Noninvasive Measurement of Microvascular Leakage in Patients with Dengue Hemorrhagic Fever

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Dengue shock syndrome (DSS) is a potentially lethal complication of dengue virus infection associated with hypotension and leakage of plasma water into the extravascular space. To determine whether the underlying pathophysiology of DSS is distinct from that in milder forms of the disease, we assessed microvascular permeability, by use of strain gauge plethysmography, in Vietnamese children with DSS (n = 19), or dengue hemorrhagic fever (DHF) without shock (n = 16), and in healthy control children (n = 15). At admission and after fluid resuscitation, the mean coefficient of microvascular permeability (Kf) for the patients with dengue was ~50% higher than that for the control patients (P = .02). There was no significant difference in Kf between the 2 groups of patients with dengue; this suggests the same underlying pathophysiology. We hypothesize that in patients with DSS, the fluctuations in Kf are larger than those in patients with DHF, which leads to short-lived peaks of markedly increased microvascular permeability and consequent hemodynamic shock.

During the past 20 years, dengue hemorrhagic fever (DHF) has become an important cause of morbidity and mortality in many parts of Asia and South and Central America [1]. Dengue shock syndrome (DSS) is a potentially lethal manifestation of dengue virus infection that predominantly affects children. In Vietnam, DSS is responsible for many thousands of pediatric hospital admissions each year, and in many areas, it is the main cause of pediatric admission during the rainy season. Severe manifestations of dengue, such as hemorrhage and shock, are believed to result from the phenomenon of immune enhancement, whereby low-circulating antibody titers from a primary infection predispose the patient to develop more severe disease when reinfected with a second dengue serotype [2].

The pathophysiological mechanisms whereby this leads to the characteristic clinical presentation of hemodynamic shock with bleeding into the skin and other sites remain unclear. It is also unclear why DHF and DSS are common in some dengue epidemics but rare or absent in others. Patients with DSS have hemoconcentration and often develop ascites and pleural effusions that suggest leakage of plasma into the extravascular space. With iv fluid resuscitation, the hematocrit level falls, and most patients recover rapidly without the need for inotropic support. Although, as yet, there is no direct proof in humans that a sudden increase in microvascular permeability is responsible for DSS, the characteristic clinical picture and limited evidence from
animal models strongly suggest that it is responsible [3].

Microvascular permeability can be assessed noninvasively in humans by use of mercury-in-silastic strain gauge plethysmography (MSG). The technique, first described by Whitney [4], has been adapted for investigation of the microcirculation [5]. Analysis of a continuous recording of limb circumference, obtained during a specific venous congestion pressure (VCP) protocol, enables the quantitation of the changes in peripheral microvascular parameters, including permeability and blood flow [6]. Although there is a large body of control data from healthy adults available for comparison, to our knowledge, this technique has not previously been used in children.

Only a small proportion of patients with dengue fever develop DSS. It is not clear whether this results from the extreme end of a distribution of increased vascular permeability found in all patients or whether the pathology of DSS is distinct from the pathology of milder forms of the disease. The aim of this study was to determine whether there is increased capillary leakage across the whole spectrum of DHF in humans or whether DSS represents a discrete clinical and pathological entity.

METHODS

Study site. The study was performed in a designated study area of the Paediatric Intensive Care Unit at the Centre for Tropical Diseases, Ho Chi Minh City, in southern Vietnam.

Patients. Children aged >5 and <15 years who were consecutively admitted to the hospital with clinically suspected DHF were entered into the study on an intention-to-treat basis if they had not received iv fluids during the current episode of illness and if their parents or guardians gave informed consent. The severity of the DHF was graded I–IV, according to World Health Organization (WHO) guidelines [7]. Shock was defined as a pulse pressure of ≤20 mm Hg with accompanying tachycardia, peripheral vasoconstriction, and prolonged capillary refill time (DHF grade III); if the blood pressure was unrecordable and the patient obtunded, the severity of the infection was considered to be grade IV. Girls who were menstruating were excluded because baseline capillary permeability is altered in this group of patients [8]. Acute dengue infection was confirmed subsequently by use of a capture ELISA to detect elevated dengue-specific IgM on paired serum samples that were obtained at admission and at discharge [9].

Clinical methods. On admission to the study, a brief history was taken and a full clinical examination was performed. In children with shock, a double-lumen central venous catheter (Cook) was inserted into the right femoral vein using the standard aseptic technique, so that the tip of the catheter lay within the inferior vena cava below the level of the diaphragm. One catheter port was connected via a transducer to a monitor (Merlin; Hewlett-Packard), to provide continuous central venous pressure measurements. Blood samples were obtained at the time of admission and were tested for hematocrit levels and full blood count, plasma lactate levels, plasma biochemistry, colloid osmotic pressure, and dengue serology. Patients were then resuscitated immediately with 0.9% saline solution, in accordance with WHO guidelines [7] and hospital policy. As soon as the patient was hemodynamically stable, the first MSG measurement was initiated. Further episodes of shock were treated with iv colloid (Dextran 70) as required.

