

Clinical and Laboratory Features That Differentiate Dengue from Other Febrile Illnesses in an Endemic Area—Puerto Rico, 2007–2008

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Abstract. Dengue infection can be challenging to diagnose early in the course of infection before severe manifestations develop, but early diagnosis can improve patient outcomes and promote timely public health interventions. We developed age-based predictive models generated from 2 years of data from an enhanced dengue surveillance system in Puerto Rico. These models were internally validated and were able to differentiate dengue infection from other acute febrile illnesses with moderate accuracy. The accuracy of the models was greater than either the current World Health Organization case definition for dengue fever or a proposed modification to this definition, while requiring the collection of fewer data. In young children, thrombocytopenia and the absence of cough were associated with dengue infection; for adults, rash, leucopenia, and the absence of sore throat were associated with dengue infection; in all age groups, retro-orbital pain was associated with dengue infection.

INTRODUCTION

Dengue is the world's most common mosquito-borne viral infection and a leading cause of morbidity throughout the tropics and subtropics.¹ Although at least 50–100 million infections are known to occur worldwide each year, the true burden of disease is unknown.² Illness caused by one of the four dengue virus serotypes can range from nonspecific febrile illness to classic dengue fever (DF), which may then progress to severe disease (e.g., dengue hemorrhagic fever or dengue shock syndrome in the 1997 dengue classification system). Countries often report only the most severe cases, many cases of DF are not diagnosed or reported and case-fatality rates between countries are not currently comparable.

The diagnosis of dengue infection early in the course of illness, before development of severe manifestations of the disease, can be challenging. Serologic tests, the mainstay of laboratory diagnosis, are unreliable early in infection (during the first 3 days after symptom onset) and usually require collection of paired acute- and convalescent-phase samples. Polymerase chain reaction (PCR) testing, which is more sensitive early in dengue infection, is usually unavailable in the countries with the highest burden of disease, and no rapid diagnostic test is in widespread clinical use. Diagnosis, therefore, typically relies on identification of clinical features consistent with the World Health Organization (WHO) case definitions.³ Unlike dengue hemorrhagic fever, which is solely a clinical diagnosis, the diagnosis of DF relies on recognition of clinical features along with serologic confirmation or an epidemiologic link to a confirmed case. For practical purposes, in dengue-endemic regions, the latter two caveats are often ignored and a diagnosis of DF made solely on the basis of clinical features consistent with the WHO case definition. However, the sensitivity and specificity of this definition have been questioned and a proposed revised case definition is under consideration.^{4–8}

Although early laboratory diagnosis is often not feasible, it has critical implications for the care of individual patients and improving public health. Early diagnosis can improve patient outcomes by enabling more timely assessment and initiation of supportive clinical management of patients with warning signs of severe disease.^{3,9} Identification of patients with dengue infection early, while the patient is still febrile (and viremic), can additionally help limit further transmission of dengue virus within households and communities. For these reasons, the WHO recently identified among its global research priorities the need for “clinical and laboratory indicators of early dengue.”¹⁰ A recent systematic literature review identified multiple clinical and laboratory features that could potentially differentiate dengue from other febrile illnesses, but concluded that published studies to date have been hampered by methodological limitations.¹¹ In addition, clinical features of dengue were noted to vary significantly between age groups and stages of illness.^{11–16}

An analysis performed by our group using data from the first year of our enhanced dengue surveillance system, published subsequent to the systematic review,¹¹ identified several early clinical features that were independently associated with laboratory-positive dengue infections in children and adults.¹⁷ These features varied somewhat between children to adults, and while a combination of clinical features predictive of dengue in children was identified; no similar combination of clinical features was found in adults. Recent improvements in our enhanced dengue surveillance system now enable us to systematically collect laboratory results in addition to clinical data on patients reported to the system. We therefore decided to undertake an analysis of data from the second and third years of the system to assess whether the incorporation of these laboratory results could enable us to identify a constellation of early clinical and laboratory features predictive of dengue infection in an endemic-disease area, including those that may vary by patient age. We collected data from the time of initial physician contact at an outpatient facility, rather than at hospitalization, thereby allowing identification of features present early in the disease course. We validated our model internally using bootstrap re-sampling techniques, a step not previously performed in any previous dengue prediction model to our knowledge.¹¹ We additionally sought to compare the discriminatory ability of our model with the current (1997)³ and proposed (November 2009) WHO case definitions for DF.⁸

