

THE CORRELATION OF PROCALCITONIN AND MYELOPEROXIDASE INDEX LEVELS IN SEPSIS PATIENTS

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ABSTRACT

Sepsis is still a main problem in the world. The European Society of Intensive Care Medicine (ESICM) and Society of Critical Care Medicine (SCCM) use the quick Sequential Organ Failure Assessment (qSOFA) to diagnose sepsis. An accurate laboratory examination that is faster than culture is needed, procalcitonin is a specific parameter for bacterial infection. Myeloperoxidase index (MPXI) is a new parameter to help to diagnose sepsis. This study aimed to analyze the correlation of procalcitonin with MPXI in sepsis patients. This research was a cross-sectional observational study. Seventy-one patients with sepsis were taken from the Resuscitation Room, Intensive Observation Room, and Intensive Care Unit of the Dr. Soetomo Hospital Surabaya based on the qSOFA and SIRS criteria. The methods used for examining were CLIA (ADVIA Centaur XP) for Procalcitonin, flow cytometry (ADVIA 2120i) for MPXI and PhoenixTM 100 for cultures. The level of procalcitonin is 0.01 ng/mL – 265.16 ng/mL (mean 16.1340.91 ng/mL). The values for MPXI were -25.5–4.6 (mean -7.9394.903). There was no correlation between procalcitonin levels with MPXI ($p = 0.604$ and $r = -0.063$). There was no correlation between procalcitonin levels with MPXI in positive culture results ($p = 0.675$, $r = 0.072$) and negative results ($p = 0.401$, $r = -0.147$). There was no correlation between the level of procalcitonin with MPXI in patients with sepsis.

Key words: Sepsis, procalcitonin, MPXI

INTRODUCTION

Sepsis is a systemic body response to infection.¹ The incidence of sepsis considered a systemic inflammatory response to infection, moreover, has recently increased.^{2,3} The number of hospitalized patients with a primary diagnosis of sepsis even has doubled, i.e. from 11.6 to 24.0 per 10,000 population in 2000-2008.³ This increase may occur due to several factors, such as an increase in antimicrobial resistance, increased use of invasive medical procedures, increased use of immunosuppressive drugs.

Furthermore, the incidence of sepsis per year between 50-95 cases per 100,000 is known to be increased as much as 9% per year. Sepsis mortality is also known to be decreased in developed countries by 9%, while in developing countries such as Indonesia the mortality is still high at 50-70%. The mortality rate, however, can become 80% when accompanied by septic shock and multiorgan dysfunction syndrome.⁴ Hence, sepsis is still known as a major problem in the world.⁵

Accurate and fast laboratory tests actually can help to diagnose sepsis. Several markers are used to

help diagnose sepsis, such as C-reactive protein, procalcitonin, IL-6, TNF α , and perceptions.⁶ Hematology analyzer Advia 2120i has new parameters to help diagnose sepsis, namely Myeloperoxidase Index (MPXI).^{7,8} Advia 2120i is an automatic hematology analyzer that works with flow cytometry principles using peroxidase staining and reagents to detect peroxidase activities of a cell.⁸ Myeloperoxidase (MPO) is a substance released by neutrophils, which activates the synthesis of hypochlorous acid (HOCl) from hydrogen peroxide (H₂O₂) and chloride ion (Cl⁻). HOCl plays an important role as the body's defense against infections of bacteria, fungi, and viruses.⁸

Researches on procalcitonin and MPXI as sepsis markers actually have been carried out in various places, but so far no studies have compared the levels of procalcitonin with MPXI in septic patients, especially in Indonesia. As a result, this study examined the correlation between procalcitonin and MPXI levels in septic patients.

METHODS

This study was an observational analytic study

with a cross-sectional design conducted from December 2017 - February 2018 using a consecutive sampling technique. The number of samples was 71 patients suspected of sepsis and aged ≥ 18 years old. Those subjects had to have at least two criteria for qSOFA, namely a Breath frequency of ≥ 22 x/minute, change of consciousness (Glasgow Coma Scale ≤ 13), or systolic blood pressure of ≤ 100 mmHg. They also had to have two or more SIRS criteria, namely a temperature of $> 38^{\circ}\text{C}$ or $<36^{\circ}\text{C}$, a heart rate of > 90 beats/minute, a respiration rate of > 20 /minute or Pa CO₂ <32 mmHg, a leukocyte count of $> 12,000/\text{mm}^3$, or an immature cell count (band) of $> 10\%$ approved by doctors in duty (DPJP) from the Department of Anesthesia and Reanimation, in the Emergency Room Installation (ERI), the Resuscitation Room (RES), and Intensive Observation Room (IOR) of the Dr. Soetomo General Hospital.

