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Abstract. Martinique experienced a dengue outbreak with co-circulation of DENV-2 and DENV-4. In an emergency department-based study, we analyzed whether the clinical presentation and outcome of adult patients were related to serotype, immune status, or plasma viral load. Of the 146 adult patients who had confirmed dengue infection, 91 (62.3%) were classified as having classic dengue fever, 11 (7.5%) fulfilled World Health Organization criteria for dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), 21 other patients (14.4%) presented with at least one typical feature of DHF/DSS [i.e., internal hemorrhage, plasma leakage, marked thrombocytopenia (platelet count ≤ 50,000 platelets/mm^3) and/or shock], and 23 further patients (15.8%) had unusual manifestations. Four patients died. Severe illness was more frequent in patients with secondary dengue infection (odds ratio, 7.18; 95% confidence interval, 3.1–16.7; $P < 0.001$). Multivariate regression analysis showed that gastrointestinal symptoms and other unusual manifestations were independently associated with DENV-2 infection, whereas cough and DHF/DSS features were independently associated with secondary immune response. A high plasma viral load was associated with DENV-2 infection, increased serum liver enzymes, and with DHF/DSS features in patients presenting after the third day of illness. The most severe cases of dengue resulted from the combined effects of DENV-2 and secondary infection.

INTRODUCTION

Dengue viruses are classified into four antigenically distinct serotypes designated DENV-1 to DENV-4. Although most infections are asymptomatic, all four DENV serotypes can cause a spectrum of disease ranging from "flu-like illness" [dengue fever (DF)] to life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).[1] The pathophysiology of severe dengue disease seems to be multifactorial, involving interactions between viral characteristics, immune features, and host genetic background.[2–5] Hyperendemicity with multiple serotypes is believed to be one of the most significant factors influencing dengue severity.[3–6]

Martinique is a Windward Island of the Caribbean. It is an overseas department of France of 1,128 km^2 with a population of ~400,000 inhabitants. Its "Creole" population is the result of mixing European and African ancestry over five centuries. During the 19th century, Indian and to a lesser extent Chinese migrants arrived in the country, and they integrated rapidly.

During the last decade, Martinique has experienced four dengue epidemics caused by DENV-2 and DENV-4 in 1995, DENV-1 in 1997, and DENV-3 in 2001.[7] Molecular epidemiologic surveillance showed that DENV-2 has persisted, whereas DENV-4 re-emerged in 2004 after a 9-year absence. The re-emerging DENV-4 clade in the French West Indies is phylogenetically related to subtype II strains isolated in the Bahamas in 1998,[8] whereas the DENV-2 clade is closely related to subtype III (Asian–American subtype).[9]

The dengue surveillance network counted ~14,500 cases of dengue fever between June 2005 and April 2006.[7] The outbreak was characterized by co-circulation of DENV-2 and DENV-4. Here we report data on adult victims. Particular attention was paid to clinical severity in view of recent developments.[10,11] The outcome was analyzed according to the dengue serotype, pre-existing heterologous antibodies, and plasma viral load.

MATERIALS AND METHODS

Study participants. The Emergency Department for adults is part of the dengue surveillance network in Martinique and focuses on early detection of severe clinical forms. All patients at least 15 years of age, with a history of acute febrile illness, admitted within 8 days of onset of fever between June 2005 and April 2006, were eligible for inclusion in this study with their informed consent. Information about patients' ethnicity was not reported because of the current strict medical ethics laws in France. Clinical data were recorded at the bedside with a computerized medical record system and a questionnaire tailored to acute febrile illness. Routine laboratory tests were performed. In addition, a 10-mL serum sample was stored in aliquots at −70°C for remote immunologic and virologic studies. All patients in whom dengue infection was shown by reverse transcriptase-polymerase chain reaction (RT-PCR) and/or anti-dengue immunoglobulin M (IgM) detection were included in the study. A daily clinical evaluation and repeated laboratory tests were performed on all hospitalized patients. Follow-up data after the first visit and triage were obtained from the medical records and/or personal phone calls after discharge.

Case definitions. Clinical forms were classified retrospectively, based on data recorded at the first visit and during follow-up. DHF and DSS were diagnosed according to the World Health Organization (WHO) classification system.[1] In keeping with earlier studies,[12–14] patients who presented with at least one typical feature of DHF/DSS [i.e., internal hemorrhage or signs consistent with plasma leakage (hematocrit > 50%, proteinemia < 50 g/L, and/or clinical evidence of serous effusion in the pleural or peritoneal cavity), marked thrombocytopenia (< 50×10^9 platelets/L), or shock (delayed capillary refill, systolic pressure < 90 mm of Hg and/or pulse pressure ≤ 20 mm of Hg)], were also included in a "DHF/DSS-like" group (Table 1). As recently proposed,[11] an "unusual manifestations" group was also created, made up of

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TABLE 1
Clinical criteria used for retrospective classification of patients with confirmed dengue fever in Martinique

