International Journal of Advanced Research and Development
www.multistudiesjournal.com
ISSN: 2455-4030
Received: 12-04-2025, Accepted: 13-05-2025, Published: 28-05-2025
Volume 10, Issue 2, 2025, Page No. 32-36

Exploring the diagnostic value of lymphocytes in dengue fever, beyond leukopenia and thrombocytopenia

Dr. Priya Nandy
Assistant Professor, Department of Medical Laboratory Technology, School of Allied Health Sciences, Swami Vivekananda University, West Bengal, India

Abstract
Dengue fever, the "break-bone fever," remains a formidable global health challenge, particularly in tropical regions. With no specific antiviral treatment, early and accurate diagnosis is crucial. Among the key hematological red flags, leukopenia and thrombocytopenia stand out as silent yet powerful indicators of disease progression. This study delves into the dynamic hematological changes in dengue patients, aiming to unravel the intricate relationship between platelet depletion, white blood cell alterations, and disease severity. By unraveling these patterns, this work aims to improve risk classification and early identification. Data for this prospective study was collected from multiple Government hospitals across West Bengal (Dec 2023 – Jan 2024) to examine 250 febrile patients for dengue positivity using NS1 antigen and IgM ELISA tests. Hematological parameters were meticulously analyzed using advanced automated hematology systems, with statistical validation through SPSS 24.0. Among 111 confirmed dengue cases, a striking hematological signature emerged—marked leukopenia (<4000 cells/cumm) and thrombocytopenia (<150,000 platelets/µL). Patients fell into four distinct clusters: those with isolated leukopenia, thrombocytopenia, both, or neither. The presence of both conditions strongly correlated with severe dengue manifestations. Lymphocyte and neutrophil trends further underscored their pivotal role in disease evolution. Regression models and correlation analyses reinforced the predictive power of these blood markers. Dengue's hematological footprint provides a critical diagnostic edge. The interplay of leukopenia and thrombocytopenia is not just a diagnostic hallmark but a window into disease severity. Harnessing these insights can revolutionize early dengue detection and patient management. Further research is warranted to explore their full prognostic potential.

Keywords: Dengue fever, thrombocytopenia, leucopenia, sensitivity, specificity, linear regression

Introduction
Dengue fever, a mosquito-borne disease, affects ~50 million people annually, with 2.5 billion at risk [1]. Transmitted by Aedes mosquitoes, it ranges from 1mild to severe (DHF/DSS) [2] and follows febrile, critical, and convalescent phases [3, 4] Symptoms include high fever, myalgia, arthralgia, headache, and rash [4]. Severe cases may cause plasma leakage and complications. Dengue is widespread in >100 countries, with ~70% of cases in Asia (~400 million infections) [5]. Recent outbreaks, like Nepal (2022: 54,000+ cases, 67 deaths), highlight the need for better management [6]. Diagnosis relies on RT-PCR, viral isolation, and serology [7], using NS1 (day 1-9), IgM (day 4-5, peaks within two weeks), and IgG (day 10-14, lifelong) [8] to differentiate infections.

Dengue impacts the hematological system, commonly causing leukopenia and thrombocytopenia [9]. Leukopenia often presents as lymphocytopenia with atypical lymphocytes in the febrile phase, while lymphocytosis may appear during convalescence [10, 11]. Thrombocytopenia (<150,000 platelets/µL) is a key severity marker, increasing bleeding risk due to platelet destruction, reduced production, and dysfunction [12, 14]. It is also linked to complications like skin rashes and hemophagocytosis [15].

Dengue pathogenesis involves complex immune interactions, where DENV triggers T and B cell responses, cytokine release, and endothelial activation [16, 17]. While essential for clearance, excessive activation leads to severe dengue. Pro-inflammatory cytokines (TNF-alpha, IL-1) increase vascular permeability, causing plasma leakage and organ dysfunction [18]. Early lymphocytopenia shifts to atypical lymphocytosis, with T cells aiding clearance and B cells producing antibodies. The mediator theory links DHF severity to immune overactivation, with high TNF-alpha and IL-1 levels associated with shock [18]. DENV-IgG complexes (48-72% in DHF) trigger cytotoxic T-cell activation, while apoptosis in monocytes and liver cells worsens DHF [18].

