A CLINICAL SCORE FOR DIAGNOSIS OF PROBABLE DENGUE IN CHILDREN IN AN ENDEMIC AREA, THAILAND

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Abstract. Dengue is one of the most important mosquito-borne diseases in tropical and subtropical regions of the world. Laboratory diagnosis is often expensive or unavailable in some endemic areas, making clinical diagnosis important for case management. In order to develop and validate the Mahidol dengue clinical score (MDCS), a predictive of dengue among children who present with acute febrile illness without localizing signs in a dengue endemic area, data on clinical and laboratory findings in a cohort study of children with acute febrile illness without localizing signs identified prospectively were analyzed and compared between those with and without laboratory-confirmed dengue. MDCS was then developed using independent clinical risk factors associated with dengue. The validity of MDCS was further evaluated by comparison to WHO dengue diagnostic criteria. In children who had acute febrile illness without localizing signs, MDCS-A version comprising of mucosal bleeding, facial flush, absence of rhinorrhea, positive tourniquet test, leucopenia, and thrombocytopenia had a diagnostic value comparable to WHO 1997 criteria, while MDCS-B version that excludes data on leukopenia and thrombocytopenia, making it more feasible in laboratory-limited settings, had a diagnostic value comparable to WHO 2009 criteria. Thus, MDCS can be used as a screening diagnostic tool for dengue infection in children in a dengue endemic area.

Keywords: clinical finding, dengue, Mahidol dengue clinical score, Thailand

INTRODUCTION

Dengue is a common cause of acute febrile illness and one of the most important mosquito-borne diseases causing significant morbidity and mortality in tropical and subtropical regions of the world (Gubler, 2002). Its clinical spectrum ranges from undifferentiated fever (UF), to fever with some signs and symptoms [*eg*, rash, myalgia, retro-orbital pain, headache-dengue fever (DF)], to dengue hemorrhagic fever (DF) (WHO, 1997) and severe dengue (WHO, 2009) and is considered as "one disease entity with

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different clinical presentations and often with unpredictable clinical evolution and outcome" (WHO, 2009). Presently there are no specific antiviral drugs for treatment of dengue. A dengue vaccine successfully completed a Phase-3 trial (Capeding *et al*, 2014) and is licensed in a number of countries (WHO, 2017). Several other candidate vaccines are undergoing clinical evaluations (Whitehead *et al*, 2007). Thus, primary prevention of dengue relies on mosquito control alone or in combination with dengue vaccination, while secondary prevention relies on early diagnosis and appropriate medical management of patients.

It is difficult to differentiate the usually non-specific signs and symptoms of dengue (*ie*, no localizing signs) from other febrile illnesses (*eg*, chikungunya, influenza, leptospirosis and other viral infection). For this reason, a high degree of under-recognition of dengue exists as shown in studies in Thailand and Cambodia, where the average under-recognition of total and inpatient dengue cases was estimated to be 8.7 and 2.6-fold, and 9.1 and 1.4 fold, respectively of the reported cases (Wichmann *et al*, 2011). Although dengue diagnostic testing using PCR to detect dengue virus (DENV) genome, detection of non-structural protein 1 (NS1) or enzyme-linked immunosorbent assay (ELISA) to detect anti-DENV IgM are highly specific and sensitive (WHO, 2009), they are expensive and often unavailable in many dengue endemic areas. Therefore, clinical diagnosis remains important, either as the sole tool or as a screening tool to identify patients requiring laboratory diagnostic testing.

World Health Organization (WHO) 1997 criteria for dengue diagnosis and treatment (WHO, 1997) have been used with some success, but limitations have been noted regarding its complexity and applicability, particularly in Latin America and in patients with severe dengue disease (Phuong *et al*, 2004; Balmaseda *et al*, 2005; Bandyopadhyay *et al*, 2006; Deen *et al*, 2006; Rigau-Perez, 2006). This led the Tropical Disease Research (TDR), WHO in 2006-7 to sponsor a multicenter study in seven countries in Asia and Latin America (Alexander *et al*, 2011), from which emerged in 2009 new WHO guidelines for dengue diagnosis that classifies the infection into probable dengue (dengue without warning sign), dengue with warning sign, and severe dengue (WHO, 2009). However, this new classification has not been validated for its usefulness.