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MATERIALS AND METHODS

Study area and population. Puerto Rico has a population of 3.8 million (2000 U.S. Census data) and is divided into 78 administrative municipalities. Passive, laboratory-based dengue surveillance has been conducted island-wide in Puerto Rico for more than 3 decades. Through this system, it was determined that rates of suspected and confirmed dengue in the rural, southeastern coastal municipality of Patillas have historically been among the highest municipality-specific rates in Puerto Rico (CDC, unpublished data). Since 2005, the Patillas Enhanced Dengue Surveillance System (P-EDSS) has been operational at the sole health center in Patillas, the Centro de Servicios Primarios de Salud de Patillas, which serves the majority of the municipality's 20,152 inhabitants (2000 U.S. Census data). This health center consists of an emergency room and an extended-hours clinic for children and adults but does not provide any inpatient care services. Details of this enhanced surveillance system have been described previously.¹⁸ Briefly, on-site Centers for Disease Control and Prevention (CDC) staff at the health center work with health-care providers to optimize identification and reporting of dengue cases and to ensure the completeness and accuracy of the dengue case identification form (DCIF), which accompanies every serum specimen submitted for testing to CDC's Dengue Branch in San Juan, Puerto Rico.

Collection of patient data. An analysis was performed using data from the P-EDSS from January 1, 2007 through December 31, 2008. All Centro de Servicios Primarios de Salud de Patillas patients suspected of having a dengue infection, as defined in the case definitions described below, were to be reported to the P-EDSS. Only patients for whom a definitive laboratory positive or negative diagnosis of dengue infection was made were included in the study. The DCIF records data on patient age, sex, days from symptom onset to specimen collection, and the provider's clinical diagnosis. Clinical data collected on the DCIF include signs and symptoms included in the 1997 WHO case definition of probable DF: headache, retro-orbital pain, myalgia, arthralgia, rash, and hemorrhage. Other signs and symptoms recorded are cough, nasal congestion, sore throat, abdominal pain, nausea, vomiting, and diarrhea. Laboratory data are collected if available, including white blood cell count, platelet count, hematocrit, albumin, and tourniquet test results. A random sample of 35 DCIFs from the study period was compared with medical records at the Centro de Servicios Primarios de Salud de Patillas to assess the accuracy and completeness of captured data. Data reported on the DCIF for this sample were 96% accurate and 96% complete compared with information available in the medical record (CDC, unpublished data).

Case definitions. *Suspected dengue case.* Documented fever of $\geq 38^{\circ}\text{C}$ or history of fever lasting 2–7 days and two or more of the following symptoms or signs: headache, rash, retro-orbital pain, myalgia, arthralgia, hemorrhage, hypotension, hemoconcentration (hematocrit elevated $\geq 20\%$ of population mean),¹⁹ thrombocytopenia (platelet count $< 100,000$ platelets/ mm^3), or attending physician suspicion of clinical dengue infection in a patient who does not meet the above criteria. Patients with a clinical diagnosis of infection by another pathogen (e.g., chickenpox, measles) were excluded.

Laboratory-positive dengue case. A case of suspected dengue with anti-dengue IgM seroconversion or single anti-dengue

IgM positivity or dengue virus identification through serotype-specific reverse transcriptase-polymerase chain reaction (RT-PCR).

Laboratory-negative dengue case. A suspected dengue case that is negative for anti-dengue IgM antibodies in a convalescent-phase specimen and with neither dengue virus nor anti-dengue IgM detected in the acute specimen.

Laboratory-indeterminate dengue case. A suspected dengue case negative for anti-dengue IgM antibodies and RT-PCR for dengue in an acute-phase specimen and for which a convalescent-phase specimen is not available.

Laboratory testing. Blood specimens were collected from all suspected dengue cases and initially centrifuged and serum separated in Patillas. Extensive efforts were undertaken to collect both acute and convalescent specimens when possible (i.e., patient education about the importance of collecting the convalescent sample, reminder flyers, and follow-up reminder phone calls). Serum samples were stored at 4°C until transported once a week by CDC staff to the CDC Dengue Branch laboratory. Serum samples collected within 5 days of onset of symptoms (acute-phase samples) were tested by serotype-specific RT-PCR for dengue virus^{20,21} and by dengue-specific IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA).²² Serum samples collected 6 or more days after symptom onset (convalescent-phase samples) were tested by MAC-ELISA. All samples with positive PCR results or seroconversion of IgM between acute- and convalescent-phase samples were tested by quantitative IgG ELISA to differentiate between primary and secondary dengue infections.

