OPEN ACCESS

IJABR

International Journal of Advanced Biotechnology and Research
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-17, Issue2, 2026, pp1-8

Research Article

Study of High Fluorescent Lymphocyte Count (HFLC) in the Sysmex XN-350 Hematology Analyzer in Dengue Infection

Subiksha R[1], Vinoth Kumar Ganesamoorthy[1], Diego Edwin[2] and Priya Banthavi S[1]

Article Info

[1]Department of Pathology, Trichy SRM Medical College Hospital & Research Centre, Tamil Nadu, India
[2]Department of Microbiology, Trichy SRM Medical College Hospital & Research Centre, Tamil Nadu, India

Corresponding Author:
Dr. Vinoth Kumar Ganesamoorthy,
Department of Pathology, Trichy SRM Medical College Hospital & Research Centre, Tamil Nadu, India

---

Received: 17/04/2026
Accepted: 11/06/2026
Published: 15/06/2026

DOI: 10.5281/zenodo.20696076

Abstract

Background: Dengue fever is a rapidly emerging mosquito-borne viral disease endemic to tropical regions. Accurate and early laboratory diagnosis is crucial to reduce morbidity and mortality. High Fluorescent Lymphocyte Count (HFLC), a research parameter generated by the Sysmex XN-350 haematology analyser, reflects activated lymphocytes seen in viral infections.

Aim: To evaluate HFLC levels in serologically confirmed dengue patients and analyse their relationship with platelet count and disease recovery.

Methods: A prospective study was conducted among in-patients of Trichy SRM Medical College Hospital & Research Centre (TSRMMCH & RC) between September 2024 and March 2025. Patients positive for NS1 antigen and/or dengue IgM were included. HFLC% values from the XN-350 analyser and correlation with platelet counts were analysed.

Results: A total of 220 patients were included. Mean HFLC% in dengue patients was 3.82 (SD ± 1.26) which was significantly higher compared to controls ($p < 0.001$). A significant inverse correlation was observed between HFLC% and platelet count ($\rho = -0.919$, $p < 0.001$).

Conclusion: HFLC is a rapid, and inexpensive screening biomarker for early diagnosis and prognosis of dengue infection, particularly in resource-limited clinical settings.

Keywords: Dengue, HFLC (High Fluorescent Lymphocyte Count), Sysmex XN-350, Thrombocytopenia, NS1 Antigen, IgM

Introduction:

Dengue fever is one of the most widespread mosquito-borne viral infections affecting humans and continues to be a major public health concern in tropical and subtropical regions [1]. The disease is caused by the dengue virus (DENV), a member of the Flaviviridae family consisting of five antigenically distinct serotypes (DENV-1 to DENV-5) transmitted primarily through the bite of Aedes aegypti mosquitoes. Globally, dengue incidence has risen sharply in the last two decades due to rapid urbanization, climatic shifts, international travel, and inadequate vector control. Current estimates indicate that nearly 390 million dengue infections occur annually, of which around 96 million results in clinical disease, emphasizing the urgency of developing rapid and accessible diagnostic support tools. The clinical presentation of dengue varies from asymptomatic infection to classical dengue fever and, in a subset of patients, can progress to severe forms characterized by plasma leakage, haemorrhage, and shock [2]. Although most cases are self-limiting, the rapid decline in platelet count (thrombocytopenia) and associated vascular complications represent critical features that require timely monitoring. Laboratory abnormalities such as leukopenia and thrombocytopenia are common and are therefore valuable for supportive diagnosis [3]. However, because early symptoms are nonspecific and overlap with other febrile illnesses such as malaria, influenza, and chikungunya, laboratory confirmation plays a central role in clinical decision-making during initial evaluation.

