


Comparison of Immature Platelet Fraction (IPF) and Procalcitonin (PCT) as Biomarkers of Infection Severity in Relation to NS1 Antigen in Patients with Dengue Hemorrhagic Fever (DHF)

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ABSTRACT

Background: Dengue hemorrhagic fever (DHF) is an infectious disease caused by the dengue virus (DENV) and transmitted by the *Aedes aegypti* and *Aedes albopictus* mosquito vectors. This disease remains a major global health problem, particularly in tropical regions such as Indonesia. Clinical indicators of DHF severity include thrombocytopenia and shock, which are associated with high viremia levels and an increase in dengue NS1 antigen in the vascular system. This study compares immature platelet fraction (IPF) and procalcitonin (PCT) levels as biomarkers of infection severity in relation to DENV NS1 antigen positivity. **Methods:** This study included 35 patients with DHF and 35 healthy controls. IPF values and PCT levels were measured. Differences between the two groups were analyzed using an independent t-test. **Results:** *p*-values for both IPF and PCT were < 0.05, indicating statistically significant differences between DHF patients and healthy controls. These findings suggest that higher IPF values are associated with thrombocytopenia, whereas elevated PCT levels may reflect more severe infection, including dengue shock syndrome. **Conclusion:** There are significant differences in IPF and PCT levels between DHF patients and controls, depending on dengue NS1 antigen positivity. IPF may serve as an indicator of thrombocytopenia, while PCT may be a marker of infection severity, including dengue shock syndrome, in patients with dengue hemorrhagic fever.

Keywords: Dengue hemorrhagic fever (DHF), immature platelet fraction (IPF), NS1, procalcitonin (PCT), thrombocytopenia.

ABSTRAK

Latar Belakang: Demam berdarah dengue (DBD) adalah penyakit infeksi yang disebabkan oleh virus dengue (DENV) dan ditularkan melalui vektor nyamuk *Aedes aegypti* dan *Aedes albopictus*. Penyakit ini masih menjadi masalah kesehatan global yang signifikan, terutama di wilayah tropis seperti Indonesia. Indikator klinis keparahan DBD adalah trombositopenia dan syok, yang berhubungan dengan tingginya tingkat viremia serta peningkatan konsentrasi protein NS1 dengue dalam sistem vaskular. Penelitian ini bertujuan untuk menyelidiki perbandingan antara nilai *immature platelet fraction* (IPF) dan kadar prokalsitonin (PCT) sebagai indikator tingkat keparahan infeksi pada pasien dengan antigen DENV NS1 yang positif. **Metode:** Penelitian ini melibatkan 35 DBD dan 35 kontrol yang sehat. Nilai IPF dan kadar PCT dilakukan pengukuran. Perbedaan antara kedua kelompok dianalisis menggunakan uji t independen. **Hasil:** Nilai *p* untuk IPF dan PCT keduanya < 0,05 yang menunjukkan perbedaan yang bermakna secara statistik antara pasien DBD dan kontrol sehat. Temuan ini menunjukkan bahwa peningkatan nilai IPF berhubungan dengan trombositopenia, sedangkan peningkatan kadar PCT dapat mencerminkan tingkat keparahan infeksi yang lebih tinggi termasuk sindrom syok dengue. **Simpulan:** Terdapat perbedaan yang bermakna pada kadar IPF dan PCT antara pasien DBD dan kontrol pada positivitas antigen NS1 dengue. IPF dapat digunakan sebagai indikator trombositopenia, sedangkan PCT dapat menjadi penanda tingkat keparahan infeksi termasuk sindrom syok dengue pada pasien demam berdarah dengue. **Ichwan Baihaki, Gita Aprilicia, Mutmainnah, Heru Purwanto Nugroho. Perbandingan *Immature Platelet Fraction* (IPF) dan Prokalsitonin (PCT) sebagai *Biomarker* Keparahan Infeksi Terkait Antigen NS1 pada Penderita Demam Berdarah Dengue (DBD).**

Kata Kunci: Demam berdarah dengue (DBD), *immature platelet fraction* (IPF), NS1, prokalsitonin (PCT), trombositopenia.