The basic procedure was identical for patients with DHF but without shock, with the exception that an iv cannula with a 3-way tap, instead of a central venous catheter, was inserted into a large forearm vein. The cannula was used both for the administration of 0.9% saline and for drawing blood samples. If any of the patients in this group developed shock, central venous pressure monitoring was undertaken as outlined above.

In all patients with DHF, blood pressure and pulse were measured either every 30 min or more frequently if indicated clinically. Blood for measurement of levels of plasma lactate and hematocrit was obtained again at 2, 4, 6, and 24 h. The MSG recording was repeated at 6 h and 24 h and again at discharge. Repeat full blood counts were collected at 24 h and then at discharge, when the full admission blood sample tests were repeated. Central venous pressure lines were removed at 24 h unless the patient remained hemodynamically unstable.

Strain gauge plethysmography was performed on age-matched control patients. Two separate recordings were made 2 weeks apart. Fully informed consent for this noninvasive procedure was given before the study by the patients’ parents or guardians.

Strain gauge plethysmography. The MSG apparatus and protocol have been described fully elsewhere [5], so we present only a brief outline. The children laid down snugly in an orthopedic mattress filled with polystyrene beads for >15 min, so that they would remain as still as possible, before data were recorded. Air was evacuated from the mattress with a foot pump after preparation was completed. The MSG assessments were made unilaterally, and the position of the chosen leg was adjusted so that the midcalf was at the same level as the right atrium. A pediatric thigh occlusion cuff was fitted to the ipsilateral leg, and the strain gauge was fixed around the left calf at a site of known circumference. At the start of each recording session, the gauge was stretched to a standard tension, so that both increases and decreases in circumference would be measured within the linear part of the gauge’s range, and then calibrated. The study protocol comprised a 3–5-min control recording, followed by the application of a series of small (8–10 mm Hg) cumulative increases in VCP. Each step lasted 4–5
Figure 1. Principles of analysis of strain gauge plethysmography recordings. A, Strain gauge output response to a cuff pressure increase of ~9 mm Hg. The initial rapid increase in limb volume \( V_a \) is attributable to vascular filling, whereas the slope \( J_v \) reflects fluid filtration. B, Values of \( V_a \) from successive cuff pressure increases are plotted against cuff pressure. The intercept with the X-axis of the fitted line represents the calf venous pressure. C, Values of \( J_v \) measured at successive cuff pressures are plotted against cuff pressure. The slope of the regression line represents microvascular permeability \( (K_f) \), and the intercept with the X-axis represents the isovolumetric venous pressure \( (P_{vi}) \). Reprinted with permission from [28].

The initial rate of volume change after the application of a subdiastolic VCP step reflects calf arterial blood flow \( (Q_a) \) [6]. Calf arterial blood flow was assessed in a subset of patients with dengue and in age-matched control patients by measurement of the slope of the first 3 s of the volume response to a 30-s duration, 40-mm Hg VCP step. Signals reflecting cuff pressure, measured by means of a pressure transducer, and changes in limb circumference were recorded continuously for later off-line “blind” analysis. The duration of a complete study was usually 35–40 min.

The same MSG protocol was used for both patients with dengue and control patients. Room temperature was kept at 24°C–26°C, by means of an air conditioning unit, so that skin temperature remained at 30°C–31°C; the latter was monitored continuously from the contralateral calf.

**MSG analysis.** The principles underlying the analysis have been described in detail elsewhere [5]. Once the ambient venous pressure in the limb is exceeded, each additional pressure increment causes a rapid change in limb volume attributable to vascular filling \( (V_a \text{ units} = \text{milliliters per 100 mL}) \) (figure 1a). When the congestion cuff pressure exceeds the value reflecting the microvascular equilibrium pressure (the isovolumetric venous pressure \( [P_{vi}] \)), and after the completion of venous filling, the steady-state change in volume \( (J_v \text{ units} = \text{mL/min per 100 mL}) \) reflects fluid filtration (figure 1a). Because the time courses of the \( V_a \) and \( J_v \) components are so different, the components...
can be readily differentiated by means of computer analysis. At pressures greater than $P_{cv}$, the relationship between VCP and $J_v$ is linear, and the slope (the filtration capacity [$K_f$]) is an index of microvascular permeability to water (figure 1b). The curvilinear relationship between $J_v$ and VCP is shown in figure 1c. The intercept of the $V_f$/VCP curve, which is the pressure that needs to be exceeded to induce the first change in limb volume, represents the local venous pressure [10].