We developed a Mahidol dengue clinical score (MDCS) using data obtained from laboratory- confirmed dengue patients in a dengue endemic area of Ratchaburi Province, Thailand among a cohort of children with prospectively identified acute febrile illnesses without localizing signs. In this report we compared the utility of MDCS with WHO 1997 and 2009 dengue diagnostic criteria in predicting laboratory-confirmed dengue.

MATERIALS AND METHODS

Study population

This was a retrospective study nested in a prospective study of the epidemiology of dengue in a cohort of primary school children in Ratchaburi Province, Thailand conducted from 2006 to 2009 (Sabchareon *et al*, 2012). Every acute febrile child with no localizing source of infection (*eg*, abscess, bacterial meningitis, exudative tonsillitis, malaria, pneumonia, and urinary tract infection) was allocated a diary card to record daily symptoms until illness recovery. Physical examination was performed by a pediatrician

and clinical laboratory investigations (*eg*, blood chemistry and complete blood count) were performed at the attending pediatrician's discretion.

Laboratory investigations

DENV infection was confirmed by detection of rising DENV-specific IgM/IgG by capture ELISA of serum specimens from patients with acute and convalescent illness (Innis *et al*, 1989), or by being DENV RT-PCR positive of specimens from those with acute illness (Sabchareon *et al*, 2012). Dengue virus serotype was determined by RT-PCR or inoculation into *Toxorhynchites splendens* mosquito with detection and serotyping by immunofluorescence.

The Ethical Review Committee for Research in Human Subjects, Ministry of Public Health, Thailand and the Institutional Review Board, International Vaccine Institute, Seoul, Korea approved the study protocol. Informed consent signed by at least one parent or legal guardian and assent form signed by the children >7 years of age were obtained prior to enrollment in the study.

Illness classification

All illness data were reviewed. Laboratory-confirmed dengue episodes were classified as DF, DHF or dengue shock syndrome (DSS) according to the 1997 WHO criteria (WHO, 1997). Episodes not meeting the criteria for DF, DHF or DSS were classified as UF. Illness also was classified as probable dengue, dengue with warning sign and severe dengue according to the 2009 WHO criteria (WHO, 2009). Children with a febrile illness who were negative for markers of DENV infection were classified as non-dengue febrile illness. Subjects with inconclusive results from dengue diagnostic testing (*eg*, positive IgM but no rising antibody titer and negative RT-PCR) were excluded from analysis.

Data analysis

Clinical and laboratory findings comparing dengue and non-dengue febrile illness were analyzed using univariate analysis. Findings with p-value <0.20 were included in multivariate analysis for independent risk factors of dengue. MDCS was developed using these independent risk factors. The presence of each risk factor is given a score of 1. The diagnostic validity of MDCS was then evaluated using receiver operating characteristic (ROC) curve and optimal cut-off points of MDCS data were assigned. Sensitivity, specificity, positive and negative predictive value were compared to WHO 1997 and WHO 2009 criteria. Data were analyzed using Statistical Package for the Social Sciences (SPSS) program version 17.0 (IBM, Armonk, NY). Frequency and median or mean values were used where appropriate. Corrected chi-square test or Fischer-exact test was used for comparing categorical variables and Student's t-test or Mann-Whitney U test for comparing continuous variable as appropriate. A p-value <0.05 is considered significant.