Standard laboratory diagnostics for dengue include detection of non-structural protein 1 antigen (NS1), IgM and IgG serology, and viral nucleic acid amplification. While NS1 antigen testing and IgM antibody detection are reliable, their availability, cost, and turnaround time can limit use during outbreaks and in resource-constrained environments [4]. In many healthcare settings, a complete blood count (CBC) is the first test performed for febrile patients, and thus additional useful information derived from hematology analysers could support earlier suspicion of dengue even before serological results are available. Technological advances in hematology automation have produced novel leukocyte indices that extend beyond traditional white cell differential counts. The Sysmex XN-350, a six-part automated hematology analyser, incorporates a dedicated channel capable of identifying High Fluorescent Lymphocyte Count (HFLC). HFLC represents activated or immunoreactivity lymphocytes, including plasmacytoid lymphocytes and immunoblasts, which contain higher nucleic acid content, resulting in stronger fluorescence staining. These activated lymphocytes typically emerge during viral infections due to antigenic stimulation and immune activation, making HFLC a potentially meaningful biomarker of viral illness [5]. Growing evidence suggests that HFLC levels rise significantly during dengue infection [6,7]. This highlights its potential value as an accessible and cost-free parameter derived from routine CBC that could support early diagnosis in primary and emergency care. Furthermore, HFLC may reflect the intensity of immune activation and has been linked to the severity of thrombocytopenia in some investigations. Patients exhibiting severe platelet decline have been observed to present with higher HFLC levels than those with milder thrombocytopenia, suggesting that HFLC may play a role not only in diagnostic screening but also in prognostic assessment.

If correlations between HFLC, dengue positivity, and thrombocytopenia are validated, HFLC could serve as a simple tool to enhance laboratory-based risk stratification and optimize early intervention. Understanding HFLC patterns among serologically confirmed dengue patients may support the integration of this index into routine hematology reporting and strengthen early screening strategies. The present study aims to analyse HFLC generated by the Sysmex XN-350 analyser in dengue-positive cases and evaluate its association with platelet count. Through this investigation, we seek to explore the clinical utility of HFLC as a diagnostic and prognostic marker in dengue

fever, with potential to improve patient monitoring and outcomes in dengue-affected regions.

Materials and Methods

Study design and setting: A prospective observational study was conducted at Trichy SRM Medical College Hospital and Research Centre between September 2024 and March 2025.

Study population: Patients who underwent dengue serological testing during the study period were assessed for eligibility. Individuals demonstrating positivity for NS1 antigen and/or dengue IgM antibody were included in the analysis, whereas cases with confirmed alternative viral infections were excluded. The non-dengue febrile control group included patients with undifferentiated acute febrile illnesses evaluated during the same study period. High fluorescence lymphocyte cell (HFLC) percentages along with lymphocyte scattergram findings were generated using the Sysmex XN-350 automated hematology analyzer. HFLC values were generated automatically by the Sysmex XN-350 analyzer under routine laboratory operational settings without additional manual gating modifications. Platelet counts corresponding to the same sample were retrieved and evaluated. Relevant demographic characteristics and available clinical details were documented for all study subjects. The study was approved by the Institutional Ethics Committee of Trichy SRM Medical College Hospital and Research Centre (Approval No:147/2024)

Statistical analysis:

Statistical evaluation was conducted using IBM SPSS Statistics. Descriptive measures were applied to summarize demographic variables, platelet counts, and HFLC parameters. Depending on the distribution pattern, continuous variables were presented either as mean with standard deviation or as median with interquartile range. Differences in HFLC values between dengue-positive and non-dengue groups were analyzed using the Mann–Whitney U test. The relationship between HFLC values and platelet counts was examined using Spearman rank correlation analysis. Receiver operating characteristic (ROC) curve analysis was performed to assess the diagnostic utility of HFLC. Statistical significance was defined as a p-value below 0.05.

Results:

The study included 220 participants comprising 120 serologically confirmed dengue cases and 100 non-dengue febrile controls. Among dengue patients, IgM positivity was observed in 96 cases (80.0%), NS1 antigen positivity in 72 (60.0%), and combined NS1 + IgM positivity in 61 (50.8%). Secondary infection serological pattern (IgM + IgG positivity) was noted in 34 cases (28.3%). Minimal serological positivity was observed in the non-dengue group (Table 1).

Most dengue patients presented between days 3–6 of fever [90/120 (75.0%)], whereas non-dengue cases showed a broader distribution (Table 2).

Thrombocytopenia was significantly more frequent in dengue patients. Moderate thrombocytopenia (50,000–100,000/μL) was observed in 46 cases (38.3%) and severe thrombocytopenia (<50,000/μL) in 37 cases (30.8%). Mean platelet count was lower in dengue patients (78,200 ± 32,500/μL) compared with non-dengue controls (162,600 ± 48,900/μL) (Table 3).

HFLC values were significantly elevated in dengue infection. Mean HFLC was 3.82 (SD ± 1.26) in dengue cases compared with 1.14 ± 0.41 in non-dengue controls, while median values were 3.75 and 1.05, respectively (Table 4). Elevated HFLC (>1.5%) was detected in 102 dengue cases (85.0%) and 18 non-dengue cases (18.0%) (Table 5).