Statistical analysis. Persons with laboratory-indeterminate dengue cases and infants (i.e., children < 365 days old) were excluded from our study analysis. Using laboratory-positive and laboratory-negative dengue cases, we performed univariate analysis to identify clinical and laboratory features associated with dengue positivity. Categorical variables were compared using the χ^2 test or Fisher's exact test as appropriate, and continuous variables were compared using Student's *t* test and the Mann-Whitney *U* test when applicable.

Variables with a *P* value of < 0.1 on univariate analysis, or those features that are part of the 1997 WHO clinical case definition of probable DF,³ were entered into a multivariate logistic regression analysis using backward elimination with 95% confidence intervals calculated using Bonferroni correction to account for multiple comparisons. Age and sex were forced into the model as potential confounders. Stratified analyses were additionally performed for young children aged 1–9 years, adolescents aged 10–19 years, and adults aged ≥ 20 years. Receiver-operator characteristic (ROC) curves were generated for the overall logistic regression model and for the age-stratified models.

We dichotomized the continuous variable platelet count into a dichotomous variable, using a threshold of $< 240,000$ platelets/ mm^3 as "low platelet count." This threshold was determined by ROC curve analysis as the level that best discriminated between the presence and absence of laboratory-positive dengue infection (data not shown). The ROC curve analysis of white blood cell count did not show a threshold level that clearly discriminated between the presence and absence of dengue, and therefore the commonly used definition of leukopenia (i.e., a total white blood cell count of $< 5,000/\text{mm}^3$) was used.

We additionally created prediction models using the current WHO case definition for probable DF and a revised probable dengue case definition proposed by an international working group of dengue experts and included in the third edition of the WHO’s “Dengue guidelines for diagnosis, treatment, prevention and control” (Table 1).^{3,8} For the purpose of this analysis, the non-specific term “aches and pains” was considered to refer only to generalized musculoskeletal complaints (i.e., arthralgia and myalgia as collected on the DCIF) and not to localized pain like headache or retro-orbital pain. The ROC curves were generated from these models according to the methods described previously. The accuracy of these models as measured by the area under the ROC curve (AUC) was compared with the AUC of our prediction model. Diagnostic accuracy was considered to be high if the AUC was > 0.9, moderate if in the range of 0.7 to 0.9, and low if between 0.5 and 0.7.^{23,24} We subsequently used bootstrap resampling methods to assess the significance of the difference in predictive ability between the models.²⁵ One thousand bootstrap samples equal in size to the original dataset of 1,734 individuals were drawn (with replacement) and the various models fit to these bootstrap samples. Differences in AUCs (Δ AUC) between the models were compared using the percentile confidence intervals method to evaluate if Δ AUC was significantly different than zero. All analyses were performed using SAS version 9.2 (SAS Institute, Cary, NC).

Ethical review. The study protocol was reviewed and approved by the Human Subjects Institutional Review Board of the Centers for Disease Control and Prevention.

RESULTS

Data were collected for 3,270 patients with suspected dengue infections from January 1, 2007 through December 31, 2008. One hundred seventy-eight infants and 1,137 persons with laboratory-indeterminate cases were excluded from analysis. Of the remaining 1,955 cases, 108 (5.5%) were dengue laboratory-positive and 1,847 (94.5%) were laboratory-negative. Of the laboratory-positive cases, 60 (55.5%) were confirmed by positive PCR results (2 DENV-1, 33 DENV-2, 24 DENV-3, 1 DENV-4), 15 (13.9%) by seroconversion from negative to positive IgM status, and 33 (30.5%) by a positive IgM result on a single specimen. Patients with laboratory-

positive dengue infections were older (median 18.8 years, range 1–91 years) than patients who were laboratory-negative for dengue (median 11.5 years, range 1–95 years) (Table 2). Persons with laboratory-confirmed dengue were more likely to be male than those who were laboratory-negative for dengue, (52% versus 46%, respectively) but this difference did not reach statistical significance. Patients, regardless of diagnosis, presented to the health-care center a median of 1 day after fever onset (range, 0–17 days all patients; 0–8 days for laboratory-positives). There were 1,810 (92.6%) patients seen within 3 days of symptom onset. Only 11 (0.005%) of the 1,955 patients required hospitalization for this illness, and that included five laboratory-positive patients and six laboratory-negative patients.

