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Correlation between Total Monocyte, Lymphocyte and Basophil Counts, as well as Monocyte-Lymphocyte Ratio with Hematocrit as Indicators of Plasma Leakage in Dengue Virus Infection

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ABSTRACT

Background: Severe dengue virus (DVI) infection is characterized by plasma leakage (PL) due to the release of vasoactive cytokines by immunocytes such as monocytes, lymphocytes, and basophils. The response to Dengue Virus (DENV) also affects the kinetics of monocytes, lymphocytes, and basophils, thus changing the examination results of leukocyte types and monocyte-lymphocyte ratio (MLR). MLR has been used as a biomarker and outcome predictor in various diseases but not to predict PL in DVI. *Objectives: To see the correlation of monocytes, lymphocytes, basophils, and MLR with hematocrit (Hct) which is a PL indicator. Methods: Correlative analytical research with a cross-sectional approach was conducted on 71 pediatric DVI patients at PKU Muhammadiyah Sampangan Hospital, Surakarta, in February-April 2024. The diagnosis of DVI was confirmed by a pediatrician according to WHO 2009 criteria. DVI patients who had a history of allergies, worms, malignancies, and DVI with secondary infections were excluded from the study. Absolute monocyte (AMC), lymphocyte (ALC), and basophil (ABC) count values , as well as MLR and Hct, were obtained from the results of a complete blood examination using a hematology analyzer. The Spearman Rho's correlation test was used to determine the relationship between variables with values of $p<0.05$ and $r>0.4$ considered significant. Results: research obtained AMC=median 313 (IQR 192) mm-3, ALC= median 1225 (IQR 1039) mm-3, ABC=0 mm-3, MLR=0.22 (0.23). The results of the correlation analysis of AMC, ALC, and MLR with Hct are AMC ($r=0.01$, $p=0.918$), ALC ($r=0.351$, $p=0.003$), and MLR ($r=0.41$, $p<0.001$), while the correlation between ABC and Hct could not be done due to no data variation. Conclusion: There is a significant correlation between MLR and Hct, but not with AMC, ALC, and ABC and Hct. MLR can potentially predict plasma leakage in patients with dengue virus infection.*

KEYWORDS:
Dengue Virus Infection, Plasma Leakage, Monocyte-lymphocyte ratio, Hematology Profile

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INTRODUCTION

Dengue virus infection is an infection caused by DENV types 1-4. The World Health Organization (WHO) reports that 3.5 billion people are living in DVI endemic areas, including Indonesia. DVI cases in Indonesia in 2022 reached 142,294 people with the deaths of 1,227 people, while until February 2023, there were 710 cases reported with the deaths of 6 people. These figures are expected to continue to increase due to high population growth rates, inadequate water supplies, poor sanitation and hygiene, increased global trade and tourism, global warming, and the development of hyperendemicity in urban areas (1,2).

Dengue virus infection does not always cause symptoms. Of all people infected with DENV, only 40% have mild to severe symptoms. Mild symptoms of DVI are flu-like fever, while severe symptoms of DVI are mainly bleeding and shock due to plasma leakage (3).

Plasma leakage in DVI causes the transfer of plasma fluid from the intravascular to the tissue, which increases Hct levels (4). The increase in Hct occurred on the third day due to PL to various degrees (5).

The immune response to DENV causes plasma leakage. The immune response to DENV is carried out by various immunocytes, such as monocytes, lymphocytes, and basophil cells (6,7). Monocytes migrate to inflammatory tissue and transform into dendritic cells (8). Monocyte-derived dendritic cells (mo-DC) are infected with DENV. Migration of infected mo-DCs triggers viremia (9). Infected monocytes can undergo cell death through apoptosis and pyroptosis (3). Monocytes in DVI, apart from being targets of infection, also act as effectors against DENV by producing tumor necrosis factor α (TNF-α) and interleukin 1β (IL-1β) as well as recognizing and processing DENV antigens to be presented to T cells. Tumor necrosis factor α and IL -1β trigger PL, while DENV recognition triggers an adaptive immune response that activates TCD4+, CD8+, and B cells. T cells produce macrophage inflammatory protein 1β (MIP1β), TNF-α, and interferon γ (IFN-γ). MIP1β increases the synthesis of TNF-α and IFN-γ, causing PL. Activated B cells secrete IL-6 and TNF-α. Both cytokines are involved in PL events (6,7,10,11).

The PL process is thought to be enhanced by the response of basophil cells to DENV, considering that there are reports that basophil cells also play a role in viral infections (12). Basophilia, reflected in absolute basophil count (ABC), and pseudo basophilia are common in DVI (7,13). However, DENV exposure to basophil cells increased the production of IL-1β and IL-6. These cytokines are known to be involved in the pathogenesis of PL (14).

DENV infection triggers immunocyte kinetics (15). Monocytes undergo migration from the bone marrow to the blood (16,17). Intermediate monocytes increase in number in the blood, while classical monocytes decrease in DVI (18). Some monocytes remain in the blood, while others enter the infected tissue (8). $TCD4^+$ and $CD8^+$ cells migrate to tissues, while activated B cells are found in the blood as plasma cell and atypical lymphocyte cells (plasmacytoid lymphocyte or lymphocytoid plasma cell) (19–21). Migration of monocytes from the bone marrow tends to increase the number of monocytes, while migration to tissue has the opposite effect. It affects AMC (22). Migration of $TCD4^+$ and $CD8^+$ cells tend to decrease the number of lymphocytes in the blood, while atypical lymphocyte cells tend to increase. This condition causes changes in ALC. Monocyte kinetics and lymphocyte kinetics as well as changes in lymphocyte composition in the blood of DVI patients will change the MLR (23,24).

