Classification of Dengue Illness Based on Readily Available Laboratory Data

James A. Potts, Stephen J. Thomas, Anon Srikiatkachorn, Pra-on Supradish, Wenjun Li, Ananda Nisalak, Suchitra Nimmannitya, Timothy P. Endy, Daniel H. Libraty, Robert V. Gibbons, Sharone Green, Alan L. Rothman,* and Siripen Kalayanarooj

Center for Infectious Disease and Vaccine Research, University of Massachusetts Medical School, Worcester, Massachusetts; Department of Virology, United States Army Medical Component–Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand; Queen Sirikit National Institute of Child Health, Bangkok, Thailand; State University of New York, Upstate Medical University, Syracuse, New York

Abstract. The aim of this study was to examine retrospective dengue-illness classification using only clinical laboratory data, without relying on X-ray, ultrasound, or percent hemoconcentration. We analyzed data from a study of children who presented with acute febrile illness to two hospitals in Thailand. Multivariable logistic regression models were used to distinguish: (1) dengue hemorrhagic fever (DHF) versus dengue fever (DF), (2) DHF versus DF + other febrile illness (OFI), (3) dengue versus OFI, and (4) severe dengue versus non-severe dengue + OFI. Data from the second hospital served as a validation set. There were 1,227 patients in the analysis. The sensitivity of the models ranged from 89.2% (dengue versus OFI) to 79.6% (DHF versus DF). The models showed high sensitivity in the validation dataset. These models could be used to calculate a probability and classify patients based on readily available clinical laboratory data, and they will need to be validated in other dengue-endemic regions.

INTRODUCTION

Dengue is an emerging infectious disease throughout the world and is endemic in tropical and subtropical areas. Recent estimates are that 3.6 billion people (55% of the global population) are at risk of dengue infection and that 70–500 million dengue virus (DENV) infections occur annually, 2.1 million of which are severe dengue illnesses with ~21,000 deaths.¹ DENV is spread by mosquito vectors, usually *Aedes aegypti* or *Ae. albopictus*. Dengue illnesses are usually classified as two distinct entities: dengue fever (DF) and dengue hemorrhagic fever (DHF), with the most severe cases of DHF classified as dengue shock syndrome (DSS). Patients diagnosed with DF typically have a mild febrile illness with two or more of the following: headache, myalgia, arthralgia, rash, hemorrhagic manifestations, and leukopenia.² DHF is defined by four diagnostic criteria established by the World Health Organization (WHO): fever, thrombocytopenia (platelet count < 100,000 cells/mm³), bleeding tendency (positive tourniquet test or spontaneous bleeding), and plasma leakage (evidence of pleural effusion, ascites, or > 20% hemoconcentration).³ Some patients with DF may exhibit severe illness but do not meet all four WHO DHF criteria. Likewise, some patients meeting diagnostic criteria for DHF have relatively mild illness, with minimal evidence of plasma leakage and bleeding diathesis not requiring medical intervention.

Previous studies have shown limited agreement between a physician's diagnosis of severe dengue illness and strict adherence to the WHO definition of DHF, and these studies have placed emphasis on a simpler definition of severe dengue illness.^{4–7} Dengue endemic regions often have limited hospital resources and may not have the capability to perform chest X-rays or ultrasounds to detect pleural effusion or ascites, making it more difficult to fulfill the WHO criteria for the diagnosis of DHF. Changes in hematocrit may be influenced by early fluid resuscitation. In addition, baseline, convalescent, and/or reference hematocrit values are needed to show hemoconcentration; these values are often missing. Given these scenarios, patients with a severe dengue infection may

be classified as DF if WHO criteria are consistently applied, which may underestimate the global severity of dengue illness. Additionally, resource-poor areas lack essential laboratory support and may be unable to differentiate a DENV infection from other febrile illness (OFI). Previous studies suggest that other or additional indicators not in the WHO definition can distinguish patients with DHF from DF or patients with dengue from patients with OFI.⁸ However, among the studies with multivariable models, all of the final models produced had limited ability to make generalizations, and none of these models were statistically validated.

The aim of this study was to assess the retrospective value of laboratory measures that physicians use to classify dengue illnesses. Using more readily available clinical laboratory measures, we developed, evaluated, and validated different models of dengue-illness classification based on a large, prospectively collected dataset and compared our models with the WHO classification system and an experienced physician's diagnosis. We also assessed whether laboratory parameters alone could appropriately classify severe versus non-severe dengue illnesses.

MATERIALS AND METHODS

Study setting. A longitudinal observational study was conducted at two hospitals in Thailand: (1) the Queen Sirikit National Institute of Child Health (QSNICH) in Bangkok during the years 1994–1997, 1999–2002, and 2004–2007 and (2) Kamphaeng Phet Provincial Hospital (KPPPH) during the years 1994–1997. The study methods have been described elsewhere.⁹ In brief, children between the ages of 6 months and 15 years, presenting with temperature $\geq 38.5^{\circ}\text{C}$ for ≤ 72 hours and no localizing symptoms, were eligible for the study. Exclusion criteria included: signs of shock at presentation, chronic disease, or an initial alternate non-dengue diagnosis. Children were admitted to the hospital and monitored throughout their hospital stay until 24 hours after their fever subsided. Written parental informed consent was obtained before enrollment. The study protocol was approved by the Institutional Review Boards of the Ministry of Public Health, Thailand, the US Army, and the University of Massachusetts Medical School.