Primary or secondary infection status was determined for 51 (47%) of the 108 patients with laboratory-positive dengue; 17 had primary and 34 had secondary infections. Patients with primary infection were significantly younger than patients with secondary infection (12.1 versus 25.2 years; $P = < 0.01$) and were more likely to be male (76% versus 44%; $P = 0.04$). Those with primary and secondary infection did not differ significantly in time from symptom onset to presentation for care. On univariate analysis, patients with primary infections were more likely to have a rash and less likely to have symptoms of body ache, joint pains, or nausea and vomiting.

There were several differences in the frequency of presenting clinical features between patients with laboratory-positive dengue and patients that were dengue laboratory-negative (Table 2). Compared with dengue laboratory-negative patients, patients with laboratory-positive dengue infections were significantly more likely to have retro-orbital pain, rash, joint pain, and body aches. In contrast, patients with laboratory-positive dengue infections were significantly less likely than laboratory-negative patients to have symptoms of upper respiratory tract infection, such as sore throat, nasal congestion, or cough. There was no significant difference between groups in the proportion of patients reporting headache, nausea and vomiting, or hemorrhagic manifestations.

Laboratory and clinical data from 1,734 (88.6%) patients were sufficient to be included in the logistic regression model (97 laboratory-positive patients and 1,637 laboratory-negative patients). Five variables were found to be independently associated with a laboratory-positive dengue infection:

TABLE 1
Current and proposed World Health Organization (WHO) dengue case definitions

Current (1997) WHO case definition ³	Proposed (2009) WHO case definition ⁸
<p>An acute febrile illness with two or more of the following:</p> <ul style="list-style-type: none"> • Headache • Retro-orbital pain • Myalgia • Arthralgia • Rash • Leukopenia • Hemorrhagic manifestations <p>(includes the tourniquet test)</p> <p>And</p> <ul style="list-style-type: none"> • Supportive serology • Occurrence at the same location and time as other confirmed cases of dengue fever 	<p>Live in or travel to endemic area Plus Fever and two or more of the following:</p> <ul style="list-style-type: none"> • Nausea/vomiting • Rash • Aches and pains • Tourniquet test positive • Leukopenia • Any warning sign <ul style="list-style-type: none"> - Abdominal pain or tenderness - Persistent vomiting - Clinical fluid accumulation - Mucosal bleed - Lethargy; restlessness - Liver enlargement > 2 cm - Increase in hematocrit concurrent with rapid decrease in platelets

TABLE 2

Characteristics of dengue laboratory-positive and laboratory-negative patients, Enhanced Dengue Surveillance System, Patillas, Puerto Rico, January 1, 2007–December 31, 2008

	Dengue-positive (N = 108)	Dengue-negative (N = 1847)	P value*
Age in years, median (range)	18.8 (1–91)	11.5 (1–95)	< 0.01
Age 1 to 9, no.	23	796	
Age 10 to 19, no.	35	397	
Age 20 and over, no.	50	654	
Sex, no. (%)			
Male	56 (52)	847 (46)	NS
Female	52 (48)	1000 (54)	NS
Fever, no. (%)	101 (93)	1719 (93)	NS
Headache, no. (%)	77 (71)	1201 (65)	NS
Retro-orbital pain, no. (%)	38 (35)	277 (15)	< 0.01
Body aches, no. (%)	71 (66)	923 (50)	< 0.01
Joint pain, no. (%)	48 (44)	591 (32)	0.02
Rash, no. (%)	23 (21)	74 (4)	< 0.01
Nausea or vomiting, no. (%)	32 (30)	517 (28)	NS
Diarrhea, no. (%)	11 (10)	160 (9)	NS
Reported bleeding, no. (%)	4 (4)	15 (1)	NS
Sore throat, no. (%)	41 (38)	1,034 (56)	< 0.01
Cough, no. (%)	41 (38)	1,145 (62)	< 0.01
Nasal congestion, no. (%)	38 (35)	1,090 (59)	< 0.01
Leukocyte count, † median (no. with data available)	6.9 (N = 53)	8.4 (N = 1102)	< 0.01
Platelet count, † median (no. with data available)	201 (N = 61)	263 (N = 1,132)	< 0.01
Hematocrit value (%), median	41.3 (N = 47)	39.6 (N = 824)	< 0.01

* χ^2 test for categorical variables, Mann-Whitney U test for continuous variables; NS = not significant.

