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ORIGINAL ARTICLE

Laboratory diagnostics of dengue fever: An emphasis on the role of commercial dengue virus nonstructural protein 1 antigen rapid test

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Dengue;
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Background/Purpose(s): In 2008, the Dengue NS1 Ag STRIP (Bio-Rad Laboratories, Marnes-la-Coquette, France) was introduced to routine dengue diagnostics in Taiwan, in addition to real-time reverse-transcription polymerase chain reaction (PCR), virus isolation, and capture immunoglobulin (Ig)M/IgG enzyme-linked immunosorbent assay (ELISA). This study aimed to evaluate the benefit of this assay and factors influencing the results of these diagnostic tests.

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Outbreak surveillance;
RT-PCR

Methods: Retrospectively, the authors enrolled laboratory-confirmed adult dengue patients from July 2008 to January 2012 in a tertiary hospital. The sensitivities of each test alone and in combination were analyzed by the duration of illness (early stage: day 0-day 3 and late stage: day 4-day 8). The factors influencing sensitivity of the Dengue NS1 Ag STRIP were examined.

Results: There were 392 patients enrolled. The overall sensitivity of the Dengue NS1 Ag STRIP was 68.37% and PCR was 71.94%. With the assistance of the Dengue NS1 Ag STRIP, a diagnosis was made in 10.97% of patients without the need for second convalescent samples, and 4.34% more cases were detected. Independent factors for reduced Dengue NS1 Ag STRIP sensitivity were dengue virus (DENV) IgG seropositivity and a sample taken after the fifth day of illness. At the early stage, the PCR and the Dengue NS1 Ag STRIP combination had the highest sensitivity rate than other combinations. At the late stage, a combination of the Dengue NS1 Ag STRIP and capture IgM/IgG ELISA had better sensitivity rates. PCR and capture IgM/IgG ELISA in combination had sensitivity above 90% through the course of illness.

Conclusion: Dengue NS1 Ag STRIP is a useful tool for early dengue diagnosis. Its use can increase the diagnostic sensitivity and decrease the need of convalescent samples. Seeking treatment late (days postonset $>$ 4) and DENV IgG seropositivity independently decrease the sensitivity of the Dengue NS1 Ag STRIP.

Introduction

Approximately 50 million people are infected by dengue annually and approximately 2.5 billion people live in dengue-endemic countries. The diagnosis of acute dengue infection is important for patient care and outbreak control. In recent years, the geographic distribution of dengue has expanded and the incidence has increased rapidly.[1,2] Dengue outbreaks have occurred in Taiwan almost every year since 1987.[3] All four dengue virus (DENV) serotypes were present in recent outbreaks in Taiwan,[4] and the occurrence of dengue cases was not limited in southern Taiwan. To constrain dengue outbreak, the faster the diagnosis, the better for the patient and outbreak control measures.

Nonstructural protein 1 (NS1) is a glycoprotein secreted by DENV infected mammalian cells.[5] Because the soluble form of NS1 can be detected in the bloodstream, tests such as antigen-capture enzyme-linked immunosorbent assay (ELISA), lateral flow antigen detection, and measurement of NS1-specific immunoglobulin (Ig)M and IgG responses have been developed.[5]

Dengue is a notifiable infectious disease in Taiwan, and patients suspected to have dengue should be notified within 24 hours.[3] The sera are sent to reference laboratories and are tested by real-time reverse-transcription polymerase chain reaction (RT-PCR), capture IgM/IgG ELISA, and virus isolation.[3] Since August 2008, the Dengue NS1 Ag STRIP (Bio-Rad Laboratories, Marnes-la-Coquette, France), an immunochromatographic test (ICT), has been used as one of the diagnostic tools for outbreak surveillance.[6,7] This test has proven useful for on-site detection of imported cases at Taiwan airports and it allows early detection of dengue cases.[8] However, the benefit of adding Dengue NS1 Ag STRIP has not yet been investigated among nationwide transmission control in Taiwan.

We conducted a hospital-based retrospective study to compare the dengue diagnostic tests routinely used in Taiwan. The usefulness of the Dengue NS1 Ag STRIP and factors influencing its sensitivity were also evaluated.

Materials and methods

Study design and patients

Adult patients (age 18 years or older) with laboratory-confirmed dengue presenting to Kaohsiung Medical University Hospital (KMUH) during July 2008 to January 2012 were enrolled. KMUH is a 1700-bed tertiary referral center in southern Taiwan but also provides primary health care.

