and PAI-1 remained significant. In the unadjusted analysis, total TFPI levels were moderately elevated during the acute phase of disease, but not after correction for hematocrit. There were no differences in free TFPI levels over time in either the basic or the adjusted analyses.

Considering the day 2 results, for which the largest number of suitable samples were available, levels of protein S ($P < .01$) and antithrombin III ($P < .01$) were significantly lower in patients with more severe shock, who showed significantly elevated levels of thrombomodulin ($P = .02$) (figure 2). Higher levels of PAI-1 ($P = .01$) and lower levels of protein S ($P = .04$) were associated with greater severity of bleeding.

Children resuscitated with a colloid solution had significantly lower levels of protein S and antithrombin III than did children resuscitated with Ringers' lactate ($P < .01$). Because patients with more-severe clinical manifestations at presentation were resuscitated with a colloid solution, and because only children with less severe disease were eligible to receive the crystalloid, the 2 effects may be linked. There was no evidence that the type of fluid used had an effect on any of the other parameters.

DISCUSSION

In the present study, DSS in children was associated with consistent alterations in the procoagulant, anticoagulant, and fibrinolytic pathways, but relatively minor changes in basic co-

agulation screening test results. Clinically significant bleeding was unusual. Levels of individual proteins demonstrated 2 distinct patterns of change with time. Levels of the anticoagulant proteins were normal or depleted at the time of admission to the hospital and decreased significantly during the subsequent 24 h. Levels of all of the remaining proteins, except TFPI, were elevated significantly at admission and also decreased during the next 24 h.

Increased vascular permeability is one of the cardinal features of DHF and is the cause of shock. Hypoalbuminemia occurs as a result of capillary leakage and correlates with severity [21]. All the coagulation proteins whose levels were measured in this study have molecular weights of 30,000–70,000 Da, which are close to the molecular weight of albumin (69,000 Da). When plasma leaks out of the intravascular compartment, it is probable that many of these proteins also leak. On day 2, leakage continues, and this, combined with the dilutional effect of fluids administered intravenously, may explain the documented decrease in plasma levels of coagulation proteins. With respect to the anticoagulant proteins C, S, and antithrombin III, which are predominantly synthesized in the liver, the low circulating levels probably reflect capillary leakage alone. An increase in the rate of consumption of these proteins may be a contributing factor, but the prompt clinical improvement in most patients and the relatively mild abnormalities in the results of basic coagulation screening tests do not support this view.