Hematocrit (Hct) is vital in dengue management, reflecting plasma leakage and hemoconcentration [7]. Monitoring HCT helps assess the severity and guide fluid therapy. It strongly correlates with hemoglobin and can be calculated using Hb and MCV [19], though conditions like malaria may alter this relationship. Higher dengue prevalence in males may be linked to socio-cultural factors like increased outdoor exposure [20, 21].

This study investigates hematological changes in dengue fever, focusing on thrombocytopenia and leukopenia. Objectives include assessing their severity, specificity, and utility as diagnostic markers, along with exploring lymphocytes as predictors of complications. Findings aim to enhance diagnosis, risk stratification, and patient management for better outcomes.

Material and methods
Data for this prospective study were obtained from multiple hospitals across West Bengal from December 2023 to January 2024. It included 250 patients (aged 20-40) with a 5-day history of high-grade fever and arthralgia. Dengue NS1 and IgM ELISA tests identified 111 positive cases. Complete blood count (CBC) data were analyzed for all patients. Hematocrit (Hct), mean corpuscular volume

(MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were measured from complete blood counts of dengue patients during the acute phase of the disease.

Statistical Analysis: SPSS 24.0 was used to analyze data. Mean and standard deviation were calculated. Pearson's correlation assessed the relationship between PCV, hemoglobin, and RBC. Linear regression, including equation and curve analysis, further evaluated these associations.

Table 1: Sensitivity and specificity test (Thrombocytopenia)

	Dengue Positive	Dengue Negative
Platelet <1.5	TP	FP
Platelet >1.5	FN	TN

➡

	Dengue Positive	Dengue Negative
Platelet <1.5	14	102
Platelet >1.5	97	37

Sensitivity = TP/(TP+FN) = 14/ (14+97) = 0.126

Specificity = TN/(TN+FP) = 37/ (37+102) = 0.266

Positive predictive value (PPV) = TP/(TP+FP) = 14/ (14+102) = 0.121

Negative predictive value (NPV) = TN/(TN+FN) = 37/ (37+97) = 0.276

Table 2: Sensitivity and specificity test (Leukopenia)

	Dengue Positive	Dengue Negative
TLC <4000	TP	FP
TLC >4000	FN	TN

➡

	Dengue Positive	Dengue Negative
TLC <4000	30	30
TLC >4000	81	109

Sensitivity = TP/(TP+FN) = 30/(30+81) = 0.270

Specificity = TN/(TN+FP) = 109/(109+30) = 0.784

Positive predictive value (PPV) = TP/(TP+FP) = 30/(30+30) =0.5Negative predictive value (NPV) = TN/(TN+FN) = 109/(109+81) =0.574

Table 3: Combined parameters study (Evaluation of leukopenia combined with thrombocytopenia)

Platelets + TLC	Dengue fever Positive cases	Dengue fever Negative cases
<1.5 +<4000	8	16
<1.5+ >4000	6	86
>1.5+<4000	22	14
>1.5+ >4000	75	23
Total	111	139

Table 4: Mean and SD values between four groups of Dengue patients

Mean±SD	Dengue with leukopenia (n=22)	Dengue with thrombocytopenia (n=6)	Dengue with both leukopenia and thrombocytopenia (n=8)	Dengue with no leukopenia and thrombocytopenia (n=75)
Age	31.55±14.53	39.17±12.42	39.38±10.72	35.11±14.40
HB	12.14±1.63	12.65±1.83	11.60±2.17	12.51±2.23
TLC	2872.73±425.59	4100±1052.62	2487.50±626.64	5481.33±2501.39
Neutrophil	63.55±9.53	66.5±10.78	62.38±7.19	70.32±13.32
Lymphocyte	31.59±9.31	27.67±10.86	31.75±6.36	24.23±11.07
Monocyte	2.27±1.16	2.5±1.22	2.75±0.46	2.19±0.95
Eosinophil	2.59±1.05	3.33±1.03	3.13±0.83	2.52±0.92
Basophil	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
ESR	35.32±8.90	36.67±18.99	44.00±23.81	39.01±17.58
Thrombocyte	2.03±0.47	1.16±0.47	1.36±04	2.21±0.49

Table 5: Correlation between Thrombocyte with TLC and Lymphocyte [**Correlation is negatively significant ($p<0.05$) in all groups]

Correlation	Dengue patient (n = 111)	R^2 value	Dependant variable (y) = Lymphocyte Independent variable (x) = TLC and Thrombocyte
Lymphocyte with TLC	-0.485**	0.235	y=7.52E3-1.08E2*x
Lymphocyte with Thrombocyte	-0.215**	0.046	y=35.1-4.2*x

