Short Report: Relationship between Nonstructural Protein 1 Detection and Plasma Virus Load in Dengue Patients

Laurent Thomas,* Fatiha Najoullah, Olivier Verlaeten, Jenny Martial, Ségolène Brichler, Stéphane Kaidomar, Victor Moravic, André Cabié, and Raymond Césaire

Centre Hospitalier Universitaire, Fort-de-France, Martinique; Centre d’Investigation Clinique et d’Épidémiologie Clinique Antilles Guyane (CIC-EC INSERM U802), Fort-de-France, Martinique

Abstract. We report data from a prospective observational study performed in Martinique during a co-epidemic of dengue virus serotype 2 (DENV-2) and serotype 4 (DENV-4). Among 70 serum samples from patients with DENV-2 (n = 21) or DENV-4 (n = 49) infections, 47 (67.1%) were positive for dengue nonstructural protein 1 (NS1). Antigenemia correlated with plasma virus load and was independent of immune status and the time of sampling. Increased viremia 4–6 days after onset of illness was associated with NS1 positivity, secondary infection, and severe disease. Testing for NS1 could help identify the potentially most severely ill patients during the critical phase of dengue.

Dengue nonstructural 1 protein (NS1) is secreted by cells infected with dengue virus (DENV).1 This glycoprotein is highly conserved for all DENV serotypes and is strongly immunogenic. The NS1 antigen and NS1-specific antibodies may play a central role in the pathogenesis of dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).2 It has been suggested that high plasma levels of NS1 could help identify patients at risk for plasma leakage.3–5 Commercial enzyme-linked immunosorbent assays (ELISAs) for detection of DENV NS1 in human serum samples have been proposed for dengue diagnosis,6–7 but the value of NS1 detection in predicting clinical severity remains to be evaluated.

In a previous study conducted during a co-epidemic of DENV serotype 2 (DENV-2) and serotype 4 (DENV-4), blood samples were obtained from patients with acute dengue infection and stored in a serum bank.8 These serum samples provided an opportunity to screen serum samples from dengue patients for NS1 and to explore the relationships between NS1 antigenemia, plasma virus loads, dengue serotypes, immune status, and outcomes of illnesses.

The data reported were derived from a prospective observational study. All patients who came to an emergency department in Martinique with a history of acute febrile illness were invited to participate in the study after providing written informed consent. Clinical data were recorded at the bedside in a computerized medical record system by means of a standardized questionnaire. The final severity of illness was diagnosed on the computerized medical record system by means of a standardized questionnaire. The final severity of illness was diagnosed on the computerized medical record system by means of a standardized questionnaire. The final severity of illness was diagnosed on the computerized medical record system by means of a standardized questionnaire.

Blood was obtained by venous puncture at admission time, and serum aliquots were stored at ~70°C for virologic studies. Laboratory methods for the diagnosis of dengue infections and plasma virus load measurements have been described.8 Briefly, a semi-nested reverse transcription–polymerase chain reaction (RT-PCR) was carried out with DENV generic and serotype specific primers, as described by Lanciotti and others.9 Quantitative real-time PCR was performed using generic primers, the iQ SYBR Green Supermix Kit, and the iCycler iQ Real Time PCR Detection System (Bio-Rad, Marne la Coquette, France). Plasma virus load was derived from standard curves obtained by serial dilution of titrated DENV-2 and DENV-4 supernatants and were expressed as plaque-forming unit (PFU) equivalents per milliliter. In addition, an ELISA for detection of NS1 was performed using PLATELLA™ Dengue NS1Ag Kits (Bio-Rad, Marne la Coquette, France) according to the manufacturer’s recommendations. Dengue-specific antibodies were detected using IgM capture, IgG capture, and IgG indirect ELISA kits (Panbio, Brisbane, Queensland, Australia). A positive IgG capture test result for a serum sample obtained within six days of the onset of fever indicated a secondary infection.10 Serum samples with negative results by IgG capture and IgG ELISA indicated a primary infection.