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INTRODUCTION

Dengue hemorrhagic fever (DHF) is an infectious disease caused by the dengue virus (DENV), which is transmitted by the mosquito vectors *Aedes aegypti* and *Aedes albopictus*.[1] Indonesia is one of the countries with endemic mosquito vectors. Based on data from the Indonesian Ministry of Health, from the first week of 2024 until the 17th week of 2024, 88,593 cases of DHF were reported with 621 deaths in 174 districts/cities in 28 provinces.[2] DENV is a member of the *Flaviviridae* family which is a positive sense RNA virus of around 11,000 nucleotides, its genome is composed of an open reading frame (ORF) region that encodes 3 structural polyproteins such as capsid (C), membrane (prM/M) and envelope (E) and 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).[3,4] Non-structural proteins are involved in viral replication. NS1 is a 46 kDa protein involved in viral replication; it is expressed on the surface of infected cells as membrane NS1 (mNS1), which does not join mature virions.[5] The level of NS1 expressed in the vascular system as secreted NS1 (sNS1) correlates with viral titer and has become a useful tool for diagnosing DENV infection.[6]

The immature platelet fraction (IPF) is a measure of platelet production, analogous to reticulocyte counts in red blood cells. IPF serves as an indicator of thrombopoiesis and reflects the percentage of immature or newly produced platelets relative to the total platelet count. This parameter can help distinguish causes of platelet increase or decrease, thereby aiding clinicians in determining appropriate treatment strategies.[7] IPF has high clinical benefits in the laboratory diagnosis and treatment of thrombocytopenia because increased IPF levels are associated with increased peripheral platelet destruction.

Procalcitonin (PCT) is a functional immune system modulator protein produced and released into the bloodstream in response to infection and/or inflammation in various tissues, especially hepatocytes and mononuclear cells (Wacker, *et al*., 2013). Clec'h, *et al.,* in 2004 reported that PCT is a biomarker for early diagnosis of sepsis in critically ill patients with a sensitivity and specificity of 77% and 79%, respectively (Clec'h, *et al*., 2004). Thanachartwet V, *et al.,* in 2016, showed that in DHF patients, PCT levels > 0.7 ng/mL were associated with shock events.[8]

This study was conducted to determine IPF values and PCT levels in DHF patients with positive NS1 results, as biomarkers of DHF infection severity, the occurrence of thrombocytopenia and shock syndrome, and as a reference for clinicians treating DHF patients.

METHODS

This is an analytical observational study with a case-control design to investigate whether IPF values and PCT levels in DHF patients can serve as biomarkers of infection severity. This study has received approval from the Department of Education and Research for the collection of stored biological samples and has been approved by the Institutional Review Board and received ethical clearance number 043/DIRUT/RSY/III/2025. The study was conducted from January 2025 to June 2025. This study used EDTA whole blood and plasma from 35 DHF patients with positive NS1 antigen results and 35 healthy controls. This study involved two groups: the patient group confirmed to have dengue hemorrhagic fever (DHF) and the control group.

The inclusion criteria for DHF patients were: positive NS1 antigen test result using a rapid diagnostic test, clinical symptoms consistent with DHF according to the WHO 2009 guidelines, laboratory evidence of thrombocytopenia, and hemoconcentration. All clinical and laboratory data, including IPF and PCT measurements, were collected at the time of confirmed NS1 positivity. The day of illness at presentation was recorded based on the patient-reported onset of fever. The inclusion criteria for healthy controls were individuals without clinical symptoms of infection, no history of fever within the past 7 days, and no known hematological or systemic diseases. Control subjects were selected based on the absence of clinical symptoms of dengue infection and negative NS1 antigen results. The control group was matched to the DHF group by age and gender to minimize confounding variables. The data were analyzed statistically using an independent t-test to assess differences in IPF and PCT levels between NS1-positive patients and healthy controls.