*Address correspondence to Alan L. Rothman, 55 Lake Avenue North, Worcester, MA 01655. E-mail: Alan.Rothman@umassmed.edu

A blood sample was obtained on the day of enrollment and daily thereafter until 1 day after defervescence or for a maximum of five consecutive blood collections. Clinical laboratory studies included complete blood count and manual white blood cell (WBC) differential. Serological assays (immunoglobulin M/G [IgM/IgG] enzyme-linked immunosorbent assay [ELISA] and hemagglutination inhibition assay), viral isolation, and/or reverse transcription polymerase chain reaction (RT-PCR) were used to confirm all dengue cases. Patients were observed daily, and clinical and laboratory measurements were recorded using standardized data-collection forms.

On the day of defervescence, finger-stick hematocrits were measured every 6 hours for 18 hours to capture hemoconcentration. A right lateral decubitus chest X-ray was taken the day after defervescence, and a pleural effusion index (PEI) was measured as $100 \times$ (maximum width of right pleural effusion/maximum width of right hemithorax). After completion of the case record and careful review of the medical record and laboratory results, a final diagnosis of DF, DHF, or OFI was assigned by an expert physician, who was not directly involved in patient care.

Severe dengue definition. An additional category was constructed to classify patients with severe dengue versus non-severe dengue. Patients with dengue were classified as having severe dengue if they met any of the following criteria: (1) final diagnosis of DHF grade 3 or 4 (i.e., DHF with shock), (2) significant pleural effusion ($PEI > 15$), (3) required total fluid intervention (oral or intravenous) in any 24-hour period that exceeded maintenance volume + 5% volume deficit^{10,11}, or (4) required intravenous fluid (IVF) throughout hospitalization (IVF was administered only under stringent circumstances, such as poor intake of oral fluids or signs of shock).

Statistical analysis. Descriptive characteristics, such as diagnosis, age, gender, length of illness at presentation, and hospital, were evaluated using *t* test for continuous variables and Pearson's χ^2 test for categorical variables.

Logistic regression models were constructed using data from QSNICH and validated using data from KPPPH. For each outcome (DF versus DHF, DHF versus all others, any dengue versus OFI, and severe dengue versus all others), univariate logistic regression was performed on the training dataset (QSNICH) for each of the following variables: maximum values for tourniquet test (number of petechiae per square inch), hematocrit, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), percent neutrophils, percent lymphocytes, and percent monocytes and minimum platelet count and WBC count. Lowess curves were used to assess the distribution of the independent variables and determine the categorization for those with skewed distributions. If the linearity assumption held true, then the variable was used as a continuous variable.

Multivariable models were constructed for each outcome in a manual stepwise procedure based on the univariate indicators with the best area under the receiver operator characteristic (ROC) curve. For variables that were highly correlated, only the variable with the higher area under the curve (AUC) from univariate analysis was used in the multivariable modeling. Variables that did not remain significant at the $\alpha = 0.05$ level were removed from the model, and the variable with the next highest area under the ROC was added into the model. The optimal sensitivity and specificity for each final multivariable model was chosen based on a probability cutoff where the sum of sensitivity and specificity were maximized, the

maximum percent correctly classified was achieved, and sensitivity remained higher than specificity.

Sensitivity and specificity for each model were also established in the test dataset using the same coefficients and probability cutoff as from the training dataset.

The percent agreement between the physician's diagnosis of DHF or the statistical models and the current WHO definition of DHF was evaluated using κ statistics. The optimal probability cutoff from the model was used to determine the proportion of patients that would be defined as DHF, where each patient above the cutoff was considered DHF and each patient below the cutoff was considered not DHF.

RESULTS

There were 1,384 patients enrolled in the study; 1,311 of these patients had a diagnosis of DHF, DF, or OFI (presumed viral, non-dengue illness). For 65 of these patients (55 with OFI, 6 with DF, and 4 with DHF), a day of defervescence could not be assigned, and they were excluded from the analysis. Some patients with OFI were discharged from the study before defervescence because of a negative PCR. Data obtained from the day of enrollment showed no significant differences between these 65 excluded patients and the remainder of the cohort with the following exceptions: (1) patients with DF included in the study were older than those with DF that were excluded (8.7 years versus 6.0 years; $P = 0.03$), and (2) patients with OFI included in the analysis had higher percent monocytes than patients with OFI that were excluded (3.8% versus 2.4%; $P = 0.01$). An additional 19 patients did not have information on all clinical laboratory variables used in the analysis and were excluded.

There were 1,227 patients included in the analysis (228 with DHF, 386 with DF, and 613 with OFI). Patients with DHF were classified by grade as follows: grade 1 ($N = 59$), grade 2 ($N = 129$), grade 3 ($N = 39$), and grade 4 ($N = 1$). There were 1,058 patients who completed the study at QSNICH and 169 patients from KPPPH (Table 1). The number of patients included in each model is shown in Figure 1.

