ISSN 0973-2063 (online) 0973-8894 (print)

Bioinformation 22(8): 4975-4980 (2026)

BIOINFORMATION
Discovery at the interface of physical and biological sciences

BIOMEDICAL INFORMATICS

www.bioinformation.net

Volume 22(8)

Research Article

Received August 1, 2026; Revised August 31, 2026; Accepted August 31, 2026, Published August 31, 2026

DOI: 10.6026/973206300224975

SJIF 2026 (Scientific Journal Impact Factor for 2026) = 8.478
2022 Impact Factor (2023 Clarivate Inc. release) is 1.9

Edited by P Kangueane

Citation: Singh *et al.* Bioinformation 22(8): 4975-4980 (2026)

Diagnostic and prognostic utility of high fluorescent lymphocyte count (HFLC) and systemic immune-inflammatory index (SII) in dengue infection using sysmex XN-1000 analyzer

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ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 22(8): 4975-4980 (2026)

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Abstract:
Early diagnosis and prognostic assessment of dengue remain challenging in resource-limited settings because conventional serological tests are often costly, time-consuming and not readily available. High Fluorescence Lymphocyte Cell percentage (HFLC%) has emerged as a potential hematological marker for dengue diagnosis, while the utility of the Systemic Immune-Inflammation Index (SII) remains unclear. Hence, 118 serologically confirmed dengue cases and 50 healthy controls were evaluated using hematological parameters obtained from the Sysmex XN-1000 analyzer. Dengue patients showed higher HFLC% with good diagnostic accuracy of 82.2% sensitivity and 72.0% specificity. Thus, data shows that HFLC% is a rapid, cost-effective and reliable parameter for early dengue detection, whereas SII has limited diagnostic and prognostic value.

Keywords: Dengue fever, high fluorescent lymphocyte count (HFLC), systemic immune-inflammatory index (SII)

Background:
Dengue is a widespread and potentially deadly mosquito-borne disease, with the WHO estimating 100–400 million annual cases, putting nearly half the world's population at risk **[1]**. Severe complications and fatalities often occur with infections by different virus serotypes **[1]**. Diagnosing dengue is challenging due to symptom overlap with other febrile illnesses and typically requires serological tests like NS1 antigen or IgM, which are costly and less accessible in developing countries **[2]**. Dengue infection induces changes in the cellular and humoral immune systems with IgM and IgG antibodies alterations in the humoral immune arm through serological tests **[3]**. Activated lymphocytes which are characteristic of many viral infections, including dengue, are known for their high fluorescence activity (high fluorescent lymphocytes, HFL), aid in early and specific dengue diagnosis and management **[4]**. Similar atypical lymphocytes are observed in other viral infections such as herpes, infectious mononucleosis, influenza, rubella and viral hepatitis **[3]**. The Sysmex XN-1000 hematology analyzer (formerly TOA Medical Electronics, Kobe, Japan) uses optical light scatter with hydrodynamic focusing and laser detection and its WDF channel provides research parameters such as high fluorescence lymphocyte percentage (HFLC%), identified as a high-fluorescence population above lymphocyte and monocyte regions **[5]**. This approach is designed to measure and analyze cell size with precision. Notably, the analyzer identifies high-fluorescence lymphocytes (HFLC), which are indicative of activated cells, such as antibody-secreting B-lymphocytes or plasma cells **[5]**. HFLC has been studied in the identification and quantification of antibody secreting cells (activated B lymphocytes) and found to have high precision and reliability in patients without systemic hematological diseases **[4]**. The Systemic Immune-Inflammation Index (SII) and Aggregate Index of Systemic Inflammation (AISI) are low-cost prognostic markers generated from regular hemograms (neutrophils, lymphocytes, platelets) that can predict dengue severity **[6]**. These indices aid in identifying patients at a higher risk of serious disease, such as thrombocytopenia, plasma leakage and hemorrhage **[7]**. Therefore, it is of interest to investigate the association between high HFLC% in serologically diagnosed dengue cases and establishing a significance of SII (systemic immune-inflammatory index) in dengue infection.

Materials and Methods:
The study followed a cross-sectional observational design and conducted in the Department of Pathology at All India Institute of Medical Sciences (AIIMS) Rajkot. The study involves a comprehensive analysis of retrospective data, focusing on cases of Dengue fever confirmed by serological examination (NS1 antigen detection) documented over an extended period from January 2024 to the April 2025. The study will cover 168 individuals, with 118 confirmed Dengue-positive cases and 50 healthy controls. Patient information, such as age, gender and clinical features, will be obtained from the hospital information system. Blood samples were examined with the Sysmex XN-1000 automated hematological analyzer. Platelet count and high fluorescence lymphocyte count (HFLC %) will be obtained from patient records stored in the hospital's information system.