The value $K_f$ is the product of the net filtration coefficient ($L_f$), a constant that describes the permeability of the microvessels per unit surface area, and the total surface area available. Studies of adult control patients have suggested that the total microvascular surface area is available for assessment over a wide range of sympathetic discharges that range from those induced by anesthesia through to the response to a sustained 60-degree head uptilt [11]. The pressure intercept of the $K_f$ slope is the isovolumetric venous pressure ($P_{cv}$); this reflects the value that needs to be exceeded to induce net fluid filtration [12]. Because the intravascular force resisting fluid filtration is the effective colloid osmotic pressure ($\pi$), $P_{cv}$ represents the product of $p$ and the constant ($\sigma$), which is the coefficient describing the microvascular permeability to proteins [13].

The relationship between the parameters defined above is described by the Starling equation, $J_v = K_f [(P_{cv} - \sigma (p - \pi))$, where $P$ represents hydrostatic force and $\pi$ and $\sigma$ represent the intravascular and interstitial compartments, respectively. Earlier studies of adult control patients [14] have shown that neither interstitial pressure nor tissue oxygenated cytochrome aa3, which was used as an index of muscle nutritive blood flow, were influenced by the MSG protocol, providing that diastolic pressure is not exceeded [14]. These observations are supported by those that show that the cumulative VCP protocol does not interfere with limb arterial inflow [6].

The periodic changes in limb volume in the strain gauge recording were quantified by use of the analysis program and a procedure that has been described in detail elsewhere [15]. In brief, 128-s segments of the recording were submitted to analysis. Linear trends were removed to avoid the appearance of spurious low frequency peaks. The data were windowed by means of a tapering Hanning window to eliminate square wave noise. A fast Fourier transformation was then performed on the resulting data, and the frequency spectrum was constructed from 0.24 to 30 cycles/min. The computer analysis program assessed the amplitude for each frequency, calculating it as the volume change (described in milliliters per 100 mL of tissue).

**Measurement of colloid osmotic pressure.** Colloid osmotic pressure was measured by use of a Wescor Colloid Osmometer type 4400 (Wescor), fitted with PM 30 (cutoff, 30,000 Da) membranes.

**Statistical analysis.** The distribution of continuous variables was assessed for normality by use of the Shapiro-Wilks test. If possible, skewed distributions were log transformed toward normality. Normally distributed data were expressed as means with 95% CIs, and comparisons between groups and over time made by use of repeated-measures analysis of variance. Post hoc comparisons between pairs of groups were made by specifying contrasts within the repeated-measures analysis of variance (ANOVA) models and adjusting for multiple comparisons. Data that were not normally distributed were expressed as medians with exact 95% binomial CIs and were compared between groups by use of the Kruskall-Wallis test. Nominal data were compared by use of Fisher’s exact test. Statistical analyses were performed by use of the Stata (StataCorp) and SuperANOVA (Abacus Concepts) computer software packages.

**RESULTS**

**Clinical and laboratory.** From July 1994 through July 1995, 50 children were studied; 19 had DSS (group A), 16 had DHF without shock (group B), and 15 were healthy control patients (group C). None of the control children had a history of previous DHF or DSS. The mean ages in the 3 groups were similar (10.2, 9.8, and 9.2 years, respectively; $P = .43$), as were other baseline characteristics (table 1). For the patients who had DHF at the time of admission to the study, severity was

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**Table 1.** Baseline characteristics of the children enrolled in the study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>With shock ($n = 19$)</th>
<th>Without shock ($n = 16$)</th>
<th>Control patients ($n = 15$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>10.2 (9.2–11.2 [7–14])</td>
<td>9.9 (8.5–11.3 [6–14])</td>
<td>9.2 (8.0–10.4 [6–12])</td>
<td>.43</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>11 (58)</td>
<td>8 (50)</td>
<td>4 (27)</td>
<td>.19</td>
</tr>
<tr>
<td>Duration of illness, d</td>
<td>4.4 (4.0–4.8 [3–6])</td>
<td>4.6 (4.1–5.1 [3–6])</td>
<td>—</td>
<td>.50</td>
</tr>
<tr>
<td>Height, cm</td>
<td>132.4 (126.2–138.5 [109–150])</td>
<td>132.0 (126.2–137.8 [112–150])</td>
<td>124.9 [117–133 [90–145]]</td>
<td>.20</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>26.6 (14.5–43.0 [3.0–30.2])</td>
<td>23.3 (15.5–38.0 [0.1–26.4])</td>
<td>26.0 (21.7–30.2 [15.0–42.0])</td>
<td>.36</td>
</tr>
</tbody>
</table>