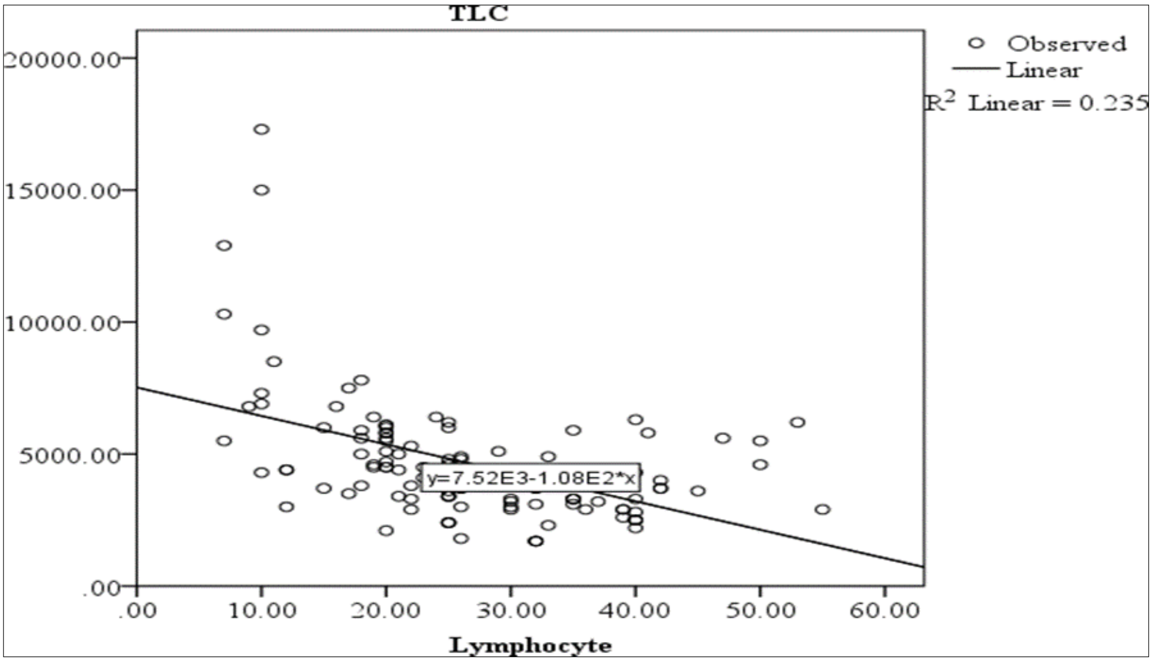

Fig 1: Linear curve between TLC and Lymphocyte

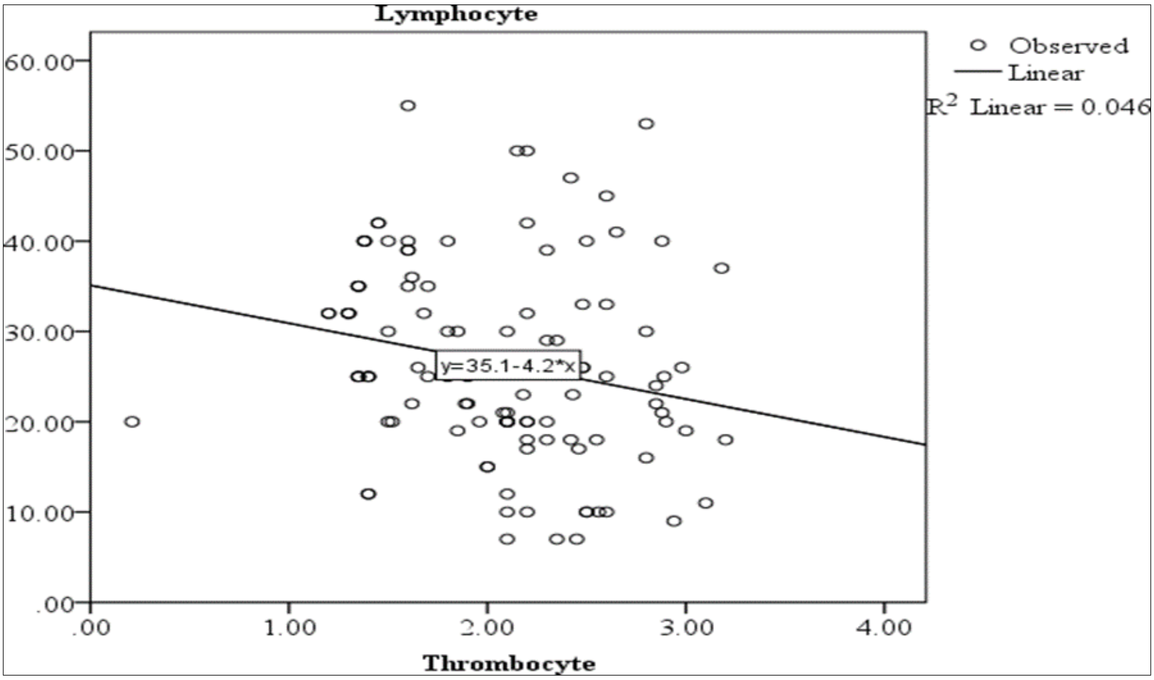

Fig 2: Linear curve between Lymphocyte and Thrombocyte

Discussion

This study evaluates leukocyte sensitivity and specificity in dengue diagnosis. Hematological data, including leukopenia (<4000 cells/cumm) and thrombocytopenia (<1.5 lac/cumm), were analyzed. These markers help differentiate DHF and assess disease severity, even in cases not meeting WHO DHF criteria [22, 24]. Sensitivity and specificity must balance false positives and negatives, as no single test confirms or rules out disease [3, 25]. PPV minimizes false positives, preventing overtreatment [26], while NPV reduces false negatives, crucial for severe or communicable diseases [27]. This study analyzed 111 dengue patients aged 30-40 years. Descriptive statistics (mean, SD) were used to assess hematological trends, including hemoglobin, leukocyte count, and thrombocytes (Table 4). Based on CBC reports, patients were categorized into four groups: (i) Dengue with leukopenia, (ii) Dengue with thrombocytopenia, (iii) Dengue with both, and (iv) Dengue without leukopenia or thrombocytopenia, highlighting the disease's dynamic hematological impact.

Dengue fever can lower hemoglobin levels, especially in Group 3, despite thrombocytopenia and leukopenia not directly affecting hemoglobin. The dengue virus infects and suppresses bone marrow, reducing RBC production and causing anemia. Increased immune-mediated RBC destruction and altered vascular function further decrease hemoglobin [28]. Plasma leakage dilutes RBCs, creating the illusion of lower hemoglobin levels [29]. Though distinct conditions, thrombocytopenia, and leukopenia often accompany hemoglobin reduction due to dengue’s impact on bone marrow and RBCs. Group 1 (dengue with leukopenia) experiences temporary bone marrow suppression, reducing total leukocyte count. Leukopenia, also seen in conditions like multiple myeloma and aplastic anemia, affects all WBC types. Group 4 (dengue without leukopenia