

Diagnostic Value of a Machine Learning-based Dengue Score in the Yumizen H500 Hematology Analyzer for Dengue Virus Infection: A Cross-sectional Diagnostic Study

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Abstract

Early detection of dengue virus infection (DVI) in healthcare settings is frequently limited by the availability and turnaround time of tests such as nonstructural protein 1 (NS1) antigen detection and Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Routine hematological evaluations are widely accessible and could be employed for screening at no additional cost. This study aims to assess the efficacy of a hematology analyzer's integrated dengue scoring system utilizing a Random Forest algorithm, referencing RT-PCR and NS1 antigen detection for diagnostic performance. A cross-sectional analysis of 119 patient samples was conducted using the Yumizen H500 hematology analyzer and a Dengue score algorithm based on Random Forest machine learning, comparing dengue scores with RT-PCR and NS1 antigen alongside immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody quantification via fluorescence immunoassay technology. Out of 119 patients (70 with confirmed DVI and 49 without), a dengue score cutoff of ≥ 50 yielded sensitivities of 90.0%, specificity of 57.1%, positive predictive value of 75.0%, negative predictive value of 79.4% and overall accuracy of 77.3%, with an area under the receiver operating characteristic curve of 0.77. The stratification of patients into risk categories based on dengue score ranges may aid in enhancing DVI diagnostic processes. The Yumizen H500 Dengue Score demonstrates substantial validity as a diagnostic screening tool for DVI, offering efficient implementation for rapid management.

Keywords: Dengue Virus Infection, Diagnostic Accuracy, Hematology Analyzer, Machine Learning, Random Forest Algorithm.

Introduction

Dengue virus infection (DVI) constitutes among the most consequential arthropod-transmitted viral diseases on a global scale, generating an estimated 390 million cases annually throughout tropical and subtropical territories (1). This mosquito-borne illness, predominantly disseminated through *Aedes aegypti* and *Aedes albopictus* vectors, has undergone substantial geographical proliferation across the preceding five decades, currently affecting more than 128 nations and exposing approximately half the global population to infection risk (2). The clinical presentation of DVI encompasses a broad spectrum, extending from subclinical cases and mild febrile episodes to life-threatening

complications such as dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS), conditions that may prove fatal without prompt recognition and appropriate therapeutic intervention (3). DVI pathogenesis involves intricate interplay between viral determinants and host immunological mechanisms. Upon inoculation via infected mosquito bite, the dengue virus—comprising four antigenically distinct serotypes (Dengue Virus 1-4 (DENV-1, DENV-2, DENV-3 and DENV-4))—initiates replication within various cellular targets including dendritic cells, monocytes and macrophages (4). Each serotype possesses the capacity to induce the complete disease spectrum. Primary infection

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confers lifelong homotypic immunity while providing only transient heterotypic cross-protection against alternate serotypes (5). Subsequent infection with a different serotype substantially elevates the probability of developing severe manifestations, a phenomenon attributed to antibody-dependent enhancement (ADE) mechanisms (6). The immunopathological cascade in severe DVI encompasses excessive cytokine production, complement system activation and endothelial dysfunction, culminating in plasma leakage, hemorrhagic complications and potential circulatory collapse (7). Thrombocytopenia and hemoconcentration serve as characteristic hematological hallmarks of dengue infection, reflecting both direct viral effects and immune-mediated mechanisms (8). Prompt and precise DVI diagnosis remains essential for optimal patient management and surveillance of public health. Current diagnostic methodologies include viral detection through Reverse Transcription-Polymerase Chain Reaction (RT-PCR) or nonstructural protein 1 (NS1) antigen assays during acute infection phases, alongside serological antibody detection (immunoglobulin M (IgM) and immunoglobulin G (IgG) for later disease stages (9). Nevertheless, these diagnostic modalities present limitations regarding availability, cost considerations and turnaround time, particularly within resource-constrained healthcare environments (10). RT-PCR, while highly specific, demands specialized laboratory infrastructure and technical expertise. NS1 antigen testing, though more accessible, exhibits variable sensitivity contingent upon infection timing, serotype and immune status (11, 12). Serological assays may demonstrate cross-reactivity with related flaviviruses, including Zika virus, potentially compromising diagnostic specificity (13). Given these diagnostic challenges, considerable interest has emerged in leveraging routine hematological parameters for DVI screening. Dengue infection induces characteristic alterations in complete blood count (CBC) profiles, including leukopenia, thrombocytopenia and hemoconcentration (14, 15). These modifications typically manifest early during infection and can be readily detected through standard automated hematology analyzers available in most clinical laboratories. Recent technological advances in hematology analyzers have incorporated

sophisticated machine learning algorithms capable of analyzing complex patterns within hematological data (16, 17). The Yumizen H500 hematology analyzer (HORIBA Medical) integrates a proprietary Dengue score algorithm developed through Random Forest machine learning methodology. This algorithm processes multiple hematological parameters simultaneously to generate a composite score reflecting DVI probability. Random Forest represents an ensemble learning technique that constructs multiple decision trees during training and outputs the mode of individual tree classifications (18). This approach offers several advantages for medical diagnostic applications, including robustness against overfitting, capability to handle high-dimensional data and ability to capture non-linear relationships between variables. In the context of dengue diagnosis, Random Forest algorithms can identify subtle patterns within hematological parameters that may not be apparent through conventional analysis.

