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Correlation between Immature Platetet Fraction Value and SOFA Score in Sepsis Patient

Hesty Rhauda Ashan[1], Husni[1], Eugeny Alia[2]

[1]Department of Clinical Pathology, Faculty of Medicine, Andalas University/Dr. M. Djamil Hospital, Padang, Indonesia. E-mail: hesty.ashan@gmail.com
[2] Department of Clinical Pathology, Faculty of Medicine, Andalas University/Achmad Mochtar Hospital, Bukittinggi, Indonesia

ABSTRACT

Sepsis is a medical emergency that represents systemic inflammatory reaction to infectious process that can lead to organ dysfunction and death. Sequential Organ Failure Assesment (SOFA) score is used to assess severity of organ dysfunction in septic patients. Immature Platelet Fraction (IPF) value can be used to evaluate thrombopoiesis. Research shows that IPF can provide information regarding inflammatory activity and disease prognosis. A high IPF value in septic patient indicates the formation and recruitment of immature platelets that are triggered by infection. The aim of this study was to determine correlation between IPF value and SOFA score in septic patients. This was an analytical study with a cross-sectional design in 28 patients with sepsis who met the inclusion and exclusion criteria and conducted IPF tests at Central Laboratory of Dr M. Djamil Hospital, Padang. The study was conducted from February 2020 to March 2021. Immature platelet fraction value was performed using automated hematology analyzer with flow cytometry method and SOFA scores were assessed by clinicians and obtained from medical records. Data were analyzed by Pearson correlation test, with significance $p < 0.05$. Median value of IPF in patients with sepsis was 4.8 (1.4-15.7) % and median of SOFA score in patients with sepsis was 5.5 (2-12). Correlation test showed a strong positive correlation between IPF values and SOFA score with $r=0.684$ and $p < 0.05$. There was a strong positive correlation between IPF values and SOFA score in sepsis.

Keywords: Immature platelet fraction, sepsis, SOFA score

INTRODUCTION

Sepsis is a medical emergency that represents a systemic inflammatory reaction to an infectious process that can lead to organ dysfunction and death.[1] Early diagnosis of sepsis is important in patient management, because delay can increase mortality.[2] The World Health Organization (WHO) estimaed that there were 48.9 million cases and 11 million deaths related to sepsis worldwide in 2017, which accounted for nearly 20% of global deaths.[3]

The Sequential Organ Failure Assessment (SOFA) score was used to assess the severity of organ dysfunction in septic patients.[4,5] The SOFA score of patient was assessed on admission to the ICU and monitored every 24 hours. The SOFA score consists of six criteria that assess the function of organ systems (respiratory, cardiovascular, renal, nervous system, liver function and hemostasis system).[6]

Procalcitonin (PCT) is a marker used for the diagnosis and monitoring of sepsis. Increased PCT synthesis can occur in severe infections and is part of the systemic response to sepsis, but the increase in PCT levels can also occur in post-traumatic and surgical conditions.[7,8]

Platelets play an important role in the response to endothelial injury and to infection. The inflammatory response and activation of the coagulation cascade in sepsis may coincide. This can lead to mild thrombocytopenia to a state of Disseminated Intravascular Coagulation (DIC). Thrombocytopenia is common in septic and critically ill patients. Thrombocytopenia is also an independent risk factor for mortality in ICU patients and is a sensitive marker of disease severity, and the concept of cellular immune response suggests platelets play an important role in the body's response to infection.[9]

Inflammatory response accompanied by endothelial tissue damage in sepsis triggers platelet activation which can be stimulated by interactions with pathogens. Platelet adhesion and aggregation on damaged endothelial tissue will form a plug to maintain vascular integrity and prevent bleeding. Platelet activation also contributes to microthrombus formation and causes organ failure in systemic inflammation, especially in sepsis.[10,11]