We compared the characteristics of subjects enrolled at QSNICH and KPPPH. A higher proportion of patients with DHF were enrolled at KPPPH compared with QSNICH (30.8% versus 16.6%; $P < 0.001$). Patients presenting to KPPPH were also older than those presenting to QSNICH (8.9, 95% confidence interval [CI] = 8.4–9.3 versus 7.7, 95% CI = 7.5–7.9; $P < 0.001$). There were no differences between the hospitals with regards to gender and days ill at presentation. However, at QSNICH, patients with DHF presented later than patients with DF or OFI (2.3 versus 2.1 and 1.7, respectively; $P < 0.001$), and patients with DF presented later than patients with OFI (2.1 versus 1.7, respectively; $P < 0.001$). Additionally, at QSNICH, patients with DHF and patients with DF were older than patients with OFI ($P < 0.001$). At KPPPH, a lower percentage of males was seen among patients with DF compared with patients with DHF or OFI ($\chi^2 P = 0.03$ and $\chi^2 P = 0.02$, respectively).

Univariate analysis. Table 2 shows the results of univariate logistic regression modeling using the QSNICH data. Clinical laboratory variables distinguishing each of the different diagnostic categories included minimum platelet count, maximum daily hematocrit (Hct), $AST > 100$, maximum ALT, and a positive tourniquet test (> 20 petechiae). Among

TABLE 1
Study sample characteristics

Hospital	N (%)	Gender		Age (years)		Days ill at presentation	
		Males	Females	Mean	95% CI	Mean	95% CI
QSNICH	1,058 (86.2)	579	479	7.7	7.5, 7.9	2.0	1.9, 2.0
DHF	176	102	74	8.8	8.3, 9.3	2.3	2.2, 2.4
DF	324	175	149	8.6	8.2, 8.9	2.1	2.0, 2.2
OFI	558	302	256	6.9	6.6, 7.1	1.7	1.7, 1.8
KPPPH	169 (13.8)	102	67	8.9	8.4, 9.3	2.0	1.8, 2.0
DHF	52	35	17	9.1	8.3, 9.9	2.0	1.8, 2.2
DF	62	29	33	9.3	8.5, 10.0	2.2	2.0, 2.4
OFI	55	38	17	8.3	7.5, 9.1	1.9	1.8, 2.1
Total	1,227	681	546	7.9	7.7, 8.1	1.9	1.9, 2.0

CI = confidence interval; DF = dengue fever; DHF = dengue hemorrhagic fever; OFI = other febrile illness; QSNICH = Queen Sirikit National Institute of Child Health.

these variables, unit decreases in minimum platelet count and increases in maximum Hct, AST, and ALT and having a positive tourniquet test were associated with having the more severe outcome in each of the models. The AUC for these variables ranged from 0.92 to 0.54. Additional variables of decreasing age and decreasing minimum WBC count distinguished between all categories except DHF versus DF and were associated with having the more severe outcome in each model. The maximum percent lymphocytes and percent neutrophils distinguished patients with DHF versus DF and those with dengue versus OFI.

Multivariable analysis. Table 3 shows the results of multivariable analysis using QSNICH data. Maximum AST and ALT were found to be highly correlated (Pearson correlation = 0.86), and therefore, only maximum AST was used in the modeling. Incremental decreases in minimum platelet count (one unit = 25,000 cells/mm³) and a maximum AST > 100 were found to be associated with the more severe outcome in each of the final multivariable models. Additionally, increases in the maximum daily Hct were associated with having a

diagnosis of DHF compared with DF or DF + OFI. Increases in maximum percent neutrophils, age, and incremental decreases in minimum WBC count (one unit = 500 cells/mm³) were associated with a diagnosis of DHF versus DF. All variables except maximum percent lymphocytes and percent neutrophils showed an association with having severe dengue illness compared with non-severe dengue or OFI. In addition to distinguishing patients with severe dengue, a tourniquet test of petechiae ≥ 20 was also included in the model for distinguishing patients with serologically confirmed dengue from those with OFI. Age is included in the models of DHF versus DF and severe dengue versus all others and shows that younger patients have higher odds of DHF or severe dengue after adjusting for all other variables in the model.

The probability cutoff for each multivariable model was defined as the cutoff that gave the highest percent of patients correctly classified where the sensitivity remained higher than the specificity. Figure 2 illustrates the tradeoff between sensitivity and specificity for each probability cutoff for all of the multivariable models and shows the optimal cutpoint.

FIGURE 1. Flow chart of study. Boxes show the total number of patients enrolled in the study, reasons for exclusion from the analysis, and number of patients from the training dataset (QSNICH) and test dataset (KPPPH) used in each model. DF = dengue fever; DHF = dengue hemorrhagic fever; KPPPH = Kamphaeng Phet Provincial Hospital; OFI = other febrile illness; QSNICH = Queen Sirikit National Institute of Child Health.

