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Original Article

Early diagnostic value of dengue virus NS1 antigen and nucleic acid testing

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Abstract: Objective: To analyze laboratory test results and clinical characteristics of dengue patients, providing a reference for early diagnosis and treatment. Methods: A retrospective analysis was conducted on the serological and pathogenetic results of 118 suspected dengue cases from 2017 to 2024. The study evaluated testing methods and examined patients' medical histories. Participants were grouped by age into three categories: youth (≤43 years, n=39), middle-aged (44-63 years, n=25), and elderly (>63 years, n=16). Clinical features and lab outcomes were documented and compared. Results: Among the 118 suspected dengue cases, 80 serum specimens tested positive for immunoglobulin M (IgM), NS1 antigen, or viral nucleic acid in single or multiple tests. The sensitivity and specificity of the NS1 assay (colloidal gold) compared to real-time PCR (RT-PCR) were 83.78% (62/74) and 33.33% (2/6), respectively. ROC analysis showed AUCs of 0.931 for NS1, 0.963 for RT-PCR, and 0.894 for NS1 + RT-PCR. Both NS1 antigen and nucleic acid detection rates were high during early (1-3 days) and middle (4-14 days) infection stages. Clinical symptom analysis revealed significant differences between the three groups in fatigue, vomiting, abdominal pain, and diarrhea (all P<0.05). Youth had less fatigue but more vomiting and abdominal pain than the middle-aged and elderly (all P<0.05). The middle-aged group experienced more diarrhea (P<0.05). Laboratory results showed significant differences in platelet counts, with the highest platelet counts in the middle-aged group and the lowest in the elderly (P<0.05). Conclusion: NS1 antigen and dengue virus nucleic acid are highly detectable during early to mid-infection stages. Most young and middle-aged patients show mild symptoms, while the elderly tend to have more severe forms.

Keywords: Dengue fever, clinical features, viral NS1 antigen detection (colloidal gold method), RT-PCR assay

Introduction

Dengue fever is the most widespread arboviral disease worldwide, primarily transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes [1]. There are four dengue virus (DENV) serotypes: DENV1, DENV2, DENV3, and DENV4. The main clinical manifestations include fever, headache, generalized muscle and joint pain, marked fatigue, rash, lymphadenopathy, and leukopenia [2, 3]. Dengue occurs mainly in tropical and subtropical regions, particularly in over 100 countries across Southeast Asia, the Pacific Islands, and the Caribbean [4].

With climate warming, increased international travel, and expanding trade and tourism, dengue fever is spreading more widely [5, 6], evolving from a sporadic illness into a major public health threat [7, 8]. It is estimated that approximately 390 million people are infected annually, of which 96 million develop symptomatic disease [9]. Dengue fever caused by DENV remains a significant global health challenge. Despite advances in understanding its pathogenesis, effective prognostic tools and targeted treatments are lacking due to its complex clinical presentation and the limitations of current diagnostic methods [10]. Currently, no widely applicable preventive vaccine or effective antiviral therapy is available [11].

The atypical and variable symptoms of dengue frequently result in missed or delayed diagno-

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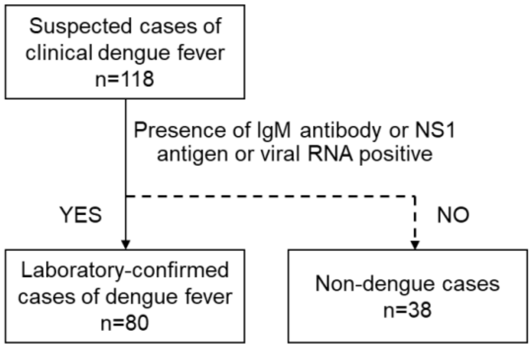

Figure 1. Laboratory diagnostic process. Note: IgM: immunoglobulin M; NS1: Non-structural Protein 1.

sis, and its clinical and epidemiological features differ across regions [12-14]. In this study, we retrospectively analyzed immunoglobulin M (IgM), NS1, and nucleic acid test results from 118 suspected cases and compared the clinical features of 80 confirmed cases across different age groups to provide data that may inform improved strategies for dengue prevention and control.

Materials and methods

Study design and patient enrollment

This retrospective study included 118 patients with suspected dengue infection, confirmed by laboratory testing. Electronic medical records from the First People's Hospital of Chun'an County, covering the period from January 2017 to December 2024, were reviewed.