Mann–Whitney U analysis showed a significant difference in HFLC values between the two groups ($U = 435.5$, $p < 0.001$) (Table 6). At an HFLC cut-off of >1.5%, sensitivity, specificity, PPV, NPV, and diagnostic accuracy were 85.0%, 82.0%, 85.7%, 81.2%, and 83.6%, respectively (Table 7).

ROC curve analysis showed very good diagnostic performance of HFLC for dengue detection with an AUC of 0.880 (Figure 1). Serial CBC analysis was performed in 34 dengue patients comprising 115 follow-up visits. HFLC% demonstrated a strong inverse relationship with platelet count, with increasing HFLC values associated with progressive thrombocytopenia. Patients with higher HFLC percentages generally showed moderate to severe thrombocytopenia. Spearman's correlation analysis showed a significant negative correlation between HFLC% and platelet count ($\rho = -0.919$, $p < 0.001$, $n = 115$) (Figure 2)

Table 1: Serological Profile of Participants

Serological Marker	Dengue Positive (n = 120)	Non-Dengue (n = 100)
NS1 Antigen Positive	72 (60.0%)	0 (0.0%)
IgM Positive	96 (80.0%)	4 (4.0%)
IgG Positive	42 (35.0%)	6 (6.0%)
Dual Positive (NS1 + IgM)	61 (50.8%)	0 (0.0%)
Secondary Infection Pattern (IgM + IgG)	34 (28.3%)	2 (2.0%)

Table 2: Day of Fever at Time of Testing

Day of Fever at Blood Sampling	Dengue (n = 120)	Non-Dengue (n = 100)
Day 1–2	18 (15.0%)	24 (24.0%)
Day 3–4	52 (43.3%)	36 (36.0%)
Day 5–6	38 (31.7%)	26 (26.0%)
Day ≥ 7	12 (10.0%)	14 (14.0%)
Mean ± SD (days)	4.6 ± 1.7	4.1 ± 2.0

Table 3: Platelet Count Distribution

Platelet Count Category	Dengue (n = 120)	Non-Dengue (n = 100)
>150,000 / µL (Normal)	14 (11.7%)	68 (68.0%)
100,000 – 150,000 / µL (Mild Thrombocytopenia)	23 (19.2%)	22 (22.0%)
50,000 – 100,000 / µL (Moderate)	46 (38.3%)	9 (9.0%)
<50,000 / µL (Severe)	37 (30.8%)	1 (1.0%)
Mean ± SD (cells/µL)	78,200 ± 32,500	162,600 ± 48,900

Table 4: Descriptive statistics of HFLC values

Parameter	Dengue (n = 120)	Non-dengue (n = 100)
Mean	3.82	1.14
Median	3.75	1.05
Standard Deviation (SD)	1.26	0.41
Range (min – max)	1.20 – 7.10	0.40 – 2.10
Interquartile Range (IQR)	2.95 – 4.60	0.90 – 1.35

Table 5: Frequency of elevated HFLC

HFLC category	Dengue (n=120)	Non-dengue (n=100)
Elevated HFLC (> 1.5%)	102 (85.0%)	18 (18.0%)
Normal HFLC (≤ 1.5%)	18 (15.0%)	82 (82.0%)

Table 6: Comparative statistics: HFLC in dengue vs non-dengue

Test	Statistic	p-value
Mann–Whitney U	435.5	< 0.001

Table 7: Diagnostic performance indices

Metric	Value
Sensitivity	85.0%
Specificity	82.0%
Positive Predictive Value (PPV)	85.7%
Negative Predictive Value (NPV)	81.2%
Diagnostic Accuracy	83.6%