**NOTE.** Data denote the mean (95% CI [range]) of each variable, unless otherwise indicated.

* For age, illness duration, height, and weight, $P$ was calculated by use of analysis of variance; for sex, Fisher’s exact test was used.
Table 2. Symptoms in 35 children with dengue hemorrhagic fever at the time of admission to the hospital.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Children with dengue</th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With shock (n = 19)</td>
<td>Without shock (n = 16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>19 (100)</td>
<td>16 (100)</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>5 (26)</td>
<td>4 (25)</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myalgia</td>
<td>2 (11)</td>
<td>2 (13)</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthralgia</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>9 (47)</td>
<td>6 (38)</td>
<td>0.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>13 (68)</td>
<td>10 (63)</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>3 (16)</td>
<td>0 (0)</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>14 (74)</td>
<td>5 (31)</td>
<td>0.018</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epistaxis</td>
<td>3 (16)</td>
<td>7 (44)</td>
<td>0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other bleeding</td>
<td>3 (16)</td>
<td>0 (0)</td>
<td>0.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>2 (11)</td>
<td>4 (25)</td>
<td>0.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lethargy</td>
<td>12 (63)</td>
<td>3 (19)</td>
<td>0.016</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Short of breath</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous iv fluids</td>
<td>10 (53)</td>
<td>2 (13)</td>
<td>0.017</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

a By Fisher’s exact test.
b Gastrointestinal tract and gums.

Table 3. Clinical signs in 35 children with dengue hemorrhagic fever at the time of admission to the hospital.

<table>
<thead>
<tr>
<th>Sign</th>
<th>Children with dengue</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With shock (n = 19)</td>
<td>Without shock (n = 16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>37.0 (37.0–37.0 [36.8–37.5])</td>
<td>37.5 (37.3–38.4 [37.0–40.0])</td>
<td>.0008</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>117 (101–120 [93–140])</td>
<td>120 (100–120 [84–120])</td>
<td>.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory rate, breaths/min</td>
<td>24 (24–28 [24–34])</td>
<td>24 (23–24 [8–24])</td>
<td>.004</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>20 (15–20 [10–24])</td>
<td>30 (30–32 [25–54])</td>
<td>.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatomegaly, no. (%)</td>
<td>17 (89)</td>
<td>14 (88)</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal tenderness, no. (%)</td>
<td>13 (54)</td>
<td>11 (46)</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold peripherally, no. (%)</td>
<td>15 (88)</td>
<td>2 (13)</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petechiae, no. (%)</td>
<td>19 (100)</td>
<td>12 (75)</td>
<td>.035</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purpura, no. (%)</td>
<td>1 (5)</td>
<td>0 (0)</td>
<td>1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capillary hematocrit level, %</td>
<td>51 (48.7–62.4 [44–57])</td>
<td>43 (40.9–44.6 [36–48])</td>
<td>&lt;.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Venous lactate, µM/L</td>
<td>1.0 (0.8–1.2 [0.2–1.8])</td>
<td>0.64 (0.5–0.8 [0.2–1.2])</td>
<td>.007</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelets, ×10¹²cells/mm³</td>
<td>29,000 (20,000–32,830 [12,000–90,000])</td>
<td>64,000 (40,000–108,966 [20,000–150,000])</td>
<td>.0003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC count, ×10⁹cells/mm³</td>
<td>4,325 (3,443–7,026 [2,000–14,700])</td>
<td>3,425 (2,855–4,748 [1,700–5,200])</td>
<td>.065</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colloid osmotic pressure, mm Hg</td>
<td>22.7 (19.0–24.7 [10.8–30.0])</td>
<td>26.6 (23.5–28.7 [16.3–34.8])</td>
<td>.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Data for continuous variables denote the median (95% CI [range]) of the clinical sign value, unless otherwise indicated, and were compared by use of the Kruskal-Wallis test. Nominal data were compared by use of Fisher’s exact test.

a Normally distributed data are expressed as mean (95% CI [range]). Data compared by use of t tests.

graded as follows: grade I in 2 patients; grade II in 14; grade III in 18; and grade IV in 1. Other than a higher frequency of abdominal pain and lethargy in children in group A, reported symptoms in the 2 groups were similar (table 2). Treatment was not delayed while we waited for study equipment to be set up, so 10 patients in group A and 2 in group B had been given 0.9% saline before baseline blood tests were performed; the median fluid volume administered in these cases was 4.0 mL/kg (interquartile range, 2.1–6.5 mL/kg; range, 1.5–25.0 mL/kg).

The clinical and laboratory findings in the 2 patient groups at the time of admission to the study are shown in table 3. Patients in group A had a higher median respiratory rate, narrower pulse pressure, higher venous hematocrit and lactate levels, and lower platelet counts and colloid osmotic pressure. They were also more likely to exhibit bleeding into the skin than were those in group B. Patients in group B were more likely to be febrile.