Inclusion criteria: (1) Diagnosis consistent with the Diagnostic Criteria for Dengue Fever (WS216-2008) and the Guidelines for the Diagnosis and Treatment of Dengue Fever (2nd ed.) [15], with the first day of fever onset defined as day 1. (2) Availability of complete clinical data.

Exclusion criteria: (1) Severe hepatic or renal insufficiency; (2) Malignant tumors.

Based on these criteria, 118 eligible patients were enrolled, including 63 males and 55 females. The study protocol was approved by the Ethics Committee of the First People's Hospital of Chun'an County (No. 2025020626).

Data collection and grouping

Diagnostic testing: Laboratory test records, including methods and results from serum samples, were collected to compare the diagnostic performance of different techniques. The diagnostic process is illustrated in **Figure 1**. IgM and NS1 antigens were detected by colloidal gold immunochromatographic assay (GICA), and dengue virus nucleic acids were detected by real-time RT-PCR.

Patient grouping and clinical data: Confirmed patients were stratified into three age groups: young (≤43 years, n=39), middle-aged (44-63 years, n=25), and elderly (>63 years, n=16). General information was recorded, including age, sex, onset time, and changes in clinical symptoms and signs. Laboratory findings included routine blood tests, coagulation profiles, and biochemical examinations.

Statistical methods

All data were analyzed using SPSS 27.0 software. Continuous variables were expressed as mean ± standard deviation (x±sd), and compared among groups using one-way ANOVA, with LSD post hoc tests for pairwise comparisons. Categorical variables were expressed as n (%) and compared using the χ^2 test or Fisher's exact test. The diagnostic accuracy of different methods was assessed by ROC curve analysis. A two-sided $P<0.05$ was considered statistically significant.

Results

Laboratory testing overview

Of the 118 suspected dengue cases, 80 serum specimens tested positive for IgM antibody, NS1 antigen, or viral nucleic acid, either individually or in combination, yielding a positivity rate of 67.80% (80/118). Among the 80 positive samples, the detection rates were 42.50% (34/80) for IgM antibody, 86.25% (69/80) for NS1 antigen, and 92.25% (74/80) for RT-PCR. Based on epidemiological history and clinical presentation, 80 cases were confirmed as dengue fever (**Table 1**).

IgM antibody detection was low in the early stage (15.63%, 5/32) but increased to 83.33% (5/6) after 15 days. NS1 antigen detection was highest within 1-3 days of illness (90.63%, 29/32), decreased to 83.33% (5/6) after 15 days, and further declined to 66.67% (4/6) thereafter. Viral nucleic acid detection was 96.88% (31/32) within 1-3 days and declined to 84.62% (11/13) during days 8-14. Combined

Table 1. NS1 antigen and nucleic acid test results of 80 laboratory-diagnosed cases of dengue fever with different disease stages

Days of illness (d)	n	IgM-positive [n (%)]	NS1-positive [n (%)]	Nucleic acid positive [n (%)]	NS1 and nucleic acid positive [n (%)]
1-3	32	5 (15.63)	29 (90.63)	31 (96.88)	28 (87.50)
4-7	29	14 (48.28)	24 (82.76)	25 (86.21)	23 (79.31)
8-14	13	10 (76.92)	10 (76.92)	11 (84.62)	8 (61.54)
≥15	6	5 (83.33)	4 (66.67)	5 (83.33)	3 (50.00)
Total	80	34 (42.50)	69 (86.25)	74 (92.50)	62 (77.50)

Note: IgM: immunoglobulin M; NS1: Non-structural Protein 1.

Table 2. Nucleic acid results of 80 cases of dengue laboratory diagnosis GICA test for NS1 antigen and RT-PCR test for nucleic acid

Ns1 Antigen	Nucleic Acid		Total
	Positive	Negative	
Positive	62	3	65
Negative	12	3	15
Total	74	6	80

Note: NS1: Non-structural Protein 1; GICA, gold immunochromatographic assay.

Table 3. ROC curve analysis of NS1 antigen, nucleic acid and co-diagnosis

	AUC	S.E	*P*	95% CI
NS1 antigen	0.931	0.024	0.000	0.885, 0.978
RT-PCR	0.963	0.018	0.000	0.928, 0.997
NS1 antigen + RT-PCR	0.894	0.029	0.000	0.836, 0.951

Note: RT-PCR: real-time PCR; NS1: Non-structural Protein 1; GICA, gold immunochromatographic assay.

detection of NS1 antigen and viral nucleic acid was positive in 87.50% (28/32) within 1-3 days, but dropped to 50.00% (3/6) after 15 days.