Signs and symptoms	$N = 146$	Clinical forms of severity
Acute febrile illness with at least two of the following: headache, myalgia, vomiting, abdominal pain, asthenia. No bleeding or mild external spontaneous bleeding or positive tourniquet test. No apparent plasma leakage. Platelet counts $> 50 \times 10^9$/L	91	Classic DF ($N = 91$, 62.3% of patients)
Above clinical features and at least one of the following		
Symptomatic orthostatic hypotension	11	
Hepatitis	6	
Rhabdomyolysis	3	
Encephalopathy	5	Unusual manifestations group
Myocarditis	1	
Pericarditis	1	($N = 23$, 15.8% of patients)
Above signs and symptoms of DF with or without unusual manifestations, and at least one of the following: internal hemorrhage, evidence of plasma leakage, platelet counts $< 50 \times 10^9$/L, shock		
DHF according to strict WHO requirements	9	DHF/DSS-like group
DSS according to strict WHO requirements	2	($N = 32$, 21.9% of patients)
Platelet counts $< 50 \times 10^9$/L only	6	
Miscellaneous overlapping symptoms	15	

patients not meeting the above DHF/DSS-like criteria but having at least one of the following features: encephalopathy, symptomatic postural hypotension, dehydration, serum electrolyte or acid-base disorders, hepatitis (10-fold increase in aminotransaminases), rhabdomyolysis (20-fold increase in creatine kinase), or elevated cardiac enzymes (troponin-1c > 1 μg/L). Patients included in the DHF/DSS-like and unusual manifestations groups were considered to have severe illness. Patients who presented with acute febrile illness alone or associated with isolated mild external hemorrhage (positive tourniquet test, petechiae, mucosal bleeding) were diagnosed as having classic DF.

Dengue RNA detection and genotyping. RT-PCR determination was performed on material sampled on admission to the emergency room. Viral RNA was extracted from 140 μL of serum using the High Pure Viral Nucleic Acid reagent set (Roche Molecular Biochemicals, Meylan, France) according to the manufacturer's instructions. RT-PCR was carried out with dengue consensus primers D1 and D2, followed by semi-nested PCR with D1 and serotype-specific primers TS1, TS2, TS3, and TS4, as described by Lanciotti and others.[15] This procedure was verified through participation in a multicenter quality control study conducted by the National Reference Center for Arboviruses, Institut Pasteur, French Guiana.

Preparation of dengue virus–titrated controls. Local isolates of DENV-2 and DENV-4 were propagated in *Aedes pseudoscutellaris* (AP61) cells. The culture supernatants were titrated on vero cell monolayers using a standard plaque-forming assay. Stock suspensions of the two dengue virus serotypes were stored at –80°C until use. Tenfold serial dilutions were made in negative human plasma to obtain concentrations ranging from 10^6 to 10 plaque forming units (PFU)/mL.

Dengue plasma viral load measurement. Quantitative real-time PCR was carried out in sera collected on admission to the emergency room using primers D1 and D2 and intercalation of SYBR Green I as the fluorescence reporter (Verlaeten and others, unpublished data). Briefly, RNA was reverse-transcribed with the SuperScript II Reverse Transcriptase kit (Invitrogen, Cergy-Pontoise, France). Real-time PCR was performed with the iQ SYBR Green Supermix kit (Bio-Rad, Marne la Coquette, France) using the iCycler iQ Real Time PCR detection system (Bio-Rad). Threshold cycles (C_T) were calculated, and melting curve analysis was done for each PCR product. Standard curves were obtained with titrated DENV-2 and DENV-4 supernatants serially diluted from 10^6 to 10 PFU/mL. The standard curves obtained by serial dilution of titrated DENV-2 and DENV-4 supernatants had similar slopes, the PCR efficiencies being consistently above 90% and the correlation coefficients $R^2 > 0.997$. The detection limit was estimated at 10 PFU equivalents/mL.

Antibody responses. Dengue-specific antibodies were detected in sera collected on admission to the emergency room by using IgM capture, IgG capture, and IgG indirect ELISA kits (Panbio, Brisbane, Australia). A serum-to-calibrator absorbance ratio ≥ 1.1 was defined as positive for IgM capture and IgG indirect tests, whereas a ratio ≥ 2.2 was needed for IgG capture, as recommended by the manufacturer. The elevated cut-off for the IgG capture test has been shown to discriminate between primary and secondary IgG responses in a single acute phase serum sample.[16,17] A positive IgG capture test on serum collected within 7 days of the onset of fever was considered to indicate a secondary infection. Sera negative by IgG capture were tested by the IgG indirect ELISA. If this was positive, the case was also classified as a secondary infection. If both tests were negative, the infection was diagnosed as primary.

Statistical analysis. Data were analyzed using StatView 4.5. Duration of illness at admission was defined as the time elapsed from the date and hour of onset of chills and fever to the date and hour of clinical examination and blood sampling. Each time period from 0 to 24 hours was rounded up to 1 day of illness. Values, where indicated, are expressed as the median and 25–75% interquartile range (25–75 IR). Data were compared across groups using non-parametric tests. Multiple logistic regression analysis was used to assess whether independent association could be shown between variables of clinical interest and serotype and/or immune status. Odds ratios (ORs) were generated and expressed with 95% CIs. Two-tailed $P \leq 0.05$ was considered statistically significant.

RESULTS

Spectrum of disease. A total of 389 questionnaires were completed. Of these, 175 (45%) were assigned alternative

diagnoses, 68 (17.5%) to indeterminate serologies, and 146 (37.5%) to confirmed dengue infections. DENV-2 was shown in 39 patients, DENV-4 in 64 patients, DENV-3 in 6 patients, and DENV-1 in 1 patient. No dual infections were observed. In another 36 patients, the diagnosis of dengue fever was based on a positive IgM capture test only. The median age of the patients with dengue fever was 35 years (25–75 IR, 26 years). There were 63 males and 83 females (female to male ratio 1.3). Most patients (115/146) had been given various doses of paracetamol at home (median maximum dose, 30 mg/kg/d; 25–75 IR, 30 mg/kg/d). Twelve patients had notable associated pathologies. Pregnancy was mentioned in two other patients. After the initial assessment, 97 (66.5%), 25 (17.1%), and 24 (16.4%) patients, respectively, were classified in the DF, DHF/DSS-like, and unusual manifestations groups. The median time between onset of fever and the first visit was 3 days (25–75 IR, 4 days) and was 2 (25–75 IR, 3 days), 4 (25–75 IR, 2.5 days), and 6 days (25–75 IR, 3 days) in the DF, unusual manifestations, and DHF/DSS-like groups, respectively ($P < 0.001$ by Kruskal-Wallis test).