Previous investigations have documented characteristic hematological changes associated with dengue infection, including decreased platelet counts, elevated hematocrit values and alterations in white blood cell populations (19, 20). However, individual parameters often lack sufficient sensitivity and specificity for reliable diagnosis. Machine learning approaches offer the potential to integrate multiple parameters into a unified diagnostic score with enhanced performance characteristics (21).

Despite the promising potential of machine learning-based hematological screening for DVI, limited published evidence exists regarding the diagnostic performance of such systems in clinical settings. Furthermore, the relationship between machine learning-derived dengue scores and established reference standards (RT-PCR and NS1 antigen detection) requires systematic evaluation. Additionally, the performance of these scoring systems across different infection types (primary versus secondary) and disease severity classifications warrants investigation.

This study addresses these knowledge gaps by evaluating the diagnostic performance of the Yumizen H500 Dengue score algorithm in a clinical cohort of patients with suspected dengue infection. We compared the Dengue score against RT-PCR and NS1 antigen detection as reference standards

and examined its performance in relation to infection type and disease severity. Our findings may inform the potential utility of machine learning-enhanced hematology analysis as a rapid, cost-effective screening tool for DVI in clinical practice. This study serves as an external validation of the existing proprietary Yumizen H500 Dengue score algorithm to evaluate its diagnostic performance in Indonesian population. The specific objectives of this investigation were:

- (a) to assess the diagnostic accuracy (sensitivity, specificity, positive predictive value, negative predictive value) of the Yumizen H500 Dengue score for DVI detection using RT-PCR and NS1 antigen as reference standards;
- (b) to evaluate the discriminatory capacity of the Dengue score through receiver operating characteristic (ROC) curve analysis;
- (c) to examine the relationship between Dengue score values and infection type (primary versus secondary); and
- (d) to investigate the association between Dengue score categories and disease severity classifications according to World Health Organization (WHO) criteria.

Methodology

Study Design and Setting

This cross-sectional diagnostic study was conducted at Airlangga University Academic Hospital (approximate GPS coordinates 7°15'44.8" S, 112°47'32.8" E) between June to October 2025. The study was designed as an external validation to evaluate the diagnostic accuracy of the Yumizen H500 Dengue score system by comparing it against established reference standards (RT-PCR and NS1 antigen detection) in a clinically relevant population. The study protocol was reviewed and approved by the institutional ethics committee (approval number UA-02-25074) and all participants provided written informed consent prior to enrollment. The study adhered to the Standards for Reporting of Diagnostic Accuracy Studies (STARD) guidelines as shown in Figure 1. The protocol is as follows:

- (a) eligible patients presenting with fever ≤ 5 days at the Emergency or Inpatient Department were enrolled after informed consent;
- (b) venous blood was collected via standard phlebotomy into K2-EDTA tubes (5 mL) for complete blood count and Dengue score

analysis and plain tubes (5 mL) for serological and molecular testing;

- (c) EDTA samples were processed within 8 hours on the Yumizen H500 hematology analyzer using impedance and DHSS flow cytometry, which automatically generated the Dengue score (0–100);
- (d) serum samples were tested for NS1 antigen using fluorescent immunoassay (Standard F2400) and for IgM/IgG Dengue antibodies;
- (e) remaining serum was stored at -80°C until batch-processed for RT-PCR using the Standard M10 Arbovirus Panel (multiplex real-time RT-PCR detecting DENV-1 through DENV-4);
- (f) RT-PCR results were interpreted as positive when $\text{Ct} \leq 36.0$ with valid internal control ($\text{Ct} \leq 23-27$).

Quality assurance was performed on all instruments prior to analysis, including calibration and internal quality controls

Study Population

This investigation involved patients with acute febrile illness, requiring inclusion criteria of ages 18-65, fever or recent febrile history, informed consent and blood sample provision, while excluding individuals with specific hematological disorders, medications affecting blood counts, or those who are pregnant as shown in Figure 1. The sample size was formally calculated using the formula for comparing two proportions (22), with the following parameters: $\alpha = 0.05$ ($Z = 1.96$), $\beta = 0.10$ ($Z = 1.282$), $P1 = 0.64$ (proportion of DVI) (23) and $P2 = 0.36$ (proportion of DVI among febrile patients) (24). The minimum calculated sample size was 65 subjects. With a 2:1 (DVI: non-DVI) ratio, the total required sample was 98, which was increased by 10% to account for potential dropouts, yielding a minimum of 108 subjects. The final enrollment of 119 patients (70 DVI and 49 non-DVI) exceeds this requirement. Consecutive sampling was employed.

Blood Sample Collection and Processing

Venous blood samples were obtained from each participant using standard phlebotomy techniques and processed within 8 hours of presentation. Blood was collected into the following tubes:

- (a) Ethylenediaminetetraacetic acid (EDTA) tubes for complete blood count analysis;

(b) plain tubes for RT-PCR testing; and serological testing (NS1, IgM, IgG).

All samples were labeled with unique identifiers and processed according to standardized protocols.