Results of GICA detection of NS1 antigen and RT-PCR detection of nucleic acid

Among the 80 confirmed serum samples, 62 were positive by both GICA for NS1 antigen and RT-PCR for nucleic acid, while 3 were negative by both. The sensitivity and specificity of GICA relative to RT-PCR were 83.78% (62/74) and 50.00% (3/6), respectively. A significant difference was observed between the two methods (χ^2=4.158, P=0.041), with a kappa value of 0.200 (**Table 2**).

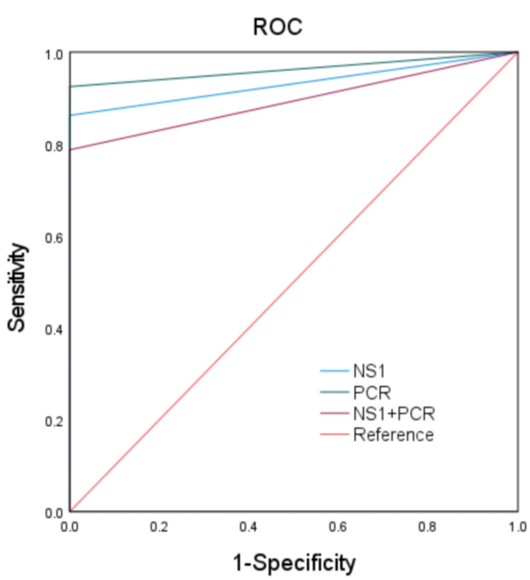

Figure 2. ROC curves for NS1 antigen, nucleic acid and co-diagnosis. Note: NS1: Non-structural Protein 1; RT-PCR: real-time PCR.

Diagnostic efficacy analysis

ROC curve analysis compared the diagnostic efficacy of NS1 antigen and nucleic acid testing. As shown in **Table 3** and **Figure 2**, the AUCs were 0.931 for NS1 antigen detection, 0.963 for nucleic acid detection, and 0.894 for combined positivity by both methods.

Comparison by illness stage

No significant difference was observed between NS1 antigen detection (GICA) and nucleic acid detection (RT-PCR) at different stages of illness (P>0.05) (**Table 4**).

Epidemiological analysis

The peak incidence of the 80 confirmed cases between 2017 and 2024 occurred from August

Table 4. Comparison of NS1 antigen detection by GICA and nucleic acid results by RT-PCR in 80 laboratory-diagnosed cases of dengue fever

	1-3 d			4-7 d			8-14 d			≥15 d		
	+	-		+	-		+	-		+	-	
NS1 antigen	29	3		26	3		10	3		4	2	
RT-PCR	31	1		27	2		11	2		5	1	
P			1.067			0.219			0.248			0.444
x^2			0.302			0.640			0.619			0.505

Note: NS1: Non-structural Protein 1; RT-PCR: real-time PCR.

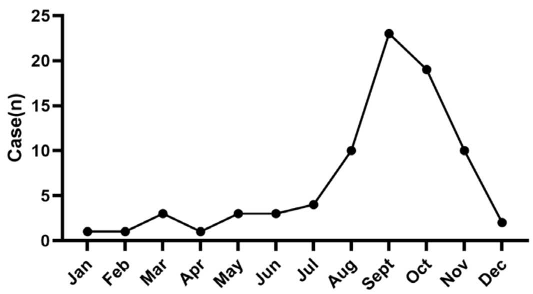

Figure 3. Time of onset for dengue patients in 2017-2024.

to November (**Figure 3**). There were 41 males (51.25%) and 39 females (48.75%), with a male-to-female ratio of 1:0.95. The age of onset ranged from 12 to 77 years (mean 45.22±17.08). The distribution by age group was 48.75% (39/80) youth, 31.25% (25/80) middle-aged, and 20.00% (16/80) elderly.

Clinical signs and symptoms

Analysis of clinical manifestations across age groups (**Table 5**) revealed significant differences in fatigue, vomiting, abdominal pain, and diarrhea (P<0.05).

Fatigue: The youth group had significantly less fatigue than the middle-aged group (P<0.05), but there was no difference compared with the elderly (P>0.05).

Vomiting and abdominal pain: The incidence was significantly higher in the youth group than that in the elderly (P<0.05); no difference from the middle-aged group (P>0.05).