Subsequently, serum procalcitonin (PCT tube) was examined using the CLIA method with the ADVIA Centaur XP device based on the insert kit procedure from Siemens Healthcare Corporation. MPXI (EDTA) analyzed by flow cytometry method with the 2120i Hematology analyzer ADVIA. Next, the examination of culture was conducted using BD PHOENIX TM 100 equipment. The samples then were examined in the Clinical Pathology Department of the Dr. Soetomo Hospital, R & D and Immunology Department, as well as the Laboratory of the Emergency Installation (IRD) at the Dr. Soetomo Hospital.

Afterward, a statistical analysis, Pearson correlation test, was performed if Procalcitonin and MPXI data were normally distributed. Meanwhile, the Spearman test using SPSS 16 software with a significance level of $p < 0.05$ was carried out if the data were not normally distributed. This study was approved by the Ethics Committee of the Dr. Soetomo General Hospital with ethical clearance number 730/Panke.KKE/XII/2017.

RESULTS AND DISCUSSION

The results of the study showed that there were four samples excluded from 75 samples since their PCT levels were less than 200 L (minimum sample limit). The results of the study also revealed that the study subjects were mostly male as many as 37 people (52.1%). Besides, the age range of the subjects was known to be from 23 years old to 77 years old, with a mean age of 46.5 years (SD=14.3). The demographic and clinical characteristics of the 71 suspected sepsis subjects included in this study are listed in Table 1.

Table 1. Characteristics of research subjects in patients suspected of sepsis

Sample characteristics	Total
Sex n (%)	
Male	37 (52.1)
Female	34 (47.9)
Room	
ICU	11
ROI	42
RES	18
Age (years) mean \pm SD	46.5 \pm 14.3
Systolic blood pressure (mmHg)	99 \pm 14.5
Leukocyte count (thousand/ mm^3)	18.2 \pm 10.8
Breath Frequency (x /minute)	25 \pm 2.5
Duration of treatment (days)	3.4 \pm 2.1

The basis of this finding may be related to hormonal differences between the sexes that can contribute to the difference in the inflammatory response and the occurrence of sepsis.⁹

Similarly, several clinical and experimental studies have reported significant effects of sex hormones on cellular and humoral immune responses. The male hormone (androgens) appears to be immunodepressive, while the female hormone

Table 2. Data on number and type of samples, as well as culture, results in sepsis patients

	Culture Results			Types of Bacteria	
		+	-	Gram-positive Bacteria (%)	Gram-negative Bacteria (%)
Culture samples	112	45 (40)	67 (60)	19 (42)	26 (58)
Blood	52	18 (35)	34 (65)	12 (67)	6 (33)
Urine	28	15 (54)	13 (46)	2 (13)	13 (87)
Sputum	28	12 (43)	16 (57)	5 (42)	7 (58)
Pus	4	0 (0)	4 (100)	0 (0)	0 (0)

Table 3. Types of bacteria found in samples with positive culture result

Blood samples (n=18)		Urine samples (n=15)		Sputum samples (n=12)	
Gram-positive bacteria		Gram-positive bacteria		Gram-positive bacteria	
<i>S.hemolyticus</i>	4	<i>C. non-urealyticum</i>	1	<i>S.viridans</i>	5
<i>S.aureus</i>	4	<i>S.epidermidis</i>	1		
<i>S.hominids</i>	3				
<i>E.faecalis</i>	1				
<i>E.coli</i>	4	<i>E.coli</i>	8	<i>E.coli</i>	3
<i>E.cloacal</i>	1	<i>K.pneumonia</i>	1	<i>P.aeruginosa</i>	1
<i>K.pneumonia</i>	1	<i>P.aeruginosa</i>	1	<i>K.pneumonia</i>	1
		<i>A.baumanii</i>	1	<i>A.baumanii</i>	1
		<i>S.indologenes</i>	1	<i>S.maltophilia</i>	1
		<i>S.marcescens</i>	1		

