Association of Immature Platelet Fraction and Trombopoietin Levels Based on the Grades of Sepsis Severity

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Abstract

Sepsis is one of the major causes of morbidity and mortality around the world. Organ dysfunction in sepsis can be identified using *Sepsis-related Organ Failure Assessment*. The dysfunction of the coagulation cascade, the number of pro-inflammatory cytokines, and endothelial dysfunction promote the consumptive thrombocytopenia in sepsis patients, which triggers a compensatory response in the body by increasing thrombopoietin release. As a result, it increases platelet production which can be assessed by immature platelet fraction (IPF). This study is a preliminary research to see the relationship between IPF values and serum thrombopoietin (TPO) levels in terms of the clinical severity of patients with sepsis based on SOFA scores. A cross-sectional design was performed in this study with 49 samples of patients with sepsis, collected during June-July 2019, at Dr. Wahidin Sudirohusodo Hospital in Makassar. The study was conducted in August 2019. IPF values were measured by the fluorescent flow cytometry method. Meanwhile, serum thrombopoietin levels were calculated by the enzyme-linked immunosorbent assay (ELISA) method using human Thrombopoietin ELISA (RayBioTech, Australia). The results showed that the IPF value increased along with the SOFA score, followed by a decrease in platelet ($p = 0.014$). TPO levels presented an upward along with SOFA scores with a decrease in platelet ($p = 0.008$). There was a significant correlation between IPF values and TPO levels ($r = 0.606$, $p < 0.001$) where the Immature Platelet Fraction score and thrombopoietin levels increased with SOFA scores. In addition, there was also a positive correlation between IPF values and TPO levels in sepsis patients.

***Keywords**: Sepsis, SOFA score, immature platelet fraction, thrombopoietin, platelets*

Introduction

Sepsis is a systemic inflammation as a response of the body to infection, potentially leading to organ dysfunction or death. Sepsis is one of the major causes of morbidity and mortality around the world. In 2013, the World Health Organization (WHO) reported that the number of sepsis cases worldwide reached 30 million cases with mortality rates in developing countries at 60-80%. Subroto and Loehoeri (2003) stated that the incidence of sepsis in several referral hospitals in Indonesia was around 15-37.2% with a mortality rate of 37-80%.[1-4]

Disorders of hemostasis in sepsis are caused by several things: malfunctioning of the coagulation cascade, the number of pro-inflammatory and anti-inflammatory cytokines released by mononuclear cells and endothelial cells, thrombus formation in the advanced stages, and stimulation of plasminogen and antithrombin-III activation that cause fibrinolysis. As a result, it causes clot formation and bleeding associated with disseminated intravascular coagulation (DIC). Disseminated intravascular coagulation results in increased consumption of platelets, causing thrombocytopenia in patients with sepsis.[5,6]

Consumptive thrombocytopenia in sepsis triggers a compensatory response from the body. The liver increases thrombopoietin release, stimulating the production and differentiation of megakaryocytes in the bone marrow which increases platelet production. The increased platelet production promotes an increase in the number of immature platelets in the peripheral characterized by

high RNA in the cytoplasm of platelets.[7]

Immature platelet fraction (IPF) is a recent parameter that measures immature platelets in peripheral blood by staining RNA in the cytoplasm of immature platelets and measured through laser light. IPF examination is used to distinguish thrombocytopenia due to bone marrow failure from consumptive thrombocytopenia.[8]

Many studies of organ malfunction in sepsis are still being carried out especially in the failure of hematopoiesis. This study aims to analyze the correlation of IPF values and thrombopoietin levels with clinical severity based on SOFA scores in patients with sepsis.

Method

A cross-sectional study was performed to analyze the value of IPF and thrombopoietin levels in patients with sepsis at the Inpatient Installation of Dr. Wahidin Sudirohusodo General Hospital, Makassar. This research was conducted during June-July 2019. The samples in this study were the adult patients diagnosed with sepsis based on the clinical symptoms and examinations. Excluded criteria were those with incomplete laboratory data, hematological malignancies, bone marrow failure before the diagnosis of sepsis, and those with lysis/ lipemic that could not be re-sampled. All samples were examined for IPF values by using the Sysmex XN-1000 automatic hematological analysis device and for TPO levels by the ELISA (Enzyme Linked Immunosorbant Assay) method in the research unit of the medical faculty of Universitas Hasanuddin/Hasanuddin University Hospital. The study subjects were observed during inpatient at Wahidin Sudirohusodo General Hospital to investigate the patient outcomes.