Patient information and clinical data from medical records were reviewed under the approval of the KMUH institutional review board. The diagnosis sheet provided by the Taiwan Centers for Disease Control (Taiwan CDC) includes results of real-time RT-PCR and serotype, if available, as well as Dengue NS1 Ag STRIP and capture IgM and IgG ELISA. If the result in a single serum sample is undetermined, a second sample is requested for capture IgM/IgG ELISA during the convalescent phase to make the final diagnosis. To compare the diagnostics used in outbreak surveillance, only records with a single sample tested by all three methods (RT-PCR, NS1 rapid test, and capture IgM/IgG ELISA) were enrolled in our analysis.

Definition

Day 0 indicates the day of symptom onset.[9] We analyzed patients who received diagnostic tests during the period of day 0 to day 8 of illness. To evaluate the influence of duration of illness at the time of sample collection on sensitivity of diagnostics, day 0 to day 3 is defined as the early stage of illness and day 4 to day 8 as the late stage. We compared demographic characteristics and sensitivity of diagnostics by stratum of duration of symptoms (0—3 days vs. 4—8 days). To investigate the factors influencing

the sensitivity of NS1, we defined sample taken after the fifth day of illness onset as delayed detection phase (day 5 to day 8). A previous study found the sensitivity of NS1 declined obviously after the fifth day of illness.[10] The elderly are defined as age 65 years or older.

Dengue diagnosis

The reporting system for dengue in Taiwan was described in a previous study.[3] The routine diagnotic tools included real-time RT-PCR, Dengue NS1 Ag STRIP, capture IgM and IgG ELISA, and virus isolation.[3,11,12] According to the guidelines for dengue control in Taiwan, all methods are used before the eighth day of illness.[4] A confirmed dengue case is defined as a single sample that tests positive by RT-PCR, virus isolation, or Dengue NS1 Ag STRIP. Paired serology (samples collected in acute and convalescent phase) revealing four-fold increase or seroconversion of dengue virus—specific IgM or IgG is also defined as a confirmed case.[4] The result of virus isolation was not included for analysis, because it took more than 1 week to obtain a result and therefore was less applicable for early diagnosis. We use the terms PCR, NS1, IgM, and IgG to mean real-time RT-PCR, Dengue NS1 Ag STRIP, and capture IgM and IgG ELISA, respectively.

Statistical analysis

Pearson chi-square and Fisher exact test were used for categorical variables. Continuous variables between groups were compared using the Wilcoxon rank-sum test. The logistic regression was performed to identify independent determinants. The sensitivity of diagnostics was calculated as [(positive cases tested)/(confirmed cases tested)] $\times$ 100%. All statistical analyses were performed using JMP software, version 9.0.0 (SAS Institute Inc. Cary, NC, USA). Significance was assigned at $p < 0.05$ for all parameters and all analyses were two-tailed.

Results

Characteristics of the study population

During the study period, 888 suspected dengue cases were notified and 471 people had laboratory-confirmed dengue at KMUH. Among these, 442 (93.84%) were adults, and 392 patients were tested with all three diagnostic methods within 9 days postonset (DPO) of illness (day 0 to day 8) and were enrolled for analysis. From years 2008 to 2011, the numbers of cases in each year were 15, 48, 171, and 158, and the dominant serotypes (DENV) were DENV1, DENV3, DENV3, and DENV2, respectively (Fig. 1A). The number of dengue patients peaked in the 50- to 54-year age group (13.52%), whereas the elderly (age 65 years and older) accounted for 16.33% of the total cases (Fig. 1B). The median age was 48 years (quartile 32—58.75), and females comprised 55.36% of all cases (Table 1). There was no difference in age, sex, or incidence of underlying diseases by stage of illness (0—3 days and 4—8 days). DENV2 and DENV3 were predominant serotypes during the study period