Activated platelets can mediate cell-to-cell interactions and play a role in innate immune response in response to infection. Platelet response can be seen in Figure 1. Platelets express receptors

on their surface, such as toll-like receptors. Expression of platelet toll-like receptor causes activated platelets to bind to and capture pathogens, and can directly kill pathogens by producing thrombocidins or by aggregating around pathogens and trapping them as a process of pathogen elimination by phagocytic cells.[12,13]

Cluster of Differentiation (CD)154, also known as CD40 ligand (CD40L), is an immunomodulator initially described in activated CD4-positive T cells and later found to be expressed on other types of cells such as basophiles, mast cells, activated CD8-positive T cells and platelets. CD154 protein is also known to be expressed by activated platelets, which are derived from granules. Platelet-expressed CD154 can interact with CD40 present on endothelial cells and induce the release of chemokines that trigger leukocyte recruitment to sites of inflammation. Soluble CD154 released by platelets can also interact with vascular cells (including endothelial cells). This soluble form is usually released from activated T cells and platelets by proteolytic cleavage.[14,15]

Platelets have the ability to interact with various cells, especially neutrophils. Platelet attachment to neutrophils is important in the formation of NETs. Neutrophil Extracellular Traps (NETs) are structures consisting of chromatin, histones, anti-microbial proteins that function as traps and kill bacteria, viruses and fungi. Platelets also interact with cells, especially leukocytes and secrete cytokines and chemokines that attract neutrophils to the damaged endothelium.[13] Activated platelets can also activate monocytes and Dendritic Cells (DCs). This leads to an increase in antigen presentation to T cells, which can enhance the adaptive immune response.[12]

Inflammatory pathways and hemostasis are associated with sepsis due to simultaneous activation of the inflammatory and coagulation cascades. The consequences of this interaction range from mild thrombocytopenia to DIC. Consumptive thrombocytopenia in sepsis triggers the body's compensatory response. Liver will increase the release of thrombopoietin, stimulate production and differentiation of megakaryocytes in bone marrow, which will increase production of platelets. Increased production of platelets will increase the number of immature platelets in the periphery, which have a lot of RNA in the cytoplasm of platelets. High IPF values in septic patients indicate infection-induced formation and recruitment of young platelets.[16,17]

The wide range of conventional and innovative parameters offered by the modern age of hematological analyzers typically include Complete Blood Count (CBC), reticulocyte (RET) and differential leukocyte count and recently IPF provides a more clear cut assessment of red blood cells and platelet production. The evaluation of IPF gives significant data for the analysis and development of patients with sepsis. Moreover, IPF% corresponds with the positivity of blood cultures and in general surge before the beginning of sepsis. It is the main marker whose qualities appear to shift autonomously from those of ordinary coagulation tests. According to the newer studies, IPF may be a valuable prognostic factor to assess the seriousness of the disease and mortality in patients with sepsis.[2]

De Blasi *et al.*, in Italy showed that IPF was able to predict the occurrence of sepsis up to 2 days before the onset of clinical sepsis, with an IPF value > 4.7%, a specificity of 90% and a sensitivity of 56.2% in patients who were admitted to the ICU (p=<0.001).[2]

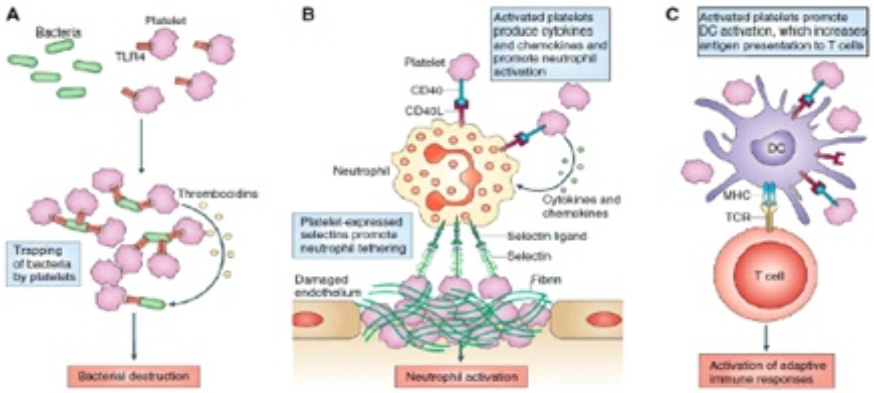

Figure 1. Platelet response to infection[12]