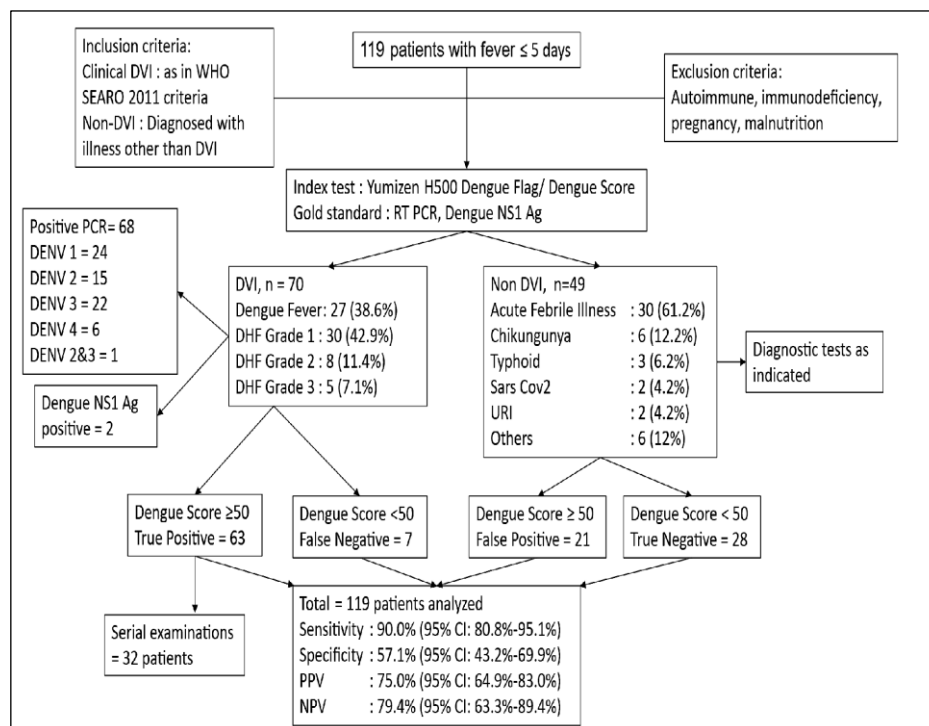


Figure 1: Research Workflow

Note: RT = Real Time Polymerase Chain Reaction, DHF = Dengue Hemorrhagic Fever, DVI = Dengue Virus Infection, URI = Upper Respiratory Tract Infection, PPV = Positive Predictive Value, NPV = Negative Predictive Value.

Hematological Analysis and Dengue Score Calculation

Complete blood count analysis was performed using the Yumizen H500 hematology analyzer (HORIBA Medical, France) according to manufacturer specifications. The analyzer automatically generated standard CBC parameters including white blood cell (WBC) count and differential, red blood cell (RBC) parameters, platelet count and indices and cell population data patterns. Additionally, the integrated Dengue score algorithm automatically calculated a probability score based on the Random Forest model.

The Dengue score algorithm utilizes multiple hematological parameters including but not limited to: WBC count and differential, platelet count, hematocrit, atypical lymphocyte percentage and various cell distribution parameters. The algorithm outputs a score ranging from 0 to 100.

RT-PCR for Dengue Virus Detection and Serotyping

RT-PCR served as the principal reference standard for confirming DVI and determining serotypes using an Arbovirus panel (SD Biosensor) with

primers tailored for the four dengue serotypes (DENV-1, DENV-2, DENV-3, DENV-4).

NS1 Antigen Detection

NS1 antigen testing was performed using fluorescent immunoassay (FIA) methodology with Dengue NS1 Ag FIA (SD Biosensor). This assay identifies the dengue NS1 protein released by infected cells during the acute infection phase, with testing conducted per the manufacturer's guidelines and results categorized as positive or negative based on predetermined thresholds. NS1 testing served as an additional reference standard, particularly for early-phase infections.

Serological Testing (IgM and IgG)

Dengue-specific IgM and IgG antibodies were identified through FIA techniques utilizing Anti Dengue IgM/IgG FIA (SD Biosensor). These tests help distinguish primary from secondary dengue infections based on antibody patterns. In primary infections, IgM appears first and reaches higher levels than IgG, while in secondary infections, IgG rises rapidly and reaches higher levels than IgM. Testing was performed according to manufacturer protocols and results were recorded as positive or negative as shown in Table 1.

Table 1: Specimen and Instruments

Specimen	Parameter	Instrument	
Whole Blood K2 EDTA Serum	Dengue Flag/ Dengue Score	Yumizen H500	Horiba Medical
	PCR Dengue	M10 Platform	SD Biosensor
	Dengue NS1 Ag	Standard F-2400	
	IgM and IgG Dengue	Standard F-2400	

Note: K2 EDTA = Dipotassium Ethylenediamine Tetraacetic Acid, PCR = Polymerase Chain Reaction.

Clinical Assessment and Disease

Classification

Clinical data were collected for all participants, including detailed history of present illness, past dengue infections, travel history and physical examination findings. Disease severity was categorized in accordance with the dengue guidelines established by the WHO in 2011 (9). Infection classification (primary or secondary) was established through serological analysis, where primary infection was indicated by negative IgG and secondary infection by positive IgG.

Data Management and Statistical Analysis

Data collected during the study were systematically documented on standardized case report forms and subsequently entered a secure electronic database, ensuring patient confidentiality through unique identifiers. Data integrity was upheld via dual-entry verification and routine cleaning processes. Descriptive statistics summarized demographic and clinical features, with continuous variables represented as mean $\pm$ standard deviation or median with interquartile range, while categorical variables were expressed as frequencies and percentages.

All statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corporation, Armonk, NY, USA), including diagnostic accuracy calculations (sensitivity, specificity, PPV, NPV, likelihood ratios), ROC curve analysis with AUC computation, 95% confidence interval estimation, normality testing (Kolmogorov-Smirnov and Shapiro-Wilk), Chi-square tests and Spearman's rank correlation. A p-value <0.05 was considered statistically significant. Youden Index was used to

determine the optimal cut-off point on the ROC curve. Individual AUC calculations for DENV-1, DENV-2, DENV-3 and DENV-4 with 95% CI.