Diarrhea: More common in the middle-aged group than in the youth group (P<0.05); no difference compared with the elderly group (P>0.05). No significant difference was found between the youth and elderly groups (P>0.05).

Mild vs. severe clinical manifestations

Symptoms were classified as mild or severe according to clinical severity [16] (**Table 6**). The five most common mild symptoms were fever, chills, malaise, headache, and musculoskeletal pain.

In the youth group, malaise was less frequent (P<0.05), while vomiting and abdominal pain were more frequent (P<0.05) compared with the other groups.

In the middle-aged group, diarrhea was significantly more frequent than that in the other two groups (P<0.05).

Severe symptoms were relatively uncommon.

In the youth group, subcutaneous petechiae/hematoma was most frequent (12.8%), with conjunctival congestion and chest tightness/palpitations also observed.

In the middle-aged group, chest tightness/palpitations were most frequent (12.0%), followed by conjunctival hemorrhage, subcutaneous petechiae/hematoma, and dyspnea.

In the elderly group, all severe symptoms recorded in the study were present, with an incidence rate of 6.25% for each (**Table 7**).

Laboratory findings

Comparison of laboratory results across age groups (**Table 8**) revealed a significant difference in platelet counts (P<0.05): highest in the middle-aged group and lowest in the elderly group. No significant differences were observed for other laboratory parameters (P>0.05).

Discussion

Dengue fever is mainly prevalent in tropical and subtropical regions, primarily transmitted

Table 5. Clinical signs and symptoms of dengue fever in patients of different ages [n (%)]

Clinical Symptoms	Youth Group (n=39)	Middle-age Group (n=25)	Senior Group (n=16)	*t*	*P*
Fever	37 (94.87)	24 (96.00)	15 (93.75)	0.833	0.659
Chills/frigidity	31 (79.49)	20 (80.00)	14 (87.50)	0.515	0.773
Fatigue	28 (71.79)	23 (92.00)	15 (93.75)	6.061	0.048
Headache	36 (92.31)	24 (96.00)	15 (93.75)	0.354	0.838
Bone/muscle pain	36 (92.31)	24 (96.00)	15 (93.75)	0.354	0.838
Flushing of the face/chest	13 (33.33)	7 (28.00)	5 (31.25)	0.202	0.904
Skin rash	11 (28.21)	5 (20.00)	3 (18.75)	0.842	0.656
Pruritus	6 (15.38)	4 (16.00)	2 (12.50)	0.103	0.950
Sore throat/cough	9 (23.08)	5 (20.00)	5 (31.25)	0.701	0.704
Nauseate	11 (28.21)	4 (16.00)	2 (12.50)	2.271	0.321
Vomit	11 (28.21)	2 (8.00)	1 (6.25)	6.061	0.048
Abdominal pain	13 (33.33)	3 (12.00)	1 (6.25)	6.832	0.033
Constipation	2 (5.13)	7 (28.00)	2 (12.50)	6.122	0.047
Retro-orbital pain	4 (10.26)	0 (0.00)	1 (6.25)	2.735	0.255
Lumbago	1 (0.00)	3 (12.00)	1 (6.25)	2.315	0.314
Lymph gland enlargement	0 (0.00)	0 (0.00)	1 (6.25)	4.051	0.132
Disorders of consciousness	0 (0.00)	0 (0.00)	1 (6.25)	4.051	0.132
Conjunctival hyperemia	3 (7.69)	1 (4.00)	1 (6.25)	0.354	0.838
Chest tightness/palpitations	1 (2.56)	3 (12.00)	1 (6.25)	2.735	0.255
Subcutaneous petechiae/hematoma	5 (12.82)	1 (4.00)	1 (6.25)	1.641	0.440
Cavernous hemorrhage	0 (0.00)	0 (0.00)	1 (6.25)	4.051	0.132
Hepatosplenomegaly	0 (0.00)	0 (0.00)	1 (6.25)	4.051	0.132
Shortness	0 (0.00)	1 (4.00)	1 (6.25)	2.154	0.341
Shock	0 (0.00)	0 (0.00)	1 (6.25)	4.051	0.132

by Aedes aegypti mosquitoes. Consequently, its incidence shows seasonal variation [17], typically occurring between May and December, with a peak from July to September [18]. Dengue is widespread in endemic areas, with most cases reported in adults [19]. The incubation period is generally 3-14 days, with a mean of 4-7 days [20]. Following an outbreak, atypical clinical symptoms and inapparent infections complicate differential diagnosis from other infectious diseases [21]. Therefore, rapid and accurate multi-indicator laboratory testing is crucial for early detection, enabling timely isolation of infection sources and prevention of further spread [22].