TLR=Toll Like Receptor; CD40=Cluster of Differentiation 40; CD40L=Cluster of Differentiation 40 ligand; DC=Dendritic Cell; MHC=Major Histocompatibility Complex; TCR=T Cell Receptor

Muronoi *et al.*, in Japan found that an increase in IPF played a role as a predictor of mortality within 28 days in sepsis patients (p=0.0007).[18]

Wu *et al.*, in China showed that elevated percentage of Reticulated Platelet (RP%) is associated with increased mortality in septic shock patients. Percentage of reticulated platelet, also known as IPF, was shown to be higher in patients who died with sepsis compared to patients who survived with sepsis. The sensitivity was 88% and specificity was 84% between survivors and non-survivors.[2]

Tiro *et al.*, have described correlation between IPF and SOFA scores in sepsis patients. The study found that the IPF value increased along with the SOFA score, followed by a decrease in platelet (p=0.014). The study subjects were divided into 3 groups based on the SOFA scores: group 1 with the score at 2-6, group 2 at 7-9, and group 3 at >9. The mean of the IPF values increased with increasing SOFA scores.[17]

Research on correlation between IPF value and SOFA score in septic patients has never been conducted at Dr. M. Djamil Hospital, Padang. Based on the background, authors were interested to analyze the correlation between IPF value and SOFA score in sepsis patients at Dr. M. Djamil Hospital.

METHODS

This study was an analytical study with a cross-sectional design of 28 samples. The research was conducted at the Central Laboratory Installation Dr. M. Djamil Hospital, Padang and the intensive care room Dr. M. Djamil Hospital from February 2020 to March 2021. Population was patients who had been diagnosed as sepsis by clinicians and were admitted to Dr. M. Djamil. The sample was part of population, which met the inclusion and exclusion criteria. Sampling was carried out by consecutive sampling method with inclusion criteria: samples delivered to laboratory for hematology examination with diagnosis of sepsis, age ≥ 18 years, and patients who were admitted to the hospital in 24-48 hours with diagnosis of sepsis. Patients with hematologic malignancies, aplastic anemia, liver disease, and kidney disease, patients who received chemotherapy for malignancies, and patients with incomplete SOFA score data.

The blood sample was whole blood with anticoagulant K2EDTA. The IPF examination was carried out in a central laboratory using automatic hematology analyzer with flow cytometry method on PLT-F channel. The reagent will perforate platelet cellular membrane and nucleic acid will be stained by fluorescent dye (oxazine).[19]

The SOFA score was determined based on an assessment of six organ systems: respiratory, cardiovascular, liver, coagulation, renal, and neurological systems, each of, which was rated from 0 to 4 (Table 1). These scores were accumulated to establish a total SOFA score with a maximum value of 24. A SOFA score ≥ 2 was defined as sepsis. The SOFA score assessment was carried out by the clinician. SOFA score data for this study were obtained from patient medical records.[4,20]

The research data was reported in frequency distribution tables and diagrams. Data were analyzed using normality test method of Saphiro-Wilk. The data were not normally distributed and transformed using logarithms, followed by the Pearson correlation test after the data were normally distributed. Data were analyzed using a computer program. The p-value <0.05 showed significant correlation. The positive correlation showed that higher IPF score led to increased SOFA score.

This study was approved by the Ethics Committee for Health Studies Dr. M. Djamil Hospital, Padang with number 04/KEPK//2021.