RESULTS

The prevalence of laboratory-confirmed dengue was determined for 10,128 (5,106 male and 5,022 female) person-year of observation among 3-13-year old children with active fever surveillance. During the study period there were 1,467 febrile episodes without localizing signs that had available clinical data. Two hundred and ninety-seven (20.2%) episodes were proven to be DENV infection and 1,154 (78.7%) were non-dengue. Sixteen episodes (1.1%) had inconclusive results and were excluded from the analysis. Among dengue episodes, 108 (36%), 71 (24%), 48 (16%), and 17 (6%) were infected

Table 1
Clinical diagnosis of dengue infection in Ratchaburi Province, Thailand from 2006 to 2009 based on WHO 1997 and 2009 criteria.

Criteria	Clinical diagnosis	Confirmatory laboratory diagnosis of dengue	
		Dengue (n = 297) Number (%)	Non-dengue (n = 1,154) Number (%)
WHO 1997	Dengue		
	Dengue fever	140 (47)	88 (7.6)
	Dengue hemorrhagic fever	38 (13)	3 (0.3)
	Dengue shock syndrome	7 (2)	0
	Non-dengue	112 (38)	1,063 (92.1)
WHO 2009	Dengue		
	Probable dengue	48 (16)	234 (20.3)
	Positive warning signs	203 (68)	607 (52.6)
	Severe dengue	27 (9)	6 (0.5)
	Non-dengue	19 (6)	307 (26.6)

with DENV serotype 1, -2, -3, and -4, respectively. DENV serotype could not be identified in 53 (18%) patients. Clinical diagnosis of dengue among 1,451 febrile episodes without localizing signs had a sensitivity, specificity, positive predictive value and negative predictive of 62.3%, 92.1%, 67.0%, and 90.5%, respectively using WHO 1997 criteria (WHO, 1997) and 93.6%, 26.6%, 24.7% and 94.2%, respectively using WHO 2009 criteria (WHO, 2009) (Table 1).

Headache, anorexia and vomiting were the most common clinical findings in dengue episodes while headache, anorexia, and rhinorrhea were the most common findings in non-dengue episodes (Table 2). Significantly higher proportion of dengue episodes have anorexia, vomiting, positive tourniquet test, myalgia, abdominal pain, facial flush, rash, diarrhea, and mucosal bleeding, but a lesser proportion have rhinorrhea compared to non-dengue episodes. Dengue episodes also have significantly lower white blood cell count and platelet count compared to non-dengue episodes. Regression analysis showed seven independent risk factors for dengue infection, namely, rash, leucopenia (WBC <4,000 cell/μl), thrombocytopenia (platelet <150,000 cell/μl), positive tourniquet test, facial flush, mucosal bleeding, and absence of rhinorrhea (Table 3).

Because almost all cases of dengue have rash in the convalescent phase, therefore the presence of rash may not be helpful for early diagnosis and management and rash was not included in the MDCS. Two MDCS versions were developed: MDCS-A that includes six risk factors, namely, absence of rhinorrhea, facial flush, leucopenia, mucosal bleeding, positive tourniquet test, and thrombocytopenia; and MDCS-B that includes four risk factors, namely, absence of rhinorrhea, facial

Table 2
Demographic data, clinical and laboratory findings of dengue and non-dengue febrile children in Ratchaburi Province, Thailand from 2006 to 2009.