Previous studies have been done regarding the relationship between immunocytes and hematocrit.

However, the relationship between immunocytes, such as monocytes and lymphocytes, with hematocrit is primarily indirect. In general, previous research reported that there was a relationship between monocytes and lymphocytes and the degree of severity, assuming that the degree of severity of DVI was identical to PL, which was characterized by an increase in Hct (25–28). It is not appropriate because the WHO diagnostic criteria for DVI are underrated when estimating PL (4). Meanwhile, previous research regarding the correlation of basophils with hematocrit in DVI has never been carried out.

A number of studies report the use of Neutrophil-lymphocyte ratio (NLR) as a predictor or indicator of severe DVI. However, the results reported regarding the NLR cutoff as a predictor are not uniform and require serial NLR examinations (29–31). Other methods need to be developed to overcome this problem. One potential method is MLR. Monocyte Lymphocyte Ratio is a cheap and easy biomarker of infection and inflammation (32). Monocyte Lymphocyte Ratio has been used as a biomarker and predictor of outcomes in various diseases such as cancer (33), cancer metastases (34), heart disease (35), chronic kidney failure (36), retinal occlusion (37), endophthalmitis (38), osteoarthritis (39), gout arthritis (39), Corona Virus Disease-19 (40), Influenza (41), malaria (42), and pneumonia (43). For DVI cases, there have been no reports of the use of MLR as a predictor of PL.

Research on the correlation between lymphocytes, monocytes, and PL was based on the assumption that in DVI, PL was found along with the severity of the degree of DVI. Meanwhile, previous research regarding the correlation of basophils and MLR with hematocrit in DVI has never been carried out. Based on this background, research that correlates AMC, ALC, ABC, and MLR with Hct necessarily needs to be carried out.

METHODS

The research uses a correlative observational analytical research type with a cross-sectional design. The research was conducted at PKU Muhammadiyah Sampangan Hospital, Pasar Kliwon, Surakarta, from 1 February – 31 April 2024. The target population was all pediatric DVI patients, while the actual population was pediatric DVI patients treated at PKU Sampangan Hospital, Surakarta. Inclusion criteria were a diagnosis of DVI by a pediatrician according to WHO 2009 criteria, suffered from DVI on days 3 – 5, and age < 15 years. The exclusion criteria were having another infectious disease established at the time of the study, a history of allergies, a history of malignancy, a history of worm infections, never having received a dengue vaccine, and not being willing to be involved in the research.

The research sample was 71 patients. The sample size was calculated using the sample size formula for research on correlative analysis of numerical data. Sampling was carried out using consecutive non-probability sampling. The research independent variables are AMC, ALC, ABC, and MLR, while the dependent variable is Hct.

The research procedure follows: Blood from patients who meet the restriction criteria is taken using an aseptic phlebotomy technique. Blood was collected in a blood collection tube with K_3EDTA anticoagulant (OneMed, Indonesia). The blood was taken to the Clinical Pathology Laboratory at PKU Muhammadiyah Hospital Sampangan Surakarta for a complete blood test using a Mindray BC-5000 Hematology analyzer (Shenzhen Mindray Bio-Medical Electronics Co., Ltd, China). The results of the examination are used to calculate AMC, ALC, ABC, MLR, and Hct with the formula: AMC = number of leukocytes x % monocytes, ALC = number of leukocytes x % lymphocytes, ABC = number of leukocytes x % basophils, while MLR = divide the number of AMC by ALC.

The data obtained were processed using Jeffreys's Amazing Statistics Program software version 0.16.1.0 (Amsterdam University). A p-value <0.05 with r>0.4 was considered significant.

The research has received approval from the Health Research Ethics Committee of the Faculty of Medicine, Universitas Muhammadiyah Surakarta, with number 5189A/B.2/KEPK-FKUMS/II/2024.

RESULT AND DISCUSSION

The research was conducted at PKU Muhammadiyah Hospital Sampangan Surakarta from 1 February to 31 March 2024. It involved 71 research subjects, whose characteristics are presented in Table 1.

Table 1. Subject Characteristics

Subject Characteristics	Result
Sex	
Male (%)	48
Female (%)	52
Age *	7 (7,5)
Temperature (°C) **	37,68 (0,5)
Weight (kg) *	23,1 (23,75)
Bleeding Manifestation (%)	39,45
Epistaksis (%)	15,49
Peteki (%)	28,12
Abdominal pain (%)	52,11
Muscle pain (%)	28,17
Disease Severity	
Dengue without WS (%)	32,39
Dengue with WS (%)	57,75
Severe Dengue (%)	9,86
Hematocrit (%) *	38,3 (7,2)
Leucocyte (mm^{-3})	3290 (1575)
AMC (mm^{-3}) *	313 (192)
Monocytopenia (%)	19,72
Normal (%)	74,65
Monocytosis (%)	5,63
ALC (mm^{-3}) *	1225 (1039)
Limfocytopenia (%)	63,38
Normal (%)	35,21
Limfocytosis (%)	1,41
ABC (mm^{-3}) *	0
MLR (mm^{-3}) *	0,22 (0,23)

Note: (°C) degrees Celsius, (kg) kilograms, (/mm3) per cubic mm, * data presented as median (IQR), categorical data presented as percentages, ** data presented as mean (SD), VS-MPR (Vovk-Sellke Maximum p-Ratio), WS (warning sign).