Figure 1: ROC curve and optimal cut-off

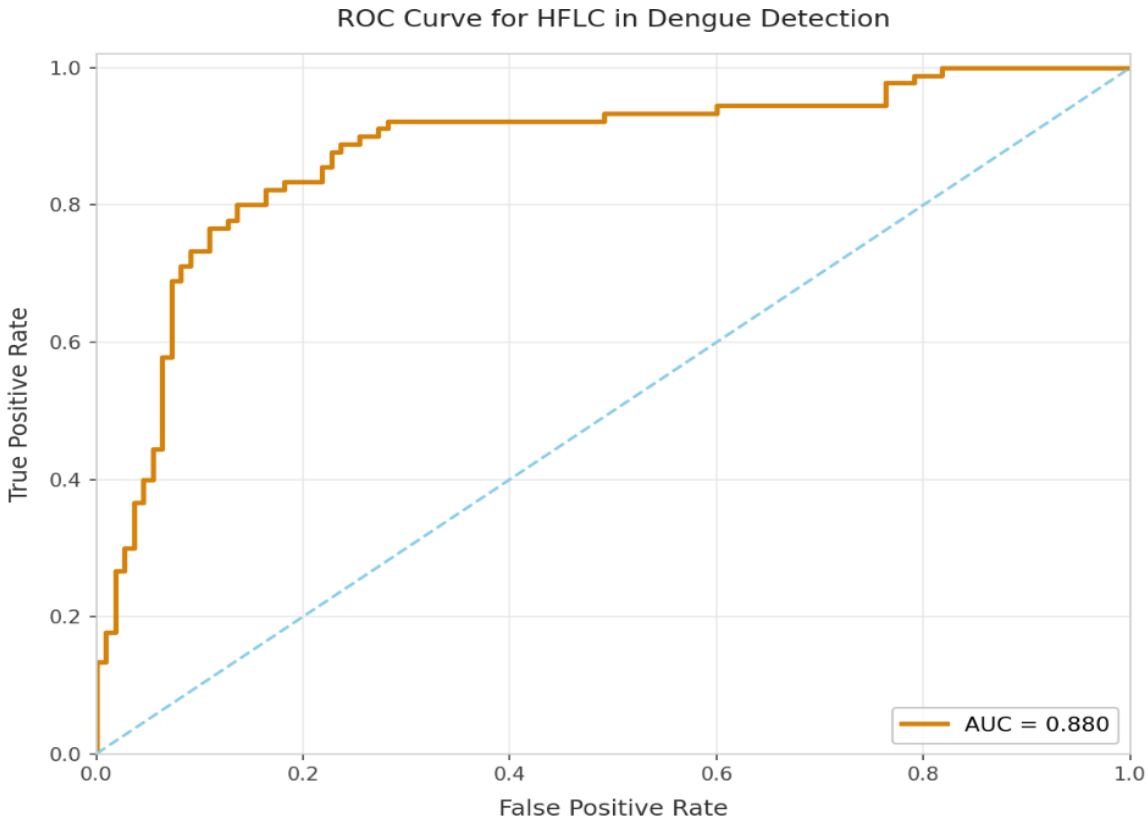
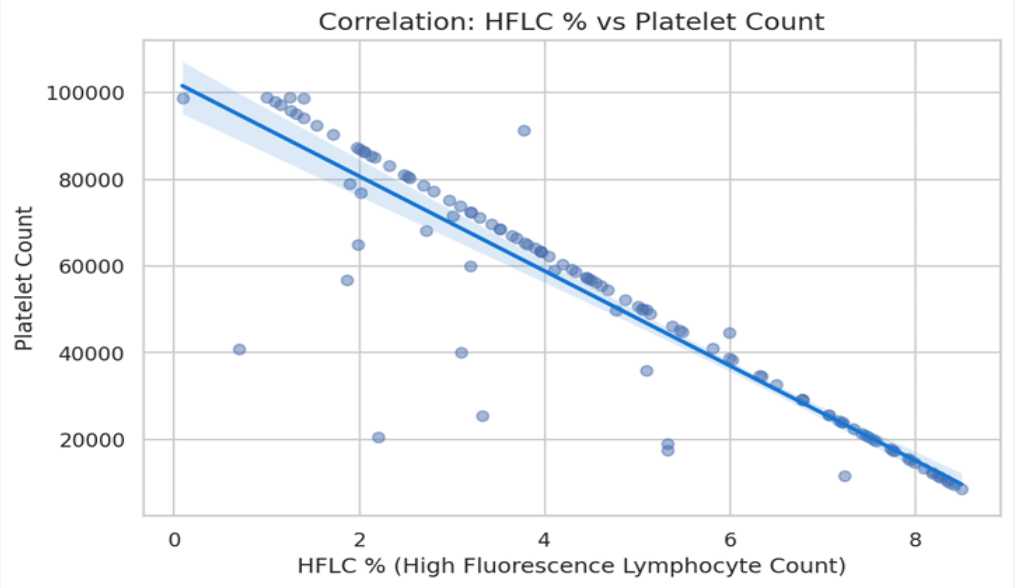


Figure 2: Correlation of HFLC% vs platelet count.