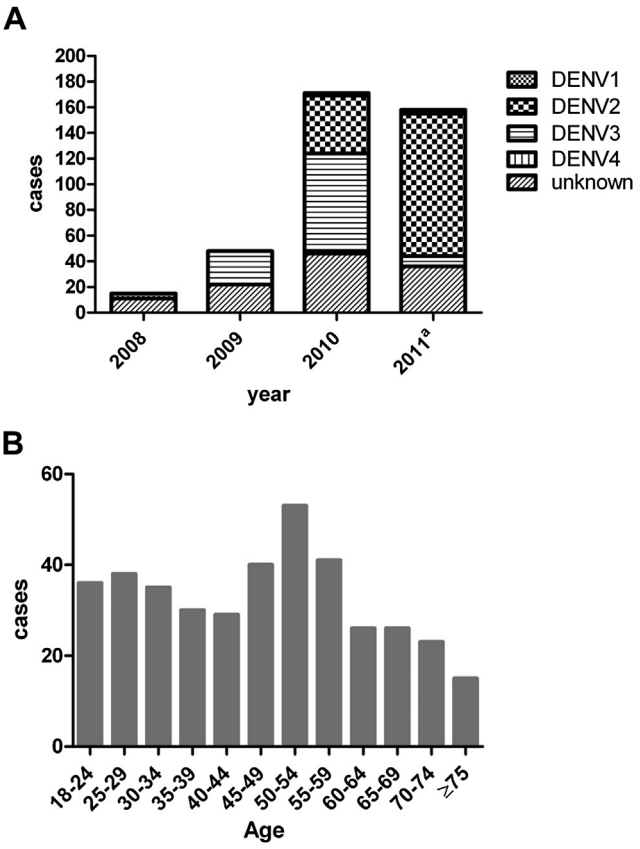

Figure 1. (A) Serotype distribution during 2008 to 2011. (B) Age distribution of dengue cases. [a]One case was notified in January 2012.

based on PCR results, but patients with unknown serotype accounted for 29.34% of cases (Table 1).

Sensitivity of diagnostics

The overall sensitivities of PCR, NS1, and capture IgM/IgG ELISA were 71.94%, 68.37%, and 40.05%, respectively. The capture IgM/IgG ELISA had higher sensitivity at the late stage (10.05% vs. 74.32%, $p < 0.0001$) (Table 2). The PCR and NS1 double positive rate was 53.06% and the difference between the early and late stages was significant (68.9% vs. 34.97%, $p < 0.0001$) (Table 2).

Among the three combinations of any two diagnostic methods, the combination of PCR and capture IgM/IgG ELISA had the best sensitivity at both stages. The combination of PCR and NS1 had a sensitivity of 99.52% (only one case missed) at the early stage; however, the sensitivity dropped to 73.22% at the late stage ($p < 0.0001$). In contrast, the sensitivity was better at the late stage (96.17%) than the early stage (78.95%) when NS1 and capture IgM/IgG ELISA were combined to make a diagnosis ($p < 0.0001$) (Table 2). The sensitivity of these diagnostic tests and any two in combination by illness day are shown in Fig. 2.

Role of PCR

In approximately 13% of patients, diagnosis was made by PCR solely and most of them were at the early stage (PCR+/

Table 1 Comparison of demographic characteristics and dengue serotypes of 392 patients in early and late stage of dengue infection

Variable	Stages			Total cases (n = 392) n (%)
	Days 0–3 (n = 209) n (%)	Days 4–8 (n = 183) n (%)	p	
Median age[a]	48 (32–59.5)	48 (33–58)	0.9309	48 (32–58.75)
Sex				
Male	95 (45.45)	80 (43.72)	0.7297	175 (44.64)
Female	114 (54.55)	103 (56.28)		217 (55.36)
Diabetes mellitus	33 (15.79)	32 (17.49)	0.6522	65 (16.58)
Hypertension	40 (19.14)	38 (20.77)	0.6874	78 (19.90)
Chronic kidney disease[b]	13 (6.25)	6 (3.28)	0.1727	19 (4.86)
Hepatitis B and/or C	28 (13.40)	15 (8.20)	0.1002	43 (10.97)
Serotype				
DENV1	5	4		9 (2.30)
DENV2	117	39		156 (39.80)
DENV3	73	38		111 (28.32)
DENV4	0	1		1 (0.26)
Unknown	14	101		115 (29.34)

[a] Quartile in parentheses.
[b] At the early stage, one patient did not have renal function data.
DENV = dengue virus.

serology−/NS1−, early stage vs. late stage: 21.05% vs. 3.83%, $p < 0.0001$) (Table 2). Age older than 65 years, hepatitis B/C, and early stage (days 0–3) were factors associated with isolated PCR positivity in univariate analysis (p = 0.0082, 0.0385, and < 0.0001, respectively) (Table 3). Age older than 65 years and sample collected at the early stage were independent factors in multivariate analysis (adjusted odds ratio [OR]: 2.421, 6.407, respectively) (Table 3).