The results of the correlation test between research variables are presented in table 2.

Table 2. Spearman's Rho correlation test results

Corelation	r-value	p-value	VS-MPR
AMC - Hct	0,01[a]	0,918*	1
ALC - Hct	0,351[b]	0,003**	23,03
ABC – Hct	Na	Na	Na
MLR - Hct	0,41[c]	<0,001**	117,89

Note: [a] is not correlated, [b] is weakly correlated, [c] is moderately correlated, Na = Not available, *not statistically significant, **statistically significant.

Hematocrit is an essential parameter in DVI because Hct is one of the benchmarks for the presence of PL (4). Table 1 shows that the median Hct level of research subjects were 38.3%. Several previous studies reported that Hct levels in DVI patients were generally <40% (44–48), although there are also research results that reported >40% (49). The exciting thing is that whatever the Hct level reported, DVI patients admitted to the hospital generally have experienced PL (5). Hct levels >45% indicate the presence of PL (50), although it does not mean that a normal Hct in a DVI patient indicates the absence of PL. The underlying reason is that bleeding in DVI patients causes a decrease in Hct despite PL (51).

The number of leukocytes from the research was 3290/mm^3. This number is below the reference value, so it is said that the research subjects experienced leukopenia (52). This study's results align with previous research (53–55). Leukopenia is common in DVI (51) and in most other viral infections (56,57). It is due to the suppression of hematopoiesis in the bone marrow (58). It was reported that leukopenia began on day 2.5 (44) and further stated that leukopenia is a predictor of severe DVI in children (59).

Table 1 shows that the median AMC was 313 (192)/mm^3. These results indicate that the amount of AMC is average because it is between 200-800/mm^3 (60). Comparison of AMC results with AMC reference range shows that 74.65% of AMC are categorized as normal, 19.72% have monocytopenia, and only 5.64% have monocytosis. The results of this research are in accordance with previous studies which reported that monocytes in DVI patients were within the normal range (53,61,62). However, previous research results also reported monocytosis (28,63) and monocytopenia (55). The cause of the difference in results is the difference in data collection time and degree of severity. Patients with severe DVI and collection time during the critical period tend to produce monocytosis (63), while non-severe DVI (dengue without WS and with WS) tend to produce normal monocytes (53,61,62). The normal monocyte results in this study were due to two reasons. First, the loss of monocytes from the circulation is offset by a supply from the bone marrow. Monocytes migrate from the circulation to sites experiencing inflammation due to DVI (8). Many monocytes undergoing apoptosis and pyroptosis due to infection with DENV or exposure to TNFα (3,64). Both of these reduce the number of monocytes, but high levels of CCL2 in the DVI cause the release of monocytes from the bone marrow into the circulation (65–69). Second, there is a change in the percentage of monocyte subsets in the blood. In DVI, there was an increase in the intermediate monocyte subset $CD14^{++}$ $CD16^{+}$ but a decrease in the classical monocyte subset $CD14^{++}CD16^{-}$ (18,70).

Other research states that in DVI, there is an increase in classical and intermediate monocytes but a decrease in non-classical monocytes (71). Both types of monocyte subset changes caused the number of AMCs to remain unchanged.

Based on table 2, it was found that AMC was not correlated with Hct (r = 0.01, p = 0.918). The VS-MPR value = 1, it indicates that there is no correlation between AMC and Hct. This is caused by intermediate and classical monocytes produce cytokines that trigger PL (72), whereas non-classical monocytes produce cytokines that cause vasoconstriction (71). Increases in intermediate monocytes and decreases in non-classical monocytes lead to increases in cytokines that promote PL but decrease levels of cytokines that protect endothelial integrity (71), causing an increase in vascular permeability that triggers PL, characterized by an increase in Hct (4). The number of AMCs does not change with an increase in Hct due to cytokines produced by monocytes, so there is no direct correlation between AMC and Hct. Based on this, AMC does not have the potential to be used as a PL indicator.

Lymphocytes are essential immunocytes in the immune response to DENV (73). Table 1 showed that the median ALC was 1225 (1039)/mm^3. In detail, the lymphocytes from the research results can be categorized into three categories, namely 63.38% lymphopenia, 35.21% normal, and 1.41% lymphocytosis. Based on the ALC reference figures, which range from 1,500-7,000/mm^3 (74), the study results are included in the lymphopenia category. The detailed lymphocyte categories confirmed the conclusion of lymphopenia, which showed that 63.38% of patients had lymphopenia. Lymphopenia occurs due to infection of hematopoietic progenitor cells by DENV, activation of T cells, infection of bone marrow stroma by DENV, and migration of lymphocytes to infected tissue (75,76). The results of this study are in line with previous research (61), although there are research results that differ. Setiawan et al. (2024) reported that lymphocytosis occurred in DVI patients (77), while Rai et al. (2019) reported that the number of lymphocytes in DVI patients was normal (62). This difference is due to the restriction criteria and DVI classification (61,62,77).

The results of the statistical analysis of the correlation between ALC and Hct showed a weak correlation (r=0.351) that was statistically significant (p=0.003). The VS-MPR value = 23.03 supports statistical significance, it indicates that there is a correlation between ALC and Hct.