Where indicated, values are expressed as the median and 25–75% interquartile range [IQR]). Data were compared across groups by using non-parametric tests. Logistic regression analysis was used to identify independent association between NS1, plasma virus load, dengue serotype, immune status, and other variables of clinical interest. Variables were entered into multivariate analysis by using a backward selection algorithm. All analyses were done in Statview version 4.5 software (Abacus Concepts, Berkeley, CA).

In the initial study, 110 (28.6%) of 384 patients with acute febrile illness had confirmed dengue infection by RT-PCR. The semi-nested procedure identified 64 DENV-4 infections, 39 DENV-2 infections, 6 DENV-3 infections, and 1 DENV-1 infection.8 Testing for NS1 and measurement of virus load were performed in 70 (68%) of 103 DENV-2 and DENV-4 cases, for which sufficient serum was available. Although serum samples tested were not randomly allocated, the distribution of the clinical variables between the DENV-2 patients and the DENV-4 patients was similar to that observed in the original population.8

The median age of the patients was 30 years (25–75% IQR = 23 years), and the male-to-female ratio was 0.61:1. The median time from the onset of fever to admission to the emergency department (time of presentation and sampling) was 2 days (25%–75% IQR = 2 days) in DENV-4 patients and 3 days (25–75% IQR = 3 days) in DENV-2 patients (P = 0.024, by Mantel-Cox log-rank test). Serologic testing indicated a primary dengue infection in 9 (42.9%) of 21 DENV-2 patients and 43 (82.7%) of 49 DENV-4 patients (P = 0.001, by Fisher’s exact test). Regarding the final severity of illness, uncomplicated dengue fever was diagnosed in 39 (79.6%) of 49 DENV-4 patients and 11 (47.6%) of 21 DENV-2 patients (P = 0.041, by Fisher’s exact test). However, the trend towards more DHF/DSS
in DENV-2 patients than in DENV-4 patients was not statistically significant (7 [33.3%] of 21 versus 9 [18.4%] of 49; \( P = 0.218 \), by Fisher’s exact test).

The median plasma viral load was 5,790 PFU equivalents/mL (25–75% IQR = 45,248 PFU equivalents/mL), and values ranged from 5.6 to 4,940,000 PFU equivalents/mL (Figure 1, top panel). Viremia levels were significantly higher in patients with DENV-2 infections than in those with DENV-4 infections (Figure 1, middle panel). Plasma virus loads decreased significantly over the time of sampling in patients with primary infections (\( P = 0.001 \), by Mantel-Cox log-rank test), but remained increased until the sixth day of illness in those with secondary infections (\( P = 0.915 \), by Mantel-Cox log-rank test). During the first three days of the disease, the distribution of virus loads was not different between patients with primary infections and those with secondary infections (Figure 2A).

**Figure 1.** Percentile distribution of plasma virus loads for 70 patients with symptomatic dengue (DENV) infections on admission to an emergency department in Martinique. PFU = plaque-forming unit; NS1 = non-structural protein 1. \( P \) values were calculated by using the Mantel-Cox log-rank test.

**Figure 2.** Distribution of plasma virus loads in 70 patients with symptomatic dengue infections on admission to an emergency department, Martinique. \( P \) values were calculated using the Mantel-Cox log-rank test.
and between those with uncomplicated illness and those with severe illness (Figure 2B). During days 4–6, patients with secondary infections or with severe illnesses had higher virus loads than those with primary infections or uncomplicated illnesses (Figure 2C and D).