NS1 Antigen Test

This test is qualitative, using a membrane-based Right Sign immunochromatography method to detect the dengue NS1 antigen in serum or plasma.[9] The test is performed by dropping 3 drops of serum or plasma into the sample hole, adding 1 drop of buffer to the cassette, and then reading the results after 10 minutes. Positive results are indicated by the appearance of a pink to red line on the control and test lines. Negative results are indicated by the appearance of a pink to red line in the control area. The results are invalid if no line appears in the control area.

Immature Platelet Fraction (IPF) Test

IPF measurement on the Sysmex XN-550 uses flow cytometry with two fluorescent dyes, namely polymethine and oxazine, which penetrate the platelet cell membrane and stain RNA, allowing two populations to be distinguished.[10] Mature platelets appear as blue dots, while immature platelets show increased volume and fluorescence intensity compared to mature platelets and are seen as green dots. IPF is the percentage of immature platelets to the total number of platelets (IPF%). Normal value for IPF is < 7%.

Procalcitonin (PCT) Level Test

This test uses the Biotime rapid quantitative test lateral flow immunoassay method.[11] If the sample is inserted into the cartridge, the PCT in the sample will bind to the fluorescence-labeled mouse anti-PCT monoclonal antibody, forming an antigen-antibody complex. During the test, the complex will bind to the nitrocellulose membrane in the test area coated with a specific anti-PCT monoclonal antibody, forming an antibody-antigen-antibody complex in that area. The intensity of the fluorescence signal produced is proportional to the PCT levels in the sample. The fluorescence signal will be detected and used to calculate the PCT concentration in the sample using a calibration curve. This test is carried out by adding 50 uL of serum/plasma to the provided buffer, then *homogenizing*. Take 80 uL of the mixture and drop it into the cartridge. Insert the cartridge into the Biotime device. The results are normal if the PCT level is < 0.5 ng/mL.

RESULT

The study used secondary data from laboratory examination results. The study


included 35 patients who tested positive for dengue infection based on NS1 antigen results and 35 healthy patients as controls.

Immature Platelet Fraction (IPF)

Among 35 patients with confirmed DHF, 71% (25 patients) demonstrated abnormal IPF values (> 7%) **(Figure 1).** An independent t-test was performed to compare IPF values between DHF and non-DHF patients **(Table 1).** The mean IPF value in DHF patients was 9.7 ± 3.69%, while in non-DHF patients it was 4.2 ± 2.14%. This difference was statistically significant ($p = 0.001$), with a mean difference of 5.5% between the two groups.

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Procalcitonin Levels

Among 35 patients with confirmed DHF, 91% (32 patients) had high PCT levels **(Figure 2**). An independent t-test was performed to compare PCT levels between DHF and non-DHF patients **(Table 2).** The mean PCT level in DHF patients was 12.57 ± 13.30 ng/mL, whereas in non-DHF patients it was 0.61 ± 0.53 ng/mL. This difference was statistically significant ($p < 0.05$).

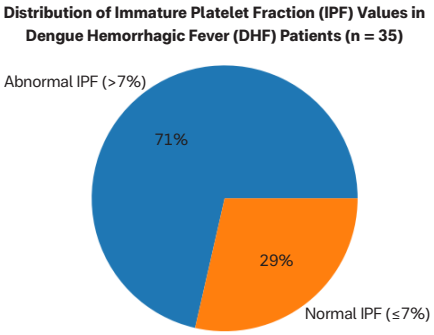

Figure 1. IPF levels in 35 patients confirmed with DHF.

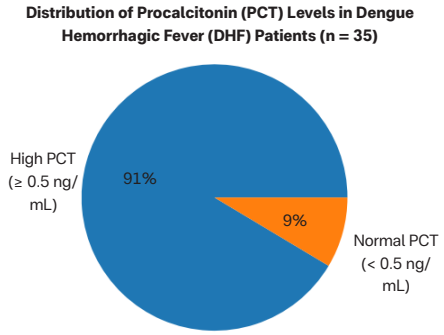

Figure 2. PCT levels in 35 patients confirmed with DHF.