Lymphopenia has been used as a predictor of DVI diagnosis (78) and even a predictor of DVI severity (75). However, the research showed a mild correlation between ALC and Hct (r=-0.351, p=0.003, and VS-MPR = 23.03). These results are not like the criteria established to determine the

existence of a correlation between ALC and Hct, so there is no correlation between ALC and Hct. Lymphocytes play a role in the immune response to DENV (73). Cytokines produced due to lymphocyte activation play a role in PL (6,7,10,11). Based on this, it is strongly suspected that the parameters that correlate well with Hct are cytokines produced by lymphocytes and not the lymphocytes themselves. From the research results, it can be concluded that lymphocytes independently are not good enough to be used as predictors of PL, even though it is said that lymphocytes are a predictor of the severity of DVI. The research results of Rodrigo et al. (2021) support this statement(4). According to their research, the WHO DVI criteria are overrated for detecting the severity of DVI but underrated for detecting PL (4).

The absolute basophil cell count as a result of research findings is 0/mm^3. The reference value for ABC is 0-150/mm^3 (79). These results indicate that the ABC found is still within the range of the ABC reference value. The number of ABC in all samples was 0, causing no variation in the results of measuring the number of basophils, so the correlation between ABC and Hct could not be carried out. However, logically, the absence of basophils in the circulation indicates no correlation between ABC and Hct. The results of this study are different from previous research by Malathesa et al. in 2014, which stated that 52.9% of DVI patients experienced basophilia (7), but are in line with the results of research by Manuel et al. in 2012, which reported that high basophils are pseudo basophilia not due to increase in absolute basophils. It occurs because atypical lymphocyte cells are resistant to lysis, so the hematology analyzer machine counts them as basophils (13). Another explanation for why there is no increase in basophils is that there is a shift in the count of leukocyte types to the right in dengue virus infection so that mononuclear levels are relatively more abundant than polymorphonuclear basophils and eosinophils (80,81). In addition, basophil cells can be infected by DENV. DENV infection of basophils triggers the autophagy process in basophil cells. DENV needs the autophagy process to support its life cycle. The effect of autophagy that occurs in basophil cells is basophil cell apoptosis (82). Basophil cell death causes a decrease in the number of basophil cells. Since the number of basophil cells in the circulation is only 0-0.5%, basophil cell apoptosis causes the number of basophil cells to decrease further. It may not be detected on CBC examination using a hematology analyzer.

Previous findings indicate that basophil cells are involved in the immune response to DENV. It is possible because there is evidence that high levels of anaphylatoxins (C3a and C5a) and immunoglobulin E (IgE) are found in DVI patients. Complement and IgE are known to be factors that

activate basophil cells. Activated basophil cells release vasoactive substances such as leukotrienes and chymase, which cause PL. DENV-infected basophil cells secrete vasoactive cytokines such as IL-1β and IL-6, which are known to induce PL. In addition, active basophil cells release histamine. High histamine levels in the blood and urine of DVI patients correlate with the degree of DVI disease. It further strengthens the fact that basophil cells are involved in DVI immunopathogenesis (12,82–84). However, the absence of basophil cells in the blood of DVI patients in this study means that basophil cells still do not have the potential to be used as a PL indicator.

Table 1 shows that the median MLR value is 0.22 (0.23). Until now, there is no definite reference value for MLR, but referring to the normal reference values of AMC and ALC, an estimate of the normal range for MLR can be made, around 0.11-0.13 (60,85). Based on the estimated range of reference values, it can be seen that there has been an increase in the MLR value. Increased MLR has been reported to occur in DVI, even early in the infection (23). Monocyte Lymphocyte Ratio in severe DVI is slightly higher than in mild DVI (24). It was further reported that MLR can be used as an initial reference to differentiate malaria from DVI (86). The increase in MLR in this study occurred due to a decrease in lymphocytes, considering that the number of monocytes was relatively stable.

Monocyte-lymphocyte ratio correlated with Hct with a moderate correlation (r=0.41) and was statistically significant (p=<0.001). The VS-MPR results (117.89) it indicates that there is a correlation between MLR and Hct. It makes MLR potentially useful as a PL predictor. MLR is better than NLR because it is based on AMC and ALC calculations which are often affected by DENV infections (87,88), the immune response to DVI is generally characterized by the polarization of Th1 cells which affects monocytes more than neutrophils (89), and NLR is more useful in predicting the severity of other viral infections such as COVID-19 than dengue fever (90).

There are several limitations in this research. Among the limitations of this study is that it used DVI patients without grouping them into degrees of DVI and did not use baseline data. The implication obtained from the research is that MLR needs to be considered when treating DVI patients and has the potential to be used as a predictor of PL. The results and limitations of the research led to further research being carried out on the same topic but adjusted to the degree of severity, considering baseline data and using MLR as a predictor of PL in DVI using diagnostic studies.

CONCLUSION

The research results show a significant correlation between MLR and HCT but no correlation between AMC, ALC, ABC, and HCT.