Patient outcome. After the first evaluation, 68 (46.6%) of the 146 patients were hospitalized. Ten of the other patients returned to the hospital within 3 days. Of these, seven patients had confirmed DF, but three patients were subsequently hospitalized for symptomatic postural hypotension, hepatitis, and DHF. Among the patients who were hospitalized after initial presentation, three patients initially diagnosed with DF were upgraded during hospitalization: two to the unusual manifestations group and one to the DHF/DSS-like group. Five patients initially diagnosed with unusual manifestations were subsequently included in the DHF/DSS-like group. The final classification of clinical severity is shown in Table 1. Symptomatic postural hypotension was documented in 14.4% of patients (47.8% of patients with unusual manifestations and 31.2% of those with DHF/DSS-like features). Hepatitis was recorded in 7.5% of patients (26.1% of patients with unusual manifestations and 15.6% of patients with DHF/DSS-like features). Rhabdomyolysis was recorded in 4.8% of patients (13% of patients with unusual manifestations and 12.5% of patients with DHF/DSS-like features).

Three previously healthy patients infected with DENV-2 virus and included in the DHF/DSS-like group at presentation died within 2 weeks after onset of fever: a 62-year-old woman with intracranial bleeding (platelet count 14×10^9/L) died of irreversible coma; a 53-year-old man with fulminant hepatitis (aspartate aminotransferase, 12,770 U/L; platelet count, 17×10^9/L; prothrombin time, 11% of normal) developed hepatic coma and died despite attempted liver transplantation; and a 41-year-old woman with acalculous gangrenous cholecystitis developed *Proteus mirabilis* septicemia and died of septic shock despite attempted cholecystectomy. In addition, a previously healthy 35-year-old woman was diagnosed with acute severe myocarditis 6 days after onset of fever (EKG abnormalities; troponin-1c = 21.9 μg/L; elevated dengue IgM antibodies; no serotype available). She refused heart transplantation and died of cardiogenic shock 2 weeks later. No other fatal cases were recorded by the surveillance network in Martinique.

Differences in severity between primary and secondary dengue infections. Analysis of the immune response indicated a primary dengue infection in 78 patients (66.7%) and a secondary infection in 39 patients (33.3%). A secondary immune response was significantly associated with severe illnesses (OR, 7.18; 95% CI, 3.1–16.7; $P < 0.001$, Fisher test), and all fatal cases involved secondary infection. Patients with secondary infection were more likely to be hospitalized (OR, 3.38; 95% CI, 1.5–7.6; $P < 0.01$, Fisher test). Cough and gastrointestinal symptoms were more frequent in patients with secondary infection (41% versus 19.2%, respectively, $P = 0.02$; 78.9% versus 41%, $P < 0.001$, Fisher test) and so was purpura (13.5% versus 1.3%; $P < 0.05$, Fisher test; Figure 1) and hepatitis (16.2% versus 1.3%; $P < 0.01$, Fisher test). Several biochemical and hematologic parameters recorded at presentation in the emergency room differed between patients with primary and secondary dengue infection (Table 2; Figure 2, A and B).

Differences in clinical presentation between primary DENV-2 and DENV-4 infections. Primary infection was diagnosed in 17 of 36 DENV-2 patients (47.2%) and 52 of 59 DENV-4 patients (88.1%; $P < 0.001$, Fisher test). In these patients, the median time between onset of fever and the first visit was 2 days (25–75 IR, 2 days). This was significantly different from the time of presentation of patients with secondary infections (median time, 4 days; 25–75 IR, 2 days; $P < 0.001$, Mann-Whitney test). Sore throat was mentioned in 19 DENV-4 patients (36.5%) and in 1 DENV-2 patient (5.9%; $P = 0.015$, Fisher test). Gastrointestinal symptoms were recorded in 11 DENV-2 patients (64.7%) and in 17 DENV-4 patients (32.7%; $P = 0.025$, Fisher test). Unusual manifestations were observed mostly in DENV-2 patients, whereas rates of DHF/DSS-like features were identical in both serotypes (Table 3). Patients with DENV-2 infections showed higher serum creatine kinase and aspartate aminotransferase levels.

Differences in clinical presentation between secondary DENV-2 and DENV-4 infections. The distribution of all variables of clinical interest recorded in the emergency room and the clinical forms of severity observed during follow-up were not significantly different between patients with secondary DENV-2 or DENV-4 infections.

Relationship between the plasma viral load and the serotype. The viral loads in serum ranged from 6 to $461{,}000 \times 10^3$ PFU equivalents/mL, with a median of 7.16×10^3 PFU equivalents/mL (25–75 IR, 68.29×10^3 PFU equivalents/mL), and fell from day 1 to day 6 of illness in both the DENV-2 and DENV-4 groups (Figure 2, C and D). DENV-2–infected patients had higher viral loads than DENV-4–infected patients [32.65×10^3 (25–75 IR, 396.25×10^3 PFU equivalents/mL) versus 5.2×10^3 PFU equivalents/mL (25–75 IR, 33.22×10^3 PFU equivalents/mL), respectively; $P = 0.015$, Mann-Whitney test]. However, this difference in viral loads between serotypes only held true for primary infections (Table 3).

No correlation was observed between plasma viral load and sex, age, and most physiological variables. A noteworthy exception was that higher viral loads were seen in patients with aspartate aminotransferase levels at least 5-fold the normal value [45.9×10^3 (25–75 IR, $2{,}367 \times 10^3$ PFU equivalents/mL) versus 5.8×10^3 PFU equivalents/mL (25–75 IR, 47.98×10^3 PFU equivalents/mL), respectively; $P = 0.05$, Mann-Whitney test]. The highest plasma viral load ($461{,}000 \times 10^3$ PFU equivalents/mL) was recorded in the patient with secondary DENV-2–associated fatal fulminant hepatitis.