TABLE 2
Univariate analysis of maximum or minimum clinical laboratory variables using the QSNICH data

	DHF vs. DF			DHF vs. all others			Dengue vs. OFI			Severe dengue vs. all others		
	OR	95% CI	AUC	OR	95% CI	AUC	OR	95% CI	AUC	OR	95% CI	AUC
Age (years)	1.03	0.97, 1.09	0.52	1.15	1.09, 1.21*	0.62	1.21	1.16, 1.27*	0.66	1.13	1.07, 1.20*	0.60
Gender†	1.17	0.81, 1.70	0.52	1.17	0.84, 1.62	0.52	1.05	0.83, 1.34	0.51	1.22	0.86, 1.72	0.52
Minimum platelets‡	0.46	0.39, 0.54*	0.84	0.42	0.37, 0.48*	0.92	0.54	0.50, 0.58*	0.90	0.49	0.43, 0.55*	0.90
Minimum WBC count§	1.01	0.94, 1.09	0.52	0.75	0.70, 0.80*	0.74	0.56	0.52, 0.60*	0.90	0.72	0.66, 0.78*	0.76
Maximum Hct (%)	1.29	1.21, 1.37*	0.73	1.39	1.32, 1.47*	0.79	1.24	1.19, 1.29*	0.69	1.39	1.32, 1.47*	0.78
Maximum monocytes (%)												
≤ 5¶												
> 5/≤ 10	0.85	0.57, 1.28		0.83	0.58, 1.19		0.97	0.70, 1.18		0.84	0.57, 1.24	
> 10	0.67	0.39, 1.14	0.54	0.87	0.54, 1.41	0.52	1.37	0.95, 1.96	0.53	1.25	0.78, 2.02	0.54
Maximum lymphocytes (%)												
≤ 40¶												
> 40/≤ 50	0.58	0.34, 1.01		1.18	0.76, 1.86		2.15	1.52, 3.04*		1.88	1.16, 3.03¶	
> 50/≤ 60	0.42	0.24, 0.73¶		1.08	0.67, 1.73		2.99	2.08, 4.29*		1.37	0.81, 2.31	
> 60	0.52	0.30, 0.88¶	0.58	1.14	0.74, 1.77	0.52	2.39	1.70, 3.34*	0.61	1.32	0.80, 2.15	0.56
Maximum neutrophils (%)												
≤ 60¶												
> 60/≤ 75	2.04	1.23, 3.38¶		1.46	0.93, 2.31		0.70	0.49, 0.99¶		1.21	0.75, 1.97	
> 75	1.61	1.001, 2.58	0.57	0.92	0.60, 1.42	0.55	0.45	0.33, 0.62*	0.58	0.92	0.59, 1.44	0.53
Maximum AST**												
≤ 100¶												
> 100	4.61	3.12, 6.83*	0.68	14.10	9.72, 20.45*	0.77	39.36	20.54, 75.41*	0.70	10.71	7.3, 15.64*	0.75
Maximum ALT**												
≤ 50¶												
> 50/≤ 100	3.02	1.87, 4.87*		6.97	4.49, 10.83*		8.71	5.09, 14.88*		4.98	3.11, 7.98*	
> 100	3.28	1.99, 5.43*	0.63	9.05	5.61, 14.59*	0.70	21.68	9.35, 50.27*	0.65	12.47	7.66, 20.28*	0.71
Maximum tourniquet test††												
≤ 20¶												
> 20	1.59	1.01, 2.50¶	0.54	6.02	4.03, 9.00*	0.70	10.22	7.70, 13.57*	0.76	6.27	4.06, 9.70*	0.70

OR = odds ratio; CI = confidence interval; ALT = alanine aminotransferase; AST = aspartate aminotransferase; DF = dengue fever; DHF = dengue hemorrhagic fever; Hct = hematocrit; OFI = other febrile illness; QSNICH = Queen Sirikit National Institute of Child Health; WBC, white blood cell.

* $P < 0.001$.

† Reference group is females.

‡ 25,000 cells/mm³.

§ 500 cells/mm³.

¶ Reference group.

|| $P < 0.05$.

** Units/dL.

†† Number of petechiae per square inch.

Validation of multivariable models. The frequency distribution of each diagnosis (DHF, DF, and OFI) according to the optimal probability cutoff for each model is given in Figure 3, showing the distribution of diagnoses in both the training and test datasets. The sensitivity/specificity analysis,

including AUC, positive and negative predictive values, and percent correctly classified, and validations with the test dataset are indicated in Table 4. When applying each model to the test dataset, the sensitivity decreased by 2.7% (DHF versus DF) to 8.9% (DHF versus all others). The specificity