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 data were statistically analyzed using the *Kruskal-Wallis* Test to assess the differences in IPF values and TPO levels based on the SOFA scores. *Spearman* correlation test was performed to assess the correlation between IPF values and TPO levels. The results of the analysis were significant with a p value at <0.05.

Results

Table 1. The characteristics of the subjects of the study

Variables	n (%)	Median (Min-Max)
Age (years)		57 (24-80)
Genders (n=49)		
Men	24 (48,9)	
Women	25 (51,1)	
SOFA Scores (n=49)		
Group I (2-6)	33 (67,3)	
Group II (7-9)	11 (22,5)	
Group III (>9)	5 (10,2)	
PLT (103/mm3)		158 (8-501)
IPF (%)		6,6 (0,6 – 23,5)
TPO (pg/mL)		792,9 (447,1-4839,5)
Mortality (n=47)	31 (66)	
Group I	21 (67)	
Group II	7 (64)	
Group III	3 (60)	

*Source: Primary data

49 subjects met the inclusion and exclusion criteria with a median of age at 57 years old. There was no difference in the category of gender. 33 subjects had SOFA scores at 2-6, while 11 subjects were at 7-9, and 5 of them were at >9. The median of platelet rates from all samples was 158,000/mm3 with the lowest platelet rates of 8,000/mm3 and the highest platelet rates of 501,000/ mm3. The median of IPF values was 6.6% with the lowest IPF value of 0.6% and the highest of 23.5%. The median of TPO levels in the study subjects was 792.9 pg/mL with the lowest levels of 447.1 pg/mL and the highest levels of 4893.5 pg/mL.

2 out of 49 patients requested termination of care at the request of the family. The number of deaths in the study population was 31 of 47 patients (66%). The mortality rate for the three groups reached more than 60%.

Table 2. The comparison of IPF rates based on the SOFA scores

Groups	SOFA scores	IPF (%)	PLT (103 /mm3)	p*
I	2-6	6,8±4,8	205,55±115,37	
II	7-9	8,3±5,7	130,09±112,39	0,014
III	>9	13,9±2,8	34,20±11,21	

Source: Primary data

* *Kruskal-Wallis* test

Table 2 shows the IPF rates in the three groups of study subjects based on SOFA scores. The Kruskal-Wallis test indicated that there were significant differences (p = 0.014) of the mean of IPF values in the three groups of the research subjects. The mean of IPF values increased along with SOFA scores, while the platelet rates decreased with an upward in SOFA scores. The increase in IPF value was inversely proportional to the platelet rates; the lower the platelet rates, the higher the mean IPF value. The results indicated a significant association of increased SOFA score with platelet rates and IPF values. The difference in IPF values based on SOFA scores is shown in Figure 1.