Relationship between the plasma viral load and the immune status. Viral loads were not significantly different be-

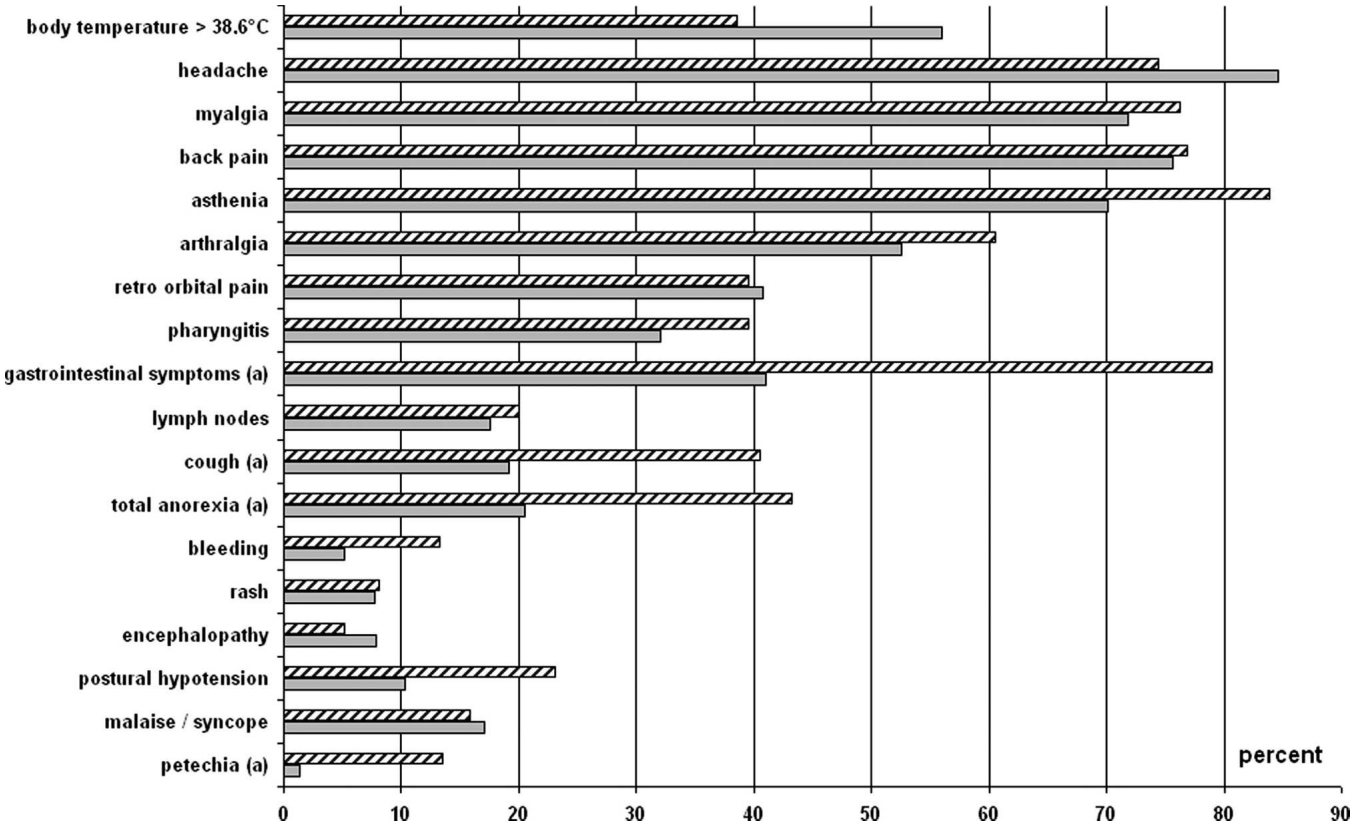

FIGURE 1. Frequency of basic symptoms reported on admission to the emergency room in adult patients diagnosed with primary (gray bars) or secondary dengue infections (hatched bars). (a) $P < 0.05$, Fisher test.

TABLE 2

Data recorded on admission to the emergency room and final clinical severity diagnosed during follow-up in patients with confirmed primary or secondary heterologous dengue infections

Data*	Primary infection ($N = 78$)	Secondary dengue infection ($N = 39$)	P
Age (years)	34 (27)	35 (22)	0.924
Male/female (N)	32/46	21/18	0.238
Body temperature (°C)	38.8 (1.3)	37.8 (2.5)	0.013†
Pulse pressure (mm of Hg)	49 (22.5)	48.5 (16)	0.479
Heart rate (beats/min)	95 (28)	82 (14)	0.018†
Natremia (mmol/L) [135–145]‡	137 (4)	135 (4)	0.088
Chloremia (mmol/L) [90–105]	99 (3)	97.5 (6)	0.002†
Kaliemia (mmol/L) [3.5–4.5]	3.9 (0.6)	3.9 (0.6)	0.318
HCO_3^- (mmol/L) [22–30]	24 (4)	25 (4)	0.369
Proteinemia (g/L) [60–80]	73 (6.8)	72 (10.5)	0.477
Hematocrit (%) [36–44]	39 (6)	41 (6.8)	0.005†
Hemoglobinemia (g/L) [105–135]	132 (24)	141 (24)	0.001†
Platelets ($\times 10^9$/L) [150–350]	168 (78.8)	82 (97)	<0.001†
Polymorphonuclear cells ($\times 10^9$/L) [1.5–8.5]	4.17 (2.5)	3.11 (2.4)	0.002†
Lymphocytes ($\times 10^9$/L) [1–4]	0.56 (0.39)	0.83 (0.79)	<0.001†
AST (U/L) [4–37]	30.5 (21)	130 (209)	<0.001†
ALT (U/L) [4–50]	19 (21)	78 (155)	<0.001†
CK (U/L) [20–170]	153 (148)	200 (364)	0.187
CRP (mg/L) [0–10]	9.6 (38.7)	6.6 (28)	0.208
APTT(s) [25–40]	37 (3.3)	41.5 (7)	0.001†
Prothrombin level (%) [70–100]	73 (17)	82 (17)	0.007†
Viral load ($\times 10^3$ PFU equivalent/mL)	10.7 (72.6) $N = 59$	2.5 (9.4) $N = 19$	0.23
Classic dengue fever [N (%)]	61 (78.2)	13 (33.3)	<0.001§
DHF/DSS-like group¶ [N (%)]	4 (5.1)	16 (41.1)	<0.001§
Unusual manifestations** [N (%)]	13 (16.7)	10 (25.6)	0.32
Hospitalized [N (%)]	29 (37.2)	26 (66.7)	0.003§