Role of NS1

Without NS1, we may miss the diagnosis in approximately 4% of patients at both early and late stages (PCR−/serology−/NS1+, 4.31% vs. 4.37%, p = 0.9747). A second sample would be needed for capture IgM/IgG ELISA for approximately one-tenth of patients (10.97%) to make a definite diagnosis, particularly when patients presented

Table 2 Sensitivities of diagnostics and laboratory results of confirmed cases, the results were compared by early and late stage

Variable	Stages			Total cases (n = 392) n (%)
	Days 0–3 (n = 209) n (%)	Days 4–8 (n = 183) n (%)	p	
PCR sensitivity	196 (93.78)	86 (46.99)	<0.0001	282 (71.94)
NS1 sensitivity	156 (74.64)	112 (61.2)	0.0043	268 (68.37)
IgM/IgG (+)	21 (10.05)	136 (74.32)	<0.0001	157 (40.05)
Both RT-PCR and NS1 positive	144 (68.9)	64 (34.97)	<0.0001	208 (53.06)
PCR and/or NS1 positive	208 (99.52)	134 (73.22)	<0.0001[a]	342 (87.24)
PCR and/or IgM/IgG positive	200 (95.69)	175 (95.63)	0.9747	375 (95.66)
NS1 and/or IgM positive	158 (75.6)	165 (90.13)	0.0002	323 (82.40)
NS1 and/or IgM/IgG positive	165 (78.95)	176 (96.17)	<0.0001	341 (86.99)
PCR (+), IgM/IgG (+), NS1 (+)	9 (4.31)	32 (17.49)	<0.0001	41 (10.46)
PCR (+), IgM/IgG (−), NS1 (+)	135 (64.59)	32 (17.49)	<0.0001	167 (42.60)
PCR (+), IgM/IgG (+), NS1 (−)	8 (3.83)	15 (8.2)	0.0663	23 (5.87)
PCR (+), IgM/IgG (−), NS1 (−)	44 (21.05)	7 (3.83)	<0.0001	51 (13.01)
PCR (−), IgM/IgG (+), NS1 (+)	3 (1.44)	40 (21.86)	<0.0001[a]	43 (10.97)
PCR (−), IgM/IgG (−), NS1 (+)	9 (4.31)	8 (4.37)	0.9747	17 (4.34)
PCR (−), IgM/IgG (+), NS1 (−)	1 (0.48)	49 (26.78)	<0.0001[a]	50 (12.76)

[a] Fisher exact test.
IgG = immunoglobulin G; IgM = immunoglobulin M; NS1 = Dengue NS1 Ag STRIP; PCR = polymerase chain reaction; RT-PCR = reverse transcriptase-polymerase chain reaction.

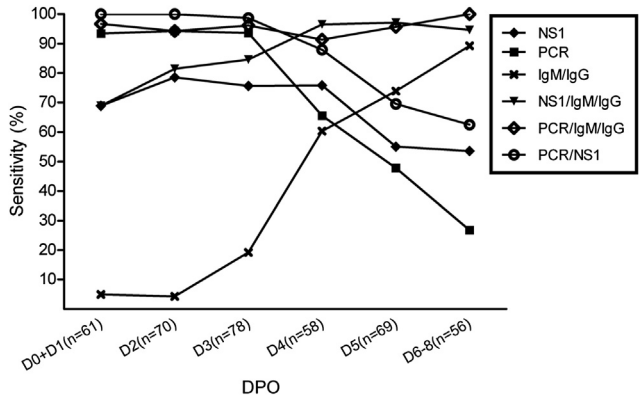

Figure 2. Sensitivities of Dengue NS1 Ag STRIP (NS1), polymerase chain reaction (PCR), and immunoglobulin M/immunoglobulin G (IgM/IgG) and their combination. DPO = days postonset.

at the late stage (PCR−/serology+/NS1+, 1.44 vs. 21.86%, $p < 0.0001$) (Table 2).