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REFERENCES

1. Direktorat Jenderal Pencegahan dan Pengendalian Penyakit. Informasi dengue 2023 [Internet]. Kementrian Kesehatan Indonesia. 2023 [cited 2023 Sep 28]. Available from: https://p2pm.kemkes.go.id/publikasi/infografis/informasi-dengue-2023
2. World Health Organiization. Dengue in the South-East Asia [Internet]. World Health Organiization. 2023 [cited 2023 Sep 28]. Available from: https://www.who.int/indonesia/health-topics/dengue-and-severe-dengue
3. Castillo JA, Naranjo JS, Rojas M, Castaño D, Velilla PA. Role of Monocytes in the Pathogenesis of Dengue. Arch Immunol Ther Exp (Warsz). 2019;67(1):27–40.
4. Rodrigo C, Sigera C, Fernando D, Rajapakse S. Plasma leakage in dengue: a systematic review of prospective observational studies. BMC Infect Dis. 2021 Oct;21:1082.
5. Talukdar S, Thanachartwet V, Desakorn V, Chamnanchanunt S, Sahassananda D, Vangveeravong M, et al. Predictors of plasma leakage among dengue patients in {Thailand}: A plasma-leak score analysis. PLoS One. 2021 Jul;16(7):e0255358.
6. Srikiatkhachorn A, Mathew A, Rothman AL. Cytokine storm. Semin Immunopathol. 2017;39(5):563–74.
7. Malathesa M, Ashwini H. Hematological Manifestations in Dengue Fever – An Observational Study. J Evol Med Dent Sci. 2014;3(9):2245–50.
8. Rathore APS, St John AL. Immune responses to dengue virus in the skin. Open Biol. 2018;8:180087.
9. Duangkhae P, Erdos G, Ryman KD, Watkins SC, Falo LD, Marques ETA, et al. Interplay between Keratinocytes and Myeloid Cells Drives Dengue Virus Spread in Human Skin. J Invest Dermatol. 2018;138(3):618–26.
10. Imad HA, Phumratanaprapin W, Phonrat B, Chotivanich K, Charunwatthana P, Muangnoicharoen S, et al. Cytokine expression in dengue fever and dengue hemorrhagic fever patients with bleeding and severe hepatitis. Am J Trop Med Hyg. 2020;102(5):943–50.
11. Alsaffar H, Martino N, Garrett JP, Adam AP. Interleukin-6 promotes a sustained loss of endothelial barrier function via janus kinase-mediated STAT3 phosphorylation and de novo protein synthesis. Am J Physiol - Cell Physiol. 2020;314(5):C589–602.
12. Zhang N, Zhang ZM, Wang XF. The roles of basophils in mediating the immune responses. Eur J Inflamm. 2021;19(36):1–7.
13. Manuel K, Ambroise MM, Ramdas A, Varghese RG. Pseudobasophilia as a Screening Tool in Dengue: A Single Center Study. J Lab Physicians. 2021;13(02):156–61.
14. King CA, Marshall JS, Alshurafa H. Release of Vasoactive Cytokines by Antibody-Enhanced Dengue Virus Infection of a Human Mast Cell/Basophil Line. J Virol. 2000;74(15):7146–50.
15. NaranjoGómez JS, Castillo JA, Rojas M, Restrepo BN, Diaz FJ, Velilla PA, et al. Different phenotypes of nonclassical monocytes associated with systemic inflammation, endothelial alteration and hepatic compromise in patients with dengue. Immunology. 2018 Nov;156(2):147–63.
16. Kratofil RM, Kubes P, Deniset JF. Monocyte conversion during inflammation and injury. Arterioscler Thromb Vasc Biol. 2017;37(1):35–42.
17. Teh YC, Ding JL, Ng LG, Chong SZ. Capturing the fantastic voyage of monocytes through time and space. Front Immunol. 2019;10(MAR):1–9.
18. Lertjuthaporn S, Keawvichit R, Polsrila K, Sukapirom K, Chuansumrit A, Chokephaibulkit K, et al. Kinetic changes of peripheral blood monocyte subsets and expression of co-stimulatory molecules during acute dengue virus infection. Pathogens. 2021;10(11):1–16.
19. Clarice CSH, Abeysuriya V, de Mel S, Thilakawardana BU, de Mel P, de Mel C, et al. Atypical lymphocyte count correlates with the severity of dengue infection. PLoS One. 2019;14(5):1–11.
20. Manh DH, Weiss LN, Thuong N Van, Mizukami S, Dumre SP, Luong QC, et al. Kinetics of CD4+

T Helper and CD8+ Effector T Cell Responses in Acute Dengue Patients. Front Immunol. 2020;11(September):1–11.