TABLE 3
Multivariable models among QSNICH data

	DHF vs. DF		DHF vs. all others		Dengue vs. OFI		Severe dengue vs. all others	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Age (years)	0.90	0.83, 0.99*					0.88	0.81, 0.96†
Minimum platelets‡	0.53	0.45, 0.62†	0.51	0.44, 0.59†	0.68	0.63, 0.74†	0.61	0.53, 0.69†
Minimum WBC count§	1.19	1.05, 1.34*			0.68	0.63, 0.74†	0.88	0.80, 0.98†
Maximum Hct. (%)	1.19	1.10, 1.29†	1.17	1.09, 1.25†			1.23	1.14, 1.32†
Maximum neutrophils (%)								
≤ 60¶								
> 60/≤ 75	2.10	1.10, 3.99*			0.56	0.31, 0.99*		
> 75	1.89	1.03, 3.49*			0.55	0.33, 0.93*		
Maximum AST								
≤ 100¶								
> 100	2.32	1.43, 3.78†	3.40	2.14, 5.40†	8.99	4.07, 19.85†	2.31	1.45, 3.68†
Maximum tourniquet test**								
≤ 20‡								
> 20					3.80	2.51, 5.75†		

OR = odds ratio; CI = confidence interval; AST = aspartate aminotransferase; DF = dengue fever; DHF = dengue hemorrhagic fever; Hct = hematocrit; OFI = other febrile illness; QSNICH = Queen Sirikit National Institute of Child Health; WBC = white blood cell.

* $P < 0.05$.

† $P < 0.001$.

‡ 25,000 cells/mm³.

§ 500 cells/mm³.

¶ Reference group.

|| Units/dL.

** Number of petechiae per square inch.

FIGURE 2. Sensitivity and specificity of multivariable logistic regression models from the training dataset. Sensitivity is indicated by the solid blue line, specificity is indicated by the solid red line, and the optimal probability cutoff is indicated by a solid vertical line. (A) DHF versus DF with an optimal probability cutoff = 0.35. (B) DHF versus all others with an optimal probability cutoff = 0.19. (C) Dengue versus OFI with an optimal probability cutoff = 0.45. (D) Severe dengue versus all others with an optimal probability cutoff = 0.17. DF = dengue fever; DHF = dengue hemorrhagic fever; OFI = other febrile illness.

increased for each validation except severe dengue versus all others, where specificity decreased and sensitivity increased. The percent correctly classified decreased by 0.7% (DHF versus DF) to 4.3% (severe dengue versus all others). The final model distinguishing between patients with dengue and patients with OFI performed the best, giving the highest AUC, specificity, and percent correctly classified in both the training and test datasets.

Outside of a research setting, the entire spectrum of a patient's illness may not be available. Therefore, each model was applied to the test dataset using data only at defervescence and/or at 24 hours after defervescence, the period of greatest risk for plasma leakage when patients are most likely to require hospitalization. When using data from the test dataset obtained in these later stages of illness, the sensitivity for most models decreased. However, using data at 24 hours after

FIGURE 3. Distribution of calculated probabilities among each diagnosis for each model. Blue = DHF; red = DF; gold = OFI. In each section, the top panel represents the distribution of probabilities in the training dataset (QSNICH), and the bottom panel represents the distribution of probabilities in the test dataset (KPPPH). (A) DHF versus DF where the optimal probability cutoff = 0.35. (B) DHF versus all others where the optimal probability cutoff = 0.19. (C) Dengue versus OFI where the optimal probability cutoff = 0.45. (D) Severe dengue versus all others where the optimal probability cutoff = 0.25. DF = dengue fever; DHF = dengue hemorrhagic fever; KPPPH = Kamphaeng Phet Provincial Hospital; OFI = other febrile illness; QSNICH = Queen Sirikit National Institute of Child Health.

TABLE 4
Validation of QSNICH multivariable models to KPPPH data using optimal probability cutoff

	DHF vs. DF		DHF vs. all others		Dengue vs. OFI		Severe dengue vs. all others	
	QSNICH	KPPPH	QSNICH	KPPPH	QSNICH	KPPPH	QSNICH	KPPPH
AUC	0.87	0.86	0.94	0.91	0.96	0.93	0.92	0.86
Sensitivity	79.6	76.9	85.8	76.9	89.2	81.6	83.0	83.3
Specificity	79.3	80.6	85.4	85.5	88.4	90.9	82.3	76.7
(+) Predictive value	68.0	76.9	53.9	70.2	87.1	94.9	44.3	49.2
(-) Predictive value	87.8	80.6	96.8	89.3	90.1	70.4	96.6	94.4
Correctly classified	79.6	78.9	85.4	82.8	88.7	84.6	82.4	78.1

AUC = area under the curve; DF = dengue fever; DHF = dengue hemorrhagic fever; KPPPH = Kamphaeng Phet Provincial Hospital; OFI = other febrile illness; QSNICH = Queen Sirikit National Institute of Child Health.

defervescence, it remained moderately high for DHF versus DF, increasing from 76.9% to 86%, and DHF versus all others, at 71%.

Figure 4 shows the four validated multivariable models. Coefficients for the calculation of probability for each model are obtained by taking the natural logarithm of the odds ratio for each variable in Table 3.

Classification from models compared with WHO and expert-physician diagnosis of DHF. Table 5 shows the percent agreement and κ statistics between the model's classification of DHF, the physician's diagnosis of DHF, and the WHO diagnosis of DHF. When applying the optimal probability cutoffs, the percent agreement between the model and the WHO classification of DHF compared with DF was 80.0% ($\kappa = 0.58$; $P < 0.001$). The percent agreement between the model and the WHO definition of DHF improved to 86.2% compared with all others (DF + OFI; $\kappa = 0.60$; $P < 0.001$). In both cases, the model had a higher percent agreement with a WHO diagnosis of DHF than the physician's diagnosis of DHF.