Sensitivity of NS1 related to serotypes

The sensitivity of NS1 was different between DENV2 and DENV3 significantly (65.38% vs. 88.78%, $p = 0.0008$). When we compared the NS1 sensitivity between DENV2 and DENV3 by days of illness, the difference was only significant on day 2 (65.71% vs. 89.29%, $p = 0.0385$) and on day 3 was borderline significant (64.1% vs. 87.1%, $p = 0.0522$) (Fig. 3).

Sensitivity of NS1 related to IgM/IgG status and PCR results

There was a significant difference in NS1 sensitivity between the IgG-positive and -negative patients (28.57% vs. 75.99%, $p < 0.0001$). This difference was also observed in DENV IgM status (IgM+ vs. IgM−: 58.65% vs. 73.36%, $p = 0.003$). However, when we added the effect of DENV IgG, the effect of DENV IgM on the NS1 sensitivity reduction disappeared (IgM+/IgG− vs. IgM−/IgG−: 70.21% vs. 78.30%, $p = 0.1209$). The sensitivity of NS1 was lower in patients with PCR-negative results than in those with positive results (54.55% vs. 73.76%, $p = 0.0005$).

Factors influencing the sensitivity of NS1

The univariate analyses for factors influencing the sensitivity of NS1 revealed that older age (55 years and older vs. 18−34 years old, OR: 2.23 [95% CI: 1.43−3.48], $p = 0.0004$), delayed detection (days 5−8 vs. days 0−4, OR: 2.50 [95% CI: 1.60−3.92], $p < 0.0001$), DENV IgG seropositivity (OR: 7.91 [95% CI: 4.40−14.76], $p < 0.0001$), and patients with diabetes mellitus (OR: 1.82 [95% CI: 1.05−3.14], $p = 0.0313$) or hypertension (OR: 2.35 [95% CI: 1.41−3.92], $p = 0.001$) are more likely to associate with negative results. In multivariate analysis, delayed detection (days 5−8 vs. days 0−4, adjusted OR: 1.93 [95% CI: 1.16−3.20], $p = 0.0105$) and DENV IgG seropositivity (adjusted OR: 6.07 [95% CI: 3.24−11.72], $p < 0.0001$) were independently associated with negative NS1 results in dengue patients (Table 4).

Discussion

Our results reveal NS1 to be a useful tool for early dengue diagnosis. For patients who are DENV IgG seropositive and seekingtreatment late (DPO > 4), the results of NS1 need to be judged carefully. The results are more likely to be negative in these patients despite dengue virus infection.

In the current study, the sensitivity of NS1 was 68.37%, which was lower than in a previous study in Taiwan that used the test for imported case detection.[8] In that study, most of the samples were taken early (DPO < 5) (19 of 22, 86.36% vs. 68.11% in the current study), and the DENV2 percentage was lower than ours (3 of 22, 13.64% vs. 39.80%). In previous reports, the sensitivity and specificity of NS1 were between 61.6-90.4% and 94.4-100%, respectively.[9] Unlike other reports using stored sera or plasma to evaluate the sensitivity of diagnostics,[7,9,13−18] samples in the current study had been tested within 24 hours after being collected for outbreak surveillance. We believe our results better reflect real-world practice.

Osorio et al[9] found the factors influencing the reduced sensitivity of NS1-based diagnostic tools included samples

Table 3 Factors influencing PCR positivity, with negative results in both NS1 and capture IgM/IgG ELISA ($n = 392$)

Variable	Univariate analysis				Multivariate analysis			
	Crude odds ratios	Lower 95% CI	Upper 95% CI	*p*	Adjusted odds ratios[b]	Lower 95% CI	Upper 95% CI	*p*
Age 65 y and older	2.483	1.238	4.805	0.0082	2.421	1.168	4.875	0.0147
Diabetes mellitus	1.091	0.475	2.275	0.8264				
Hypertension	1.453	0.709	2.821	0.2856				
Chronic kidney disease[a]	1.844	0.509	5.336	0.2947				
Hepatitis B and/or C	2.276	1.001	4.825	0.0385	2.111	0.897	4.678	0.0735
Sample in early stage	6.705	3.122	16.667	<0.0001	6.407	2.960	16.017	<0.0001

[a] One patient did not have renal function data.
[b] Adjustment for age, sex, hepatitis B and/or C, and stage in multivariate logistic regression.
CI = confidence interval; ELISA = enzyme-linked immunosorbent assay; IgM/IgG = immunoglobulin M/immunoglobulin G; NS1 = Dengue NS1 Ag STRIP.