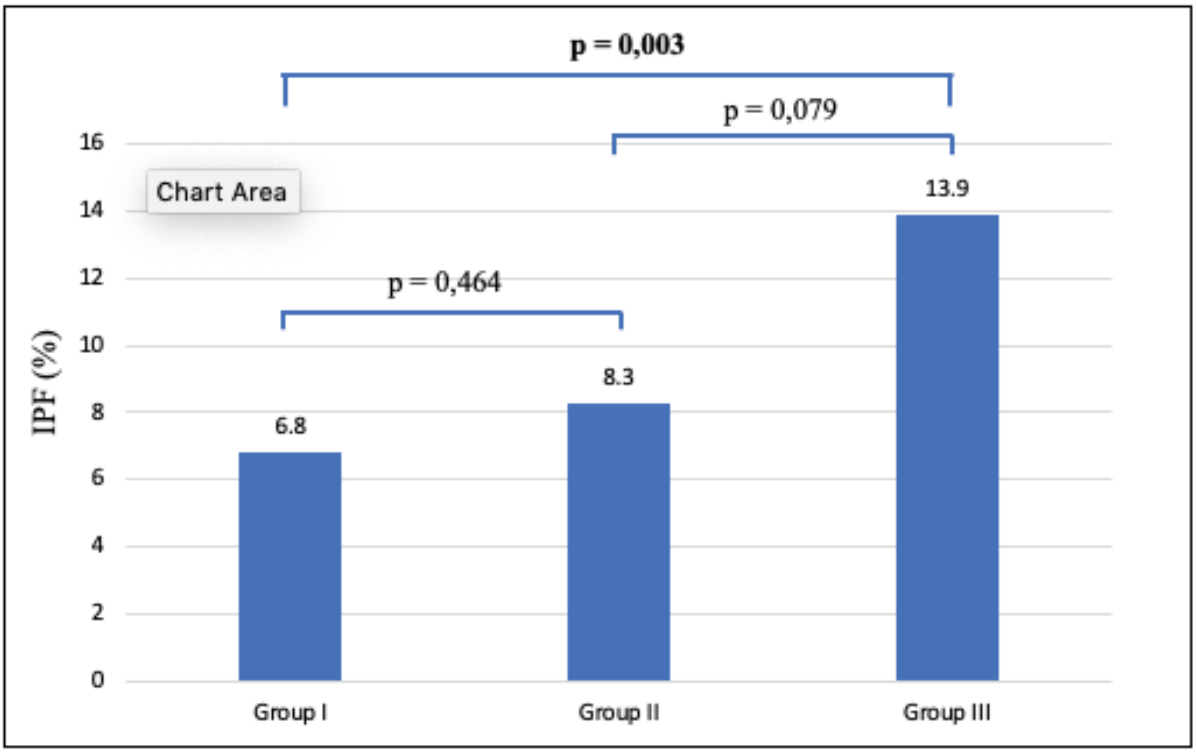

Figure 1. The comparison of IPF rates based on the groups of SOFA scores

On the post hoc test, the IPF value was significantly different between group I (mean IPF value = 6.8 ± 4.8%) and group III (mean IPF value = 13.9 ± 2.8%) with a value of p = 0.003. Meanwhile, IPF values between groups I and II and between groups II and III did not show significant differences (p = 0.464 and p = 0.079).

Table 3. The comparison of TPO levels based on the SOFA scores

Groups	SOFA scores	TPO (pg/mL)	PLT (10^3 /mm^3)	p*
I	2-6	976,4±804,1	205,55±115,37	
II	7-9	1166,1±494,8	130,09±112,39	0,008
III	>9	1638,3±94,7	34,20±11,21	

Source: Primary data

* *Kruskal-Wallis* test

Table 3 presents the levels of TPO in the three groups of study subjects based on SOFA scores. The *Kruskal-Wallis* test indicated that there were significant differences (p = 0.008) of the average TPO levels in the three groups of study subjects. The mean of TPO levels rose along with SOFA scores. The increase of TPO levels was inversely proportional to the platelet rate; the lower the platelet rate, the higher the mean of TPO levels. These results indicated an association of increased SOFA scores with platelet rate and TPO levels. The difference in TPO levels based on SOFA scores is shown in Figure 2.