The model of DHF versus DF classified 42.0% (258/614) of patients with dengue as having DHF. The physician diagnosed 37.1% (228/614) of patients with dengue as DHF, and strict adherence to the WHO definition would have diagnosed 33.1% (203/614) patients with dengue as DHF. The model of DHF versus all others classified 51.6% (317/614) of patients

with dengue as DHF. The model of severe dengue versus all others classified 49.2% (302/614) of patients with dengue as having a severe dengue illness, including 203 patients diagnosed by the physician as DF. Only 6.5% (40/614) of patients with dengue were diagnosed by the physician as DHF grade 3 or 4.

DISCUSSION

In this study, we developed models using clinical laboratory indicators to find associations with an expert physician's final diagnosis and WHO criteria of DHF and DF. Although these models rely on laboratory results, these tests are part of standard clinical practice, and the models do not rely on more costly chest X-ray or other measures of capillary leakage. In addition, we established a category of severe dengue illness using indicators known to be associated with DSS. We found that a large percentage of patients with dengue would be classified as having a severe dengue illness given their clinical laboratory values throughout their hospitalization. To our knowledge, our study involves the largest set of systematically collected data to address these questions.

An important aspect of this study is the use of a validation dataset. Previous studies have shown that strict adherence to WHO criteria does not identify all severe dengue disease. Some studies have used different definitions that may be more suitable for identifying severe disease and less confusing for physicians^{4,12-16}; however, these different definitions have not been validated. The validation dataset in our study involved a different hospital, with a distinct and more rural catchment area (approximately 350 km northwest of Bangkok). Although patients at KPPPH were older and more patients were diagnosed with DHF, our models were still a robust fit for these data, with little change in the AUC compared with the training dataset. KPPPH is also in a region where Japanese encephalitis (JE) virus is known to cocirculate, and there is routine vaccination against JE.^{17,18} This diversity adds to the validation of the models presented here.

There is controversy surrounding the classification of DHF using the WHO definition. The previous WHO definition of DHF requires strict adherence to four criteria, which includes ambiguous definitions of bleeding tendency and hemoconcentration.³ Comparing our models with an expert physician's diagnosis of DHF and the WHO definition of DHF could help to alleviate ambiguity by finding other objective indicators that do not necessarily depend on convalescent visits or use of expensive tests in resource-limited areas. Although the diagnosing physician used chest X-rays to determine the final

A Equation 1

$$\ln \frac{\hat{p}}{1-\hat{p}} = -6.85 + .74(\text{Neuts}_{>60\&=75}) + .64(\text{Neuts}_{>75}) - .10(\text{Age}) \\ - .64(\text{Platelets}/25,000) + .84(\text{AST}_{>100}) + .18(\text{Hct}) + .17(\text{WBC}/500)$$

B Equation 2

$$\ln \frac{\hat{p}}{1-\hat{p}} = -5.82 - .67(\text{Platelets}/25,000) + 1.22(\text{AST}_{>100}) + .16(\text{Hct})$$

C Equation 3

$$\ln \frac{\hat{p}}{1-\hat{p}} = 4.61 - .39(\text{Platelets}/25,000) - .38(\text{WBC}/500) + 2.20(\text{AST}_{>100}) \\ + 1.33(\text{Positive TT}) - .59(\text{Neuts}_{>60\&=75}) - .60(\text{Neuts}_{>75})$$

D Equation 4

$$\ln \frac{\hat{p}}{1-\hat{p}} = -6.79 - .50(\text{Platelets}/25,000) - .12(\text{WBC}/500) + .21(\text{Hct}) \\ + .83(\text{AST}_{>100}) - .13(\text{Age})$$

FIGURE 4. Validated multivariable probability models for classifying patients with dengue. (A) DHF versus DF. (B) DHF versus all others. (C) Dengue versus OFI. (D) Severe dengue versus all others. Note that categorical variables (percent neutrophils, percent monocytes, positive tourniquet test, AST, and gender) are 0/1. AST = aspartate aminotransferase; Hct = hematocrit; ln = natural logarithm; neuts = percent neutrophils; $\hat{p}$ = predicted probability; WBC = white blood cell.

TABLE 5
Comparison of percent agreement and κ statistics between final models, WHO DHF criteria, and physician's final diagnosis

	Model vs. WHO			Model vs. physician			Physician vs. WHO		
	Agreement (%)	κ	P value	Agreement (%)	κ	P value	Agreement (%)	κ	P value
DHF vs. all others									
Probability cutoff = 0.19	86.2	0.60	< 0.001	85.5	0.59	< 0.001	92.4	0.74	< 0.001
DHF vs. DF									
Probability cutoff = 0.35	80.0	0.58	< 0.001	79.5	0.57	< 0.001	84.6	0.66	< 0.001

DF = dengue fever; DHF = dengue hemorrhagic fever; WHO = World Health Organization.

diagnosis, our models show high sensitivity in distinguishing DHF from DF and DHF from all others without including chest X-ray or hemoconcentration. Our models did not show an improved percent agreement over the physician's diagnosis or the WHO definition of DHF. However, this is not surprising when considering that the physician used PEI as an indicator for plasma leakage and the majority of patients diagnosed with DHF had evidence of pleural effusion. Nevertheless, our validated model of DHF versus DF did show a high percent agreement with both the physician's diagnosis of DHF and the WHO definition (79.5% and 80.0%, respectively).