21. Rivino L, Lim MQ. CD4+ and CD8+ T-cell immunity to Dengue – lessons for the study of Zika virus. Immunology. 2017;150(2):146–54.
22. Wan S-W, Wu-Hsieh BA, Lin Y-S, Chen W-Y, Huang Y, Anderson R. The monocyte-macrophage-mast cell axis in dengue pathogenesis. J Biomed Sci. 2018 Nov;25(1):77.
23. Soh JXJ, Loh J, Zheng S. A Low Lymphocyte: Monocyte Ratio Provides A Clue To Diagnosing Early Dengue Fever. J Infect. 2020;80(2):232–54.
24. Retnoningrum D, AP P. Monocyte Lymphocyte Ratio in Dengue Hemorrhagic Fever. Indones J Clin Pathol Med Lab. 2018;23(2):111–3.
25. Chunhakan S, Butthep P, Yoksan S, Tangnararatchakit K, Chuansumrit A. Vascular leakage in dengue hemorrhagic fever is associated with dengue infected monocytes, monocyte activation/exhaustion, and cytokines production. Int J Vasc Med. 2015;2015:1–9.
26. Hunais MM, Shiffana S, Udayangani WGN. Indonesian Journal of Medical Laboratory Science and Technology. Indones J Med Lab Sci Technol. 2024;6(1):9–17.
27. Zargari Marandi R, Leung P, Sigera C, Murray DD, Weeratunga P, Fernando D, et al. Development of a machine learning model for early prediction of plasma leakage in suspected dengue patients. PLoS Negl Trop Dis. 2023;17(3):e0010758.
28. Chaloemwong J, Tantiworawit A, Rattanathammethee T, Hantrakool S, Chai-Adisaksopha C, Rattarittamrong E, et al. Useful clinical features and hematological parameters for the diagnosis of dengue infection in patients with acute febrile illness: A retrospective study. BMC Hematol. 2018;18(1):1–10.
29. Rv N, Premkumar L, Jayakumar S. Correlating RDW , hematocrit , and neutrophil to lymphocyte ratio with patient outcomes in dengue : A retrospective hospital-based study. Int J Mosq Res. 2024;11(3):28–31.
30. Ishaque N, Usman M, Imran A, Malik N. Utilization of Neutrophil to Lymphocyte Ratio to Assess Recovery in Patients with Dengue. J Haematol Stem Cell Res. 2023;22(2):77–80.
31. Yuditya DC, Sudirgo I. The Relation between Neutrophil Lymphocyte Count Ratio (NLCR) and Dengue Infection Grade of Severity in Adult Patients in RS Muhammadiyah Ahmad Dahlan Kediri in January 2019. Str J Ilm Kesehat. 2020;9(1):20–5.
32. Russell CD, Parajuli A, Gale HJ, Bulteel NS, Schuetz P, de Jager CPC, et al. The utility of peripheral blood leucocyte ratios as biomarkers in infectious diseases: A systematic review and meta-analysis. J Infect. 2019;78(5):339–48.
33. Fang L, Yan FH, Liu C, Chen J, Wang D, Zhang CH, et al. Systemic inflammatory biomarkers, especially fibrinogen to albumin ratio, predict prognosis in patients with pancreatic cancer. Cancer Res Treat. 2021;53(1):131–9.
34. Starzer AM, Steindl A, Mair MJ, Deischinger C, Simonovska A, Widhalm G, et al. Systemic inflammation scores correlate with survival prognosis in patients with newly diagnosed brain metastases. Br J Cancer. 2021;124(7):1294–300.
35. Tudurachi BS, Anghel L, Tudurachi A, Sascău RA, Stătescu C. Assessment of Inflammatory Hematological Ratios (NLR, PLR, MLR, LMR and Monocyte/HDL–Cholesterol Ratio) in Acute Myocardial Infarction and Particularities in Young Patients. Int J Mol Sci. 2023;24(18).
36. Qiu C, Liu S, Li X, Li W, Hu G, Liu F. Prognostic value of monocyte-to-lymphocyte ratio for 90-day all-cause mortality in type 2 diabetes mellitus patients with chronic kidney disease. Sci Rep. 2023;13(1):1–8.
37. Üçer MB, Cevher S. Assessment of systemic inflammatory response index and other inflammatory indicators in retinal vein occlusion. Med (United States). 2023;102(49):E36512.
38. Niu R, Yan Z, Wang Y, Li Y, Feng W, Liu J, et al. Study of clinical traits and systemic immune inflammation index assessments in patients with endogenous endophthalmitis over the last ten years. BMC Ophthalmol. 2024;24(1):1–9. A
39. Zhou Q, Liu J, Xin L, Hu Y, Qi Y. Systemic inflammation response index as an emerging biomarker in osteoarthritis patients: a bibliometric and large sample retrospective investigation. Clin Exp Rheumatol. 2024;42(1):92–103.
40. Ghobadi H, Mohammadshahi J, Javaheri N, Fouladi N, Mirzazadeh Y, Aslani MR. Role of leukocytes and systemic inflammation indexes (NLR, PLR, MLP, dNLR, NLPR, AISI, SIR-I, and SII) on admission predicts in-hospital mortality in non-elderly and elderly COVID-19 patients. Front Med. 2022;9.
41. Cunha BA, Connolly JJ, Irshad N. The clinical usefulness of lymphocyte:monocyte ratios in differentiating influenza from viral non-

influenza-like illnesses in hospitalized adults during the 2015 influenza A (H3N2) epidemic: the uniqueness of HPIV-3 mimicking influenza A. Eur J Clin Microbiol Infect Dis. 2016;35(1):155–8.