The kinetics of viral nucleic acid and NS1 antigen clearance and persistence after dengue virus infection represent a dynamic process [23]. The dengue virus genome is a positive-sense single-stranded RNA, which can be directly detected in serum by targeting viral RNA [24]. In this study, viral nucleic acid in the serum of 80 confirmed dengue cases was detected by fluorescent RT-PCR, with an overall positive rate of 92.50%. The positive rate reached 96.88% within 3 days of symptom onset, indicating high sensitivity and specificity of RT-PCR for early diagnosis, making it a preferred method for rapid detection.

NS1 protein is a highly conserved non-structural glycoprotein of the dengue virus [25]. High serum concentrations of NS1 antigen in the acute phase of both primary and secondary infections make it an ideal marker for early diagnosis [26, 27]. In this study, the overall positive rate of NS1 antigen was slightly lower than that of RT-PCR, but the difference was not statistically significant across illness stages, suggesting that both NS1 antigen and nucleic acid detection are suitable for laboratory screening. ROC analysis confirmed that RT-PCR had the highest diagnostic efficacy.

The age of onset among the 80 confirmed cases ranged from 12 to 77 years, with youth and middle-aged groups accounting for 80.00%

Table 6. Mild clinical manifestations of dengue fever in patients of different ages [n (%)]

Clinical Symptoms	Youth Group (n=39)	Middle-age Group (n=25)	Senior Group (n=16)	*t*	*P*
Fever	37 (94.87)	24 (96.00)	15 (93.75)	0.833	0.659
Chills/frigidity	31 (79.49)	20 (80.00)	14 (87.50)	0.515	0.773
Fatigue	28 (71.79)	23 (92.00)	15 (93.75)	6.061	0.048
Headache	36 (92.31)	24 (96.00)	15 (93.75)	0.354	0.838
Bone/muscle pain	36 (92.31)	24 (96.00)	15 (93.75)	0.354	0.838
Flushing of the face/chest	13 (33.33)	7 (28.00)	5 (31.25)	0.202	0.904
Skin rash	11 (28.21)	5 (20.00)	3 (18.75)	0.842	0.656
Pruritus	6 (15.38)	4 (16.00)	2 (12.50)	0.103	0.950
Sore throat/cough	9 (23.08)	5 (20.00)	5 (31.25)	0.701	0.704
Nauseate	11 (28.21)	4 (16.00)	2 (12.50)	2.271	0.321
Vomit	11 (28.21)	2 (8.00)	1 (6.25)	6.061	0.048
Abdominal pain	13 (33.33)	3 (12.00)	1 (6.25)	6.832	0.033
Constipation	2 (5.13)	7 (28.00)	2 (12.50)	6.122	0.047
Retro-orbital pain	4 (10.26)	0 (0.00)	1 (6.25)	2.735	0.255
Lumbago	1 (2.56)	3 (12.00)	1 (6.25)	2.315	0.314
Lymph gland enlargement	0 (0.00)	0 (0.00)	1 (6.25)	4.051	0.132

Table 7. Severe clinical manifestations of dengue fever in patients of different ages [n (%)]

Clinical Symptoms	Youth Group(n=39)	Middle-age Group (n=25)	Senior Group (n=16)	*t*	*P*
Disorders of consciousness	0 (0.00)	0 (0.00)	1 (6.25)	4.051	0.132
Conjunctival hyperemia	3 (7.69)	1 (4.00)	1 (6.25)	0.354	0.838
Chest tightness/palpitations	1 (2.56)	3 (12.00)	1 (6.25)	2.735	0.255
Subcutaneous petechiae/hematoma	5 (12.82)	1 (4.00)	1 (6.25)	1.641	0.440
Cavernous hemorrhage	0 (0.00)	0 (0.00)	1 (6.25)	4.051	0.132
Hepatosplenomegaly	0 (0.00)	0 (0.00)	1 (6.25)	4.051	0.132
Shortness	0 (0.00)	1 (4.00)	1 (6.25)	2.154	0.341
Shock	0 (0.00)	0 (0.00)	1 (6.25)	4.051	0.132