We showed high sensitivity and specificity in classifying patients with severe dengue defined by shock or need for fluid resuscitation. Some indicators used in the WHO definition of DHF are affected by early hydration and detecting hemoconcentration and plasma leakage can be difficult.¹³ We defined a less-subjective category of severe dengue, which considered the amount of fluid resuscitation needed, and produced a model with high sensitivity and specificity without using a chest X-ray or hemoconcentration. Although early hydration can still affect the values of hematocrit used in our models, we have removed the requirement for baseline or convalescent hematocrits that may not always be available outside of a research setting. Furthermore, we applied our models to the validation dataset using data obtained at 24 hours after defervescence only and still achieved high sensitivity and specificity for DHF versus DF and DHF versus all others. This suggests that our models will be applicable outside of a research setting and perhaps, generalizable to patients who present later in illness; however, further studies are needed to test the generalizability.

Bleeding tendency is often indicated by a positive tourniquet test, but the test method is not always harmonized among treatment centers (Thomas SJ, unpublished data) and confusion arises as to which cutoff should be used to indicate a positive test.¹³ We found that a cutoff ≥ 20 petechiae yielded a higher AUC compared with a cutoff of ≥ 10 petechiae (data not shown). However, across all multivariable models, a positive tourniquet test showed an association only in the patients with dengue compared with patients with OFI. This supports use of this indicator to identify patients with dengue, whereas the tourniquet test has performed less well for distinguishing DHF from DF.^{9,16,19,20}

Minimum platelet count and maximum Hct were associated with DHF and are part of the WHO definition. Although platelet count and Hct are included in the model, not all patients diagnosed by physicians with DHF had a platelet count below 100,000, and most patients with DHF had plasma leakage detected by pleural effusion rather than by hemoconcentration. Our models have no thresholds for particular variables but instead, use a combination of clinical laboratory variables to calculate a probability that can be used to classify patients.

The main limitation to our study is the exclusion of children who presented later in illness. This study design limits the number of patients who developed severe dengue illness. Our study was also limited to Thailand's pediatric population. However, this reflects what is seen in southeast Asia, where the majority of dengue cases are in the pediatric population.

Our study identified clinical indicators that could be used to calculate a probability of DHF or severe dengue illness. From our models, patients with DF or non-severe dengue illness and OFI have a uniformly low probability of DHF or severe dengue. The probability calculated from our models could be used to classify patients when other indicators, such as a chest X-ray or convalescent sera, are unavailable. These models are not meant to guide clinical management but can be used for retrospective classification of dengue illness in the absence of standard indicators of plasma leakage.

Received March 1, 2010. Accepted for publication June 29, 2010.

Financial support: This work was supported by National Institutes of Health Grant NIH-P01AI34533, Centers for Disease Control and Prevention Office of the Director Grant 1R36CK00123-01, and the Military Infectious Disease Research Program. The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the view of the US Government. The authors have no conflicting financial interests.

Authors' addresses: James A. Potts, Anon Srikiatkachorn, Wenjun Li, Daniel H. Libraty, Sharone Green, and Alan L. Rothman, Center for Infectious Disease and Vaccine Research and Department of Medicine, University of Massachusetts Medical School, Worcester, MA, E-mails: James.Potts@umassmed.edu, Anon.Srikiatkachorn@umassmed.edu, Wenjun.Li@umassmed.edu, Daniel.Libraty@umassmed.edu, Sharone.Green@umassmed.edu, and Alan.Rothman@umassmed.edu. Stephen J. Thomas, Ananda Nisalak, and Robert V. Gibbons, Department of Virology, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, E-mails: stephen.thomas@afirms.org, anandan@afirms.org, and robert.gibbons@afirms.org. Pra-on Supradish, Suchitra Nimmannitya, and Siripen Kalayanaroj, Queen Sirikit National Institute of Child Health, Bangkok, Thailand, E-mails: praonsu@yahoo.com, sujitrn@health.moph.go.th, and sirip@health.moph.go.th. Timothy P. Endy, Department of Medicine, University of New York, Upstate Medical University, Syracuse, NY, E-mail: endyt@upstate.edu.