Results

Study Population and Baseline

Characteristics

A cohort of 119 patients was recruited and successfully underwent all necessary laboratory assessments. This population included individuals diagnosed with DVI (n=70) and a control group with non-dengue febrile illness (n=49), with their demographic and clinical attributes detailed in Table 2. The median age of participants was 29 years, with a range of 18-62 years. The gender distribution showed 51.3 % male and 48.7 % female participants. The median day of fever at presentation was 3 days (median: 1-5 days). Among the confirmed DVI cases, disease severity classification revealed: dengue fever (38.6%), DHF Grade 1 (42.9%), DHF Grade 2 (11.4%), DHF Grade 3 (7.1%). Infection type analysis showed primary infection in 89.9% and secondary infection in 8.6% of cases.

The 49 non-dengue patients in Table 2 were diagnosed with the following conditions: Acute Febrile Illness of unspecified etiology (n=30, 61.2%), Chikungunya (n=6, 12.2%), Typhoid fever (n=3, 6.2%), COVID-19 (n=2, 4.2%), Upper Respiratory Tract Infection (n=2, 4.2%), gangrene (n=1, 2%), tonsillitis (n=1, 2%), sepsis (n=1, 2%), Plasmodium vivax malaria (n=1, 2%), urinary tract infection (n=1, 2%) and influenza (n=1, 2%). These alternative diagnoses were confirmed through appropriate laboratory testing (IgM serology, PCR, blood smear, urine analysis, chest X-ray, etc.) as described in the inclusion criteria.

Table 2: Characteristics of Study Population

Characteristics	n (%) (N=119)	Median (Min – Max)
Age (years)		29.0 (18.0 – 62.0)
< 50	108 (90.1%)	
≥ 50	11 (9.9%)	
Gender		

Male (%)	61 (51.3%)	
Female (%)	58 (48.7%)	
DVI patients	70 (58.8%)	
Dengue fever	27 (38.6%)	
DHF Grade 1	30 (42.9%)	
DHF Grade 2	8 (11.4 %)	
DHF Grade 3	5 (7.1%)	
Non DVI patients	49 (41.2%)	
Acute Febrile Illness	30 (61.2%)	
Chikungunya	6 (12.2%)	
Tifoid	3 (6.2 %)	
Covid	2 (4.2 %)	
Upper Respiratory Infections	2 (4.2 %)	
Gangren	1 (2%)	
Tonsilitis	1 (2%)	
Sepsis	1 (2%)	
Plasmodium vivax	1 (2%)	
UTI	1 (2%)	
Influenza	1 (2%)	
Fever Duration at Presentation		3 (1 – 5)
1 day	10 (8.4%)	
2 days	21 (17.6%)	
3 days	33 (27.8%)	
4 days	27 (22.7%)	
5 days	28 (23.5%)	
PCR result (Arbovirus Panel)	119	
DENV 1	23 (19.3%)	
DENV 2	15 (12.6%)	
DENV 3	22 (18.5%)	
DENV 4	6 (5.1%)	
DENV 2 and 3	1 (0.8%)	
DENV 1 and WNV	1 (0.8%)	
CHIKV	6 (5.1%)	
Negative	45 (37.8%)	
Dengue NS1 Ag		
Positive	58 (48.8%)	
Negative	60 (50.4%)	
No Data	1 (0.8%)	
Infection type		
Primary	62 (89.9%)	
Secondary	6 (8.6%)	
Unclassified	1 (1.5%)	
Dengue Flag		
Positive	83 (69.7%)	
Negative	36 (30.3%)	
Dengue Score		56 (18 – 91)
WBC (10 ³ cells/mm ³)		4.3 (1.5 – 20.6)
PLT (10 ³ cells/mm ³)		137 (20 – 435)
≥ 150	53 (44.6%)	
100-149	38 (31.9%)	
< 100	28 (23.5%)	
LYM (%)		22.4 (1.5 – 61.6)
ALY (%)		0.4 (0.2 – 8.6)

Note: DVI = Dengue Viral Infection, UTI = Urinary Tract Infection, PCR = Polymerase Chain Reaction, DENV = Dengue Virus, CHIKV = Chikungunya Virus, WNV= West Nile Virus, Ag = Antigen, WBC = White Blood Cell, PLT = Platelet, LYM = Limfosit, ALY = Atypical Lymphocyte.

Notably, 5 of 6 Chikungunya patients (83.3%) showed positive Dengue flags with scores ranging from 54–82, while 1 patient with *P. vivax* malaria correctly showed a negative Dengue flag (score 25) with a positive *P. vivax* flag. The results of our findings were

- (a) the full diagnostic performance metrics including sensitivity (90.0%), specificity (57.1%), PPV (75%), NPV (20%), LR+ (2.09), LR– (0.18) and accuracy (77.3%);
- (b) the risk stratification across four Dengue score categories with corresponding PPVs (18%, 50%, 74% and 100%);
- (c) per-serotype diagnostic performance (DENV-1: sensitivity 95.7%, AUC 0.835; DENV-2: sensitivity 86.7%, AUC 0.764; DENV-3: sensitivity 90.9%, AUC 0.837; DENV-4: sensitivity 83.3%, AUC 0.743);
- (d) temporal analysis showing optimal performance on fever days 2–3 with differentiated cut-offs for early (≤ 3 days) versus later presentation; and
- (e) significant hematological differences between DVI and non-DVI groups (leukopenia, thrombocytopenia, relative lymphocytosis).