All patients survived. Eight children in group A had >1 episode of shock (1 patient had 5 separate episodes), whereas 2 children in group B developed shock during the course of the study. Nine children from group A and 1 from group B needed colloid therapy for recurrent episodes of shock, and 1 patient in each group required inotropic support for a short period (table 4). Patients in group A were more likely to bleed from iv cannula sites, but there was no difference in overall rates of hemorrhage between the groups. Duration of hospital stay was similar for patients in the 2 groups. At discharge, patients in group A still had a lower median platelet count and colloid osmotic pressure and a higher median venous lactate than did those in group B.

MSG. A total of 136 recordings were made, 117 of which could be analyzed; for the remaining 19, the patients were restless, and movement artifact made analysis impossible. In 1 patient from group A, the MSG equipment failed and no recordings could be made. In those children who were able to...
stay still, the procedure was very well tolerated, and no child complained of discomfort or became distressed in any way.

**Microvascular leakage (Kf).** Values of microvascular permeability (Kf) that we obtained are presented in table 5 and are illustrated in figure 2. Healthy control patients were observed to have mean Kf values approximately twice as high as those of healthy adults observed in previous studies that used identical equipment for measurement and analysis. At admission, geometric mean Kf was 8.9 (7.3–10.8) mL/min/mm Hg/100 mL × 10−3 in the patients with dengue, compared with 6.4 (5.2–7.9) for the first measurement in the control group (P = .04). By use of repeated-measures ANOVA and after correction for multiple comparisons, geometric mean Kf values in both group A and group B were found to be significantly higher than those in healthy control patients (P < .02 in both cases, and P < .02 for the overall effect of subject group). Time had no effect on the significant difference between the children in the control group and the children in group A and group B (P = .45 for patients with DSS and P = .80 for patients with DHF). Between the 2 groups of patients with dengue, there was no significant difference in geometric mean Kf at admission (P = .97). A comparison of the 2 groups of patients with dengue over the whole study period showed no effect of either time (P = .91) or group (P = .19). Children in both group A and group B continued to have similarly increased microvascular permeability throughout the duration of their hospital stay.

**Isovolumetric venous pressure (Pvi).** Mean Pvi, at admission was 25.6 (95% CI, 24.1–29.2) in the patients with dengue, compared with 17.5 (95% CI, 14.8–20.2) in the first set of measurements for control patients (P = .0001). Repeated-measures ANOVA was used to compare the 3 groups both at the time of admission and at discharge; these showed that both groups of patients with dengue had significantly higher mean values of Pvi at the time of admission than did healthy control patients (P = .001 for patients with DSS and P = .004 for patients with DHF, after correction for multiple comparisons) (table 5; figure 3). As with Kf, time had no effect on the significant difference in Pvi between the children in the control group and the children in groups A and B (P = .49 for DSS and P = .95 for DHF). Comparing the children in groups A and B over the whole study with repeated-measures ANOVA showed no effect of either group (P = .91) or time (P = .19). As with Kf, Pvi remained similarly elevated in both groups of children with dengue at the time of discharge.

**Volumotion.** The most striking and unexpected finding we saw in the MSG records of all patients with DHF was the phenomenon of volumotion. This is a slow oscillating signal from the gauge that indicates cyclical variation in limb circumference. It occurred with an average frequency of 5.4 cycles/min and an amplitude of 0.09 mL/100 mL; it was present at each stage of the study, from admission to discharge. Neither the amplitude nor the frequency was influenced by either the level of congestion pressure used in the protocol or the stage in the disease. Volumotion, as observed by use of strain gauge plethysmography, may reflect periodic changes in arteriolar resistance (vasomotion) [15]. Although it is known to occur in

Table 4. Complications and outcome markers in 35 children with dengue hemorrhagic fever.

<table>
<thead>
<tr>
<th>Complication</th>
<th>With shock (n = 19)</th>
<th>Without shock (n = 16)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shock, No. (%)</td>
<td>8 (42)</td>
<td>2 (13)</td>
<td>.07</td>
</tr>
<tr>
<td>Required colloid, No. (%)</td>
<td>9 (47)</td>
<td>1 (7)</td>
<td>.02</td>
</tr>
<tr>
<td>Required inotropes, No. (%)</td>
<td>1 (5)</td>
<td>1 (7)</td>
<td>1.0</td>
</tr>
<tr>
<td>Epistaxis, No. (%)</td>
<td>2 (11)</td>
<td>3 (19)</td>
<td>.64</td>
</tr>
</tbody>
</table>

Bleeding

At iv sites, No. (%) 12 (63) 4 (25) .04

Any, No. (%) 13 (68) 7 (44) .18

Days in hospital 3.8 (3.5–4.1 [2–5]) 4.0 (3.4–4.6 [2–6]) .46

Hematocrit level at discharge, % 35 (33–37 [28–43]) 36 (34–38 [31–44]) .34

Platelets at discharge, ×1012 cells/mm3 73176 (54352–92001 [16000–134000]) 102800 (78830–126771 [10000–172000]) .045

Venous lactate at discharge, μM/L 0.62 (0.42–0.82 [0.1–1.3]) 0.38 (0.24–0.52 [0.1–0.8]) .04

Colloid osmotic pressure at discharge 22.5 (20.9–25.3 [17.5–33.2]) 25.8 (22.8–28.0 [16.6–32.8]) .15

**NOTE.** Data denote the mean (95% CI [range]) of the complication value, unless otherwise indicated.

* t test for continuous data or Fisher’s exact test for nominal data.