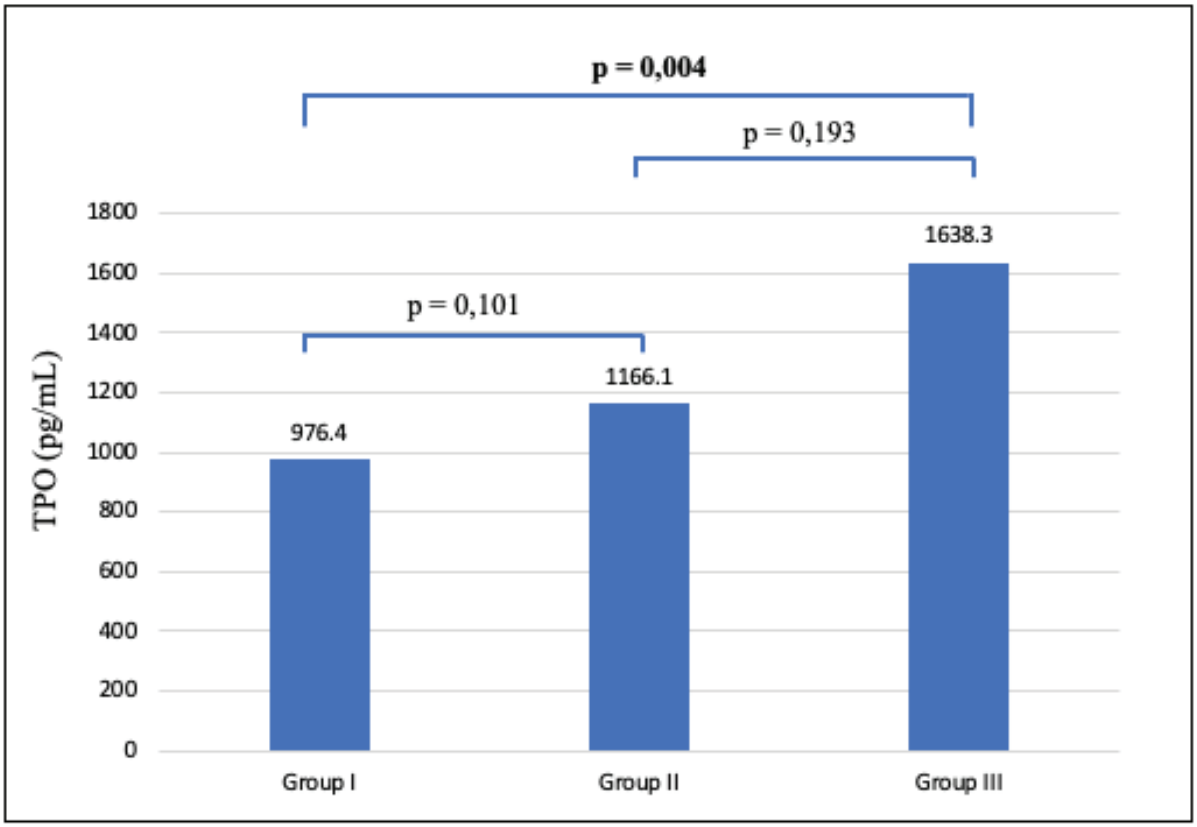

Figure 2. The comparison of TPO levels based on SOFA scores

Post hoc test showed that TPO levels were significantly different between group I (mean TPO level = 976.4 ± 804.1 pg/mL) and group III (mean TPO level = 1638.3 ± 94.7 pg/mL) with a p value = 0.004. Meanwhile, TPO levels between groups I and II and TPO levels between groups II and III did not show significant differences (p = 0.101 and p = 0.193).

Table 4. The correlation of IPF rates and TPO levels

	IPF		
	n	r	p*
TPO	49	0,606	<0,001

Source : Primary data

* Spearman Test

Measurement of correlation with IPF values and TPO levels showed a significant association. Spearman correlation test results indicated a strong positive correlation between IPF values and TPO levels (r = 0.606, p <0.001); the higher the TPO level, the higher the IPF value. The correlations of IPF values and TPO levels are shown in Table 4.

Discussion

This study showed that there was no significant difference between men (48.9%) and women (51.1%) with an average age of 54.9 years old. In 2005, an epidemiological study reported that sepsis was more frequently found in men than women with a relative risk of 1.28 in men. This difference may be caused by abnormalities of comorbid conditions in the study subjects and different lifestyles according to demographic conditions. Some comorbid conditions and lifestyle are related to sepsis in the form of immune status, heart disease, and alcohol intake.[9]

The mean of the IPF values increased with increasing SOFA scores. The mean of group I IPF was 6.8 ± 4.8%, while group II was 8.3 ± 5.7%, and group III was 13.9 ± 2.8%. The *Kruskal-Wallis* test showed that there were significant differences in the mean for the IPF of the three groups. Platelet rates are low with increasing SOFA scores. Both of these results provide a description of the condition of thrombopoiesis in the state of sepsis. Endothelial dysfunction which promotes a decrease in platelet count causes a compensatory mechanism in the body to increase thrombopoiesis characterized by an increase in IPF value. A research by Hubert et al (2015) reported the same thing as an increase in IPF values and decreased platelet rates in patients with sepsis shock compared with severe sepsis and sepsis without complications.[9]