Dengue virus NS1 was detected in 47 (67.1%) of 70 patients positive for dengue by RT-PCR. There was no difference in epidemiologic, clinical, and biological variables between NS1-positive and NS1-negative patients, with the exception of plasma virus loads, and both parameters were prolonged in secondary and severe infections. This finding suggests that NS1 testing could help in identifying the patients likely to develop severe outcomes during the so-called critical phase of dengue. This phase occurs in some patients at approximately 3–5 days of illness and is characterized by development of a vascular permeability syndrome that leads to various degrees of plasma volume loss and hypotension. In addition to warning signs such as a rapid decrease in body temperature, persistent intractable vomiting, increasing abdominal pain, increased hematocrit, and a decrease in thrombocyte counts, a positive NS1 test result would be of special interest for quickly confirming the diagnosis of dengue and suggesting a high probability of persistent viremia.

Future evaluations of the prognostic value of NS1 detection should focus on the time of sampling and clinical phase of the disease. Quantitative assays are also needed because the kinetics of NS1 antigenemia may be of interest for predicting the risk of DHF or other severe complications.

Vital signs

Body temperature, °C
38.7 [1.4] 38.7 [1.7] 0.762

Pulse pressure, mm Hg
48 [24] 49 [20] 0.626

Heart rate, beats/minute
93 [30] 89 [26] 0.684

Signs and symptoms, no. (%)

Headache
42 (89) 20 (87) 1

Retroorbital pain
18 (41) 9 (39) 1

Muscle and/or joint pain
36 (77) 15 (68) 0.559

Backache
38 (81) 17 (74) 0.545

Epigastralgia
19 (41) 9 (39) 1

Gastrointestinal signs
24 (51) 12 (52) 1

Fatigue
34 (74) 17 (74) 1

Cough
12 (26) 6 (26) 1

Rash
5 (11) 0 (0) 0.161

Bleeding
5 (11) 3 (13) 1

Postural hypotension
5 (11) 1 (4) 0.656

Presyncope/syncope
12 (26) 5 (22) 1

Biology

Hematocrit, %

Hemoglobinemia, g/L
134 [26] 135 [24] 0.435

Hematocrit, %

Thrombocyte count × 10^3/mL
154 [94] 171 [105] 0.753

Lymphocyte count/mL
3,705 [2,530] 4,580 [2,050] 0.167

ASAT (U/L)
30 [43] 43 [44] 0.270

NEAT (U/L)
3,705 [2,530] 4,580 [2,050] 0.167

Creatine kinase (U/L)
135 [235] 186 [202] 0.204

Total proteinemia (g/L)

C-reactive protein (mg/L)
6.3 [28] 10.6 [38] 0.274

Uncomplicated dengue, no. (%)
34 (69.4) 16 (69.6) 1

Hospitalized, no. (%)
20 (43) 8 (35) 0.609

Clinical data for 70 symptomatic dengue patients on admission in Martinique*

<table>
<thead>
<tr>
<th>Clinical data</th>
<th>NS1 positive (n = 47)</th>
<th>NS1 negative (n = 25)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma virus load × 10^3 plaque-forming unit equivalent/mL</td>
<td>13.1 [71.8]</td>
<td>1.06 [8]</td>
<td>0.012</td>
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<tr>
<td>Epidemiology</td>
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<td></td>
<td></td>
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<tr>
<td>Age, years</td>
<td>30 [28]</td>
<td>32 [25]</td>
<td>0.837</td>
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<td>Male:female</td>
<td>17:30</td>
<td>9:14</td>
<td>1</td>
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<tr>
<td>DENV-2:DENV-4 infection</td>
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<td>8:15</td>
<td>0.590</td>
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<tr>
<td>Primary:secondary infection</td>
<td>36:11</td>
<td>16:7</td>
<td>0.569</td>
</tr>
<tr>
<td>Sampled days 1–3:4–6</td>
<td>34:13</td>
<td>15:8</td>
<td>0.586</td>
</tr>
</tbody>
</table>

*NS1 = nonstructural protein 1; DENV = dengue virus; AST = aspartate aminotransferase; ALT = alanine aminotransferase. Continuous data are expressed as median [25–75% interquartile range].

By Fisher’s exact test (categorial data) or Mann-Whitney test (continuous data).
REFERENCES