Table 1. Independent t-test results of IPF values in DHF and non-DHF patients.

IPF Values	Mean (X)	Standard Deviation (SD)	*p*-value
DHF Patients	9.7	3.69	0.001
Non-DHF Patients	4.2	2.14	

Abbreviations: IPF: Immature platelet fraction; DHF: Dengue hemorrhagic fever.

Table 2. Independent t-test results of PCT levels in DHF and non-DHF patients.

PCT Level	X	SD	*p*-value
DHF patients	12.57	13.30	< 0.001
Control	0.61	0.53	

Abbreviations: PCT: Procalcitonin; DHF: Dengue hemorrhagic fever.

DISCUSSION

Dengue hemorrhagic fever (DHF) is an acute viral infectious disease transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes, characterized by sudden high fever, muscle and joint pain, rash, and, most typically, thrombocytopenia and increased capillary permeability. To detect dengue infection early and assess its severity, sensitive and specific biomarkers are needed. One viral component widely used for detecting dengue infection is non-structural protein 1 (NS1). NS1 is a glycoprotein released during dengue virus replication and can be detected in blood from the first day of fever.[12] Detection of NS1 is not only useful for early diagnosis, but is also associated with a high incidence of viremia and disease severity.[13] NS1 reflects viral replication and viremia levels.[14] In addition to NS1, immature platelet fraction (IPF) and procalcitonin (PCT) can serve as faster, more accurate indicators of severity. IPF is known as an indicator of megakaryocyte activity in the production of young platelets in response to thrombocytopenia.[15]

The results showed that DHF patients had higher IPF values compared to the control group (9.7% vs 4.2%), with a statistically significant difference ($p < 0.05$). This finding may suggest higher IPF levels in DHF patients than in controls. This strengthens the hypothesis that dengue infection, characterized by NS1 detection, triggers platelet destruction and stimulates bone marrow to increase platelet production, as reflected in the increased IPF value.[16] High IPF reflects the body's compensatory response to thrombocytopenia, a typical sign of DHF. This study is in line with the findings of Klinger & Jelkmann (2002) that IPF increases in thrombocytopenia due to viral infection or systemic inflammation.[17] PCT levels were also higher in DHF patients compared to controls (12.57 ng/mL vs 0.61 ng/mL), with a statistically significant difference ($p < 0.05$). This finding may suggest higher PCT levels in DHF patients than in controls. Increased PCT levels are usually associated with bacterial infections, but recent studies have shown that PCT can also increase in severe viral infections, including DHF, in response to systemic inflammation (Sani, *et al*., 2018). This increase indicates widespread immune activation, which is often seen in severe DHF cases. Srikiatkhachorn, *et al.,* (2011) stated that PCT can be a supporting biomarker in assessing the severity of dengue infection.[18] Barczak in 2025 also showed that PCT increases in severe viral infections such as dengue, due to a widespread systemic inflammatory response.[19] In severe dengue, there is a robust inflammatory response characterized by the release of pro-inflammatory cytokines, including interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6). These cytokines can stimulate PCT production in various tissues, including the liver.[20]

CONCLUSION

This study demonstrates a significant correlation between the presence of dengue virus NS1 antigen and two clinical parameters, Immature Platelet Fraction

(IPF) and Procalcitonin (PCT) levels, both of which are indicators of infection severity in DHF patients. These findings suggest that elevated IPF may serve as an early indicator of thrombocytopenia and bone marrow compensation, while increased PCT levels reflect systemic inflammatory responses that may precede dengue-induced shock. The integration of NS1 antigen testing with IPF and PCT measurements can provide a more comprehensive assessment of dengue severity and may assist clinicians in identifying patients at risk of complications.

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DECLARATIONS

There is no conflict of interest in this research.

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