For the other proteins, the situation appears to be more complicated. Most are present in low concentrations, if at all, in normal plasma but are rapidly synthesized in response to sepsis or trauma. Tissue factor, the critical procoagulant protein responsible for initiating the coagulation cascade, is synthesized predominantly by monocytes but also by endothelial cells,
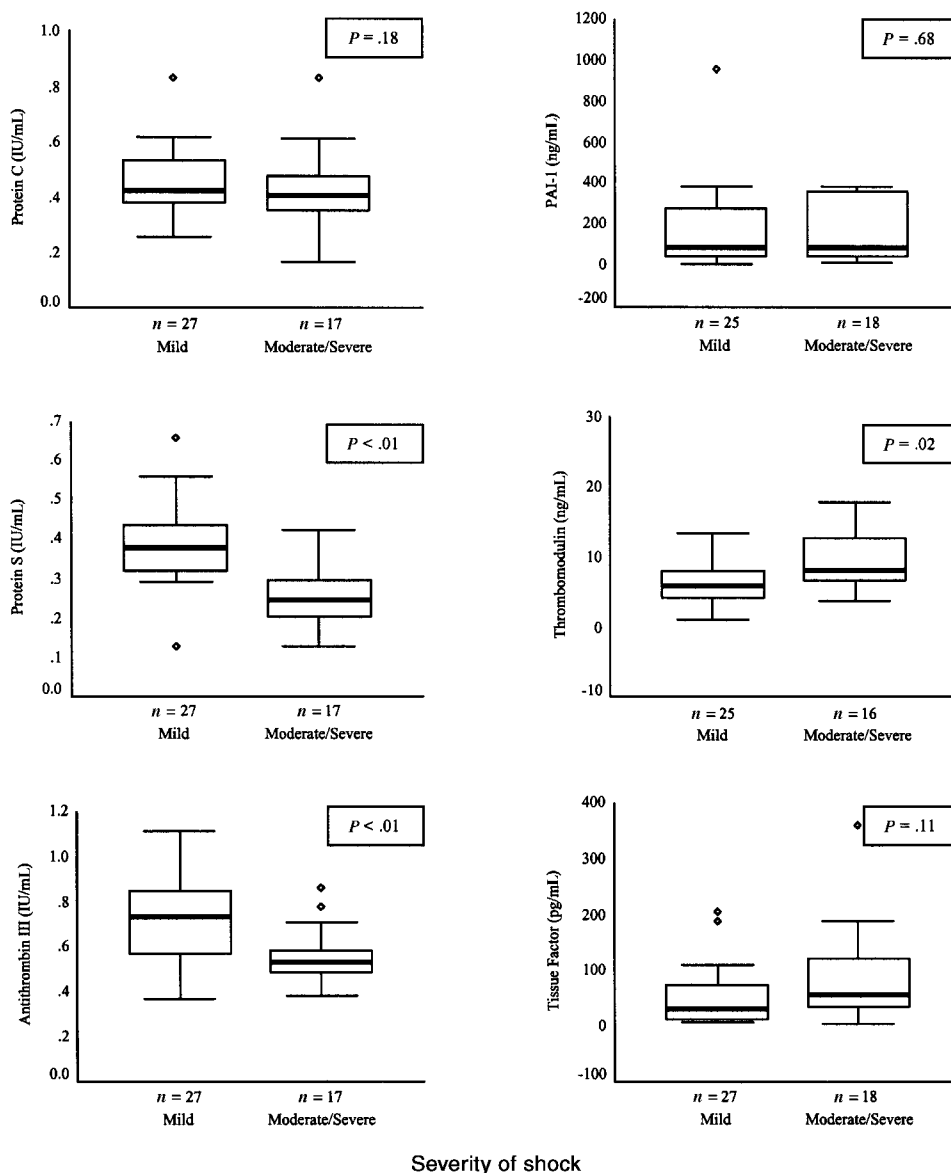
Figure 2. Box plots showing levels of specific coagulation proteins on day 2 after admission to the hospital among 167 Vietnamese children with dengue shock syndrome, according to the severity of shock. *Boxes,* median and interquartile ranges; *whiskers,* overall range, excluding outliers. Comparisons between severity groups were made with the nonparametric Kruskal-Wallis test. PAI-1, plasminogen activator inhibitor type 1.

whereas PAI-1, a potent inhibitor of fibrinolysis, is produced by both platelets and endothelial cells. The high plasma levels documented in this study may result from activation of any of these cell lines. However, the elevated levels of thrombomodulin, a protein thought to be synthesized and expressed exclusively by endothelial cells, indicate specific activation of endothelial cells. Cultured endothelial cell lines readily support dengue infection [22], but monocytes are considered the principal target of infection in vivo. Inflammatory cytokines secreted by monocytes infected with dengue virus are known to activate endothelial cells in vitro [23], and the present study demonstrates that such activation also occurs in vivo, possibly

by a similar mechanism. A complex interaction of increased production by activated endothelium, platelets, and/or monocytes, together with ongoing losses due to capillary leakage and, later, the dilutional effects of fluid resuscitation, may explain the changes observed in levels of tissue factor, thrombomodulin, and PAI-1. Interpretation of the TFPI data is difficult, because several different pools of TFPI are known to exist within the intravascular system [24], and the response to disease may involve redistribution and mobilization of TFPI from these pools.

Given the abnormalities in all the major pathways of the coagulation cascade (i.e., low levels of the natural anticoagulant

proteins and increased levels of the major procoagulant and antifibrinolytic agents), the complete absence of thrombotic complications and the relative infrequency of serious bleeding manifestations are surprising. In patients with bacterial sepsis, low levels of the anticoagulant proteins are usually associated with the development of thrombotic complications or significant laboratory evidence of disseminated intravascular coagulation and are predictors of poor outcome [25, 26]. Our findings suggest that low levels of these proteins per se do not cause disseminated intravascular coagulation. Recent work on meningococcal sepsis [27] has indicated that, in addition to low plasma levels of proteins C and S, a reduction in the endothelial expression of thrombomodulin occurs, which results in a profound deficiency of activated protein C and the development of disseminated intravascular coagulation. If the protein C activation pathway remains intact in patients with DHF, this may explain why similarly low levels of the anticoagulant proteins appear to have a negligible overall effect on coagulation.