Table 8. Laboratory findings in patients with dengue fever of different ages

Laboratory parameters	Youth Group (n=39)	Middle-age Group (n=25)	Senior Group (n=16)	*F*	*P*
WBC ($\times 10^9$/L)	3.33±1.28	3.20±1.54	3.09±0.90	0.219	0.804
GRAN ($\times 10^9$/L)	1.74±0.51	1.62±0.52	1.80±0.43	0.668	0.516
LYM ($\times 10^9$/L)	0.91±0.24	0.90±0.21	0.80±0.19	1.492	0.231
PLT ($\times 10^9$/L)	130.69±10.94	136.20±15.35	122.69±16.96	4.726	0.012
Albumin (g/L)	38.72±12.03	39.14±8.73	38.16±9.33	0.043	0.958
ALT (U/L)	66.38±15.65	62.92±12.67	60.00±12.64	1.255	0.291
AST (U/L)	49.33±12.34	49.48±14.50	44.75±12.55	0.805	0.451
LDH (U/L)	245.79±28.27	245.24±25.20	242.94±24.46	0.066	0.936
CK (U/L)	202.03±16.14	193.60±20.57	200.88±24.79	1.499	0.230
K^+ (mmol/L)	3.05±0.53	2.95±0.46	2.98±0.45	0.295	0.745
Na^+ (mmol/L)	128.11±24.50	137.42±22.15	140.14±30.82	1.753	0.180
CRP (mg/L)	6.46±1.29	6.81±1.09	6.77±1.46	0.692	0.504

Note: WBC: white blood cell; GRAN: neutrophil; LYM: lymphocyte; PLT: blood platelet; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; CK: creatine kinase; CRP: C-reactive protein.

(64/80). This aligns with reports that dengue predominantly affects young and middle-aged adults aged 20-60 years (73.72-90.30%) [28, 29]. Symptom analysis revealed that fatigue was less common in the youth group than in middle-aged and elderly patients, whereas vomiting and abdominal pain were more prevalent. This may be explained by the inclusion of some minors in the youth group, who have different perceptions of fatigue and more fragile gastrointestinal function. The five most frequent mild symptoms were fever, chills, malaise, headache, and musculoskeletal pain, consistent with previous findings [30].

Severe manifestations were relatively uncommon. In the youth group, subcutaneous petechiae/hematoma was most frequent, with conjunctival congestion and chest tightness/palpitations also observed. In the middle-aged group, chest tightness/palpitations predominated, with conjunctival hemorrhage, petechiae/hematoma, and dyspnea also reported. In the elderly group, all severe symptoms included in this study were present, though the overall comorbidity rate remained low.

Laboratory testing showed reduced leukocyte and neutrophil counts in most patients, consistent with prior reports [31]. Other findings included hypoalbuminemia, and elevated alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, creatine kinase, and creatine kinase isoenzymes were also present, suggesting hepatic and myocardial injury, in line with previous studies [32]. Electrolyte disturbances, including hypokalemia and hyponatremia, were also observed.

This study has several limitations inherent to a retrospective design. Despite applying strict inclusion and exclusion criteria, selection bias cannot be fully excluded. Furthermore, the single-center nature and limited sample size restrict the generalizability of the findings. Future multicenter studies with larger cohorts, both domestic and international, are warranted to validate and expand upon these results.

In conclusion, both dengue virus NS1 antigen and nucleic acid testing demonstrated high detection rates and consistency during the early to mid-stage of infection (1-7 days), supporting their use in early diagnosis and screening of suspected cases. The age of onset was predominantly 24-63 years, with a nearly equal male-to-female ratio. The five most common mild symptoms were fever, chills, malaise, headache, and musculoskeletal pain, whereas severe manifestations were relatively infrequent. Thrombocytopenia varied across age groups, while leukopenia and neutropenia were common in most patients.

Disclosure of conflict of interest

None.

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References

[1] Xie Y, Jiao L and Sun Q. Dengue virus and lipid metabolism: unravelling the interplay for future therapeutic approaches. Emerg Microbes Infect 2025; 14: 2477647.

[2] Ansari A, Sachan S, Ahuja J, Venkadesan S, Nikam B, Kumar V, Jain S, Singh BP, Coshic P, Sikka K, Wig N, Sette A, Weiskopf D, Mohanty D, Soneja M and Gupta N. Distinct features of a peripheral T helper subset that drives the B cell response in dengue virus infection. Cell Rep 2025; 44: 115366.