Dengue Virus Detection and Serotype Distribution

RT-PCR detected dengue virus in 68 patients (57.1% of total). Among PCR-positive cases, the serotype distribution was: DENV-1 19.3%), DENV-2 (12.6%), DENV-3 18.5%) and DENV-4 (5.1%). NS1 antigen was detected in 58 patients (48.8% of total). The combination of RT-PCR and/or NS1 positivity identified a total of 70 confirmed DVI cases.

Hematological Parameters in Dengue Patients

Hematological analysis revealed characteristic changes associated with DVI. Table 3 summarizes the key hematological parameters in dengue-positive versus dengue-negative patients. The DVI group demonstrated significantly lower white blood cell counts (3.9 ± 1.4 vs $8.2 \pm 2.6 \times 10^3/\mu\text{L}$, $p < 0.001$) and platelet counts (89.6 ± 32.4 vs $198.5 \pm 54.2 \times 10^3/\mu\text{L}$, $p < 0.001$) compared to non-DVI patients. Lymphocyte percentage was significantly higher in dengue-positive cases ($28.4 \pm 8.2\%$ vs $22.1 \pm 6.5\%$, $p < 0.001$), while hemoglobin and hematocrit showed no statistically significant differences between groups.

Table 3: Key Hematological Parameters in DVI and non-DVI Patients

Parameter	DVI (n=70)	Non-DVI (n=49)	P value
Hemoglobin (g/dL)	13.8 ± 1.9	13.2 ± 2.1	0.152
Hematocrit (%)	42.1 ± 5.6	40.3 ± 6.2	0.134
WBC ($\times 10^3/\mu\text{L}$)	3.9 ± 1.4	8.2 ± 2.6	$<0.001^*$
Platelets ($\times 10^3/\mu\text{L}$)	89.6 ± 32.4	198.5 ± 54.2	$<0.001^*$
Lymphocyte (%)	28.4 ± 8.2	22.1 ± 6.5	$<0.001^*$

Note: *Statistically significant ($p < 0.05$), DVI = Dengue Virus Infection, WBC = White Blood Cells.

Diagnostic Performance of Dengue Score

Using the manufacturer-recommended cut-off value of ≥ 50 , the Dengue score demonstrated the following diagnostic performance characteristics: Sensitivity: 90.0% (95% CI: 80.8%-95.1%), Specificity: 57.1% (95% CI: 43.2%-69.9%), Positive Predictive Value (PPV): 75.0% (95% CI: 64.9%-83.0%), Negative Predictive Value (NPV): 79.4% (95% CI: 63.3%-89.4%), Accuracy: 77.3% (95% CI: 69.0%-83.9%).

ROC curve analysis revealed an area under the curve (AUC) of 0.77 (95% CI: 0.69-0.85) as shown in Figure 2, indicating good discriminatory ability for distinguishing DVI from non-dengue febrile illness. The curve plots sensitivity against 1-specificity across all possible Dengue score thresholds, with the diagonal reference line representing chance-level discrimination. An AUC of 0.77 falls within the acceptable discrimination range (0.7–0.8), confirming the Dengue score's validity as a preliminary screening instrument in clinical settings.

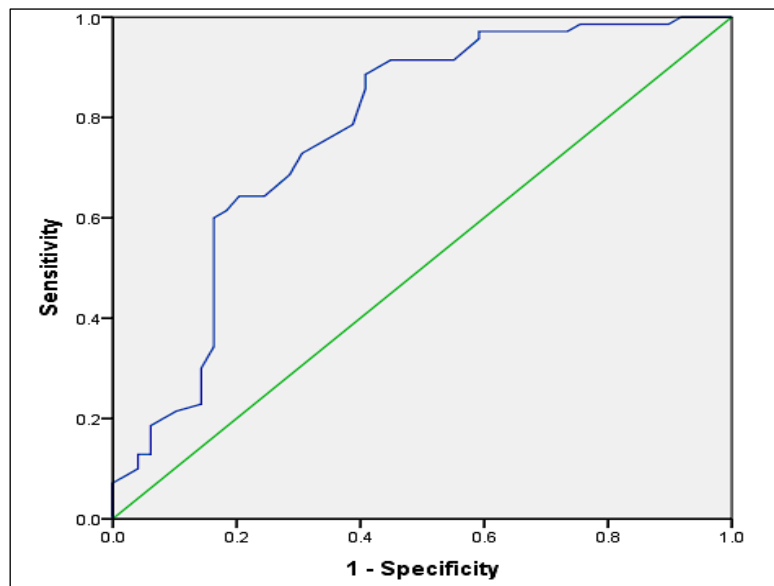


Figure 2: ROC Curve for Dengue Score

Performance Across Risk Categories

By calculating the PPV of various cut-offs, an increase in the risk of DVI can be observed along with an increase in the Dengue score, as shown in Figure 3. A total of 33 patients were categorized as low risk [0–48] with a calculated positive predictive value (PPV) of 18%, while 4 patients fell into the moderate risk category [49–50] with a PPV of 50%. Furthermore, 77 patients were classified as high risk [51–82], yielding a PPV of 74% and 5

patients were identified as very high risk [83–100], demonstrating a PPV of 100%.

Beyond binary classification, stratification into ordinal risk categories (low, moderate, high, very high) showed a monotonic relationship between score magnitude and probability of dengue confirmed by RT-PCR and/or NS1. This graded pattern supports using the reported numeric score to complement clinical judgment rather than relying only on a single cut-off.