(estrogen) increases the humoral and cellular immune response. An in-vitro study even shows that estrogen has an effect in increasing human peripheral blood mononuclear cell response to lipopolysaccharide. This initial immune response then can contribute to increased resistance to sepsis in the female.¹⁰

In addition, the incidence of sepsis was mostly found in the age range of 51-60 years old. This indicates that the incidence of sepsis is increasing in old age. The median age for septic patients in Europe is known to be around 65 years. Another study revealed that the fatality rate of sepsis patients associated with age was increased from 10% in children to 40% in patients aged > 85 years.

Moreover, the results showed that leukocyte count obtained was 18,168 per μL . This study also found that there were 22 (32%) sepsis patients with a leukocyte count of <12,000 per μL and 49 (68%) patients with a leukocyte count of $\geq 12,000$ per μL . The natural immune response to extracellular bacteria is mainly through the mechanism of phagocytosis by neutrophils, monocytes, and tissue macrophages.¹¹ Neutrophils are phagocytic cells with the main function of searching for and destroying microorganisms, resulting in an increase in leukocyte count accompanied by immature granulocytes in sepsis.¹²

The number of patients with a positive culture was 36 people, while 35 patients with negative cultures. There were 112 samples collected, consisted of 52 blood cultures, 28 urine cultures, 28 sputum cultures, and 4 pus cultures. Data on the number and type of culture sample as well as culture results can be seen in Table 2. Data on types of bacteria found in samples with positive culture results can be seen in Table 3.

Based on Table 3, there were 45 samples (40%)

with the positive culture results, while 67 samples (60%) with the negative culture results. These findings can be caused by the fact that the research subjects were taken from Dr. Soetomo Hospital, a tertiary referral hospital so that those patients had previously received antibiotic therapy.

Different from the results of this study, a research conducted by Puah *et al.* on 1001 sepsis patients in Singapore found that there were 415 (41.5%) septic patients with negative cultures and 586 (58.5%) septic patients with positive cultures. Puah *et al.* argue that culture was less sensitive to identify all types of bacteria. Polymerase Chain Reaction (PCR) examination, on the other hand, was thought to be able to improve the detection process. Thus, many patients with septic clinical symptoms still could give positive PCR results through their culture results are negative.¹³

In this study, the most positive culture results were found in patients with Gram-negative bacteria. Similarly, research conducted by Artero *et al.* finds that the most bacteria causing sepsis are Gram-negative bacteria. Gram-negative bacteria that cause sepsis are dominated by *Escherichia coli*, especially in elderly patients. Research conducted by Hefner *et al.* on 211 sepsis patients in North Carolina also states that Gram-negative bacteria are bacteria mostly causing sepsis (61%).^{14,15}

The highest level of procalcitonin in this study was 265.16 ng/mL, while the lowest one was 0.01 ng/mL with a mean SD value of 16.13 ± 40.91 ng/mL as illustrated in Table 4. On the other hand, the highest level of MPXI in this study was 4.6, while the lowest one was -25.5 with a mean \pm SD value was -7.939 ± 4.903 , as depicted in Table 4.

Schuetz *et al.* state that in septic patients, procalcitonin levels increased from tens to hundreds of times. Schuetz *et al.* also argue that a procalcitonin

Table 4. The average procalcitonin and MPXI levels

Markers	Number of samples	Mean	SD
Procalcitonin	71	16.13	40.91
MPXI	71	-7.939	4.903

level of > 0.5 ng/mL can be considered as an indicator of bacterial infection, while a procalcitonin level of <0.1 ng/mL indicates that there is no bacterial infection.¹⁶

In addition, Kibe *et al.* say that systemic procalcitonin secretion is a component of the inflammatory response that is quite specific for systemic bacterial infection.¹⁷

Meisner and Nargis, furthermore, argue that the value of PCT in healthy individuals is generally less than 0.05 ng/mL, while PCT level from 0.5 ng/mL to 2.0 ng/mL can indicate sepsis, and PCT level above 2.0 ng/mL but less than 10 ng/mL indicates a high risk for the onset of organ dysfunction.¹⁸ In this study, procalcitonin levels were as low as 0.01 ng/mL. This could be caused by a fact that samples were taken too early during the infection as reported by Shehabi *et al.* and Bouadma *et al.*^{19,20} According to Cassado the possibility of low procalcitonin level can also be related to the type of microorganism that causes and the process of severity that occurs.²¹

