Immature platelet fraction in bacterial sepsis severity assessment

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Abstract. Sepsis is an infection-induced syndrome, mostly caused by bacteria, of organ dysfunctions that caused by host response dysregulations. One of the simplest sepsis-indicator is platelet and its indexes. A new platelet parameter called immature platelet count (IPF) became the interest in this study. The study aims to see whether IPF could assess sepsis severity by procalcitonin (PCT). Sixty-four of seventy-one patients with increased PCT were included in this cross-sectional study and separated into three groups based on their PCT levels. IPF showed no significance among the three groups (p-value >0.05) while platelet count was significant (p-value <0.05). Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) showed a strong positive correlation with IPF. Higher sepsis severity based on PCT showed larger platelet count, as the result of platelet destructions caused by pro-inflammatory cytokines and endotoxins.

1. Introduction
Sepsis causes serious problems in community healthcare, such as severe diseases and mortalities.[1] Even in the United States, a developed country, >200,000 cases of sepsis-related mortalities appeared each year.[2] But the highest sepsis rate is found in low-to-middle income countries, e.g., India and Indonesia.[3] In 2013, 192 patients with sepsis were in a hospital in Bandung.[4] In Adam Malik General Hospital, Medan, there were 233 cases of sepsis reported in 2015.[5]

Infections induce pro-inflammatory responses which activate the release of cytokines and cause systemic inflammatory response syndrome (SIRS).[6] Without early detection and accurate antimicrobial treatment, infection-induced inflammation will develop into sepsis. Sepsis is a life-threatening organ dysfunction that is caused by host response dysregulation towards infection.[7] Cultures are conventional laboratory ways to detect sepsis, but this method has low specificity, time-consuming (24-48 hours) and contamination-vulnerable.[8,9]

One of the most popular laboratory parameters for suspecting sepsis is a prohormone of calcitonin called procalcitonin (PCT). During infections, mostly that are caused by gram-negative bacteria, TNF-α and IL-6 together with bacteria endotoxin will induce the release of PCT in the bloodstream.[10] Based on The National Institute for Health and Care Excellence (NICE), the normal PCT level cutoff for healthy people is <0.05 ng/mL.[11] But not all laboratory facilities in Indonesia have the devices for measuring blood PCT levels.
Another simpler parameter that has often been used in sepsis is platelet. Both pro-inflammatory cytokines and bacterial endotoxin trigger platelet activation, aggregation and adhesion, resulting in platelet sequestration and destruction.[12,13] There are several platelet indexes besides platelet count, such as plateletcrit, MPV and PDW and a new parameter that is called IPF. IPF is the percentage of reticulated platelets could be measured in blood. It is also one of the ways to detect the production rate of bone marrow platelet.[14]

The purpose of this study was to see whether IPF could assess sepsis severity by procalcitonin (PCT). By studying this, we could gain more information of platelet indexes that could be used to assess sepsis severity.

2. Method
It was a cross-sectional research with consecutive sampling, conducted from October to December 2016 in the Clinical Pathology Department of Haji Adam Malik General Hospital in Medan. Patients were from the emergency room, wards, intensive care unit, high care unit and high dependency unit of Haji Adam Malik General Hospital.

Patients that had their venous blood drawn for full blood count and had increased PCT level were included. But, the ones with dengue fever, malaria infection and <18 years old of age were excluded. The full blood count was analyzed using an automated hematology analyzer Sysmex XN-1000. The patients’ IPF were gathered from the result. Mini Vidas Brahms with enzyme-linked fluorescence assay (ELFA) principal was used to analyze the PCT levels. They were then separated into 3 groups according to their blood PCT levels (group I = ≥0.05 – <2 ng/mL; group II = ≥2 – <10 ng/mL; group III = ≥10 ng/mL).

The clinical characteristics and the differences of IPF and the other platelet indexes between the three groups were by analyzing Kruskal-Wallis test. As for the correlations between IPF and the other platelet parameters, Pearson’s correlation test was used if one of the variables had anormal distribution and Spearman’s correlation test was used if both variables were not normally distributed. The results were considered as significant if the p-value is < 0.05.

The patients received informed consent before their laboratory data were collected. This study had an ethical clearance approval from the Medical Faculty of Universitas Sumatera Utara Health Research Ethical Committee.

3. Result and Discussion
There are 64 patients totally, 40 (62.5%) were male, and 24 (37.5%) were female. The mean (SD) of the patients’ age was 49 years old (14.4 years). The mean (SD) of platelet count was 288,828.1/µL (20784.7/µL) and the median (min., max.) IPF was 4.5% (0.9%, 18.5%).

| Variable                | Value           |
|-------------------------|-----------------|
| Gender, n (%)           |                 |
| Male                    | 40 (62.5)       |
| Female                  | 24 (37.5)       |
| Age, yearsa             | 49.0 ± 14.4     |
| Procalcitoninb (ng/mL)  | 21.8 (0.17-200.0) |
| IPF (%)b                | 4.5 (0.9-18.5)  |
| Platelet count (/µL)c   | 288828.1 ± 20784.7 |
| MPV (fL)d               | 10.5 ± 1.3      |
| Plateletcrit (%)e       | 0.3 ± 0.2       |
| PDW (%)e                | 11.8 (7.7-21.4) |

*a data is presented as mean ± SD
*b data is presented as median (min, max)
The total of patients in each of the three PCT groups were 17 patients (26.5%), 23 patients (36%), 24 patients (37.5%) respectively. Differences of the IPF and the other platelet indexes among the three groups were in Table 2. The data showed that IPF, MPV, and PDW were not significant among the groups (p-value 0.644), while platelet count and plateletcrit had significant differences with p-value 0.043 and 0.038 respectively. Then we continued to analyze the variables that were significant with Post Hoc test between 2 groups, and it showed that when the group I or II were compared to group III the results was significant, but it is not significant when the group I was compared to group II.