Post hoc test showed significant differences in IPF values between group I with SOFA score 2-6 (mean IPF value = 6.8 ± 4.8%) and group III with SOFA score 9-15 (mean IPF value = 13.9 ± 2.8%). Meanwhile, the mean of group I IPF value is higher than group II, and the mean of group II IPF value is higher than group III, but there was no significant difference. These results differ from studies by 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). This difference may be due to differences in the assessment of SOFA scores and IPF scores. The assessment of SOFA scores and IPF scores in the study by Hubert et al was performed 24 hours after the subjects received intensive care while in this study the assessment of SOFA scores and IPF values was obtained when the patient was diagnosed with sepsis.[9]

The highest IPF value in this study was 23.5% in patients with congestive heart failure with complications of pulmonary edema. TPO levels in these patients were 2,291 pg / mL, platelet rates at 100,000 / mm3, and SOFA scores at 6. Despite the highest IPF values compared to other patients, TPO levels and SOFA scores in these patients were not the highest in the study sample. This might occur because thrombopoiesis was influenced by several other cytokines that were not examined in this study such as IL-3, IL-6 and IL-11.[10]

The mean of TPO levels increased along with SOFA scores and was inversely proportional to platelet rates. The mean of TPO levels in group I was 976.4 ± 804.1 pg / mL, while group II was 1166.1 ± 494.8 pg / mL, and group III was 1638.3 ± 94.7 pg / mL. The Kruskal-Wallis test showed that there were significant differences (p = 0.008) of the average TPO levels in the three groups of study subjects. This is in line with previous research by Zakynthinos et al (2004) and Segre et al (2014) who reported elevated TPO levels in sepsis shock compared with severe or uncomplicated sepsis.

Research comparing TPO levels based on SOFA scores has never been conducted.[11,12]

In line with the post hoc IPF test, differences in TPO levels were only found to be significant between group I and SOFA scores at 2-6 (the mean of TPO levels = 976.4 ± 804.1 pg / mL), and group III with SOFA scores of 9 -15 (mean TPO level = 1638.3 ± 94.7 pg / mL). There were no significant differences in TPO levels between groups I and II and between groups II and 3, but IPF values and TPO levels increased along with the SOFA score.

The correlation test results between IPF values and TPO levels in adult sepsis patients showed a positive correlation between IPF values and TPO levels (r = 0.606, p = 000). The condition of consumptive thrombocytopenia in sepsis caused the response from the body to compensate by increasing thrombopoiesis by increasing TPO production (Hitchcock IS & Kaushansky K, 2014; Larkin CM et al., 2016). The increase in TPO production promoted an increase in immature platelets in circulation with the elevation in IPF value. Significant correlation between IPF value and TPO level showed that there was no bone marrow failure in the process of thrombopoiesis in the state of sepsis.[13,14]

This research has several limitations: the lack of subjects in the study in patients with higher SOFA scores (group III) which could affect the mean of IPF values and TPO levels in this group, the assessments of IPF and TPO levels which were only performed at the beginning of diagnosis without a description of the function of thrombopoiesis in the bone marrow throughout the course of sepsis, and the absence of therapeutic analysis in research subjects that might affect the value of IPF and TPO levels.

Conclution and Suggestion

This study concludes that the value of Immature Platelet Fraction and thrombopoietin levels increases along with the SOFA scores. In addition, there is a positive correlation between the value of Immature Platelet Fraction and thrombopoietin levels in patients with sepsis. Further research is needed with better distributed research subjects in each group regarding the grades of sepsis, followed by analyses of the therapy and examinations towards the value of serial IPF and TPO following the course of sepsis.

Ethical Clearance- Taken from Health Research Ethics Commission, Medical Faculty, Hasanuddin University – RSPTN UH – RSUP Dr. Wahidin Sudirohusodo Makassar

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Conflict of Intereset- Nil

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