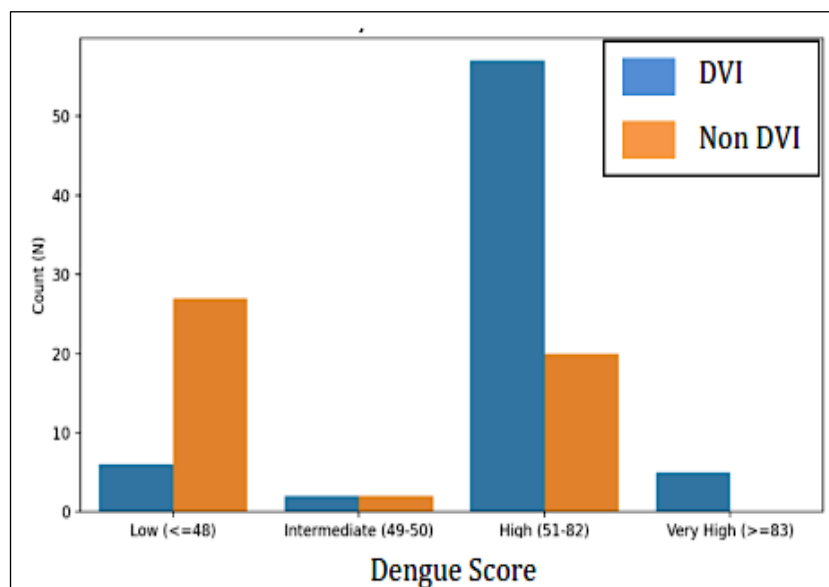


Figure 3: Dengue Virus Infection Probability According to Dengue Score.

Note: DVI = Dengue Virus Infection.

Subgroup Analysis

Primary versus Secondary Infection and Disease Severity. The Dengue score exhibited no statistically significant disparity between primary

and secondary infections ($p=0.23$) and across varying disease severities ($p=0.538$), while demonstrating diagnostic consistency across serotypes DENV-1 to DENV-4, with exploratory

analyses revealing elevated median scores in DHF grade 2 (n=4) and grade 3 (n=3) relative to dengue fever. Although these severe subgroups were small, the direction of effect suggests the score may also reflect host-response intensity and warrants prospective evaluation as an early triage marker.

At the manufacturer's cut-off of 50, the Dengue score demonstrated high sensitivity across all four serotypes: DENV-1 (95.7%, 95% CI 78.1–99.9, AUC 0.835), DENV-2 (86.7%, 95% CI 59.5–98.3, AUC 0.764), DENV-3 (90.9%, 95% CI 70.8–98.9, AUC 0.837) and DENV-4 (83.3%, 95% CI 35.9–99.6, AUC 0.743). Specificity was uniform at 60% (95% CI 44.3–74.3) as the comparator group (PCR-negative) was constant.

DENV-3 showed the best overall performance, followed by DENV-1 and DENV-2, while DENV-4 demonstrated slightly lower sensitivity, which

may be attributed to distinct hematological responses among serotypes —

DENV-2 and DENV-3 are known to cause more severe thrombocytopenia and bleeding manifestations (25). The limited DENV-4 sample (n=6) with wide confidence intervals necessitates cautious interpretation and further validation.

Temporal Analysis

The relationship between Dengue score and day of illness at presentation was analyzed to determine the optimal timing for score-based screening as shown in Figure 4. This temporal analysis illustrates how hematological parameters change across the febrile period, with the Dengue score showing peak discriminatory performance between days 2 and 4 of illness onset.

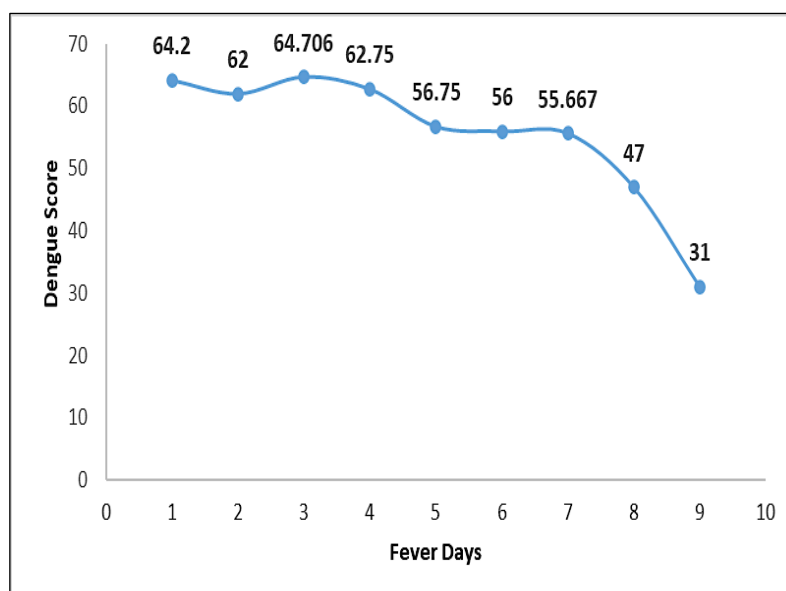


Figure 4: Dengue Score Mean by Fever Day

Longitudinal analysis demonstrated that Dengue scores peaked around day 2 of fever and subsequently declined as shown in Figure 4. The decrease in scores during the advanced phase indicates normalization of hematological parameters. These results align with other studies indicating recovery of platelets and leukocytes during the healing phase (3, 5). Previous research outlines the phases of Dengue infection, with the febrile phase (days 1-3) showing high viremia and increased Dengue scores (26). In the first three days of fever, a high score more frequently aligned with reference positivity, supporting the use of the score as an early adjunct to trigger confirmatory testing and closer monitoring.