[3] Lin H, Ngwe Tun MM, Zin YMW, Myint KT, Khine WK, Nwe KM, Thant AA, Shwe TT, Mar W, Aye KS, Aung KM, Takamatsu Y, Aung WW, Kyaw YY, Urano T, Morita K and Thu HM. Comparison of clinical and virological features in pediatric and adult dengue cases at Insein General Hospital during Myanmar's 2022 dengue season. Trop Med Health 2025; 53: 13.

[4] Li V, Mishra H, Ngai M, Crowley VM, Tran V, Painaga MSS, Gaite JY, Hamilton P, Conroy AL, Kain KC and Hawkes MT. Soluble tumour necrosis factor receptor 1 predicts hospitalization in children and young adults with dengue virus infection in the Philippines. Cytokine 2025; 190: 156911.

[5] Nasir M, Irfan J, Asif AB, Khan QU and Anwar H. Complexities of dengue fever: pathogenesis, clinical features and management strategies. Discoveries (Craiova) 2024; 12: e189.

[6] Santoso MS, Arguni E, Rana B, Adiniko ME, Denis D, Supriyati E, Indriani C, Trianty L, Ahmad RA, Noviyanti R and Sasmono RT. Sensitivity and specificity of an all-in-one cartridge-based dengue real-time reverse transcription-PCR for point-of-care detection and serotyping of Den-

gue virus in samples from Indonesian patients. Ann Lab Med 2025; 45: 503-508.
[7] Banu SS, Nahar K, Sultana N, Tony SR, Alam S, Rashed A, Karim Y, Hannan SB, Ghosh AK, Bhuiyan AH, Begum MN and Rahman M. Molecular epidemiology of circulating dengue serotypes in Dhaka, Bangladesh: 2023 outbreak. IJID Reg 2025; 14: 100597.
[8] Rufasto Goche KS, Lizarbe Castro MV, Lozano Zanelly GA, Lira Camargo WM, Ascayo Velasquez EY, Murillo Carrasco AG and Diaz-Obregón D. Epidemiological dynamics of dengue in Peru: temporal and spatial drivers between 2000 and 2022. PLoS One 2025; 20: e0319708.
[9] Liu X. Opportunities and challenges of mRNA technologies in development of dengue virus vaccine. Front Immunol 2025; 16: 1520968.
[10] Selvaraj AD, Ramaian Santhaseela A and Tamilmani E. Overview of dengue diagnostic limitations and potential strategies for improvement. Diagnosis (Berl) 2025; 12: 313-321.
[11] Rajpal A, Pannu AK, Behera A, Sharma N and Hanumanthappa MK. Utilization of local remedies among dengue patients admitted to the emergency department of a tertiary care center: an observational study. Indian J Crit Care Med 2025; 29: 148-150.
[12] Abbasi E. The impact of climate change on Aedes aegypti distribution and dengue fever prevalence in semi-arid regions: a case study of Tehran Province, Iran. Environ Res 2025; 275: 121441.
[13] de Almeida MT, Merighi DGS, Visnardi AB, Boneto Gonçalves CA, Amorim VMF, Ferrari ASA, de Souza AS and Guzzo CR. Latin America's dengue outbreak poses a global health threat. Viruses 2025; 17: 57.
[14] Anh DD, The NT, My TN, Linh LTK, Hoan NX, Kremsner PG, Toan NL, Song LH and Velavan TP. Epidemiology and genotype dynamics of dengue in hospitalized patients in northern vietnam between 2020 and 2022. Open Forum Infect Dis 2025; 12: ofae753.
[15] Zhang FC, He JF, Peng J, Tang XP, Qin CF, Lu HZ, Li XW, Liu QQ, Zhao H, Sheng JF and Wang GJ; Society of Infectious Diseases, Chinese Medical Association; Society of Tropical Medicine and Parasitology, Chinese Medical Association; Society of Emergency Medicine, China Association of Chinese Medicine. Guidelines for diagnosis and treatment of dengue in China. Zhonghua Nei Ke Za Zhi 2018; 57: 642-648.
[16] Kularatne SA and Dalugama C. Dengue infection: global importance, immunopathology and management. Clin Med (Lond) 2022; 22: 9-13.
[17] Gholami H, Gachpazan M and Erfanian M. Mathematical modeling and dynamic analysis of dengue fever: examining economic and psychological impacts and forecasting disease trends through 2030-a case study of Nepal. Sci Rep 2025; 15: 10027.
[18] Vairaperumal T, Lee PT and Liu PY. Portable point-of-care diagnosis platforms and emerging predictive biomarkers for rapid detection of severe dengue viral infection. ACS Sens 2025; 10: 3302-3316.
[19] Teutsch SM, Nunez CA, Morris A, Eslick GD and Elliott EJ. Australian Paediatric Surveillance Unit (APSU) Annual Surveillance Report 2022. Commun Dis Intell (2018) 2023; 47.
[20] Ali S, Kaisar MMM, Hengestu A, Teguh AI, Janova AM, Chriestya F, Loe L and Wijaya JW. Clinical and laboratory profiles of dengue infection in the hospitals in North Jakarta, Indonesia. IJID Reg 2025; 14: 100612.
[21] Guo J, Lu J, Du P, Cheng K, Lei C, Jiang Y, Peng M, Li Y, Sun K, Xu C, Yu Y, Gao C, Kang Q, Zhang Y, Wang R and Yang Z. Fluorescence reduction neutralization test: a novel, rapid, and efficient method for characterizing the neutralizing activity of antibodies against dengue virus. Curr Issues Mol Biol 2025; 47: 140.
[22] Dong B, Feng S and Feng X. Advances in the epidemiology, pathogenesis, diagnostic methods, and vaccine development of dengue fever: a comprehensive review. Viral Immunol 2025; 38: 53-60.
[23] Zhang Y, Xiang Y, Hou D, Fang L, Cai S, Zhang J, Wang Y, Jiang Y, Liu B, Bai J, Ding Y, Fang J, Chen S, Liu X and Ren X. A one-pot method for universal Dengue virus detection by combining RT-RPA amplification and CRISPR/Cas12a assay. BMC Microbiol 2025; 25: 163.
[24] Abera A, Tegally H, Tasew G, Wilkinson E, Ali A, Regasa F, Bitew M, Belachew M, Chabuka L, Mwanyika G, Tshiabuila D, Giandhari J, Pillay S, Poogavanan J, Moir M, Kraemer MUG, Khan K, Huber C, Tollera G, de Wit TFR, Baxter C, Lessells R, Wolday D, Beyene D and de Oliveira T; CLIMADE Consortium. Genomic characterization of circulating dengue virus, ethiopia, 2022-2023. Emerg Infect Dis 2025; 31: 516-525.
[25] Sevilla ME, Bokser V, Miño L, Oks I, Amatto MB, Nicolau V, Altschuler A and Juárez XS. Severe dengue in pediatrics: 10 cases in Buenos Aires, Argentina. Arch Argent Pediatr 2025; 123: e202410568.
[26] Prieto-Torres AE, Medina-Lozano LJ, Ramírez-Ávila JD and Faccini-Martínez ÁA. Utility of VIDAS(®) dengue diagnostic assays to differentiate primary and secondary dengue infection: a cross-sectional study in a military hospital