Discussion:

The present study evaluated High Fluorescent Lymphocyte Count (HFLC) as a diagnostic and prognostic indicator in dengue infection using the Sysmex XN-350 analyser. The findings revealed a markedly higher HFLC percentage in dengue patients compared with non-dengue febrile controls, with 85% of dengue cases showing HFLC >1.5%, whereas only 18% of non-dengue subjects exhibited elevated HFLC. This establishes HFLC as a sensitive marker of immune activation in dengue infection. The mean HFLC among dengue patients (3.82%) in this study aligns with the pattern reported by Alva et al. [7], who observed significantly increased HFLC in NS1-positive dengue patients with a mean value of 3.55%. These consistent results confirm HFLC as a reliable marker reflecting the activation and proliferation of reactive lymphocytes characteristic of viral infections, particularly flavivirus pathology. The current study also supports previous evidence linking HFLC with thrombocytopenia severity. A strong inverse association was evident: 68.1% of dengue cases with HFLC >3.5% belonged to the moderate-to-severe thrombocytopenia group. This relationship has been echoed in the work of Abeysuriya et al. (2022) [8] who reported that higher HFLC correlated with greater platelet decline during critical phases of dengue infection. The probable mechanism may involve heightened immunological stimulation, cytokine surge, and bone marrow suppression demonstrated across severe dengue pathology [9]. Regarding diagnostic performance, the obtained sensitivity (85.0%) and specificity (82.0%) were comparable with earlier studies: B. Raharjo and S. Hadi et al. (2019) reported slightly lower sensitivity (79%) but similar specificity (83%) [10].

In the present study, HFLC elevation was notable in patients presenting during days 3–6 of fever, suggesting that HFLC could serve as an early adjunctive marker during the critical phase of dengue infection, particularly before definitive serological confirmation. Similar temporal trends were reported by Samudi Raju et al., who demonstrated that HFLC% begins to rise from day 3 of illness, peaks around days 6–7, and subsequently declines during recovery [11]. A major strength of HFLC lies in its zero additional cost, fast turnaround time, and integration into routine CBC [12]. While other biomarkers such as NS1 antigen and dengue IgM offer high accuracy, they require additional assays and may be unavailable in primary care settings [13]. The use of HFLC in screening is especially relevant during dengue surges when early triage influences morbidity and mortality

outcomes. However, a subset (16%) of dengue patients in the present study did not demonstrate significant HFLC elevation, indicating that HFLC should be interpreted as an adjunctive rather than standalone diagnostic marker. Similar findings were reported by Ningombam et al., who demonstrated that although HFLC showed good sensitivity and specificity for dengue diagnosis, a proportion of serologically confirmed dengue cases did not exhibit elevated HFLC values [6]. Reduced immune activation during the very early phase of infection and altered immune responses in elderly or immunocompromised individuals may contribute to this variability. Additionally, 18% of non-dengue febrile patients demonstrated elevated HFLC values, likely reflecting activated lymphocytes associated with other viral infections and acute inflammatory conditions. It is noteworthy that four patients in the non-dengue febrile control group demonstrated isolated dengue IgM positivity. These patients had alternative confirmed diagnoses (hepatitis B, hepatitis C, and typhoid fever) and were considered false-positive dengue IgM reactions; therefore, they were retained in the non-dengue control group. Thus, although HFLC is sensitive to dengue-mediated immune activation, it is not entirely disease-specific and functions best as a screening and prognostic tool rather than a standalone diagnostic parameter [14]. Overall, this study contributes valuable regional evidence from South India, where dengue is endemic. The resemblance of trends across international studies further strengthens the argument for incorporating HFLC reporting into dengue management algorithms, especially in resource-limited healthcare settings. One limitation of the present study was the absence of detailed etiological classification among non-dengue febrile controls. Since different infectious conditions may independently influence lymphocyte activation and HFLC values, future studies including microbiologically characterized febrile control groups may provide a more accurate evaluation of HFLC performance across various infectious diseases.

CONCLUSION

HFLC measurement through the Sysmex XN-350 analyser provides a rapid, non-invasive, and cost-effective index of immune activation in dengue infection. HFLC was significantly elevated in serologically confirmed dengue cases and shows strong association with thrombocytopenia severity. With a sensitivity of 85% and specificity of 82%, HFLC demonstrates promising screening potential for early dengue detection, potentially before definitive serological confirmation. The prognostic implication of HFLC in predicting platelet recovery further enhances its clinical utility.