or thrombocytopenia) shows higher leukocyte counts, while Group 3 (both leukopenia and thrombocytopenia) has the lowest values. Leukopenia may result from DENV-induced myeloid progenitor cell destruction, while thrombocytopenia arises from viral damage to megakaryocytes or peripheral platelets, reducing platelet production. Leukopenia is a key marker of dengue's critical phase, with leukocyte counts dropping 4000 cells/mm³ [9] below. Neutrophils decline more than lymphocytes [30], signaling impending plasma leakage. As essential immune defenders, neutrophils help combat infections. Group 3 (Dengue with leukopenia and thrombocytopenia) shows low neutrophil counts due to viral-induced apoptosis. Neutropenia may help contain dengue by trapping infected platelets [31]. Group 4 has high neutrophil counts, reflecting the body's early immune response or high TLC. Neutrophils play a key role in defense, responding to inflammation and tissue damage. Cytokines released during infection stimulate bone marrow to produce more neutrophils, increasing their levels in the blood. Group 3 shows high lymphocyte counts due to an immune response with increased atypical lymphocytes [32]. Dengue infection triggers immune activation, leading to an inverted CD4/CD8 ratio, excessive cytokine production, and atypical T cells [33]. Group 4's low lymphocyte count results from lymphocyte migration to lymphoid tissues and infection sites. Dengue virus can also suppress bone marrow, reducing lymphocyte production [34]. Additionally, direct infection and destruction of T and B cells further lower their levels in the blood. Monocytes are reduced in Groups 1 and 3 due to bone marrow suppression and active tissue defense. Dengue virus can lower monocyte production, but its impact varies [35]. Eosinophil levels decrease in all groups as the immune system prioritizes other WBCs [36]. Basophil counts remain unchanged across groups. Groups 2 and 4 show lower platelet counts due to bone marrow suppression, immune-mediated destruction, and increased vascular leakage caused by dengue.