42. Warimwe GM, Murungi LM, Kamuyu G, Nyangweso GM, Wambua J, Naranbhai V, et al. The Ratio of Monocytes to Lymphocytes in Peripheral Blood Correlates with Increased Susceptibility to Clinical Malaria in Kenyan Children. PLoS One. 2013;8(2):10–2.
43. Wang JL, Lu XY, Xu XH, Zhang KJ, Gong H, Lv D, et al. Predictive role of monocyte-to-lymphocyte ratio in patients with Klebsiella pneumonia infection: A single-center experience. Med (United States). 2019;98(38).
44. Kularatnam GAM, Jasinge E, Gunasena S, Samaranayake D, Senanayake MP, Wickramasinghe VP. Evaluation of biochemical and haematological changes in dengue fever and dengue hemorrhagic fever in Sri Lankan children: A prospective follow up study. BMC Pediatr. 2019;19:87.
45. Amrita A, Rani P, Singh G, Yadav VK. Early hematological parameters as predictors for outcomes in children with dengue in Northern India: A retrospective analysis. J Cardiovasc Dis Res. 2022;13(7):155–63.
46. Prajapati X, Goswami H, Parikh U. Hematological Parameters in Dengue Positive Cases : A Study in Tertiary Care Teaching Hospital. Int J Dent Med Sci Res. 2022;4(6):779–83.
47. Majumdar A, Kumar A, Biswas S. Automated complete blood count profile as a probable indicator of dengue fever severity in children. J Sci Soc. 2022;49(2):186.
48. Jatmiko SW, Suromo L, Dharmana E. IgM-RF pada Anak Terinfeksi Virus Dengue Tidak Berkorelasi dengan Jumlah Trombosit dan Hematokrit. J Kedokt Brawijaya. 2017;29(04):306–11.
49. Sharma SK, Rathore M. Evaluation of Haematological Parameters in Dengue Patients. Asian J Med Res. 2022;11(1):1–5.
50. Mulyaningrum U, Wardani K. Clinical and Hematological Parameters as the Predictors of Shock in Dengue Infection. Glob Med Heal Commun. 2018;6(3):176–81.
51. World Health Organization. Comprehensive guidelines for prevention and control of dengue and dengue haemorrhagic fever. Revised ed. World Health Organization, Regional Office for South-East Asia. New Delhi: World Health Organization, Regional Office for South-East Asia; 2011.
52. Arpáš T, Doubek M. Differential diagnosis of leukocytosis and leukopenia. Vnitr Lek. 2022;68(7):E28–35.
53. Juliansen A, Budiputri CL, Meliani F, Muljono MP, Heriyanto RS, Chandra S, et al. Clinical characteristics and laboratory parameters in differentiating dengue from other acute febrile illnesses. Egypt Pediatr Assoc Gaz. 2022;70(1).
54. Yesmin S, Sarmin S, Ahammad AM, Rafi MA, Hasan MJ. Epidemiological Investigation of the 2019 Dengue Outbreak in Dhaka, Bangladesh. J Trop Med. 2023;2023.
55. Thapa TB, Bhattarai BR, Maharjan S, Pokhrel S, Sapkota M. Insights of hematological parameters in dengue virus-infected patients. Indian J Pathol Oncol. 2023;10(4):340–5.
56. Bisetegn H, Ebrahim H. The prevalence of thrombocytopenia and leucopenia among people living with HIV/AIDS in Ethiopia: A systematic review and meta-analysis. PLoS One. 2021;16(9 September):1–14.
57. Mart G, Malkan UY, Buyukasik Y. Determination of etiology in patients admitted due to isolated leukopenia. Med (United States). 2022;101(33):E30116.
58. Pascutti MF, Erkelens MN, Nolte MA. Impact of viral infections on hematopoiesis: From beneficial to detrimental effects on bone marrow output. Front Immunol. 2016;7(SEP):1–12.
59. Risniati Y, Tarigan LH, Tjitra E. Leukopenia Sebagai Prediktor Terjadinya Sindrom Syok Dengue Pada Anak Dengan Demam Berdarah Dengue Di RSPI. Prof. dr. SULIANTI SAROSO. Media Penelit dan Pengemb Kesehat. 2012;21(3 Sept):96–103.
60. Mangaonkar AA, Tande AJ, Bekele DI. Topical Collection On Myelodysplastic Syndromes And Mpn/Mds Overlap. Differential Diagnosis and Workup of Monocytosis: A Systematic Approach to a Common Hematologic Finding. Curr Hematol Malig Rep. 2021;16:267–75.
61. George T, Jakribettu RP, Suresh S, Pais MLJ, Baliga MS. Correlation of Clinico-Hematological Parameters in Adult Dengue with Survival and Death: Emphasis on Cause of Mortality. Med J Dr DY Patil Vidyapeeth. 2023;16(6):956–61.
62. Rai DA, Azad DS, Nautiyal DS, Acharya DS. Correlation between hematological and serological parameters in dengue patients- an analysis of 2022 cases. Trop J Pathol Microbiol. 2019;5(8):547–54.
63. Sivathanu S, Manickam K, Sampath S, Nagaraj

P, Kumar SKS, Pachaimuthu RA. To study the clinical profile of dengue fever and to evolve a prognostic marker based on hematological parameters for severe dengue. Int J Contemp Pediatr. 2017;4(2):538.