† $\times 10^3$ cells/mm³.

retro-orbital pain, rash, low platelet count (platelets < 240,000 cells per mm³), absence of sore throat, and absence of cough (Table 3). The ROC curve (Figure 1) arising from our predictive model for all age groups incorporating these variables had an AUC of 0.76. For all age groups, the AUC of the logistic regression model from the current WHO case definition was 0.67 and for the logistic regression model generated from the proposed WHO case definition was 0.66. Similarly, when strat-

TABLE 3

Multivariate predictors of laboratory-positive dengue infection by age group*

Covariate	Adjusted odds ratio	95% confidence interval
All Ages		
Retro-orbital pain	2.9	(1.8–4.9)
Rash	5.7	(3.1–10.5)
Platelets < 240,000 cells/mm ³	2.1	(1.3–3.4)
No sore throat	2.1	(1.3–3.4)
No cough	1.9	(1.1–3.2)
Age 1–9 years †		
Retro-orbital pain	5.8	(1.5–23.0)
No cough	3.4	(1.2–9.3)
Platelets < 240,000 cells/mm ³	4.0	(1.3–11.9)
Age 10–19 years ‡		
Retro-orbital pain	3.7	(1.7–8.2)
Rash	13.2	(4.8–36.1)
No cough	3.0	(1.4–6.8)
Age ≥ 20 years §		
Retro-orbital pain	2.4	(1.2–4.7)
Rash	7.0	(2.0–24.9)
No sore throat	8.1	(3.1–18.4)
Leukopenia	3.9	(1.8–8.8)

*Data from 97 laboratory-positive cases and 1,637 laboratory-negative cases.

†Data from 18 laboratory-positive cases and 667 laboratory-negative cases.

‡Data from 33 laboratory-positive cases and 371 laboratory-negative cases.

§Data from 46 laboratory-positive cases and 599 laboratory-negative cases.

ified by age group, AUCs for the predictive model exceeded AUCs from models generated from the current and proposed WHO case definitions (Figure 1). Performances of the models did not change significantly if only patients with definitive laboratory confirmation of dengue (PCR positivity or IgM seroconversion) were included (data not shown).

Clinical and laboratory features that were predictive of laboratory-positive dengue infections were found to vary by patient age (Table 3). For patients aged 1–9 years, retro-orbital pain, low platelet count, and absence of cough were independently associated with laboratory-positive dengue infection. In the 10–19-year age group, retro-orbital pain, rash, and absence of cough were predictive. For patients ≥ 20 years of age, retro-orbital pain, rash, absence of sore throat, and leukopenia were associated with laboratory-positive dengue infection.

As evaluated using bootstrap resampling, the ROC curves generated from the all-ages predictive model performed significantly better than either the model generated from the proposed WHO case definition (Table 4). When the performance of each model was examined by age group, the performance of the predictive model was statistically significantly better than models generated from either the current or proposed WHO case definitions for every age group, except in the 10–19 year age group as compared with the model generated from the current WHO case definition (data not shown).

DISCUSSION

An accurate assessment of the burden of dengue in many parts of the world is hampered by inability to make a laboratory diagnosis and difficulties in identifying less severe forms of dengue by clinical presentation alone. A clinically applicable diagnostic algorithm could improve reporting in areas where laboratory resources are limited, while simultaneously benefiting busy clinicians by identifying dengue cases so that appropriately targeted prevention messages and management can be provided. This analysis using data from our enhanced dengue surveillance system shows that a prediction model can be constructed from readily available data collected early in the disease course. A combination of four clinical features and one routine laboratory test predicted dengue positivity with moderate accuracy, and more accurately than either the current or proposed WHO dengue case definition while requiring the collection of fewer data. Additionally, our study shows that for our population the proposed WHO dengue case definition is, at best, no better than the existing WHO case definition in predicting laboratory-positive dengue.