* Median value and (25–75% interquartile range).
† $P < 0.05$ by Mann-Whitney test.
‡ Reference range is shown in brackets.
§ $P < 0.05$ Fisher's test.
¶ DHF/DSS-like group was defined as the presence of at least one of the following: internal hemorrhage, signs of plasma leakage, shock, platelet count < 50×10^9/L.
** Unusual manifestations group was made up of other severe clinical manifestations not included in the definition of DHF/DSS-like group.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase; CRP, C reactive protein; APTT, activated partial thromboplastin time; PFU, plaque forming unit.

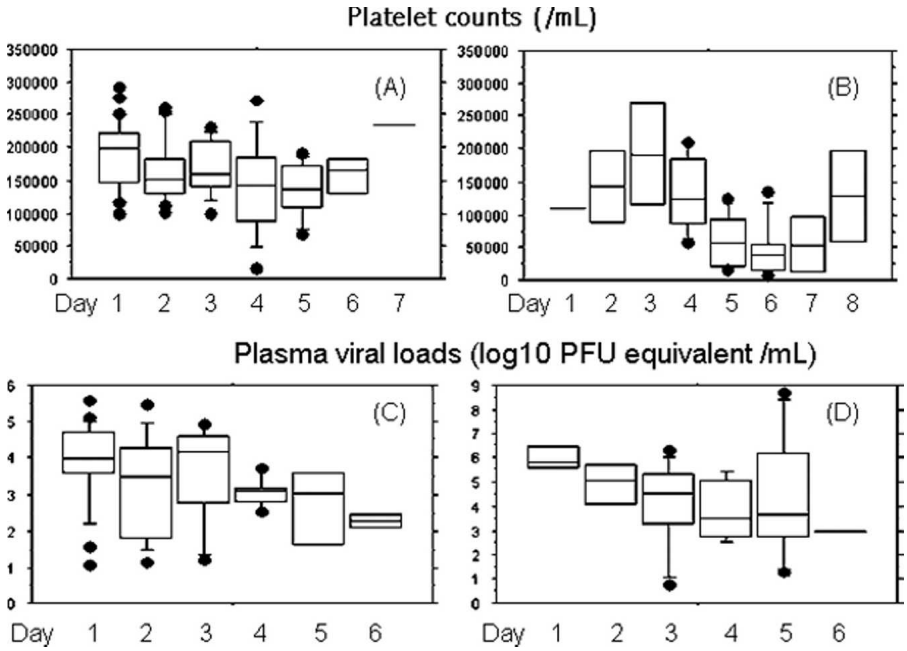

FIGURE 2. Distribution of platelet counts and plasma viral loads recorded on admission to the emergency room according to the time since onset of fever. **A**, Patients with primary dengue infection ($P = 0.01$, Spearman rank correlation test). **B**, Patients with secondary infection ($P = 0.003$, Spearman). **C**, Patients with DENV-4 infections ($P = 0.008$, Spearman). **D**, Patients with DENV-2 infections ($P < 0.05$, Spearman). Box plots show median values (horizontal line in the box), 25–75% interquartile range (lower-upper limits of the box), 90% range of data (additional bars), and outliers (circles).

TABLE 3

Data recorded on admission to the emergency room and final clinical forms of severity diagnosed during follow-up in patients with confirmed primary dengue DENV-2 or DENV-4 infections

Data*	DENV-2 ($N = 17$)	DENV-4 ($N = 52$)	P
Age (years)	27 (28)	35 (23)	0.373
Male/female (N)	8/9	19/33	0.569
Body temperature (°C)	38.9 (1)	38.7 (1.3)	0.408
Pulse pressure (mm of Hg)	43 (23)	50 (26)	0.199
Heart rate (beats/min)	86 (40)	101 (28)	0.33
Natremia (mmol/L) [135–145]†	135 (5)	137 (3)	0.194
Chloremia (mmol/L) [90–105]	99 (2)	100 (4)	0.037‡
Kaliemia (mmol/L) [3.5–4.5]	3.8 (0.5)	3.9 (0.5)	0.75
HCO_3^- (mmol/L) [22–30]	24 (4)	24 (4)	0.873
Proteinemia (g/L) [60–80]	74 (8)	73 (5)	0.109
Hematocrit (%) [36–44]	40 (8)	38 (6)	0.319
Hemoglobinemia (g/L) [105–135]	138 (18)	132 (23)	0.139
Platelets ($\times 10^9$/L) [150–350]	163 (63.7)	170 (88)	0.197
Polymorphonuclear cells ($\times 10^9$/L) [1.5–8.5]	4.07 (2.48)	4.47 (2.35)	0.479
Lymphocytes ($\times 10^9$/L) [1–4]	0.56 (0.29)	0.51 (0.35)	0.625
AST (U/L) [4–37]	39 (43)	26.5 (24)	0.011‡
ALT (U/L) [4–50]	20 (24.3)	18 (20.5)	0.136
CK (U/L) [20–170]	347 (460)	116 (101)	0.008‡
CRP (mg/L) [0–10]	28.1 (48.9)	6.9 (31.2)	0.075
APTT(s) [25–40]	37 (4.5)	37 (3)	0.218
Prothrombin level (%) [70–100]	71 (12)	73 (18)	0.492
Viral load ($\times 10^3$ PFU equivalent/mL)	180 (527) $N = 15$	5.3 (38) $N = 44$	0.003‡
Classic dengue fever [N (%)]	11 (64.7)	45 (86.5)	0.071
DHF/DSS-like group§ [N (%)]	1 (5.8)	3 (5.8)	1
Unusual manifestations¶ [N (%)]	5 (29.4)	4 (7.7)	0.035**
Hospitalized [N (%)]	6 (35.3)	17 (32.8)	1