Discussion

This study shows that the Yumizen H500 machine learning-based Dengue Score can support early identification of dengue virus infection using routine hematology, with sensitivity 90.0% and moderate specificity 57.1% at a cutoff of ≥ 50 against RT-PCR and/or NS1. Given that early dengue management depends heavily on timely recognition and monitoring for warning signs, a high-sensitivity screening tool embedded in a hematology analyzer can be operationally useful in endemic settings and during surges when confirmatory test capacity and turnaround time are constrained (1, 2).

Our findings extend the original validation of the same Yumizen H500 dengue score algorithm in Mumbai, India, reporting an AUC of 0.82, sensitivity of 79.3% and specificity of 71.5% at the same cutoff of ≥ 50 , using NS1 antigen and IgM/IgG serology as reference standards (27). The higher sensitivity but lower specificity observed in our study likely reflects the use of RT-PCR as the primary gold standard, which captures viremic patients earlier in the disease course when hematological changes may be most pronounced. This comparison suggests that the algorithm performs consistently across different populations and reference methodologies, with the trade-off between sensitivity and specificity being influenced by the reference standard chosen.

At the chosen threshold, the likelihood ratios (LR+ 2.09 and LR- 0.18) indicate that the score is better suited for triage and ruling-out than for definitive confirmation. In practice, a low score could help deprioritize dengue in non-severe febrile illness when clinical suspicion is low, while a high score can justify early dengue-focused pathways such as closer observation, repeat hematocrit and platelet monitoring and targeted confirmatory testing. This role aligns with WHO guidance that emphasizes early detection of progression risk and rational use of laboratory resources, especially where access to Nucleic Acid Amplification Test (NAAT) is limited (1).

The AUC of 0.77 obtained in this study is classified as acceptable discrimination, with the AUC values of 0.7–0.8 as acceptable and 0.8–0.9 as excellent (28). While this places our results slightly below the excellent threshold reported in India cohort (AUC 0.82) (27), the clinical utility of the score remains substantial when considered within established dengue management (9) and the comprehensive guidelines for dengue prevention and control, which emphasize the importance of early detection tools that can be deployed at the point-of-care without additional reagent costs (29). The dengue score fulfills this role by leveraging routine hematological data already generated during standard clinical evaluation.

The modest specificity is clinically plausible because several acute febrile illnesses can produce overlapping hematologic signatures including leukopenia, relative lymphocytosis and thrombocytopenia. In endemic areas, differential diagnoses such as chikungunya, other viral infections and

bacterial infections in early stages can mimic dengue clinically and hematologically, particularly before distinctive features evolve. Therefore, the score should be interpreted alongside clinical presentation, epidemiologic context and confirmatory assays rather than as a standalone diagnosis (1, 3).

The algorithm's efficacy is biologically plausible, as dengue is known to induce bone marrow suppression alongside peripheral platelet destruction or consumption, resulting in leukopenia and thrombocytopenia, while inflammatory and immune responses may alter leukocyte differentials and elevate atypical lymphocyte levels. These effects are often detectable early in illness and are routinely captured by modern analyzers. The integration of multiple parameters through a Random Forest model may therefore improve discrimination relative to single-parameter rules, consistent with broader evidence that multivariable or machine learning approaches can enhance diagnostic classification when individual markers are non-specific (1, 4, 5).

Our cohort had a median fever day of 3 at presentation, a window in which NS1 and RT-PCR tend to perform best, while serology may still be negative in primary infection. In this early phase, hematologic changes may already be evolving, making analyzer-based scoring potentially complementary to antigen or NAAT testing. However, because hematologic patterns vary by day of illness and by host immune status, the same cutoff may not be optimal across all timepoints. Future work should assess day-by-day performance and evaluate whether dynamic cutoffs improve specificity without materially harming sensitivity (1, 2).

Most cases in this study were primary infections, with smaller representation of secondary dengue. Secondary infections elevate the risk of plasma leakage and severe dengue via immunopathological mechanisms, with distinct hematologic trajectories observed in platelet and hematocrit alterations. If the score relies heavily on platelet or leukocyte features, case-mix differences could influence calibration. Stratified analyses by immune status and severity are therefore important and multicenter validation in settings with higher proportions of secondary infection would strengthen generalizability (1, 6).

All four dengue serotypes were represented, which is encouraging for external validity since hematologic manifestations are broadly shared across serotypes though severity risk may vary by serotype and by population immunity. Because algorithm performance can drift when epidemiology shifts, periodic local re-evaluation is recommended, particularly during outbreaks dominated by a single serotype (1, 6).

A practical implementation is a tiered pathway: patients with score <50 and low clinical suspicion can be managed with routine evaluation and safety-netting, while patients with score ≥50 can be prioritized for NS1/RT-PCR where available or closer monitoring when confirmatory testing is delayed. This can reduce time-to-triage decisions and may optimize limited confirmatory testing by focusing on higher pretest probability patients. Because the positive predictive value depends on prevalence, institutions should interpret PPV and NPV in light of local dengue incidence and seasonality and consider adjusting cutoffs during low-prevalence periods to reduce unnecessary follow-up testing (1, 7).