One important feature of our study, which has received relatively little attention previously, is the variation in clinical and laboratory features of dengue by patient age. Although these findings have also been reported by other groups,^{12,15} the WHO case definition makes no distinction between pediatric and adult patients. Moreover, among 19 previous studies of clinical features of dengue,^{5,12–17,26–37} only three have examined children and adults separately.^{12,17,36} It is not surprising, given the differences between children and adults in prior immunologic exposure to dengue and other viral infections, that pathophysiological responses to dengue infection^{12,38} and clinical and laboratory features may vary significantly. For example, subgroup analysis of our data shows that leukopenia, one of the manifestations of dengue listed in the current WHO

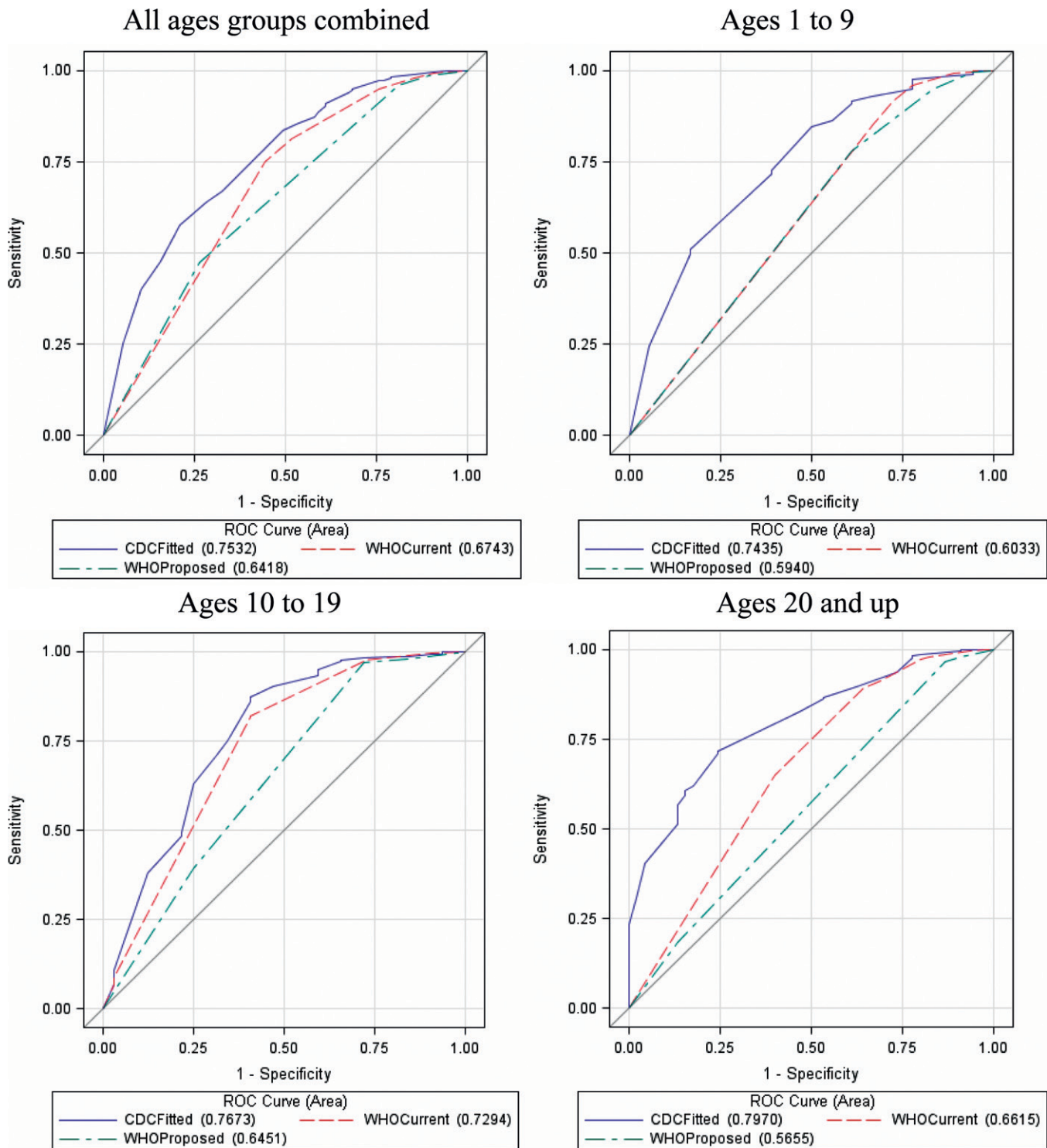


FIGURE 1. Receiver-operator characteristic (ROC) curves by age groups for Centers for Disease Control and Prevention (CDC) predictive model* and models generated from current† and proposed‡ World Health Organization (WHO) dengue case definitions. * Variables included in CDC predictive model: retro-orbital pain, rash, platelet count < 240,000 cells/mm³, no sore throat, no cough. † Variables included in current WHO case definition model: headache, retro-orbital-pain, myalgia, arthralgia, rash, leucopenia, hemorrhagic manifestation. ‡ Variables included in proposed WHO case definition model: nausea, vomiting, rash, aches and pains (myalgia or arthralgia), leucopenia, abdominal pain/tenderness, clinical fluid accumulation, mucosal bleed.

case definition, is predictive of dengue early in the course of infection only among adults aged ≥ 20 years. Leukopenia is a common clinical finding in many viral childhood infections,³⁹ and as children have an average of 6 to 8 viral infections

annually,^{40,41} the finding that this is not a good early predictor for dengue among children could be anticipated. Although a previous study from Thailand did identify leukopenia as a good early predictor of dengue infection in children,¹⁴ another

TABLE 4
Comparison of area under the ROC curves (AUCs) of different models by bootstrap resampling for all age groups

Model	AUC or Δ AUC	Standard error	Lower percentile confidence boundary*	Upper percentile confidence boundary*