* Median value and (25–75% interquartile range).
† Reference range is shown in brackets.
‡ $P < 0.05$, Mann-Whitney test.
§ DHF/DSS-like group was defined as the presence of at least one of the following: internal hemorrhage, signs of plasma leakage, shock, platelet count $< 50 \times 10^9$/L.
¶ Unusual manifestations group was made up of other severe clinical manifestations not included in the definition of DHF/DSS-like group.
** $P < 0.05$, Fisher test.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; CK, creatine kinase; CRP, C reactive protein; APTT, activated partial thromboplastin time; PFU, plaque forming unit.

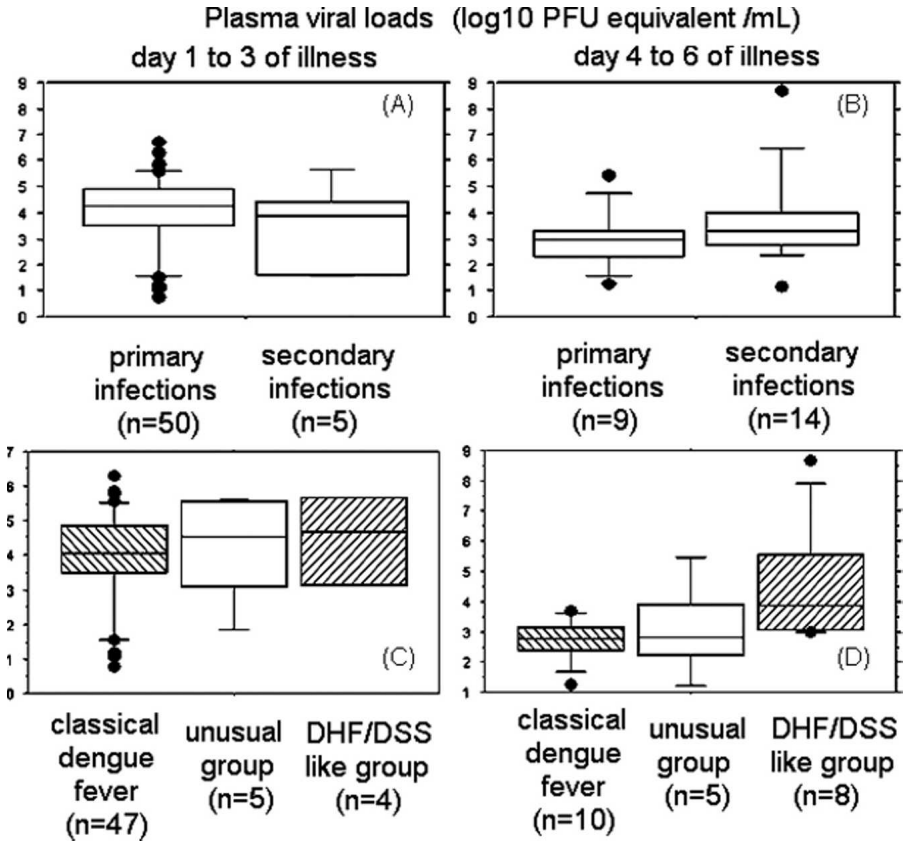

FIGURE 3. Distribution of plasma viral loads recorded in patients with dengue fever on admission to the emergency room. **Left**, Patients admitted within 3 days after onset of fever. **Right**, Patients admitted later. Box plots show median values (horizontal line in the box), 25–75% interquartile range (lower-upper limits of the box), 90% range of data (additional bars), and outliers (circles). *N*, number of patients included. [A,B,C]No significant differences between groups; [D]$P < 0.05$, Kruskal-Wallis test. DHF/DSS-like group was defined as the presence of at least one of the following: internal hemorrhage, signs of plasma leakage, shock, platelet count < 50 × 109/L. Unusual group is made up of other severe clinical manifestations not included in the definition of DHF/DSS-like group.

tween primary and secondary infections (Table 2; Figure 3, A and B).

Relationship between the plasma viral load and the severity of disease. Presentation after the third day of illness was associated with higher viral loads in the DHF/DSS-like subgroup than in the other subgroups (Figure 3, C and D).

Multiple logistic regression analysis. Analysis was performed on data from patients with DENV-2 or DENV-4 and with primary or secondary dengue infections ($N = 95$; Table 4). Analysis showed that unusual manifestations were independently associated with DENV-2 infection, whereas DHF/DSS-like features were independently associated with a secondary immune response. Occurrence of gastrointestinal symptoms was associated with DENV-2 infection regardless of the immune status. Cough was associated with a secondary immune response regardless of the dengue virus serotype. Other symptoms such as body temperature, fatigue, postural hypotension, petechiae, or bleeding did not show any association. Syncope was associated with older age ($P = 0.05$, Wald test). Increased hemoglobinemia, serum creatine kinase, and viremia levels were associated with DENV-2 infection. Increased serum liver enzymes levels, decreased platelet count, and increased activated partial thromboplastin time were associated with a secondary immune response. Decreased hematocrit and hemoglobinemia were associated with female sex ($P < 0.001$, Wald test). Some variables were associated with the time since onset of fever: platelets and polymorphonuclear cells counts and plasma viral loads decreased with time ($P < 0.01$, Wald test), whereas alanine aminotransferase, lymphocytes counts, and prothrombin levels increased with time ($P < 0.01$, Wald test).