Ethical considerations:
As the study relied solely on existing medical records without direct patient involvement, institutional review board approval

was not required. Instead, an ethical exemption certificate was obtained from AIIMS Rajkot.

Data collection:
Patient data, including demographic details such as age and gender, as well as clinical findings, will be systematically retrieved from the hospital information system. Additionally, laboratory investigations, particularly complete blood count (CBC) reports, will be obtained from the hospital database. These reports are generated using the Sysmex XN-1000 automated hematological analyzer, which provides detailed hematological profiles.

The systemic immune-inflammation index (SII) will be calculated as formula:

[Neutrophil count × Platelet count / Lymphocyte count]

Inclusion criteria:
Clinically suspected cases of Dengue fever confirmed by serological testing.

Exclusion criteria:
Cases with fever other than Dengue will be excluded from the study.

Statistical analysis:
Statistical calculations were performed using SPSS (version 27.0; SPSS Chicago, IL, USA). Descriptive statistics, frequency, percentage, median and range will be calculated and summarized. Based on normality test, parametric or non-parametric test will be used to compare the groups – dengue fever and controls. In order to compare between groups, the Mann–Whitney U test and the Kruskal–Wallis test were applied for continuous variables. The receiver operating characteristic (ROC) curve was generated and Youden's index was calculated to obtain the predictive cut-off value of HFLC% to suspect dengue. A $p < 0.05$ was determined to be statistically significant. Data will be gathered, organized and examined with Microsoft Excel.

Results:
Overall, the demographic profile indicates that dengue predominantly affected a relatively younger population in this cohort, averaging the age to 26.38 years. While sex distribution exhibits male predominance which remained similar across cases and controls. The Case group (n = 118) showed a higher mean HFLC level (Mean = 2.825, SD = 4.383) compared to the Control group (n = 50; Mean = 0.960, SD = 4.378) **(Table 1)**. The standard deviations are almost identical in both groups, indicating similar variability of HFLC values among cases and controls. The standard error of the mean (SEM) is lower in cases (0.4035) than controls (0.6191), reflecting the larger sample size in the case group. An independent samples *t*-test with Welch's correction was applied due to unequal variances (Levene's test, $P = 0.037$). The mean HFLC level was significantly higher in cases compared to controls (mean difference = 1.87; $t = 2.52$, df = 92.48; $P = 0.013$). The 95% confidence interval for the mean difference ranged from 0.40 to 3.33. The difference in HFLC levels between cases and controls demonstrated a moderate effect size. Hedges' g was 0.42 (95% CI: 0.09–0.76), indicating a clinically meaningful elevation of HFLC levels among cases. Similar estimates were obtained using Cohen's d (0.43) and Glass's Δ (0.43), confirming the robustness of the observed effect and clinical relevance of the observed elevation in HFLC levels among cases. A statistically significant reduction in neutrophil counts was observed in dengue cases ($p = 0.005$; mean difference = –1274.8/μL). This reflects typical dengue-associated neutropenia, likely due to marrow suppression and peripheral destruction during viral infection. The confidence interval (–2158.9 to –390.8) does not include zero, reinforcing a true difference between groups. The difference in lymphocyte counts between the two groups was not statistically significant ($p = 0.596$). Although dengue cases showed slightly higher mean lymphocyte counts, this variation was not large enough to reach significance, possibly due to the wide standard deviation observed in cases. Platelet levels were significantly lower in dengue cases compared with controls ($p = 0.012$; mean difference = –47,750/μL). This aligns with the hallmark thrombocytopenia seen in dengue infections, driven by bone marrow suppression, platelet destruction and increased peripheral consumption The confidence interval (–84,981 to –10,519) confirms a robust and clinically meaningful difference. No significant difference was found between cases and controls for the SII parameter ($p = 0.552$). Effect sizes (Cohen's, Hedges' correction and Glass's delta) were calculated to quantify the magnitude of differences between dengue cases and controls for each hematological parameter. Moderate effect sizes were observed for neutrophils and platelets, both decreased in dengue cases, confirming their strong diagnostic relevance. HFLC showed a small positive effect, but not statistically meaningful. Pearson's correlation analysis showed a weak but statistically significant negative correlation between HFLC and platelet count ($r = −0.222$, $p = 0.016$, $n = 118$), indicating that higher HFLC values were associated with lower platelet counts among 118 dengue cases. Normality testing using Kolmogorov–Smirnov and Shapiro–Wilk tests showed that HFLC values were not normally distributed across all platelet count categories ($p < 0.001$). Hence, non-parametric methods such as the Kruskal–Wallis test were applied for further analysis. Kruskal–Wallis test was applied due to non-normal distribution of HFLC. A *P*-value <0.05 was considered statistically significant. Kruskal–Wallis analysis showed a statistically significant difference in HFLC levels across platelet count categories ($\chi^2 = 6.59$, $p = 0.037$). Patients with platelet count <50,000 exhibited the highest mean rank (72.45), followed by the 50,000–100,000 group (60.46), while the >100,000 group had the lowest mean rank (53.07), indicating increasing HFLC levels with worsening thrombocytopenia. Receiver operating characteristic (ROC) analysis demonstrated that HFLC effectively discriminated cases from controls with an area under the curve (AUC) of 0.844 (95% CI: 0.777–0.910, $p < 0.001$), indicating good diagnostic accuracy. An optimal cut-off