**Table 2. Differences of IPF between three groups of PCT levels.**

| Variable       | Procalcitonin Groups | n   | Median (min.-max.) | P value |
|----------------|----------------------|-----|--------------------|---------|
| IPF            | I                    | 17  | 4.5 (1.1 – 10.8)   | 0.644   |
|                | II                   | 23  | 4.5 (0.9 – 18.5)   |         |
|                | III                  | 24  | 4.3 (0.9 – 10.0)   |         |
|                | I                    | 17  | 308,000 (90000-587,000) |         |
| Platelet count | II                   | 23  | 307,000 (65000-724,000) | 0.043   |
|                | III                  | 24  | 217,500 (20000-550,000) |         |
|                | I                    | 17  | 0.4 (0.1-0.6)      |         |
| Plateletcrit   | II                   | 23  | 0.3 (0.1-0.8)      | 0.038   |
|                | III                  | 24  | 0.2 (0.1-0.6)      |         |
|                | I                    | 17  | 10.3 (8.2-13.6)    |         |
| MPV            | II                   | 23  | 10.0 (8.4-15.0)    | 0.723   |
|                | III                  | 24  | 10.5 (8.5-12.3)    |         |
|                | I                    | 17  | 11.0 (7.7-19.1)    |         |
| PDW            | II                   | 23  | 10.8 (8.4-21.4)    | 0.804   |
|                | III                  | 24  | 11.8 (7.8-16.6)    |         |

Group I: PCT levels 0.05 s/d <2 ng/mL. group II: PCT levels ≥2 s/d <10 ng/mL and group III: PCT levels ≥10 ng/mL. Differences between 3 groups were by using Kruskal-Wallistest (p-value<0.05).

IPF, a new platelet index, is believed to be able to reflect the percentage of immature platelet in the peripheral circulation. This study showed that IPF has no significance in differentiating the PCT groups. Research in Brazil concluded that IPF could predict sepsis development three days before the appearance of symptoms. IPF >4.7% has 56.2% sensitivity and 90% specificity in sepsis development.[15] However, the grouping of sepsis severity was not based on PCT. The researchers in Brazil diagnosed sepsis with infection and severe sepsis with organ failure. Another study in Italy claimed that IPF did not correlate with PCT.[16]

Platelet count has been known to be one of the parameters in evaluating sepsis severity, such as Acute Physiology and Chronic Health Evaluation (APACHE) and Sequential Organ Failure Assessment (SOFA) score. Thrombocytopenia can be in sepsis-related organ failure.[17] Although platelet count showed significant differences between groups, the whole data of platelet count mostly were within normal range. It might be the result of the minimal exclusion criteria in collecting samples. In a study that was in India, patients with major trauma, operation, burnt trauma, and malignancy were excluded from the PCT study.[18] Another study in Iran excluded non-infectious diseases, such as immunodeficiency, pancreatitis, liver damage, cardiogenic shock and many others.[19]

We continued with the correlation test between IPF and the other platelet parameters, and the result showed that IPF had a strong positive correlation with MPV and PDW. MPV reflects the average platelet size while PDW is the variation of platelet morphology in a sample. Immature platelets have a tendency to be larger and could affect the MPV and PDW values.[20]
latelet count as one of the parameters for evaluating sepsis severity.

Table 3. The correlation coefficient (r) between IPF and other platelet parameters.

| Variable      | R     | p   |
|---------------|-------|-----|
| Platelet count| -.417⁴ | 0.001 |
| MPV           | .827⁵ | 0.000 |
| Plateletcrit  | -.283⁶ | 0.024 |
| PDW           | .825⁵ | 0.000 |

⁴Correlation significance of 0.01
⁵Correlation significance of 0.05
⁶The value of correlation coefficient 0.0<0.2 very weak; 0.2<0.4 weak; 0.4<0.6 moderate; 0.6<0.8 strong; 0.8-1.00 very strong.
(-) a negative correlation between the two variables.

4. Conclusion
IPF might reflect the activity of platelet production in bone marrow but had no relation with PCT in this study. On the other hand, platelet count as one of the parameters for evaluating sepsis severity showed a significant result when compared to the PCT level ≥10 ng/mL. It is a reflection of the platelet destruction process in sepsis when platelets are activated continuously by pro-inflammatory cytokines and endotoxins. The process will go on without the appropriate treatment and will fall into thrombocytopenia, in which thrombocytopenia is also popular as a marker of target organ damage in sepsis.

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