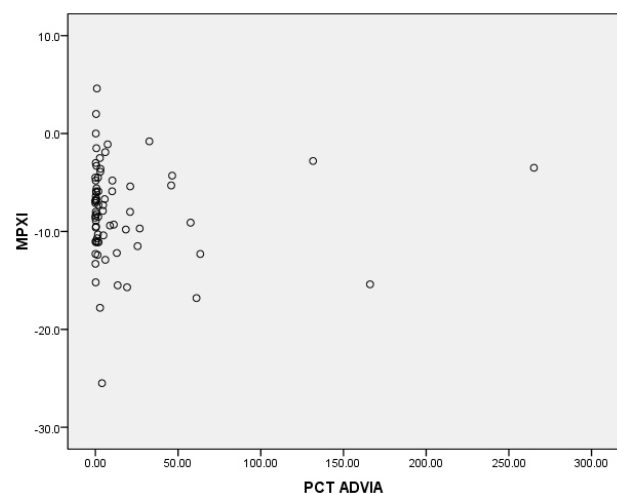
The results of the previous researches conducted by Shehabi *et al.* and Reinhart *et al.* in sepsis patients, moreover, showed that in bacterial infections the level of procalcitonin began to increase at 4 hours after the onset of systemic infection, and the peak was between 8-24 hours.^{19,22} Nevertheless, in this study, there was one sample with very high procalcitonin level (265.16 ng/mL).

In addition, the results of this research showed that MPXI level ranged from -25.5 - 4.6 (-7.939 ± 4.903). Similarly, a research conducted by Yonezawa *et al.* revealed that MPXI values decreased significantly in cases of bacterial sepsis (-2.06 ± 0.75), while in SIRS cases MPXI values decreased further (-3.18 ± 1.14). Yonezawa *et al.* also stated that MPXI values in the case of bacterial infections fluctuated during their research, depended on the cause of the disease and the medical interventions provided, including antibiotics.²³

Different from the results of this study, research conducted by Cha *et al.* showed that there was a statistically significant difference in MPXI values between sepsis and non-infectious SIRS patients. Those researchers argued that analyzing high MPXI can indicate an increase in MPO activity against

microbicide activities since MPXI levels are thought to be controlled by the MPO synthesis and release processes.⁸

Next, the results of the levels of procalcitonin and MPXI were analyzed statistically using the Spearman correlation test to determine the correlation between procalcitonin and MPXI levels. The results of the analysis showed that there was no correlation between procalcitonin and MPXI levels with a p-value of 0.604 and an R-value of -0.063 in sepsis patients (see Figure 1).

**Figure 1.** Graph of correlation between procalcitonin and MPXI levels

Based on Figure 1, there was no correlation between procalcitonin and MPXI levels in this study. The varied procalcitonin and MPXI data can be caused by heterogeneous clinical conditions of sepsis patients, such as the source of infection, the underlying disease, the severity of sepsis, and the prior antibiotic administration.

It is also known that in infectious and inflammatory conditions, proinflammatory mediators and microbial products of bacteria stimulate the expression of CALC-1 genes and induce CT-mRNA of parenchymal cells of the body to synthesize procalcitonin, resulting in procalcitonin release. Organ parenchymal cells can switch to their hormonal functions, including lung, liver, kidney, adipose, and muscle. In the conditions of sepsis and inflammation, all body cells can be said to become neuroendocrine glands, and the classical pathway becomes inactive, so the level of procalcitonin increases in sepsis.