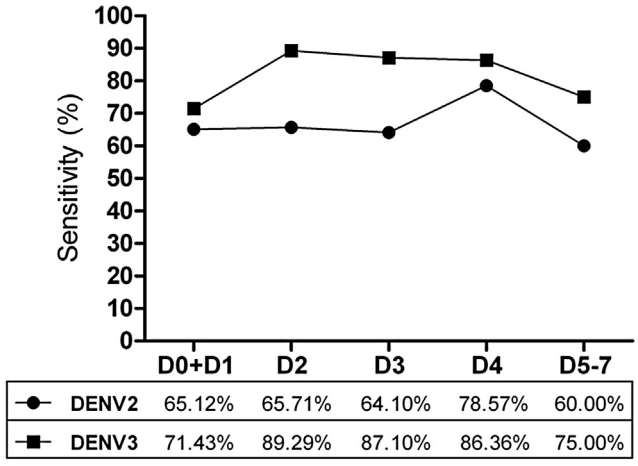

Figure 3. Sensitivity of Dengue NS1 Ag STRIP by dengue virus (DENV) 2 and 3.

taken late after illness onset (> 3 days), patients with secondary infections, and those with DENV2 and 4. We had similar findings, but the influence of duration of illness at the time of sample collection was delayed to the fifth day. In our study, the sensitivity on the fifth day (DPO 4) was still higher (75.89%) than average, and in the multivariate logistic regression analysis, delayed detection (day 5—day 8) was an independent factor for negative NS1 results. During our study period, the predominant serotypes were DENV2 and 3. There was a significant difference in NS1 sensitivity between these two serotypes. The IgM/IgG ratio was not provided on the CDC diagnostic sheet; therefore, we could not distinguish primary from secondary infection. However, a patient with secondary infection is more likely to have DENV IgG detected during acute dengue virus infection. DENV IgG seropositivity was an independent factor for a negative NS1 result in patients infected with dengue virus in our study.

To the best of our knowledge, this is the first study to investigate the influence of underlying diseases and age on the sensitivity of commercial NS1 antigen tests. In univariate analyses, older patients, patients with diabetes mellitus, and those with hypertension were more likely to test negative, but these factors were not independent in multivariate analysis. We suggest that PCR/capture IgM/IgG ELISA for dengue diagnosis would be beneficial in these patients.

The existence of DENV IgG appeared to decrease the sensitivity of NS1. A hypothesis has been proposed that the kinetics between NS1-specific IgG and DENV IgG are similar.[7] The reduced NS1 sensitivity is due to NS1 antigen binding to NS1-specific IgG to form immune complexes.[7]The existence of DENV IgM also had a similar effect on the sensitivity of NS1 in the current study. However, there was no difference in NS1 sensitivity between IgM+/IgG− and IgM−/IgG− patients. One report found this reduction of sensitivity influenced by DENV IgM was observed in the commercial NS1 ELISA test (Platelia, Bio-Rad) but not in the NS1 ICT.[14] Another study found the NS1 sensitivity in IgM+/IgG− patients was significantly higher than in IgM−/IgG − patients in another NS1 ICT test (SD Dengue Duo, Standard Diagnostics).[19] Further study designed to explore the real effect of DENV IgM is needed.

In PCR-positive patients, the sensitivity of NS1 was significantly higher than in PCR-negative patients. Other studies found NS1-positive patients had higher viremia levels,[14,19] which explained the relationship between NS1 sensitivity and PCR results.

With the assistance of NS1, approximately 4% more cases were detected with single samples at either the early or

Table 4 Logistic regression to determine the risk of Dengue NS1 Ag STRIP being negative (n = 392)