HFLC should not replace definitive dengue diagnostics; however, its integration into the initial workup of febrile patients and CBC-based triaging workflow can strengthen early recognition, facilitate prioritization of high-risk patients, and improve overall disease outcomes during dengue outbreaks. Future prospective multicentre studies with serial HFLC monitoring could help establish standardized cut-off values and pave the way for its routine use in clinical decision-making.

Authors' contributions:

Subiksha R: Investigation, writing, manuscript preparation

Vinoth Kumar. G: Supervision, Conceptualization, methodology, data curation and final editing

Diego Edwin: Data management, analysis and final editing,

Priya Banthavi. S: Co-supervision, analysis and final editing

Acknowledgement: The authors sincerely acknowledge the technicians of the Departments of Pathology and Microbiology, TSRMMCH&RC, for their valuable contribution and technical support during this study

Conflict of Interest: The authors declare that there are no conflicts of interest

Funding: This work received no specific grant from any funding agency

Declaration of Non-Use of AI: AI-assisted tools were used only for language refinement, formatting, and manuscript organization. No AI tools were used for data generation, analysis, interpretation, or scientific decision-making.

References:

1. World Health Organization. Dengue and severe dengue [Internet]. Geneva: World Health Organization; 2025 [cited 2026 May 13]. Available from: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue
2. Dash MK, Samal S, Rout S, Behera CK, Sahu MC, Das B, et al. Immunomodulation in dengue: towards deciphering dengue severity markers. Cell Commun Signal. 2024;22:451.
3. Sah R, et al. Leukopenia and thrombocytopenia in dengue patients: A cross-sectional study from tertiary hospitals in Koshi Province, Nepal. BMC Infect Dis. 2025;25:1126.
4. Jayaram A, Mathews NS, Nair SC, Geevar T, Rose W, Verghese VP, et al. High fluorescent lymphocyte cell count and scattergram patterns on the Sysmex XN series cell counters: Novel parameters in early and reliable diagnosis of dengue on a background of acute febrile illness. Int J Lab Hematol. 2021;43 Suppl 1:O156-O160.
5. Nasreen F, Khalid A, Rehan M, Shaikh A, Rehman H. High fluorescent lymphocytes in dengue infection; A navigator for hematopathologists. Prof Med J 2024;31:468–72.
6. Ningombam A, Acharya S, Sarkar A, Kumar K, Brijwal M, et al. Evaluation of sensitivity and specificity of high fluorescence lymphocyte count percentage of Sysmex XN analyzer in diagnosis of dengue. J Appl Hematol. 2022;13(3):132-8
7. Alva S, Rao PS, Pallipady A. High fluorescence lymphocyte count in dengue. Int J Health Clin Res. 2021;4(20):132-134.
8. Abeysuriya V, Choong CSH, Thilakawardana BU, et al. The atypical lymphocyte count: A novel predictive factor for severe thrombocytopenia related to dengue. Trans R Soc Trop Med Hyg. 2020;114:424-432.
9. N Chhabra G, Das B, Mishra S, Mishra B. Rapid screening of dengue fever using research parameters from new generation hematological analyzers. Int J Lab Hematol 2022;44:477–82
10. B. Raharjo and S. Hadi, "High Fluorescent Lymphocyte Count Examination in Dengue Hemorrhagic Patients With Sysmex Xn-1000 Hematology Analyzer," Indonesian Journal of Clinical Pathology and Medical Laboratory 25 (2019): 207–210,
11. Samudi Raju C, Kono M, Looi KW, Ong JX, Tan CA, Ang CS, et al. Low atypical lymphocyte score as a predictor of non-severe dengue. BMC Infect Dis. 2025;25:551.
12. Hong AV, Trung KN, Nguyen H, Viet HT. Evaluating high fluorescence lymphocyte count as a predictor of severe dengue infection. J Clin Lab Anal. 2025;39:e70083. doi:10.1002/jcla.70083
13. Tantanate C, Klinbua C. Performance evaluation of high fluorescence lymphocyte count: Comparability to atypical lymphocyte count and clinical significance. LaboratoriumsMedizin. 2018;42(6):362-368.
14. Swarna Latha P, Raj BV. Utility of newer WBC indices such as high fluorescent lymphocyte count in diagnosis and assessing the severity of dengue in the patients at a tertiary rural hospital. J Res Appl Basic Med Sci 2024;10:54–64