64. Castillo JA, Urcuqui-Inchima S. Mechanisms of monocyte cell death triggered by dengue virus infection. Apoptosis. 2018;23(11–12):576–86.
65. Mentkowski KI, Euscher LM, Patel A, Alevriadou BR, Lang JK. Inflammation: From Cellular Mechanisms to Immune Cell Education Monocyte recruitment and fate specification after myocardial infarction. Am J Physiol - Cell Physiol. 2020;319(5):C797–806.
66. Patel AA, Ginhoux F, Yona S. Monocytes, macrophages, dendritic cells and neutrophils: an update on lifespan kinetics in health and disease. Immunology. 2021;163(3):250–61.
67. Patel AA, Zhang Y, Fullerton JN, Boelen L, Rongvaux A, Maini AA, et al. The fate and lifespan of human monocyte subsets in steady state and systemic inflammation. J Exp Med. 2017;214(7):1913–23.
68. Jusof FF, Lim CK, Aziz FN, Soe HJ, Raju CS, Sekaran SD, et al. The Cytokines CXCL10 and CCL2 and the Kynurenine Metabolite Anthranilic Acid Accurately Predict Patients at Risk of Developing Dengue with Warning Signs. J Infect Dis. 2022;226(11):1964–73.
69. Williams H, Mack C, Baraz R, Marimuthu R, Naralashetty S, Li S, et al. Monocyte Differentiation and Heterogeneity: Inter-Subset and Interindividual Differences. Int J Mol Sci. 2023;24:8757.
70. Kwissa M, Nakaya HI, Onlamoon N, Wrammert J, Villinger F, Perng GC, et al. Dengue virus infection induces expansion of a CD14+CD16 + monocyte population that stimulates plasmablast differentiation. Cell Host Microbe. 2014;16:115–27.
71. Maheshwari D, Saini K, Singh P, Singla M, Nayak K, Aggarwal C, et al. Contrasting behavior between the three human monocyte subsets in dengue pathophysiology. iScience. 2022;25(6):104384.
72. Aguilar-Briseño JA, Upasani V, Ellen BM te., Moser J, Pauzuolis M, Ruiz-Silva M, et al. TLR2 on blood monocytes senses dengue virus infection and its expression correlates with disease pathogenesis. Nat Commun. 2020;11(1):1–14.
73. Tian Y, Grifoni A, Sette A, Weiskopf D. Human T Cell Response to Dengue Virus Infection. Front Immunol. 2019;10(September):1–9.
74. Paul Virgo. Children ' s Reference Ranges for FBC Q Pulse Reference N. Child ' s Ref Ranges FBC Q Pulse Ref N [Internet]. 2021;4:370. Available from: https://www.nbt.nhs.uk/sites/default/files/Childrens FBC Reference Ranges.pdf
75. Henrina J, Putra ICS, Lawrensia S, Handoyono QF, Cahyadi A. Coronavirus Disease of 2019: a Mimicker of Dengue Infection? SN Compr Clin Med. 2020;2(8):1109–19.
76. King CA, Wegman AD, Endy TP. Mobilization and Activation of the Innate Immune Response to Dengue Virus. Front Cell Infect Microbiol. 2020;10:574417.
77. Setiawan AF, Wajib YYP, Handono K, Sakti SP. Role of Lymphocytes and Atyphical Lymphocytes in Dengue Hemorrhagic Fever: A Literature Review. J Med Chem Sci. 2023;7(1):53–8.
78. Salvatory Kalabamu F, Maliki S. Use of Haematological Changes as a Predictor of Dengue Infection among Suspected Cases at Kairuki Hospital in Dar Es Salaam, Tanzania: A Retrospective Cross Sectional Study. East African Heal Res J. 2021;5(1):91–8.
79. Direktorat Jenderal Bina Kefarmasian dan Alat Kesehatan. Pedoman interpretasi data klinik. 1st ed. Kementerian Kesehatan RI. Jakarta: Kementerian Kesehatan RI; 2011. 1689–1699 p.
80. Harahap EM, Mongan AE, Memah MF. Hitung Jenis Leukosit Pada Pasien Anak Dengan Infeksi Virus Dengue Di Manado. J e-Biomedik. 2015;3(2):2013–6.
81. Milzman D, Ghai A, Hatoum Z, Timpone J. The true impact of left shift in white blood cell count:Does it determine bacterial source in sepsis. Crit Care Med. 2013;41(12):A205–A205.
82. Fang YT, Wan SW, Lu YT, Yao JH, Lin CF, Hsu LJ, et al. Autophagy facilitates antibody-enhanced dengue virus infection in human pre-basophil/mast cells. PLoS One. 2014;9(10):1–10.
83. Karasuyama H, Miyake K, Yoshikawa S, Yamanishi Y. Multifaceted roles of basophils in health and disease. J Allergy Clin Immunol. 2018;142(2):370–80.
84. Piliponsky AM, Shubin NJ, Lahiri AK, Truong P, Niino K, Tsuha AL, et al. Basophil-derived tumor necrosis factor can enhance survival in a sepsis model in mice. Nat Immunol. 2019;20(2):129–40.
85. MayoClinic Laboratory. Common labs and what they mean [Internet]. Mayo Clinic Laboratories.

2023 [cited 2024 Mar 30]. p. 1–10. Available from: https://www.parentprojectmd.org/wp-content/uploads/2021/02/Common_Labs.pdf

86. Kotepui M, PhunPhuech B, Phiwklam N, Uthaisar K. Differentiating between dengue fever and malaria using hematological parameters in endemic areas of Thailand. Infect Dis Poverty. 2017;6(1):1–9. A
87. Singla M, Kar M, Sethi T, Kabra SK, Lodha R, Chandele A, et al. Immune Response to Dengue Virus Infection in Pediatric Patients in New Delhi, India—Association of Viremia, Inflammatory Mediators and Monocytes with Disease Severity. PLoS Negl Trop Dis. 2016;10(3):e0004497.
88. Ye G, Xu Z, Yang M, Wang J, Liang J, Yin J, et al. Clinical features and transmission risk analysis of dengue virus infections in Shenzhen, During 2014–2019. Comput Struct Biotechnol J. 2023;21:3728–35.
89. Syenina A, Saron WAA, Jagaraj CJ, Bibi S, Arock M, Gubler DJ, et al. and Permeability. Viruses. 2020;12:1379.
90. Pradeep SP, Hoovina Venkatesh P, Manchala NR, Vayal Veedu A, Basavaraju RK, Selvasundari L, et al. Innate Immune Cytokine Profiling and Biomarker Identification for Outcome in Dengue Patients. Front Immunol. 2021;12:677874.