A moderate decrease in the level of fibrinogen was observed in most of our patients, as has been reported in other studies [13–15]. Because fibrinogen is an acute-phase protein, usually a considerable amount is consumed before plasma levels decrease. If the consumption of fibrinogen in DSS involved classical disseminated intravascular coagulation, greater derangement of the prothrombin and partial thromboplastin times might be expected. A recent study from South America has shown that, in vitro, dengue virus isolates can bind to and activate plasminogen directly [28], and the plasmin generated can specifically degrade both fibrin and fibrinogen in a manner similar to streptokinase. Several other studies have reported the presence of plasminogen cross-reactive antibodies in acute-phase and convalescent-phase serum samples from patients infected with dengue virus [29, 30], with evidence of a positive correlation with hemorrhage but not shock. In a study that used 125 I-fibrinogen [31], increased rates of consumption of fibrinogen were demonstrated in patients who had DHF both with and without shock or hemostatic abnormalities. A possible scenario is that dengue infection primarily activates fibrinolysis in the absence of a thrombotic stimulus, degrading fibrinogen directly and prompting secondary activation of various procoagulant homeostatic mechanisms. Thus, bleeding in patients with dengue infections may result from a combination of thrombocytopenia, dysfunctional surviving platelets, and increased fibrinolysis, rather than from classical disseminated intravascular coagulation.

We have demonstrated that, despite limited clinically significant bleeding and only mild alterations in the results of coagulation screening tests, children with DSS have significant abnormalities in all the major pathways of the coagulation cascade. The low circulating levels of proteins C, S, and antithrombin III are likely to be related to leakage of these proteins through the vascular endothelium and correlate with the severity of shock. Elevated levels of tissue factor, thrombomodulin, and PAI-1 reflect endothelial, platelet, and/or monocyte activation and may be a secondary response to direct activation of fibrinolysis by the dengue virus. In most children with DSS, these combined effects do not result in derangements severe enough to cause clinically significant bleeding. In a minority of children with severe or prolonged shock, the abnormalities may be profound and, in combination with severe thrombocytopenia and the secondary effects of hypoxia and acidosis, may result in true disseminated intravascular coagulation and major hemorrhage.

Acknowledgments

References

1. Gubler DJ. Dengue and dengue haemorrhagic fever. Clin Microbiol Rev **1998**; 11:480–96.
2. Rigau-Perez JG, Clark GC, Gubler DJ, Reiter P, Sanders EJ, Vorndam AV. Dengue and dengue heamorrhagic fever. Lancet **1998**; 352:971–7.
3. Nimmanitya S, Halstead SB, Cohen SN, Margiotta MR. Dengue and Chikungunya virus infection in man in Thailand, 1962–1964. Observations on hospitalised patients with haemorrhagic fever. Am J Trop Med Hyg **1969**; 18:954–71.
4. Monath TP. Dengue: the risk to developed and developing countries. Proc Natl Acad Sci USA **1994**; 91:2395–400.
5. Halstead SB. Antibody, macrophages, dengue virus infection, shock and hemorrhage: a pathogenetic cascade. Rev Infect Dis **1989**; 11(Suppl 4):S830–9.
6. George R, Lum LC. Clinical spectrum of dengue infection. In: Gubler DJ, Kuno G, eds. Dengue and dengue haemorrhagic fever. Wallingford, UK: CAB International, **1997**:89–113.
7. World Health Organization (WHO). Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. Geneva: WHO, **1986**.
8. World Health Organization (WHO). Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2d ed. Geneva: WHO, **1997**.
9. Dietz V, Gubler DJ, Ortiz S, et al. The 1986 dengue fever outbreak in Puerto Rico. Dengue Surveill Summ **1987**; 40:1–2.
10. Srichaikul T, Nimmanitya S, Sripaisarn T, Kamolsilpa M, Pulgate C. Platelet function during the acute phase of dengue hemorrhagic fever. Southeast Asian J Trop Med Public Health **1989**; 20:19–25.
11. Isarangkura P, Tuchinda S. The behaviour of transfused platelets in dengue hemorrhagic fever. Southeast Asian J Trop Med Public Health **1993**; 24(Suppl 1);222–4.
12. Boonpucknavig S, Vuttiviroj O, Bunnag C, Bhamarapravati N, Nimmanitya S. Demonstration of dengue antibody complexes on the surface of platelets from patients with dengue hemorrhagic fever. Am J Trop Med Hyg **1979**; 28:881–4.
13. Mitrakul C, Poshyachinda M, Futrakul P, Sangkawibha N, Ahandrik