**Table 5.** Microvascular permeability (Kf), isovolumetric venous pressure (Pvi), and volumotion in 35 children with dengue hemorrhagic fever.

| Kf values in groups of patients with dengue had significantly higher mean values of Pvi at the time of admission than did healthy control patients (P = .001 for patients with DSS and P = .004 for patients with DHF, after correction for multiple comparisons) (table 5; figure 3). As with Kf, time had no effect on the significant difference in Pvi between the children in the control group and the children in groups A and B (P = .49 for DSS and P = .95 for DHF). Comparing the children in groups A and B over the whole study with repeated-measures ANOVA showed no effect of either group (P = .91) or time (P = .19). As with Kf, Pvi remained similarly elevated in both groups of children with dengue at the time of discharge.

**Volumotion.** The most striking and unexpected finding we saw in the MSG records of all patients with DHF was the phenomenon of volumotion. This is a slow oscillating signal from the gauge that indicates cyclical variation in limb circumference. It occurred with an average frequency of 5.4 cycles/min and an amplitude of 0.09 mL/100 mL; it was present at each stage of the study, from admission to discharge. Neither the amplitude nor the frequency was influenced by either the level of congestion pressure used in the protocol or the stage in the disease. Volumotion, as observed by use of strain gauge plethysmography, may reflect periodic changes in arteriolar resistance (vasomotion) [15]. Although it is known to occur in
~10% of normal adults (unpublished data) and 6% of control limbs in patients undergoing unilateral elective limb surgery [16], it was not observed in any of the control children assessed in this study.

**Blood flow (Qa).** Calf blood flow was measured in 15 of the patients with dengue (those recruited in the latter half of the study); of these, 6 children had DHF without shock and 9 had DSS. There was no difference in blood flow between these 2 clinical groups, although the geometric mean Qa overall in the patients with dengue was significantly lower than that in the 15 control patients (2.02 [95% CI, 1.42–2.87] mL/min per 100 mL of tissue compared with 5.08 [95% CI, 4.2–6.1] mL/min per 100 mL of tissue; P = .0003). This suggests that the circulation in patients with DHF and DSS is hypodynamic, rather than hyperdynamic.

**DISCUSSION**

To our knowledge, this study is the first to directly demonstrate in humans that increased microvascular permeability occurs in patients with DHF. Although the geometric mean Kf was elevated in both groups of children with dengue, no significant difference between the groups was demonstrated. Because an MSG recording takes an average of 40 min to complete, patients who had shock were resuscitated before the first recording was made (usually 20 mL/kg over 30 min was sufficient). It is possible that if Kf could have been measured immediately at admission and that if we had used a shorter study protocol, DSS vs admission might have been much higher than those in the group of patients without shock. If DSS, which occurs in the minority of children, results from an acute and short-lived massive increase in vascular permeability, then, in any study of DHF or DSS, it would be more likely to observe children recovering from DSS than to observe the onset of the vascular leak.

A short-lived, massive increase in capillary leakage associated with signs and symptoms of hypovolemic shock occurs in patients with anaphylaxis and meningococcal shock, but is unusual in the context of other severe infections. It may result from a pulse of proinflammatory mediators released into the peripheral circulation from dengue-infected macrophages. Cytokines have been implicated in a cascade of events leading to

![Figure 2](https://example.com/figure2.png)

*Figure 2.* Mean log microvascular permeability (Kf) at 0, 6, and 24 h after admission and at discharge. Healthy control children had significantly lower mean values than the 2 groups of patients with dengue (P < .02). There was no significant difference, either at the time of admission or throughout the study, between the children comprising the dengue shock syndrome (DSS) group and those comprising the group with dengue hemorrhagic fever (DHF) without shock.