Demography/clinical finding	Dengue (n = 297) Number (%)	Non-dengue (n = 1,154) Number (%)	p-value[a]
Age [median (IQR)]	9.6 (3.3)	9.4 (3.3)	0.92
Sex (male:female)	167 :130	565:589	0.03
Headache	256 (86)	960 (83.2)	0.24
Anorexia	232 (78)	699 (60.6)	<0.001
Vomiting	215 (72)	653 (56.6)	<0.001
Myalgia	159 (53)	456 (39.5)	<0.001
Abdominal pain	139 (47)	435 (37.7)	0.005
Facial flush	130 (44)	290 (25.1)	<0.001
Rhinorrhea	107 (36)	707 (61.3)	<0.001
Retro-orbital pain	96 (32)	386 (33.4)	0.76
Rash	90 (30)	24 (2.1)	<0.001
Lethargy/restlessness	65 (22)	268 (23.2)	0.68
Diarrhea	60 (20)	174 (15.1)	0.04
Arthralgia	58 (19)	199 (17.2)	0.40
Mucosal bleeding	37 (12)	32 (2.8)	<0.001
Severe bleeding	5 (2)	7 (0.6)	0.08[b]
Positive tourniquet test	172/252 (68)	178/807 (22.1)	<0.001
Hepatomegaly	44 (15)	29 (2.5)	<0.001
Hemoconcentration	27/236 (11)	3/518 (0.6)	<0.001
Ascites	8 (3)	2 (0.2)	<0.001[b]
White blood cell count (cell/μl) [median (range)]	2,800 (94-11,170)	6380 (850-26560)	<0.001
White blood cell count <4,000 cell/μl	186/237 (78)	127/520 (24.4)	<0.001
Platelet (cell/μl) [median (range)]	124,000 (5,000-380,000)	247,000 (22,000-522,000)	0.001
Platelet count <150000 cell/μl	149/237 (62)	43/520 (8.3)	<0.001

[a]Chi-square test. [b]Fisher-exact test.

flush, mucosal bleeding, and positive tourniquet test.

The receiver operating characteristic (ROC) curve of the MDCS-A has an area under the curve (AUC) of 0.836 and inclusion of rash does not significantly increase AUC (0.844), while ROC curve of MDCS-B has an AUC of 0.774 (Fig 1).

A comparison of MDCS-A (Table 4) and MDCS-B (Table 5) with clinical severity of dengue (*ie*, UF, DF, DHF, DSS) demonstrated higher score was correlated with more severe disease for both scoring modalities. Considering that the cut-off point for diagnosis of dengue should not miss severe dengue (*eg*, DHF), the cut-off points of ≥3 was assigned for MDCS-A, and ≥1 for MDCS-B. A comparison of sensitivity, specificity, positive predictive value and negative predictive value among WHO

Table 3
Independent risks of dengue infection.

Clinical/laboratory finding	Standardized coefficient	*t*	*p*-value
Rash	0.161	6.903	<0.001
Rhinorrhea	0.135	6.554	<0.001
White blood cell count <4,000 cell/μl	0.184	6.764	<0.001
Platelet count <150,000 cell/μl	0.286	10.457	<0.001
Tourniquet test positive	0.077	3.149	0.002
Facial flush	0.061	2.874	0.004
Mucosal bleeding	0.042	1.993	0.046
Myalgia	0.038	1.796	0.07
Severe bleeding	-0.028	-1.307	0.19
Vomiting	0.019	0.887	0.36
Abdominal pain	-0.019	-0.864	0.39
Anorexia	0.012	0.538	0.59
Diarrhea	-0.003	-0.158	0.88
Hepatomegaly	0.037	1.692	0.09
Hemoconcentration	0.046	1.536	0.13
Ascites	0.009	0.433	0.67

1997 criteria, 2009 criteria, MDCS-A, and MDCS-B revealed MDCS-A had a diagnostic value similar to WHO 1997 criteria while MDCS-B a diagnostic value similar to WHO 2009 criteria (Table 6).

DISCUSSION

This study identifies clinical signs and symptoms that can be used to diagnose dengue infection among pediatric patients who have acute febrile illness without localizing signs in a dengue endemic area. This study is unique because the data were obtained via a prospective active fever surveillance of the patients with acute febrile illness. This resulted in the enrolment of a spectrum of much milder symptomatic dengue infection (*ie*, UF) while other similar studies from Thailand recruited hospitalized patients (Kalayanarooj *et al*, 1997; Potts *et al*, 2010). Because most of our patients had mild illness, CBC and tourniquet test were not performed in some episodes and the majority of the tests were performed only once during out-patient visits. Due to the mildness of disease it is reasonable to assume these patients had normal CBCs but there have been no data to confirm this assumption. Similarly, liver function tests, serum electrolyte and other clinical laboratory tests were performed in only a few patients and such data were not included in the analysis.