Variable	Univariate analysis				Multivariate analysis			
	Crude odds ratios	Lower 95% CI	Upper 95% CI	p	Adjusted odds ratios[b]	Lower 95% CI	Upper 95% CI	p
Age								
Age group 1 (18—34 y)	1							
Age group 2 (35—54 y)	0.815	0.522	1.263	0.3632	1.296	0.698	2.445	0.4159
Age group 3 (≥55 y)	2.230	1.432	3.479	0.0004	1.973	0.977	4.020	0.0588
Detected phase								
Early phase (days 0—4)	1							
Delayed phase (days 5—8)	2.502	1.600	3.923	<0.0001	1.932	1.163	3.199	0.0105
IgG seropositive	7.911	4.401	14.764	<0.0001	6.069	3.243	11.716	<0.0001
Underlying diseases								
Diabetes mellitus	1.821	1.049	3.136	0.0313	0.863	0.413	1.761	0.6907
Hypertension	2.355	1.414	3.919	0.001	1.487	0.722	3.040	0.2778
Chronic kidney disease[a]	2.011	0.780	5.120	0.1396	1.576	0.520	4.627	0.4099
Hepatitis B and/or C	1.479	0.759	2.819	0.2398	1.624	0.771	3.341	0.1925

[a] One patient did not have renal function data.
[b] Adjustment for age, sex, detected phase, DENV IgG seropositivity, and underlying disease including diabetes mellitus, hypertension, chronic kidney disease and hepatitis B and/or C in multivariate logistic regression.
CI = confidence interval; DENV = dengue virus; IgG = immunoglobulin G.

late stage. At the late stage, the diagnosis of dengue can be made in more than 20% of the patients without the need for a second sample in the convalescent phase. This result showed NS1 can help us implement environment and vector control early in an outbreak. The NS1 detected more than 50% of patients with PCR-negative samples, and this rate was higher than in a previous report (37%).[20] This may be due to more of our patients having the blood test at the late stage than the previous report (46.68% vs. 34.3%) and to the sensitivity of PCR dropping earlier than that of NS1.

The overall sensitivity of PCR was 71.94%, and the sensitivity started to decrease after day 3. This finding is similar to that of a previous study.[15] We would have missed the diagnosis in one-fifth (21.05%) of patients visiting within the first 4 days of illness if PCR was not used. Using PCR, we can detect dengue in more patients at the early stage, and control measures can be taken earlier. We also found that in the elderly (age 65 years and older) and patients presenting at the early stage, a diagnosis might be more likely with PCR.

In a previous study, the combination of Platelia Dengue NS1 Ag (Bio-Rad ELISA) and IgM (MAC-ELISA) detected 82% of cases in samples collected in the first 4 days of illness onset.[21] Our finding (NS1 and/or IgM positive: 82.4%) was consistent with this observation. When IgG was added, the sensitivity increased to 87% (NS1 and/or IgM and/or IgG positive). Of note, at the early stage the sensitivity of either combination (NS1/IgM and NS1/IgM/IgG) was below 80%, which means we may miss the diagnosis in more than 20% of patients if we use these two diagnostic combinations at the early stage.

The combination of PCR and NS1 for a single sample could detect most cases in the first 4 days (99.52%), but the sensitivity decreased at the late stage. The best strategy would be to combine PCR and capture IgM/IgG ELISA because the sensitivity was higher than 95% whether at the early or late stage. Because of the cost and the requirements for laboratory facilities and trained personnel, the PCR-based diagnostic strategy may not be a good choice in resource-limited regions.

There are some limitations in our study. First, we did not use the clinical symptoms and signs in our diagnostics analysis, which would be important especially for a resource-poor area. Although low specificity, the sensitivity of the World Health Organization (WHO) 1997 classification to define illness is 95.4% and approximately 80% when WHO 2009 classification is applied.[15] Second, in our study, the serotype was determined only by PCR and there were 29.34% of the patients having unknown serotype; therefore, we could not analyze the exact effect of serotypes on the sensitivity of NS1. Third, we used DENV IgG detection to represent secondary infection and this could not give us the real proportions of primary and secondary infections. This is more similar in a real-world scenario, because clinicians read capture IgM/IgG ELISA results without knowing whether they arise from a primary or secondary infection. Fourth, we could not calculate the specificity of NS1. When NS1 was positive, the second sample would be not requested. The possibility of false-positive results with NS1 might exist. According to a previous report,[9] the specificity of NS1 is approximately 100% and in our study, patients notified were clinically suspected dengue cases. We believe the false-positive rate is extremely low.

In conclusion, the Dengue NS1 Ag STRIP played an important role in the early diagnosis of acute dengue infection. However, it might be used with caution in patients with secondary DENV infection or serum samples being taken after the fifth day of illness. The sensitivity of Dengue NS1 Ag STRIP would be compromised under these circumstances.

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