REFERENCES

1. Beatty M, 2009. *Global Burden of Dengue: Pediatric Dengue Vaccine Initiative*. Available at: www.pdvi.org/about_dengue/GBD.asp. Accessed August 3, 2010.
2. Nimmannitya S, 1987. Clinical spectrum and management of dengue haemorrhagic fever. *Southeast Asian J Trop Med Public Health* 18: 392-397.
3. World Health Organization, 1997. *Dengue Haemorrhagic Fever: Diagnosis, Treatment, Prevention, and Control*. Geneva, Switzerland: World Health Organization.
4. Balmaseda A, Hammond SN, Perez MA, Cuadra R, Solano S, Rocha J, Idiaquez W, Harris E, 2005. Short report: assessment of the World Health Organization scheme for classification of

- dengue severity in Nicaragua. *Am J Trop Med Hyg* 73: 1059–1062.
5. Bandyopadhyay S, Lum LC, Kroeger A, 2006. Classifying dengue: a review of the difficulties in using the WHO case classification for dengue haemorrhagic fever. *Trop Med Int Health* 11: 1238–1255.
 6. Setiati TE, Mairuhu AT, Koraka P, Supriatna M, Mac Gillavry MR, Brandjes DP, Osterhaus AD, van der Meer JW, van Gorp EC, Soemantri A, 2007. Dengue disease severity in Indonesian children: an evaluation of the World Health Organization classification system. *BMC Infect Dis* 7: 22.
 7. Srikiatkachorn A, Gibbons RV, Green S, Libraty DH, Thomas SJ, Endy TP, Vaughn DW, Nisalak A, Ennis FA, Rothman AL, Nimmannitaya S, Kalayanaroj S, 2010. Dengue hemorrhagic fever: the sensitivity and specificity of the World Health Organization definition for identification of severe cases of dengue in Thailand, 1994–2005. *Clin Infect Dis* 50: 1135–1143.
 8. Potts JA, Rothman AL, 2008. Clinical and laboratory features that distinguish dengue from other febrile illnesses in endemic populations. *Trop Med Int Health* 13: 1328–1340.
 9. Kalayanaroj S, Vaughn DW, Nimmannitaya S, Green S, Suntayakorn S, Kunentrasai N, Viramitrachai W, Ratanachu-ek S, Kiatpolpoj S, Innis BL, Rothman AL, Nisalak A, Ennis FA, 1997. Early clinical and laboratory indicators of acute dengue illness. *J Infect Dis* 176: 313–321.
 10. Holliday MA, Segar WE, 1957. The maintenance need for water in parenteral fluid therapy. *Pediatrics* 19: 823–832.
 11. Custer JW, Rau RE, 2009. *The Harriet Lane Handbook: A Manual for Pediatric House Officers*. The Harriet Lane Service, Children's Medical and Surgical Center of The Johns Hopkins Hospital. Philadelphia, PA: Elsevier.
 12. Harris E, Videia E, Perez L, Sandoval E, Tellez Y, Perez ML, Cuadra R, Rocha J, Idiaquez W, Alonso RE, Delgado MA, Campo LA, Acevedo F, Gonzalez A, Amador JJ, Balmaseda A, 2000. Clinical, epidemiologic, and virologic features of dengue in the 1998 epidemic in Nicaragua. *Am J Trop Med Hyg* 63: 5–11.
 13. Rigau-Perez JG, 2006. Severe dengue: the need for new case definitions. *Lancet Infect Dis* 6: 297–302.
 14. Rigau-Perez JG, Bonilla GL, 1999. An evaluation of modified case definitions for the detection of dengue hemorrhagic fever. Puerto Rico Association of Epidemiologists. *P R Health Sci J* 18: 347–352.
 15. Thangaratham PS, Tyagi BK, 2007. Indian perspective on the need for new case definitions of severe dengue. *Lancet Infect Dis* 7: 81–82.
 16. Wills B, Tran VN, Nguyen TH, Truong TT, Tran TN, Nguyen MD, Tran VD, Nguyen VV, Dinh TT, Farrar J, 2009. Hemostatic changes in Vietnamese children with mild dengue correlate with the severity of vascular leakage rather than bleeding. *Am J Trop Med Hyg* 81: 638–644.
 17. A-Nuegoonpipat A, Panthuyosri N, Anantapreecha S, Chanama S, Sa-Ngasang A, Sawanpanyalert P, Kurane I, 2008. Cross-reactive IgM responses in patients with dengue or Japanese encephalitis. *J Clin Virol* 42: 75–77.
 18. Innis BL, Nisalak A, Nimmannitaya S, Kusalerdchariya S, Chongswasdi V, Suntayakorn S, Puttisri P, Hoke CH, 1989. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *Am J Trop Med Hyg* 40: 418–427.
 19. Cao XT, Ngo TN, Wills B, Kneen R, Nguyen TT, Ta TT, Tran TT, Doan TK, Solomon T, Simpson JA, White NJ, Farrar JJ, 2002. Evaluation of the World Health Organization standard tourniquet test and a modified tourniquet test in the diagnosis of dengue infection in Viet Nam. *Trop Med Int Health* 7: 125–132.
 20. Phuong CX, Nhan NT, Kneen R, Thuy PT, van Thien C, Nga NT, Thuy TT, Solomon T, Stepniewska K, Wills B, 2004. Clinical diagnosis and assessment of severity of confirmed dengue infections in Vietnamese children: is the World Health Organization classification system helpful? *Am J Trop Med Hyg* 70: 172–179.