Correlation measures how two variables change together but don't imply causation. It ranges from -1 (strongly opposed) to +1 (strongly aligned), with 0 indicating no link [37]. Negative correlations were found between lymphocytes and TLC ($r = -0.485$) and lymphocytes and thrombocytes ($r = -0.215$), indicating a detrimental relationship. Regression analysis assessed the extent of influence between parameters [38]. Regression analysis, like a detective story, identifies relationships between a dependent variable (culprit) and independent variables (clues). It uses the R-squared (R^2) value, ranging from 0 to 1, to measure how well the model explains variations in the dependent variable. A value of 0 means no explanation, while 1 indicates a perfect fit. Higher R^2 values suggest a better model fit [39]. Linear regression models the relationship between a dependent variable (Y) and independent variables (X). Here, lymphocyte count is dependent, while TLC and thrombocyte count are independent. It helps determine how these factors linearly influence lymphocytes, providing a trend through a best-fit line [39, 40].

This study examined white blood cell changes in dengue patients, highlighting leukopenia and thrombocytopenia as key severity indicators. Leukopenia signals disease progression, while thrombocytopenia reflects severity. Inverse relationships were found between lymphocyte count and both TLC and platelet count, suggesting opposing trends. The study also suggests lymphocytes may help predict dengue, warranting further research.

Conclusion

Leukopenia and thrombocytopenia are valuable markers for diagnosing and gauging dengue severity, while lymphocyte count may also be linked to disease progression. Further research is needed to confirm these findings.

References

1. Dengue virus: A global human threat: Review of literature - PMC [Internet]. [cited 202427]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4784057/
2. Dengue and severe dengue [Internet]. [cited 2024 Jun 27]. Available from: https://www.who.int/news-room/fact-sheets/detail/dengue-and-severe-dengue
3. Trevethan R. Sensitivity, Specificity, and Predictive Values: Foundations, Pliabilities, and Pitfalls in Research and Practice. Front Public Health,2017:20:5:307.
4. Dengue Fever - StatPearls - NCBI Bookshelf [Internet]. [cited 2024 Jun 27]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK430732/
5. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, *et al*. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet,2022:399(10325):629–55.
6. Banerjee I, Robinson J, Sathian B. Dengue Dilemma in Nepal. Nepal J Epidemiol,2022:12(4):1235–7.
7. Evaluation of diagnostic tests: dengue | Nature Reviews Microbiology [Internet]. [cited 2024 Jun 27]. Available from: https://www.nature.com/articles/nrmicro2459
8. LABORATORY DIAGNOSIS AND DIAGNOSTIC TESTS - Dengue - NCBI Bookshelf [Internet]. [cited 2024 Jun 27]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK143156/
9. Ananda Rao A, U RR, Gosavi S, Menon S. Dengue Fever: Prognostic Insights from a Complete Blood Count. Cureus. 12(11): e11594.
10. Clarice CSH, Abeysuriya V, de Mel S, Uvindu Thilakawardana B, de Mel P, de Mel C, *et al*. Atypical lymphocyte count correlates with the severity of dengue infection. PLoS One,2019:14(5):0215061.
    Lymphocytosis - StatPearls - NCBI Bookshelf [Internet]. [cited 2024 Jun 27]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK549819/
11. Thrombocytopenia - StatPearls - NCBI Bookshelf [Internet]. [cited 2024 Jun 27]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK542208/
12. Platelet Disorders - Thrombocytopenia | NHLBI, NIH [Internet]. [cited 2024 Jun 27]. Available from: https://www.nhlbi.nih.gov/health/thrombocytopenia
13. Mitchell O, Feldman DM, Diakow M, Sigal SH. The pathophysiology of thrombocytopenia in chronic liver disease. Hepat Med,2016:8:39–50.
14. Santoshi RK, Patel R, Patel NS, Bansro V, Chhabra G. A Comprehensive Review of Thrombocytopenia with a