RESULTS AND DISCUSSIONS

This study found higher number of male (71%) patients compared to female (29%) patients with sepsis. The mean age was 54.79 (12.88) years with an age range of 30–86 years. This study also found that the most basic diseases were respiratory infections (82%). The median procalcitonin level was 12.38 ng/mL with a range of 2.0-151.4 ng/mL and the median of platelets was 172x109/L with a range of 8-582x10^9/L.

The median IPF value in this study was 4.8% with the lowest value of 1.4% and the highest value of 15.7%. Immature platelet fraction values above reference value were found in 11 (39%) subjects. The median SOFA score in this study was 5.5 with the lowest score of 2 and the highest score of 12.

The total subjects in this study were 28 patients, consisting of higher number (71%) of male patients compared to female patients (29%). A study by Muronoi *et al.*, in Japan, which examined the role of IPF in predicting prognosis in sepsis patients also found that sepsis patients were predominated by males (63.4%) than females (36.6%).[18] Research by Park *et al.*, in Korea, which examined the sensitivity and accuracy of IPF in determining sepsis patients also found that 61.4% of the subjects were males.[10] Research by Koyama *et al.*, regarding the relationship between IPF and mortality in sepsis patients found that male subjects experienced sepsis more than female.[21]

Table 1. Basic characteristics of research subjects

Variable	n (%)	Average (SD)	Median (min-max)
Gender			
Male	20 (71)		
Female	8 (29)		
Age (years)		54.79 (12.88)	
Site of infection			
Respiratory tract infection	23 (82)		
Gastrointestinal infection	1 (4)		
Urinary tract infection	1 (4)		
Other infections	3 (10)		
Laboratory result data			
Procalcitonin (ng/mL)			12.38 (2.0-151.4)
Platelets (x10^9/L)			172 (8-582)

Table 2. IPF score and SOFA score

Variable	n (%)	Median (min-max)
IPF (%)		4.8 (1.4-15.7)
Below the reference range	0 (0)	
Reference range (1.1-6.1)	17 (61)	
Above reference range (> 6.1)	11 (39)	
SOFA score		5.5 (2-12)
Mild organ dysfunction (0-7)	19 (68)	
Moderate organ dysfunction (8-15)	9 (32)	
Severe organ dysfunction (=16)	0 (0)	

Research on effect of gender on immune response to sepsis has concluded that the effect of sex is due to differences in hormone levels in the body. Animal studies have shown that estrogen hormone, especially estradiol, can inhibit inflammatory responses and regulate immune system to strike a balance between inflammatory and anti-inflammatory reactions, as well as trigger the repair of damaged tissue, thereby protecting organs. Estrogens have also been shown to have a protective effect on vascular endothelial cells, inhibit endothelial cell apoptosis, induce endothelial cell proliferation and migration, and trigger regeneration of small blood vessels.[22]

The most basic disease as a cause of sepsis in this study was respiratory tract infections as much as 82%, followed by other infections [infections of diabetic ulcers (10%)], gastrointestinal infections (4%), and urinary tract infections (4%). Utama *et al*., also obtained respiratory infections as the most common cause of sepsis (75%), followed by urinary tract infections (13.2%), and gastrointestinal infections (5.9%).[23] Abe *et al*., in Japan also got respiratory tract infections as the most common cause of sepsis (31.0%), followed by gastrointestinal infections (26.3%), urinary tract infections (18.4%), and skin & soft tissue infections (10.9%).[24] This study is different from Chou *et al*., in Taiwan who got urinary tract infections as the most common cause of sepsis (36.7%).[25]

The median number of platelets in the subjects was 172 x 10^9/L with a range of 8-582 x 10^9/L. Park *et al*., obtained median platelet values of patients with sepsis 126 x 10^9/L with a range of 7-548 x 10^9/L.[10] Research by Tiro *et al*., found that median value of platelets in sepsis patients was 158 x 10^9/L with a range of 8-501 x 10^9/L. The median value of platelet counts in the subjects was still in normal range.[17] The incidence of thrombocytopenia in critically patients occurs in 20%-50% of cases and associated with poor outcomes.[9]