**Table 5. Microvascular permeability (Kf) and isovolumetric venous pressure (Pvi) in the 3 study groups over time.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Children with dengue</th>
<th>Control patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>With shock (n = 19)</td>
<td>Without shock (n = 16)</td>
</tr>
<tr>
<td>Kf</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>9.3 (6.8–12.6 [1.9–26.3])</td>
<td>8.4 (6.4–11.0 [4.0–21.9])</td>
</tr>
<tr>
<td>T6</td>
<td>7.9 (6.3–9.8 [4.7–12.5])</td>
<td>9.0 (7.2–11.2 [4.9–17.3])</td>
</tr>
<tr>
<td>T24</td>
<td>7.1 (5.6–8.9 [2.9–15.6])</td>
<td>9.5 (7.0–12.8 [3.8–25.5])</td>
</tr>
<tr>
<td>TD</td>
<td>8.7 (6.7–11.2 [4.2–20.2])</td>
<td>9.1 (7.1–11.5 [4.2–15.8])</td>
</tr>
<tr>
<td>Pvi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>28.2 (24.3–32.1 [13.0–40.6])</td>
<td>24.6 (21.4–27.8 [15.1–33.8])</td>
</tr>
<tr>
<td>T6</td>
<td>24.5 (17.8–31.1 [8.4–45.3])</td>
<td>23.4 (18.6–28.1 [13.6–44.6])</td>
</tr>
<tr>
<td>T24</td>
<td>23.0 (20.1–26.0 [15.6–34.7])</td>
<td>24.2 (20.6–27.7 [9.0–33.4])</td>
</tr>
<tr>
<td>TD</td>
<td>22.9 (18.5–27.4 [14.4–40.3])</td>
<td>23.4 (18.4–28.4 [8.7–37.7])</td>
</tr>
</tbody>
</table>

**NOTE.** Kf values in mL/min/mm Hg/100 mL × 10⁻³ are expressed as geometric mean (95% CIs [range]); Pvi (in mm Hg) are expressed as arithmetic mean (95% CIs [range]). T0, at admission; T6, 6 h after admission; T24, 24 h after admission; TD, discharge.
DSS [17], although the shock syndrome of dengue does not occur in other conditions associated with pulse cytokine release such as the paroxysms of malaria, the Jarisch Herxheimer reaction, or bacterial septic shock. If “mediators” cause DSS, then they must be qualitatively or quantitatively different from those that cause the Jarisch Herxheimer reaction and septic shock.

Patients with DHF without shock at the time of admission (group B) had a higher mean $K_i$ than did the group of patients with DSS (group A) at both 6 h and 24 h after admission, although the differences were not significant. At discharge, generally 3–4 days after admission, the mean $K_i$ in both groups of patients with dengue remained significantly higher than that in control patients. In other words, all patients recovering from dengue still had leakier microvessels than did healthy control children.

The elevated vascular permeability observed in patients with DHF may have resulted from elevated concentrations of circulating proinflammatory mediators that result from viral replication within macrophages, an immune response to this, or a more direct viral damage to endothelial cells [18]. A rapid, massive, but self-limiting, further increase in microvascular permeability may be superimposed on this background of increased permeability. This would allow plasma water to flood out of the intravascular compartment, which would lead to sudden hypovolemic shock that would result in hemoconcentration, ascites, and pleural effusions. Repeated episodes of shock could be explained by further pulses of circulating mediators. Failure to treat shock at this early stage leads to the development of grade IV disease, whereby the patient becomes hypotensive and edematous—and much more difficult to treat.

The increased level of microvascular leakiness observed in all patients with DHF, regardless of whether they had shock clinically, may explain why volumotion was present in all of the recordings independent of both disease severity and the time at which the recording was made. The term “volumotion” describes slow-wave cyclical changes in limb circumference observed by use of strain gauge plethysmography and it probably reflects underlying vasomotion, a periodic change in arteriolar diameter [16]. Vasomotion has been reported in animal models during physiological [19] as well as pathological conditions [20, 21]. It has been suggested that it plays an important role during critical reduction of tissue perfusion due to hemorrhage, local blood pressure reduction, ischemia or reperfusion injury, and hypoxic hypoxia [15, 22, 23], and it has also been demonstrated in septic shock [24]. Because previous studies have failed to demonstrate a relationship between vasomotion and either central venous pressure or arterial pressure, volumotion may be induced by local regulatory mechanisms as a result of impaired peripheral tissue perfusion [15, 16, 24]. Vasomotion may be a compensatory mechanism induced to improve tissue oxygenation and nutritive blood flow, particularly in areas that are most vulnerable to hypoxic damage [25].

If volumotion does reflect the alteration of precapillary arteriolar resistance, a coordinated (synchronous) pattern may lead to periodic transmission of higher pressure waves to “fragile” microvessels. Brief increases in capillary pressure can give rise to the transient appearance of gaps through capillary endothelial cells [26]. The required pressures are large, although agents known to influence microvascular permeability (including histamine, substance P, vascular endothelial growth factor, and the calcium ionophore A23187) achieve the same effect at lower pressures [27]. Moreover, these precapillary pressure transients may combine with an effective increase in postcapillary resistance, brought about by increased adhesion molecule expression and platelet aggregation preceding degranulation, for these events occur preferentially, although not exclusively, at venular endothelial sites. The resulting periodic rise in intracapillary pressure may facilitate the short-duration formation of transeendothelial holes, and, thus, efflux of fluid into the interstitial space.