1. Dengue Branch	0.75	0.0011		
2. Current WHO	0.67	0.0012		
3. Proposed WHO	0.64	0.0012		
ΔAUC_{1-2}	0.0830		0.0412	0.1754
ΔAUC_{1-3}	0.1160		0.0543	0.1784
ΔAUC_{2-3}^\dagger	0.0330		-0.0245	0.0876

*Non-parametric 95% confidence intervals with Bonferroni correction for multiple comparisons.

†Negligible improvement of one model over the other.

study from Nicaragua found, similar to our results, that leukopenia was significantly associated with early dengue infection in adults but not in children.¹²

Another key finding of our study was the importance of retro-orbital pain as a discriminator of dengue among all age groups in our population. This is especially important given that the proposed new WHO dengue case definition does not specifically mention this symptom in its list of clinical features.⁸ Omitting retro-orbital pain from the dengue case definition or grouping retro-orbital pain in with other possibly less specific pain syndromes in the definition may reduce clinical diagnostic accuracy. In our study when we performed an alternative analysis including headache and retro-orbital pain along with myalgia and arthralgia in the new WHO criteria of “aches and pains,” the model performed non-significantly worse than when we included only myalgia and arthralgia (data not shown). This is not surprising given that univariate analysis showed that body aches, joint pain, and retro-orbital pain were associated with dengue positivity, but headache was not (Table 1). Previous studies that have combined retro-orbital pain with headache showed no association between retro-orbital pain/headache and dengue positivity,¹¹ whereas studies that have examined retro-orbital pain separately have seen this association,^{29,33,35} although sometimes only in certain age groups.¹⁷ Retro-orbital pain has been traditionally considered a relatively nonspecific manifestation of DF, but to our knowledge, no other common acute febrile illnesses have retro-orbital pain or eye pain listed in their case definitions. Retro-orbital pain has also been criticized for lacking sensitivity in children, given the presumed limited ability of young children to express location of pain. However, of 1,211 children aged 1–14 years with laboratory-confirmed dengue in Nicaragua, 59% were documented to have retro-orbital pain.¹²