The median IPF value in the subjects was 4.8% and ranged from 1.4% to 15.7%. The median IPF value of the subjects in this study was still within the range of the reference value. This was related to the median value of the subjects'platelet counts, which still within the refence range, but there has been a tendency for an increased IPF result, where an

increasein IPF is in accordance with an increase in SOFA score. The IPF value illustrates the balance between platelet production (increased immature platelet count) and platelet consumption (decreased platelet count). Thrombocytopenia results in bone marrow compensation by releasing immature platelets to the periphery, so that the IPF value increases.[21,26]

This study found 17 subjects with an IPF in reference range and 11 subjects with an IPF above the reference range. The highest IPF value of the 11 subjects was 15.7% in patients with sepsis with Community-Acquired Pneumonia (CAP). The patient's SOFA score was 8, the patient's platelet count was $95x10^9$/L, and procalcitonin was 12.22 ng/mL.

Immature platelet fraction can measure thrombopoietic activity in Bone Marrow (BM), increased IPF can be observed in patients with thrombocytopenia owing to peripheral destruction, and decreased IPF can be observed in patients with thrombocytopenia owing to BM failure.[10,17] The normal IPF value in this study was probably due to factors that might influence thrombopoiesis, such as levels of thrombopoietin (TPO), Interleukin (IL)-3, IL-6, and IL-11, which were not measured in this study. Thrombopoietin is a regulator of megakaryopoiesis and thrombopoiesis. Several cytokines also promote megakaryopoiesis with TPO, such as IL-3, IL-6, and IL-11.[17]

The median SOFA score of the subjects was 5.5 (2-12) with a mean of 6.29 (3.39). Research by Layios *et al*., in Belgium obtained a mean SOFA score of 6.0 (3.3).[27] Safari *et al*., obtained an average SOFA score of 7.13 (2.36).[28] Research by Nair *et al*., found that the initial SOFA score can act as a predictor of mortality. Patient mortality increased significantly in the initial SOFA score ≥11.[20]

This study obtained a strong positive correlation between IPF value and SOFA score using the Pearson correlation test, with r=0.684 ($p < 0.05$). This result was similar to the results in the research of Hubert *et al*., who reported an increase in IPF values in study subjects with higher SOFA scores (SOFA ≥ 6) compared to study subjects with lower SOFA scores (SOFA <6).[17]

Excessive production of proinflammatory cytokines in sepis can damage the endothelium thereby inducing neutrophils, monocytes, macrophages and platelets to bind with vascular endothelium. These cells release proteases, oxidants, prostaglandins and leukotrienes, which cause increased permeability of blood vessel walls and trigger vasodilation. Vasodilation in sepsis occurs in all major blood vessels and microcapillaries in the body. This condition causes decreased fluid perfusion and tissue hypoxia that can lead to organ failure, which can be assessed by a SOFA score.[29,30]

Consumptive thrombocytopenia in sepsis triggers the body's compensatory response. Liver will increase the release of thrombopoietin, stimulate production and differentiation of megakaryocytes in bone marrow, which will increase production of platelets. Increased platelet production will increase the immature platelet count in periphery. The results of this study were in accordance with the references suggesting that high IPF values in sepsis patients indicate formation and recruitment of immature platelets that are triggered by infection.[16,17]

The study did not classify subjects based on degree of organ dysfunction (mild, moderate, and severe); consequently, IPF in each organ dysfunction group was not possible to corellate. IPF examination is influenced by various factors that play a role in thrombopoiesis, such as TPO, IL-3, IL-6, and IL-11. However, no examination of these factors in this study.

CONCLUSIONS AND SUGGESTIONS

The IPF value had a strong positive correlation with the SOFA score in sepsis patients who were admitted to Dr. M. Djamil Hospital, Padang. Further research needs to be carried out in subjects who are classified according to the degree of organ function by considering the factors that may influence thrombopoiesis.

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