Yonezawa *et al.*, moreover, said that in severe bacterial infections, such as sepsis, active neutrophils release large amounts of MPO for bactericidal

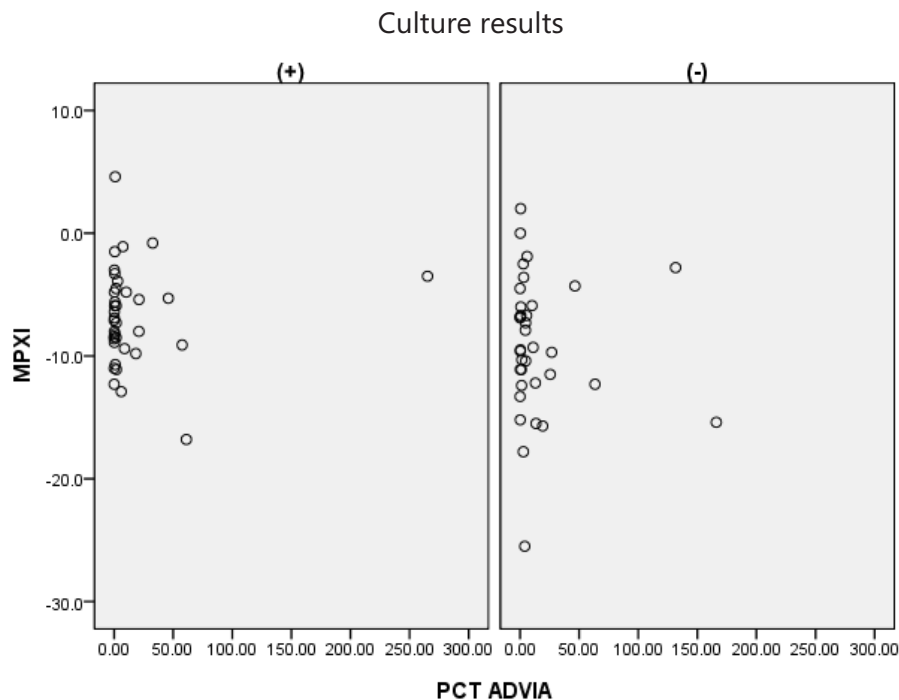


Figure 2. Graph of correlation of procalcitonin and MPXI levels on positive and negative cultures

activity, and increased degranulation of neutrophils can reduce the value of MPXI. MPXI is thought to be controlled by the balance of MPO synthesis and release. Besides, in bacterial sepsis, a cytokine storm can interfere with the natural immune system, causing neutrophil dysfunction and reducing the value of MPXI.²³ Neutrophils that are rich in MPO, according to Buffon *et al.* and Grulke *et al.*, have positive MPXI values, whereas neutrophils that lose MPO have negative values, for example, due to degranulation in systemic inflammation.^{24,25}

However, previous research conducted by Yonezawa *et al.* analyzing the correlation of CRP values and MPXI ($r = 0.58$).¹⁹ Similarly, in this study MPXI results fluctuated because of the heterogeneous causes of sepsis and the diverse medical interventions given. Consequently, MPXI cannot be used as a marker of sepsis since there was no correlation between procalcitonin and MPXI.

Next, the levels of procalcitonin and MPXI on the positive culture results were analyzed statistically using the Spearman correlation test. The results of the analysis showed that there was no correlation between procalcitonin and MPXI levels in positive culture results with a p-value of 0.675 and an r-value of 0.072 (see Figure 2).

Similarly, the levels of procalcitonin and MPXI on negative culture results were analyzed statistically using the Spearman correlation test. The results of

the analysis also showed that there was no correlation between procalcitonin and MPXI levels in the negative culture results with a p-value of 0.401 and an R-value of -0.147 (see Figure 2).

This condition is caused by MPXI values not affecting the results of positive or negative cultures. Besides, this is also because procalcitonin is produced by two mechanisms, namely direct pathways induced by lipopolysaccharide (LPS) or other toxic metabolites derived from microorganisms and indirect pathways induced by various inflammatory mediators, leading to an increase in procalcitonin level in septic patients, both caused by bacterial and non-bacterial infections.²⁶ Bone *et al.* and Puah *et al.* also states that culture examination had low sensitivity, so even though procalcitonin levels were high, negative culture results did not exclude a sepsis.^{6,13}

CONCLUSION AND SUGGESTION

In conclusion, there is no correlation between procalcitonin and MPXI levels in septic patients with both positive and negative culture results. However, this study is still only considered an initial study analyzing the levels of procalcitonin and MPXI. Hence, further researches are suggested to control the homogeneity of the basic clinical conditions of sepsis patients, such as the source of infection,

underlying disease, and severity of sepsis. Besides, further researches need to pay attention to prior antibiotic administration and the homogeneity of procalcitonin and MPXI collecting time, so they are expected to provide more perfect results.

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