S. Hemostatic and platelet kinetic studies in dengue hemorrhagic fever. Am J Trop Med Hyg **1977**;26:975–84.

14. Isarangkura PB, Pongpanich B, Pintadit P, Phanichyakarn P, Vlayasevi A. Hemostatic derangment in dengue haemorrhagic fever. Southeast Asian J Trop Med Public Health **1987**;18:331–9.

15. Funahara Y, Sumarmo, Shirahata A, Setiabudy-Dharma R. DHF characterised by acute type DIC with increased vascular permeability. Southeast Asian J Trop Med Public Health **1987**;18:346–50.

16. Maha MS, Igarashi A. The effect of nonionic detergent on dengue and Japanese Encephalitis virus antigens in antigen detection ELISA and IgM-capture ELISA. Southeast Asian J Trop Med Public Health **1997**;28:718–22.

17. Deutz-Terlouw PP, Ballering L, van Wijngaarden A, Bertina RM. Two ELISAs for the measurement of protein S, and their use in the laboratory diagnosis of protein S deficiency. Clin Chim Acta **1990**; 186;321–34.

18. Soria J, Soria C, Samama M, Nicolas G, Kisiel W. Severe protein C deficiency in congenital thrombotic disease: description of an immunoenzymological assay for protein C determination. Thromb Haemost **1985**; 53:293–6.

19. Edgar P, Jennings I, Harper P. Enzyme linked immunosorbent assay for measuring antithrombin III. J Clin Pathol **1989**;42:985–7.

20. Lanciotti RS, Calisher CH, Gubler DJ, Chang G-J, Vorndam AV. Rapid detection and typing of dengue viruses from clinical samples by using reverse transcriptase-polymerase chain reaction. J Clin Microbiol **1992**;30:545–51.

21. Nimmanitya S. Clinical spectrum and management of dengue haemorrhagic fever. Southeast Asian J Trop Med Public Health **1987**;18: 392–7.

22. Bunyaratvej A, Butthep P, Yoksan S, Bhamarapravati N. Dengue viruses induce cell proliferation and morphological changes of endothelial cells. Southeast Asian J Trop Med Public Health **1997**;28(Suppl 3):32–7.

23. Anderson R, Wang S, Osiowy C, Issekutz A. Activation of endothelial cells via antibody-enhanced dengue virus infection of peripheral blood monocytes. J Virol **1997**;71:4226–32.

24. Sandset PM, Bendz B. Tissue factor pathway inhibitor: clinical deficiency states. Thromb Haemost **1997**;78:467–70.

25. Vervloet MG, Thijs LG, Hack CE. Derangements of coagulation and fibrinolysis in critically ill patients with sepsis and septic shock. Semin Thromb Hemost **1998**;24:33–44.

26. Fourrier F, Chopin C, Goudemand J, et al. Septic shock, multiple organ failure, and disseminated intravascular coagulation: compared patterns of antithrombin III, protein C, and protein S deficiencies. Chest **1992**;101:816–23.

27. Faust SN, Levin M, Harrison OB, et al. Dysfunction of the endothelial protein C activation pathway in severe meningococcal sepsis. N Engl J Med **2001**;345:408–16.

28. Monroy V, Ruiz BH. Participation of dengue virus in the fibrinolytic process. Virus Genes **2000**;21:197–208.

29. Markoff LJ, Innis BL, Houghton R, Henchal LS. Development of cross-reactive antibodies to plasminogen during the immune response to dengue virus infection. J Infect Dis **1991**;164:294–301.

30. Chungue E, Poli L, Roche C, Gestas P, Glaziou P, Markoff L. Correlation between detection of plasminogen cross-reactive antibodies and hemorrhage in dengue virus infection. J Infect Dis **1994**;170:1304–7.

31. Srichaikul T, Nimmanitya S, Artchararit N, Siriasawakul T, Sungpeuk P. Fibrinogen metabolism and disseminated intravascular coagulation in dengue hemorrhagic fever. Am J Trop Med Hyg **1977**;26:525–32.