from Colombia. Trop Med Infect Dis 2025; 10: 40.

[27] Saisingha K, Jearanaiwitayakul T, Watterson D, Modhiran N, Ponpuak M and Ubol S. Transcriptome analysis of monocytes treated with Dengue NS1 revealed a shift in transcripts involving in self-propagated proinflammation and antiviral responses. J Infect Dis 2025; 231: e1170-e1182.

[28] Su WY, Lo HH, Wen YL, Lu JW, Yen IC, Ho YJ and Kao LT. Assessing traditional chinese medicines for anti-dengue using a national health insurance research database and bioassays. Food Sci Nutr 2025; 13: e70009.

[29] Anh DD, Vugrek L, The NT, Hafza N, My TN, Linh LTK, Loc DH, Schmidt-Chanasit J, Toan NL, Kremsner PG, Song LH and Velavan TP. Characterization of dengue patients in Vietnam: Clinical, virological, and IL-10 profiles during 2021-2022 outbreaks. PLoS Negl Trop Dis 2025; 19: e0012954.

[30] Kok SY, Abraham RE, Jauhar Ali SN, Tuang WX and Ong ELC. A retrospective study of clinical biomarkers of severe dengue in a tertiary hospital in Johor Bahru, Malaysia. Trop Med Infect Dis 2025; 10: 30.

[31] Bhuyan B, Panda B, Rout R, Mohanty L and Rajendra Prasad Rao B. Clinical profile of dengue encephalopathy: a series of twelve cases and a profile of other complications. J Family Med Prim Care 2025; 14: 447-451.

[32] Kothari D, Patel N and Bishoyi AK. Dengue: epidemiology, diagnosis methods, treatment options, and prevention strategies. Arch Virol 2025; 170: 48.