This study indicates there were many clinical features that were more common in dengue infection than in other non-localizing febrile illnesses. Kalayanarooj *et al* (1997) reported anorexia, nausea, vomiting, positive tourniquet test, lower total white blood cell count are more associated with dengue infection than other febrile illnesses. Facial flush also is commonly found in dengue infection and was used as an enrolment criterion in the study

Table 4
Clinical diagnosis of dengue in children in Ratchaburi Province, Thailand from 2006 to 2009 based on MDCS-A.

Score	Confirmatory laboratory dengue diagnosis					
	Dengue (n = 297) Number					Non-dengue (n = 1,154) Number (%)
	UF	DF	DHF	DSS	Total (%)	
0	19	1	0	0	20 (7)	382 (33.1)
1	45	7	0	0	52 (17)	524 (45.4)
2	27	17	1	0	45 (15)	172 (14.9)
3	17	26	8	2	53 (18)	58 (5.0)
4	4	56	15	2	77 (26)	15 (1.3)
5	0	31	12	2	45 (15)	3 (0.3)
6	0	2	2	1	5 (2)	0 (0)
Mean (SD)					2.9 (1.6)	1.0 (0.9)

UF, undifferentiated fever; DF, dengue fever; DHF, dengue hemorrhagic fever; DSS, dengue shcok ayndrome; MDCS; Mahidol dengue clinical score.

Table 5
Clinical diagnosis of dengue in children in Ratchaburi Province, Thailand from 2006 to 2009 based on MDCS-B.

Score	Confirmatory laboratory dengue diagnosis					
	Dengue Number (n = 297)					Non-dengue Number (%) (n = 1,154)
	UF	DF	DHF	DSS	Total (%)	
0	20	4	0	0	24 (8.1)	412 (35.7)
1	56	31	5	1	93 (31.3)	556 (48.2)
2	31	60	15	4	110 (37.0)	169 (14.6)
3	5	42	15	2	64 (21.5)	15 (1.3)
4	0	3	2	1	6 (2.0)	2 (0.2)
Mean (SD)					1.8 (0.9)	0.8 (0.7)

Table 6
Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of different clinical criteria for diagnosis of dengue.

Clinical criteria	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
WHO 1997	62.3	92.1	67.0	90.5
WHO 2009	93.6	26.6	24.7	94.2
MDCS-A (cut-off ≥3)	60.6	93.4	70.3	90.2
MDCS-B (cut-off ≥1)	91.9	35.7	26.9	94.5

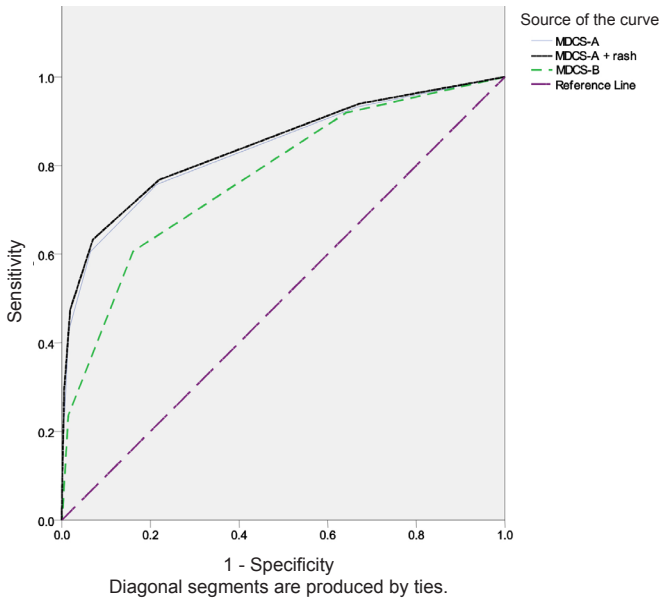

Fig 1–Receiver operating characteristic (ROC) curves of four dengue scorings for diagnosis of dengue infection.