The interpretation of these findings necessitates careful consideration of various limitations. This research was derived from a single setting, which may limit transportability and low prevalence of certain serotype (DENV 4). Future studies should test multiple centers, pediatric population and evaluate integration with simple clinical variables to improve specificity while retaining sensitivity (1, 2, 8).

Conclusion

This study makes several important contributions to the field. To our knowledge, this is the first Indonesian study to validate the Yumizen H500 dengue scoring system using real-time RT-PCR as the gold standard, in contrast to prior validation studies that relied on NS1 antigen detection or serology. The study comprehensively evaluated scoring performance across all four dengue serotypes, primary and secondary infection types, and all severity grades, providing a more complete characterisation of the score's utility than previously available. Furthermore, the introduction of a four-category risk stratification framework (Score <25, 25–49, 50–74, ≥75) offers practical guidance for clinical triage. Importantly, the scoring system operates on data generated by

routine complete blood count (CBC) analysis, requiring no additional laboratory infrastructure or cost.

The clinical implications of these findings are significant. The high sensitivity (90.0%) of the Yumizen H500 scoring system supports its use as a first-line triage tool in the acute febrile period. Patients with a score below 50—associated with a negative likelihood ratio of 0.18—may be triaged to routine outpatient monitoring with appropriate safety-netting instructions, thereby reducing unnecessary specialist referrals and resource utilisation. Conversely, patients with a score of ≥50 should receive prioritised confirmatory dengue testing (NS1 antigen or RT-PCR) in accordance with WHO (2009) and WHO SEARO (2011) guidelines, enabling timely initiation of clinical monitoring and supportive management. This approach is readily implementable in any healthcare setting already equipped with automated haematology analysers, without the need for additional diagnostic infrastructure.

This study has several limitations that should be considered when interpreting the results. First, the study was conducted at a single tertiary-care centre (RS Universitas Airlangga, Surabaya), which may limit the generalisability of findings to other clinical or geographic settings with different dengue epidemiology or patient populations. Second, serotype representation was uneven, with limited cases of DENV-4, potentially affecting the generalisability of serotype-specific analyses. Third, the timing of blood sampling was variable and not standardised to specific fever days, introducing heterogeneity in haematological findings. Fourth, no serial or longitudinal samples were collected, precluding assessment of score dynamics over the clinical course of illness. Fifth, the study enrolled adult patients only, and the results may not be directly applicable to pediatric populations given age-related differences in haematological parameters. Finally, the scoring model did not incorporate plasma leakage markers, organ function parameters, or inflammatory biomarkers, which may have provided additional discriminatory value.

Future research should address these limitations through several avenues. Multi-centre studies enrolling patients from diverse geographic regions and healthcare settings across Indonesia and South-East Asia would improve the

generalisability of the scoring system. Studies should aim for balanced representation of all dengue serotypes, particularly DENV-4, to enable robust serotype-specific performance evaluation. Prospective studies including paediatric populations with age-stratified analyses are warranted to determine whether the current cutoff score and risk categories remain applicable in younger patients. Standardised serial blood sampling at defined time points—specifically fever days 1, 3, and 5—would allow characterisation of scoring dynamics over the acute febrile course and may identify optimal testing windows. Finally, future models integrating clinical parameters such as signs of plasma leakage (e.g., pleural effusion, ascites, haemoconcentration), abdominal pain, bleeding manifestations, and organ function markers alongside haematological scoring may yield composite severity prediction models with superior diagnostic performance for the early identification of severe dengue.

Abbreviations

ADE: Antibody-dependent Enhancement, DENV 1-4: Dengue Virus 1-4, DHF: Dengue Hemorrhagic Fever, DSS: Dengue Shock Syndrome, DVI: Dengue Virus Infection, NAAT: Nucleic Acid Amplification Test, NS1: Nonstructural Protein 1, ROC: Receiver Operating Characteristic, STARD: Standards for Reporting of Diagnostic Accuracy Studies, WNV: WestNile Virus.

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Author Contributions

Hantoro Gunawan: Conceptualization, Methodology, Formal Analysis, Data Curation, Writing – Original Draft Preparation, Aryati Aryati: Conceptualization, Methodology, Investigation, Resources, Writing – Review and Editing, Supervision, Yulia Nadar Indrasari: Methodology, Writing – Review and Editing, Supervision, Nasronudin Nasronudin: Methodology, Writing – Review and Editing, Supervision, Anindya Widayarsi: Methodology, Investigation, Resources, Khalifenti Ambarsari: Methodology, Investigation, Resources, Lulut Kusumawati: Methodology,

Investigation, Resources, Brian Eka Rachman; Methodology, Supervision. All authors approve the final version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

The datasets analyzed during the current study are not publicly available due to patient privacy restrictions and ethical constraints imposed by the Airlangga University Hospital Ethical Committee. However, aggregated data are available from the corresponding author upon reasonable request.

Declaration of Artificial Intelligence (AI) Assistance

The authors acknowledge the use of generative AI tools (e.g., Scispace and ChatGPT by OpenAI) in the preparation of this manuscript, specifically for formatting, language editing and grammar correction. All content generated through AI was critically reviewed, edited and verified by the authors to ensure accuracy, originality and compliance with academic standards. The authors take full responsibility for the integrity and validity of the manuscript.

Ethics Approval

The research adhered to Good Clinical Practice standards and the Declaration of Helsinki, with ethical clearance secured from the Airlangga University Hospital Ethical Committee (approval number UA-02-25074). Informed consent was obtained from all participants, who retained the right to withdraw at any point without repercussions on their clinical treatment and no extra financial burden was imposed for study-related activities.

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