DISCUSSION

This was a descriptive clinical study of a DENV-2 and DENV-4 co-epidemic in Martinique between June 2005 and April 2006. This study defines precisely which specific clinical features were associated with these serotypes. It corroborates previous findings from other studies but also points to a new finding that a cough is associated with secondary infection. Significant results of the study include increased severity of disease seen with DENV-2 and secondary infections.

The information in this study is based on laboratory methods that allowed comparison between DENV-2 and DENV-4 cases and between primary and secondary dengue infection cases. Because blood samples were collected during the acute phase of the disease, most of the dengue cases were RT-PCR positive, which permits genotyping. The 1:3 ratio of DENV-2 versus DENV-4 infections in patients attending the hospital was similar to that recorded by the general practitioner sentinel system, in which DENV-2 and DENV-4 accounted for

TABLE 4

Clinical features and laboratory values independently associated with serotype and/or immune status in 95 patients diagnosed with DENV-2 or DENV-4 and primary or secondary dengue infections

Dependent variables*	DENV-2 (N = 36/95)	Secondary dengue (N = 26/95)
Gastrointestinal signs	**2.6 [1–7.2] (0.05)**	1.7 [0.5–5.9] (0.38)
Cough	0.6 [0.2–2.1] (0.43)	**11.7 [2.5–55] (0.002)**
Body temperature > 38.6°C	2.1 [0.7–6] (0.19)	0.6 [0.2–1.9] (0.34)
Chloremia < 99 mmol/L	0.4 [0.1–1.1] (0.07)	0.8 [0.2–3.1] (0.79)
Hemoglobinemia > 134 g/L	**7.8 [1.8–33.3] (0.005)**	0.6 [0.1–3.3] (0.60)
Hematocrit > 39%	1.9 [0.6–6.2] (0.32)	1.1 [0.3–4.7] (0.89)
Platelet count < 100×10^9/L	1.3 [0.3–9.7] (0.73)	**11.7 [1.7–83.3] (0.013)**
Lymphocytes > 0.63×10^9/L	0.8 [0.3–2.4] (0.72)	3.2 [0.9–12] (0.08)
Polymorphonuclear cells > 3.96×10^9/L	1.1 [0.4–3.2] (0.88)	1.2 [0.3–4.5] (0.81)
APTT > 38 seconds	2.3 [0.8–6.5] (0.12)	**4.8 [1.3–17.9] (0.018)**
Prothrombin level > 74%	0.4 [0.1–13.7] (0.12)	1.7 [0.4–6.3] (0.46)
CK > 163 U/L	**4.5 [1.3–15.6] (0.018)**	1.5 [0.3–6.9] (0.64)
AST > 45 U/L	2.5 [0.8–7.6] (0.11)	**12.2 [2.9–52.6] (< 0.001)**
ALT > 34 U/L	1.4 [0.5–4.3] (0.56)	**5.7 [1.4–22.8] (0.013)**
Viral load > 7,160 PFU equivalent/mL	**10 [2.4–42.9] (0.002)**	0.6 [0.1–2.9] (0.56)
DHF/DSS-like group†	1.7 [0.4–8.1] (0.49)	**10.7 [1.7–67.5] (0.012)**
Unusual manifestations group†	**4.5 [1.2–17.2] (0.029)**	1.2 [0.3–6.1] (0.78)

Values are adjusted ORs [95% CIs] (P value). Adjusted odds ratios and 95% confidence intervals were obtained by means of multiple logistic regression analysis including the serotype and immune status as independent co-variables. Odds ratios were also adjusted for age, sex, and time since onset of fever. P values were calculated by using the Wald test.

Values in bold indicate a significant independent association.

* Signs and symptoms and laboratory data were recorded on admission to the emergency room; continuous dependent variables were dichotomized around their median values, excepted for platelet count.

† Final classification of severity into DHF/DSS-like or unusual manifestations groups was based on data recorded during follow-up. DHF/DSS-like group was defined as the occurrence of at least one of the following: internal hemorrhage, signs of plasma leakage, shock, platelet count < 50×10^9/L. Unusual manifestations group was made up of other severe clinical manifestations not included in the definition of DHF/DSS-like group.

APTT, activated partial thromboplastin time; CK, creatine kinase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PFU, plaque forming unit.

28% and 70% of cases (N = 205), respectively.[7] It must be noted that primary infection was more frequent at admission in DENV-4 than DENV-2 infection. DENV-2 has been most commonly detected in secondary infection in the Americas,[6] whereas DENV-4 showed a more rapid geographic dispersal within the Caribbean basin.[18] However, the important new information here is the occurrence of overt disease with both dengue 2 and 4 viruses during primary infections in adults. A scarcity of overt disease accompanying primary dengue 2 infections in adults was noted in the 1997 outbreak in Santiago, Cuba.[19] In Thailand, primary dengue 2 and dengue 4 cases were rarely seen in hospital or out-patient pediatric populations.[20]