Recent articles have highlighted a variety of ocular manifestations associated with dengue infection, including macular and retinal hemorrhages,^{42,43} maculopathy,^{44,45} optic neuritis and neuropathy,⁴⁶ vasculitis,⁴⁷ uveitis,⁴⁸ retinal artery occlusion,⁴⁹ venular occlusion,⁵⁰ and retinal and macular edema.^{43,47} In a recent prospective study from Singapore, Seet and colleagues⁵¹ observed “clinically significant” ocular symptoms, defined as symptoms severe enough to warrant referral to an ophthalmologist, in 18% of hospitalized patients with serologically confirmed dengue. A 2006 study from India examined 134 consecutive hospitalized dengue patients and discovered ocular findings on fundoscopic examination in over 40%.⁴² Similarly, maculopathy was documented in 10% of consecutively admitted dengue patients in Singapore.⁴⁴ Based on the timing of the development of ocular signs relative to the course of dengue illness, and the association between low complement

C3 levels and “dengue-associated maculopathy,”⁴⁴ an immune-mediated process has been proposed for the ocular manifestations of dengue.^{44,45} These studies suggest that eye involvement with dengue might be more common than is appreciated and it is plausible that retro-orbital pain could be a more specific indicator of dengue infection than previously realized.

A recent systematic review identified 15 studies that have examined the differences in clinical and laboratory features between dengue and other febrile illnesses,¹¹ and since that publication at least three additional studies have been published.^{16,27,34} Our study differs from most previous studies in that data were recorded at the time of the initial clinic or emergency department visit rather than at the time of hospitalization. Hence, we were able to identify features that were predictive of dengue positivity early in the disease course, rather than mid-to-late in the course. When we reanalyzed our data excluding patients who presented more than 3 days after symptom onset (7.4% of our total patients), the only significant change in the findings was that rash was no longer associated with dengue-positivity in patients ≥ 20 years of age (data not shown). This is not surprising as the association between rash and dengue in this age group may have been driven by patients who presented for medical care at the time of the appearance of the relatively classical convalescent rash of dengue. Several of the age-specific predictive features for dengue identified in our study have been previously noted, including the presence of rash and leukopenia in adults,^{12,26,33,52} reduced platelet counts in children,^{12,14} and absence of upper respiratory symptoms in children.^{5,17}

Apart from the collection of data early in the disease course, our study has several other strengths. Unlike some previous studies, it includes only cases that could be laboratory confirmed as dengue infection or not dengue infection, thereby reducing the potential for misclassification bias. Data were collected over more than one dengue season and in a period when all four dengue virus serotypes were circulating; thereby giving a more accurate overall clinical picture than studies that took place in an outbreak setting. Finally, our study had a relatively large sample size compared with other similar studies¹¹ and we stratified by age group to reduce potential confounding by age.

Nonetheless, the study has several limitations. We used enhanced surveillance data which, while it is extremely accurate and complete overall, was missing information for certain variables such as petechiae and the results of the tourniquet test. The presence of petechiae was seen almost exclusively among patients with dengue, but because of the small numbers of patients for whom this variable was recorded, it could not be incorporated into our multivariate model. The tourniquet test is not routinely performed in Puerto Rico. Because both current and proposed WHO case definitions for probable DF include this as one possible example of the hemorrhagic manifestations criteria, absence of information on this feature may have led to an underestimation of the sensitivity of both case definitions. A prospective study is underway in Puerto Rico to evaluate the effect of inclusion of the tourniquet test on the diagnostic accuracy of the current and proposed WHO case definitions and whether including tourniquet test results would improve the performance of our predictive model. Because of the retrospective nature of our study, we were unable to assess the variation in clinical and laboratory features predictive of dengue by day of illness. Patients were seen only once or twice

in the course of their illnesses so a final determination of case severity was not possible in many cases. Therefore, this study is unable to identify early predictors that are specific to severe dengue, a pressing research need. Finally, the data are from only one region of Puerto Rico and may not be representative of other areas with different dengue transmission patterns, population demographics, underlying causes for acute febrile illness (e.g., typhoid, malaria), or where patients present for medical care later in the course of illness.

This study suggests that simple clinical and laboratory data can be used to identify early dengue infections in both adults and children. Further efforts should be made to validate these findings in other geographic settings and time periods. If the models continue to perform well over time in these settings, they could be used to develop clinical diagnostic algorithms. Future studies should also seek to detect early clinical and laboratory markers that can predict the development of severe dengue. Findings from our study and others^{11,12,15,17} suggest that separate clinical case definitions or diagnostic algorithms may be needed for children and adults. Additionally, changes to the DF case definition that deemphasize retro-orbital pain should be carefully evaluated, as this symptom was one of the strongest predictors of dengue infection in our study.

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