Mean values of isovolumetric venous pressure ($P_{\text{vi}}$) were also found to be significantly higher in patients with dengue than in healthy control patients and, as for $K_i$, these values remained elevated until discharge from hospital. The intravascular colloid osmotic pressure is the main force opposing capillary hydrostatic pressure, and $P_{\text{ci}}$ is the pressure at which these forces balance. $P_{\text{ci}}$ is influenced by the local colloid osmotic pressure and postcapillary resistance that, in adult patients with septic shock, may be raised as a result of endothelial swelling and
postcapillary white cell margination, platelet aggregation, and plugging due to microthrombi. In addition, the reduction in microvascular flow and the increase in microvascular pressure, attributable to the raised resistance, may cause hemocoagulation that, depending on the extent and duration of the increase in protein permeability, may induce an increase in local colloid osmotic pressure.

Which, if any, of these processes play a role in the pathophysiological mechanisms underlying DSS is not clear. However, the net effect would be to increase postcapillary resistance, raise local capillary pressure, and reduce capillary flow. The resulting changes in flow pattern and increased transmural fluid pressure would increase local colloid osmotic pressure by enhancing the fractional fluid extraction per unit of plasma flow along the affected capillaries. There would be a related deterioration in cellular metabolic performance that, in this study, was reflected by higher venous lactates and the presence of volumotion in patients with DHF.

Healthy Vietnamese children have values of $K_f$ approximately twice those of healthy European adults [28]. This relatively higher microvascular permeability in children may in part explain why children are prone to develop shock more rapidly than adults in conditions where increased permeability is known to be an underlying pathophysiological mechanism. The high background microvascular permeability seen in the healthy control children we studied probably resulted from the greater density—and therefore greater surface area—of growing microvessels in skeletal muscle. Growing capillaries have been demonstrated to be more permeable than mature capillaries to water and both small [29] and large [30] molecules. Recently, a better understanding has developed regarding the architecture of the contractile cytoskeletal elements of endothelial cells and the way in which they change in response to a variety of stresses [31].

In vitro evidence shows that the expression of endothelial actin cytoskeletal isoforms changes during development [32]. These data, in conjunction with the observation that the endothelial responses to histaminic H1 and H2 agonists change during in vivo development [30], provide a mechanistic basis for enhanced permeability in response to inflammatory challenges in young children. More recently, evidence has emerged showing how these cytoskeletal mechanisms may be activated. Both high intravascular pressures and challenges associated with inflammatory or regenerative responses can give rise to transient increases in microvascular permeability. The changes were associated with the transient appearance of openings through endothelial cells [33].

In figure 4, we have tried to link a hypothetical threshold for hemodynamic shock to the intravascular fluid loss attributable to DHF. The value of $K_f$ will remain stable in healthy adults, because it is influenced by the hypertrophy and hyperplasia associated with physical activity and, additionally, by the menstrual cycle in women [8]. In children, in contrast, the healthy control value will be age related [28], with the youngest children coming closer to the hypothetical threshold. In the children, DHF will increase baseline permeability from the age-dependent control value. We have shown a fluctuating baseline value in the DHF and DSS panels that possibly reflects pulsed cytokine release. Children with DHF are more likely to progress to the DSS state because their baseline microvascular permeability is higher.

Blood flow in the calf was measured in a subgroup of patients and was found to be significantly lower than it was in control patients. This is consistent with a hypodynamic, hypovolemic etiology for the reductions in systolic blood pressure and pulse pressure observed in patients with DSS. This would be consistent with the hypothesis that volumotion is an index of poor nutritive blood flow. Other shock syndromes associated with raised cytokine levels tend to be hyperdynamic in nature; a
recent study of calf blood flow in severe childhood meningo-
coccal disease has yielded mean values for Q, that are 2–3 times
higher than those in the control patients in our study (J. Gam-
ble, unpublished observations). This implies that the path-
ophysiological role of cytokines in DSS differs from that in other
“septic” shock syndromes.

In summary, this study demonstrates that increased micro-
vascular leakage occurs in children with DHF and is most pro-
nounced in children with DSS. Microvascular permeability is
constitutively higher in healthy children compared with adults,
and we hypothesize that this increased baseline permeability
renders children more susceptible to DSS than it does adults.
The underlying pathophysiological cause of the additional in-
crease in leakage associated with dengue infection may be re-
lated to mechanical, humoral, and cellular factors, but the na-
ture of these factors has yet to be determined.

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