of Kalayanarooj *et al* (1997). Gregory *et al* (2011) also found the presence of either a positive tourniquet test or leucopenia correctly identifies 94% of dengue patients. Rash, hemorrhagic manifestation and leucopenia were included in the case definition of dengue fever (WHO, 1997). Rash, positive tourniquet test, and leucopenia were included as criteria of probable dengue infection and mucosal bleeding and persistent vomiting as criteria of warning signs (WHO, 2009). A systematic review also found patients with dengue infection have lower platelet, white blood cell and neutrophil counts (Potts and Rothman, 2008). In this study, the criteria for leucopenia and thrombocytopenia were different from other studies in that the cut-off level for white blood count at <4,000 cell/μl and for platelet count at <150,000 cell/μl were better predictive values, perhaps because the majority of the patients in our study had mild dengue disease compared to DHF that is more common in the abovementioned studies.

This study identifies seven independent clinical risk factors of dengue infection; however, each of these clinical risks by itself was not specific enough and having a low positive predictive value. The combination of these clinical risk factors increased sensitivity and specificity for dengue diagnosis. MDCS-A showed a quite reasonable ROC curve. The suggested cut-off point at ≥3 was based on the assumption that this cut-off point would provide high sensitivity and specificity and not overlook severe dengue (*ie*, DHF).

The primary difference between WHO dengue diagnostic criteria and the MDCS systems is that WHO dengue diagnostic criteria are used to diagnose dengue as well as to classify dengue severity (*ie*, DF, DHF and DSS for WHO 1997 criteria, and dengue with and without warning signs and severe dengue for WHO 2009 criteria). MDCS systems were only intended to be used to diagnose whether the patient has dengue or not, and therefore much simpler to use. Although the MDCS criteria were derived from predominantly mild symptomatic dengue episodes, the diagnostic values of both MDCS systems appeared to be better for diagnosis of the more severe forms of dengue infection; however, the number of severe dengue cases was too small to perform a meaningful statistical analysis. Moreover, there are other clinical findings that more accurately predict severe dengue, such as signs of plasma leakage or shock, severe bleeding, severe organ involvement (WHO, 1997; WHO,

2009), persistent vomiting, abdominal pain, diarrhea, hepatomegaly or severe thrombocytopenia (Sirivichayakul *et al*, 2012).

MDCS should be used for screening of dengue among pediatric patients with acute febrile illness. MDCS-A is suitable for hospitals with available clinical laboratories, MDCS-B for rural health clinics where clinical laboratories are not available. MDCS also could be used in clinical triage for febrile patients to identify the patients who need CBC examination. MDCS-B could be easily taught to people in dengue endemic areas with little laboratory support. The MDCS-B predictors (absence of rhinorrhea, facial flush, mucosal bleeding, or positive tourniquet test) in a febrile child are simple criteria indicative of suspected dengue. The patients can then be transferred to a hospital for more accurate diagnosis and grading of severity. Patients who are diagnosed as probable dengue by this extended work-up or by MDCS-A can be definitively diagnosed by confirmatory laboratory tests if available and then managed accordingly. This approach should decrease the costs of confirmatory laboratory diagnosis and subsequent treatment while failing to identify a small number of patients with mild dengue.

In conclusion, based on clinical differences between dengue and other febrile illness, we suggest using the developed MDCS systems in dengue endemic areas for diagnosis of dengue infection among children with acute febrile illness without localizing signs. MDCS-B should be more appropriate in rural regions where clinical laboratories are not available and MDCS-A, with its higher specificity, more useful in hospitals where clinical laboratories are available.

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