value of ≥0.35 yielded a sensitivity of 82.2% and specificity of 72.0% **(Figure 1)**. The optimal cut-off value determined using the Youden index was ≥0.35, which provided a sensitivity of 82.2% and a specificity of 72.0%. The maximum Youden index was 0.54, reflecting a favorable balance between sensitivity and specificity. These findings suggest that HFLC may serve as a useful biomarker for differentiating cases from controls. Data analyzed using Mann–Whitney U test due to non-normal distribution of SII values. No statistically significant difference was observed between groups. The comparison of SII levels between patients with and without dengue hemorrhagic fever (DHF) was performed using the Mann–Whitney U test, as the data were not normally distributed. A total of 118 cases were analyzed, including 112 non-DHF and 6 DHF cases, with no missing data. The analysis demonstrated no statistically significant difference in SII levels between the two groups (Mann–Whitney U = 317.0, Z = –0.233, p = 0.816). The effect size was negligible (r = 0.02), indicating minimal difference between groups. These findings suggest that SII was not significantly associated with the presence of dengue hemorrhagic fever in the present study. However, the markedly small number of DHF cases may have limited the statistical power to detect a meaningful difference.

Table 1: Comparison of hematological parameters between dengue cases and controls

Parameter	Group	N	Mean	Std Deviation	*P*-value (2-tailed)
HFLC (%)	Cases	118	2.825	4.383	**0.013**
	Controls	50	0.96	4.378	
Neutrophils (/µL)	Cases	118	3,557.37	2579.56	**0.005**
	Controls	50	4,832.18	2822.28	
Lymphocytes (/µL)	Cases	118	1,837.46	2087.74	0.596
	Controls	50	1,670.80	1133.49	
Platelets (/µL)	Cases	118	140,769.49	108,514.41	**0.012**
	Controls	50	188,520.00	119,122.85	
SII(N × P/L)	Cases	118	631.13	1221.83	0.552
	Controls	50	743.16	794.39	

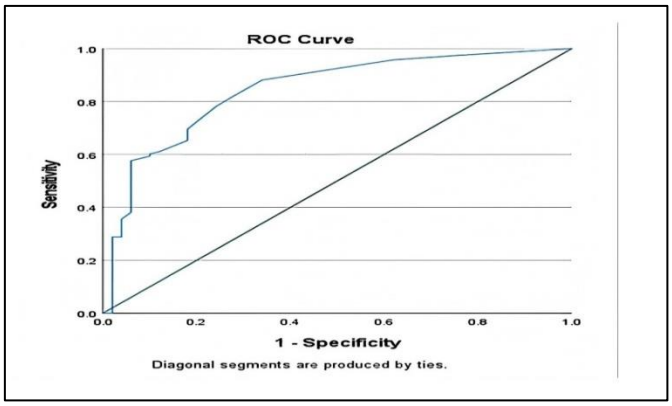

Figure 1: Receiver operating characteristic (ROC) analysis of HFLC

Discussion:

Sysmex XN-1000 automated hematological analyzer study parameters include HFLC% and count. The white blood cell differential (WDF) channel shows high fluorescence above lymphocytes and monocytes. These cells' high ribonucleic acid content causes strong fluorescence. HFLC has been shown to accurately identify and quantify antibody-secreting cells (activated B lymphocytes) in patients without systemic hematological disorders **[5, 8]**. The existence of HFLC results from the virus stimulating B lymphocytes to generate interferons against DEN antigens while also activating T lymphocytes, which then activate the complement system and produce cytokines. Activated lymphocytes can be observed in peripheral smears and identified in hematology analyzers as HFLC **[9]**. The percentage of HFLCs in the blood may therefore be proportionate to the severity of the dengue infection. The rise in HFLCs is therefore indicative of an increase in activated B lymphocytes, T lymphocytes, or monocytes and is observed during the infectious phase and decreases as the patient recovers **[10]**. As shown in our study, the case group (n = 118) showed a higher mean HFLC level (Mean = 2.825) compared to the Control group (n = 50; Mean = 0.960) which was found to be significant with p value <0.05. Dengue-related thrombocytopenia has been linked to an immune-mediated response. A larger atypical lymphocyte count is likely to indicate more immunological dysregulation, which might lead to severe thrombocytopenia. The most catastrophic manifestations of dengue, such as hemorrhage and shock, may share a same pathogenesis **[11]**. An increase in HFLC in DHF patients may indicate the activation of B-lymphocytes, T-lymphocytes, or monocytes, depending on the aetiology of the disease. Therefore, the pathophysiology of dengue hemorrhagic fever may be linked to an increase in HFLC levels **[4]**. Leucopenia and thrombocytopenia are hallmarks of dengue. Although less common, neutropenia in dengue infections has also been documented. Initiating and sustaining an immune response that destroys germs depends on neutrophils **[12, 13]**. In our study also neutrophils in dengue cases decreased statistically significantly (p = 0.005; mean difference = –1274.8/µL). This is indicative of normal dengue-associated neutropenia, which is probably brought on by peripheral damage and marrow suppression during viral infection. Since zero is excluded from the confidence interval (–2158.9 to –390.8), a real difference between the groups is supported. In our study the platelets counts were markedly reduced in dengue cases relative to controls (p = 0.012; mean difference = –47,750/µL). Higher HFLC levels were linked to decreased platelet counts in dengue cases (r = -0.222, p = 0.016, n = 118). Kruskal-analysis showed a significant variation in HFLC levels among platelet count groups (χ^2 = 6.59, p = 0.037). Patients with platelet count <50,000 had the highest mean rank (72.45), followed by 50,000–100,000 (60.46) and >100,000 (53.07), showing worsening thrombocytopenia and rise in HFLC levels. This corresponds with the characteristic thrombocytopenia observed in dengue infections, caused by bone marrow suppression, platelet destruction and heightened peripheral consumption **[14]**. The receiver operating characteristic (ROC) study revealed that

high fluorescent lymphocyte count (HFLC) was able to effectively differentiate between cases and controls, as evidenced by an area under the curve (AUC) value of 0.844 (95% confidence interval (CI): 0.777–0.910, $p < 0.001$). This indicates that HFLC possesses a high degree of diagnostic accuracy. When the cut-off value was set at a minimum of 0.35, the resulting sensitivity was 82.2%, while the specificity was 72.0%. These findings are comparable with previous study done **[15, 16]**. However no statistically significance difference found in SII in case and control group as well as between dengue fever and dengue hemorrhagic fever, which may be due to very small number of DHF cases in our study.

Conclusion:
We report serologically confirmed dengue cases showed significantly higher HFLC% than healthy controls, with 82.2% sensitivity and 72.0% specificity at a cut-off ≥0.35. HFLC% showed a negative correlation with platelet counts and increased with worsening thrombocytopenia, suggesting its association with immune activation and disease severity. Overall, HFLC% is a rapid, cost-effective parameter that can complement routine CBC for early diagnosis and risk stratification, particularly in resource-limited settings.

Limitation of study:
The study was carried out at a single center with a small sample size, which would restrict how broadly the results can be applied. Second, although there were higher HFLC levels, the study did not evaluate the use of this measure in patient treatment or clinical decision-making.

Advancement in knowledge
This study adds to the existing knowledge by showing that High Fluorescent Lymphocyte Count (HFLC %) obtained from the Sysmex XN-1000 hematology analyzer is a useful adjunctive biomarker in dengue infection. Unlike conventional serological tests, HFLC% is automatically generated during routine complete blood count analysis without additional cost or processing time, making it particularly valuable in resource-limited healthcare settings. Furthermore, HFLC% showed a significant inverse correlation with platelet count and increased progressively with worsening thrombocytopenia, suggesting its potential role as a prognostic marker for disease severity. Another important contribution of this study is the evaluation of the Systemic Immune-Inflammation Index (SII) in dengue infection. Although SII showed limited utility in the present study, it may have potential prognostic value in larger cohorts and in cases of dengue hemorrhagic fever, warranting further investigation. Overall, the study highlights HFLC% as an accessible, rapid and cost-effective parameter that may complement routine hematological evaluation for early dengue detection and risk stratification, while also clarifying the limited diagnostic significance of SII in dengue infection.

Conflict of interest: None

Research Funding: None

Article Information:
[1] Type of Article: Original Research
[2] Word Count: 3065
[3] Number of References: 16
[4] Tables: 1
[5] Figures: 1

Declarations:
We, thereby confirm, the manuscript has been read and approved by all the authors, that the requirements for the journals have been met and that each author believes that the manuscript represents honest work and this data has not been published anywhere.

Acknowledgements: None

Funding: None

Conflict of interest: None declared

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ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 22(8): 4975-4980 (2026)

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