Clinical data were recorded prospectively in the emergency room, but follow-up data were also taken into account when categorizing patients into the DF and DHF/DSS subgroups, as recommended by the WHO.[1] Recent reviews have underlined the need to revise the dengue classification system.[10,11] Phuong and others[12] and Balmaseda and others[14] suggested that dengue patients exhibiting at least one typical feature of DHF/DSS (i.e., internal hemorrhage, plasma leakage, marked thrombocytopenia, and/or shock) should be diagnosed with DHF/DSS. These authors also emphasized that sole use of the DHF/DSS classification to identify severe disease excludes a significant proportion of patients, and especially adults, with severe manifestations. This has been confirmed in a recent European study of dengue fever in travelers.[21] Once classified on the basis of at least one of the principal criteria of DHF/DSS, the patients showed characteristics usually seen with this form, including the duration of illness at initial presentation and its association with secondary dengue infection. In contrast, patients without signs of DHF/DSS but with other severe manifestations did not share these characteristics. Multivariate analysis showed that the association of DHF/DSS-like features with secondary dengue infection was independent of the serotype, whereas the association of unusual complications with DENV-2 was independent of the type of antibody response. These observations suggest that the classification proposed by Balmaseda and others[14] is clinically relevant and may correspond to different pathophysiologic processes.

Among signs recorded in the emergency room, rash was certainly under-reported.[3] This could be because of several factors including the time since clinical onset (most patients were examined at the acute febrile phase of illness) and the difficulty in observing this sign in patients mostly of mixed African ancestry. Gastrointestinal signs were reported in ~50% of patients and were more frequently observed in DENV-2 infections. Symptoms such as nausea, abdominal pain, and vomiting are frequently reported.[1,3] Sudden occurrence of an acute abdominal pain in patients with dengue fever should be considered as a sign of severity, particularly in children.[3,12,13] Cough was observed in 25% of patients. It was the only clinical sign strongly associated with secondary immune response, independently from virus serotype and time since clinical onset. Because cough could be an early clinical sign of pleural effusion or pulmonary capillary leakage, we suggest that it should be considered as an indicator of potential severity.

Our data showed that the unusual manifestations occurred earlier than DHF/DSS-like features during the course of fever. However, some clinical features showed considerable overlap between the groups. Higher serum creatine kinase levels were mostly observed in primary DENV-2 infection. Rhabdomyolysis is not well described as a complication of dengue and is probably under-reported in the medical literature.[22] Higher liver enzyme levels were associated with secondary infections, independently from virus serotype. Interestingly, a significant correlation was shown between plasma viral loads and liver enzymes levels, and it is noteworthy that the patient with DENV-2–associated fulminant hepatitis had the highest viral load. These findings are in agreement with

previous publications suggesting that increased liver enzyme levels are strong predictors of severe clinical forms of dengue fever.[23,24] Fulminant hepatitis is well documented in dengue infection and may result from a direct viral cytolysis or an adverse consequence of host immune response.[25] It has been suggested that hepatic injury may relate more to viral factors, whereas vascular permeability may be mediated predominantly by the immune response.[3] However, because most dengue patients are given paracetamol, the potential hepatic toxicity of this molecule should always be considered in dengue patients with increased liver enzymes levels.

Thrombocytopenia and coagulation disorders have been shown in severe dengue together with alterations of endothelial cells.[26] Prolongation of partial thromboplastin time was shown in our patients diagnosed with secondary immune response. In most patients, this was not associated with significant alteration in other coagulation factor levels. Thrombocytopenia was associated with secondary immune response but also decreased significantly with time, the lowest platelet counts being recorded between 4 and 7 days after onset of fever. However, some patients were diagnosed with severe thrombocytopenia without other signs and symptoms of severe illness. Identification of clinical indicators of disease severity, and redefinition of the threshold for thrombocytopenia, should be evaluated by a large multicenter descriptive study.[10,11]

DENV-2 was associated with unusual complications, regardless of immune status, which might be partly ascribed to virulence. We confirm the pathogenicity of the Asian-American DENV-2 subtype, which had previously been linked to dengue hemorrhagic fever in Cuba in 1981.[27] All the fatal cases where the serotype was known were DENV-2 secondary infections with DHF and unusual manifestations. These findings suggest that the most severe cases of dengue resulted from the combined effects of DENV-2 virulence and immune priming. Earlier studies in Thailand showed that secondary infection caused by DENV-2 was associated with more cases of DHF than was DENV-4 secondary infection.[20] It has been postulated that more efficient DENV-2 replication in primed hosts confers enhanced pathogenicity.[28] Reports on viral loads and disease severity are contradictory.[3] Molecular studies reported viral loads of higher,[29] equivalent,[30] or lower[31] magnitude in secondary dengue. DHF has been shown to be associated with higher plasma viremia.[28,30,32] There was no difference in plasma viral loads recorded in our patients with primary or secondary infection nor between the DF, DHF/DSS-like, and unusual manifestations groups. However, when the analysis was restricted to patients presenting 4 days or more after the onset of symptoms, the DHF/DSS-like subgroup was found to have higher viral loads. This is consistent with the report by Wang and others,[33] suggesting slower clearance of the virus and virus-containing immune complexes in DHF patients. These findings highlight the importance of the time since clinical onset when evaluating the significance of the plasma viral load and suggest that "original antigenic sin" and a partially misdirected humoral immune response may delay the viral clearance and create a vicious circle leading to exaggerated T-cell responses and immunopathogenesis of DHF/DSS.[34–36]

The correlation between DHF and secondary dengue infection is stronger when multiple serotypes circulate.[3,6,37] However, no increase in dengue morbidity or mortality was observed during the successive DENV-2/DENV-4, DENV-1, and DENV-3 epidemics that occurred in Martinique 4 years apart.[38] The lower rate of DHF/DSS in the Americas than in Asia is well established[5,6] and may be partly caused by differences in genetic background[39,40] and in the genetic-driven immune response. The search for a dengue resistance gene in black populations should be pursued in future.[41]

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