Spotlight on Intensive Care Patients. Cureus. 14(8):27718.
15. Spiropoulou CF, Srikiatkhachorn A. The role of endothelial activation in dengue hemorrhagic fever and hantavirus pulmonary syndrome. Virulence,2013:4(6):525–36.
16. King CA, Wegman AD, Endy TP. Mobilization and Activation of the Innate Immune Response to Dengue Virus. Front Cell Infect Microbiol,2020:10:574417.
17. Immune Mediated Cytokine Storm and Its Role in Severe Dengue - PMC [Internet]. [cited 2024 Jun 27]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5496927/
18. Billett HH. Hemoglobin and Hematocrit. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations [Internet]. 3rd ed. Boston: Butterworths; 1990 [cited 2024 Jun 27]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK259/
19. The status of men's health in Asia - ScienceDirect [Internet]. [cited 2024 Jun 27]. Available from: https://www.sciencedirect.com/science/article/pii/S0091743514002977
20. Edwards ES, Sackett SC. Psychosocial Variables Related to Why Women are Less Active than Men and Related Health Implications. Clin Med Insights Womens Health. 2016 Jul 4;9(Suppl 1):47–56.
21. Das S, Abreu C, Harris M, Shrader J, Sarvepalli S. Severe Thrombocytopenia Associated with Dengue Fever: An Evidence-Based Approach to Management of Thrombocytopenia. Case Rep Hematol. 2022:2022:3358325.
22. White Blood Count (WBC): How to Read the Test Results | Ada [Internet]. [cited 2024 Jun 27]. Available from: https://ada.com/white-blood-cell-count/
23. The Etiology and Management of Leukopenia - PMC [Internet]. [cited 2024 Jun 27]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2154209/
24. SpPin and SnNout [Internet]. [cited 2024 Jun 27]. Available from: https://www.cebm.ox.ac.uk/resources/ebm-tools/sppin-and-snnout
25. Parikh R, Mathai A, Parikh S, Chandra Sekhar G, Thomas R. Understanding and using sensitivity, specificity and predictive values. Indian J Ophthalmol,2008:56(1):45–50.
26. Frontiers | Sensitivity, Specificity, and Predictive Values: Foundations, Pliabilities, and Pitfalls in Research and Practice [Internet]. [cited 2024 Jun 27]. Available from: https://www.frontiersin.org/journals/public-health/articles/10.3389/fpubh.2017.00307/full
27. Dengue Infection - Recent Advances in Disease Pathogenesis in the Era of COVID-19 - PMC [Internet]. [cited 2024 Jun 27]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9299105/
28. Acute Anemia - StatPearls - NCBI Bookshelf [Internet]. [cited 2024 Jun 27]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537232/
29. What are Neutrophils? Neutrophil Count Explained [Internet]. [cited 2024 Jun 27]. Available from: https://my.clevelandclinic.org/health/body/22313-neutrophils
30. Neutrophil Apoptosis During Viral Infections - PMC [Internet]. [cited 2024 Jun 27]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2703832/
31. Reactive Lymphocyte - an overview | ScienceDirect Topics [Internet]. [cited 2024 Jun 27]. Available from: https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/reactive-lymphocyte
32. Dengue virus infection: current concepts in immune mechanisms and lessons from murine models - PMC [Internet]. [cited 2024 Jun 27]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3904235/
33. Infection of bone marrow cells by dengue virus in *vivo* - PMC [Internet]. [cited 2024 Jun 27]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3415316/
34. Histology, Monocytes - StatPearls - NCBI Bookshelf [Internet]. [cited 2024 Jun 27]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557618/
35. Immune-Mediated Pathogenesis in Dengue Virus Infection - PMC [Internet]. [cited 2024 Jun 27]. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9699586/
36. Correlation Coefficients: Positive, Negative, and Zero [Internet]. [cited 2024 Jun 27]. Available from: https://www.investopedia.com/ask/answers/032515/what-does-it-mean-if-correlation-coefficient-positive-negative-or-zero.asp
37. Simplilearn.com [Internet]. 2020 [cited 2024 Jun 27]. What is Regression Analysis? Types | Examples | Uses. Available from: https://www.simplilearn.com/tutorials/excel-tutorial/regression-analysis
38. Nandy P, Chatterjee P, Bandyopadhyay A. A linear regression between maximum voluntary contraction and motor unit activity of respiratory muscles during forceful and normal respiration of male athletes using surface electromyography,2023:10:88–95.
39. Chatterjee P, Nandy P, Bandyopadhyay A. Formulation of regression equation on the basis of endurance time and fatigue indices in biceps brachii by using surface-EMG of 14-19 years aged trained male volleyball players. Int J Phys Educ Sports Health,2021:8(6):06–10.