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ANALYTICAL PERFORMANCE OF PROCALCITONIN LEVEL BETWEEN CHEMILUMINESCENCE AND QUANTITATIVE IMMUNOCHROMATOGRAPHY METHODS IN SEPSIS PATIENTS

Mario[1], Betty Agustina Tambunan[1], Hardiono[2]

[1] Department of Clinical Pathology, Faculty of Medicine, Airlangga University/Dr.Soetomo Hospital, Surabaya, Indonesia. E-mail: betty_pksurabaya@yahoo.co.id
[2] Department of Anesthesiology and Reanimation, Faculty of Medicine, Airlangga University/Dr.Soetomo Hospital, Surabaya, Indonesia.

ABSTRACT

Sepsis is a public health problem in many countries. The latest diagnosis criteria are quick Sequential Organ Failure Assessment (qSOFA). Procalcitonin (PCT) can be used to aid the diagnosis of sepsis. This study aimed to determine the diagnostic value of PCT between Chemiluminescence Immunoassay (CLIA) and quantitative immunochromatography tests in sepsis patients. Samples were obtained from the resuscitation room, intensive observation room, and Intensive Care Unit (ICU) Dr. Soetomo General Hospital between December 2017-February 2018. One hundred and one subjects were examined and classified into sepsis group (n=71) and healthy group (n=30), based on qSOFA and SIRS criteria. Procalcitonin test with CLIA and quantitative immunochromatography method were performed in all subjects, followed by culture examination in sepsis group using PhoenixTM 100. The diagnostic value of the two methods was analyzed by a 2x2 table with a Confidence Interval (CI) of 95%. There were significant differences of procalcitonin level between CLIA and quantitative immunochromatography method between the sepsis group (p=0.009) and the healthy group (p=0.002). The diagnostic value of procalcitonin level by CLIA method with a cut-off value ≥ 0.27 ng/mL had the same sensitivity but higher specificity, PPV, and NPV rather than by quantitative immunochromatography method. Procalcitonin examination with CLIA had better diagnostic value than quantitative immuno-chromatography method.

Key words: Sepsis, procalcitonin, CLIA, quantitative immunochromatography, the diagnostic value

INTRODUCTION

Sepsis is a clinical syndrome as a results of the interaction between host and infectious agents, and it is characterized by systemic activation of multiple inflammatory pathways, including cytokine network and coagulation. Three major signs of sepsis are inflammation, an increase of coagulation activity, and suppression of the fibrinolytic process. Inflammation and coagulation cascades in sepsis can cause hypoxia and tissue ischemia that leads to organ dysfunction.[1]

The incidence rate of sepsis has been estimated at around 300 cases per 100,000 population in the United States.[2] Data from the Center for Population Health Sciences reported that incidence rates of sepsis between 56-91 cases per 100,000 people, with a mortality rate of 30%.[3] The incidence of sepsis in the Dr. Soetomo General Hospital Surabaya was 2446 cases in 2013 and 3060 cases in 2014. Microbial culture examination can aid the diagnosis of sepsis but has several limitations, such as needing a longer time, low sensitivity, and susceptible to contamination. Some biomarkers were used to aid the diagnosis of sepsis, such as C-Reactive Protein (CRP), Procalcitonin (PCT), Interleukin-6 (IL-6), Tumor Necrosis Factor α (TNFα), and presepsin.[4]

Since the mid-1990s, there has been increasing use of PCT measurement in identifying systemic bacterial infection.[5] Procalcitonin has a short half-life (25-30 hours in plasma), coupled with its virtual absence in health and specificity for bacterial infections, gives it a clear advantage over the other markers of bacterial infection.[6] Studies also have shown that an increase in PCT levels is minimal in viral infections, while levels increase rapidly after a single injection with endotoxin.[7] Most of the PCT measurement use serum as a sample. Nowadays, there is a PCT measurement that uses whole blood EDTA as a sample, with the quantitative immunochromatographic test method. This study aimed to analyze the difference in the diagnostic value of procalcitonin between CLIA and quantitative immunochromatography tests in sepsis patients.

METHODS

The study was conducted in December 2017-February 2018 using a cross-sectional design, and samples were taken consecutively. Subjects with age ≥ 18 years old, consisted of sepsis group (n=71) and healthy group (n=30) based on quick Sequential Organ Failure Assessment (qSOFA) and Systemic Inflammatory Response Syndrome (SIRS) criteria from the Emergency Department (ED), Resuscitation Room (RR), and Intensive Care Unit (ICU) Dr. Soetomo General Hospital Surabaya. Procalcitonin measurement was done by Chemiluminescence Immunoassay (CLIA) from Siemens ADVIA Centaur® BRAHMS PROCALCITONIN and quantitative Immunochromatography Test (ICT) from RAMP® Procalcitonin (Response Biomedical) to both groups. The microbial culture was done for sepsis group by BD PHOENIXTM 100. All Laboratory examinations were performed in the Clinical Pathology Laboratory, Dr. Soetomo General Hospital Surabaya (Table 1). The Ethics Comitee approved this study of the Dr. Soetomo General Hospital with ethical clearance number 723/panke.KKE/XII/2017.

Statistical analysis was performed using SPSS ver. 16.0. Differences of PCT levels between methods were analyzed by paired-T test if the data of each group were normally distributed or analyzed by Wilcoxon signed-rank test if the data of each group were not normally distributed. P-value <0.05 was considered statistically significant, with a 95% confidence interval.

RESULTS AND DISCUSSION

The results of this study revealed that 101 subjects were divided into sepsis group that fit qSOFA and SIRS criteria (n=71) and healthy group (n=30). All of the subjects were willing to participate in this study. In the sepsis group, 11 subjects were from ICU, 42 subjects from ED, 18 subjects from RR. Based on sex, in the sepsis group, there were 37 males and 34 females with mean age of 46.5 years old; in the healthy group, there were 12 males and 18 females with mean age of 31.3 years old. Mean systolic blood pressure, leukocyte count, and respiratory rate was significantly different between the sepsis group and healthy group ($p < 0.0001$). Baseline characteristics of subjects in this study could be seen in Table 2.

This study showed that in the sepsis group with CLIA methods, the highest PCT level was 265.16 ng/mL, and the lowest was 0.01 ng/mL. In the quantitative ICT method, the highest PCT level was 200 ng/mL, and the lowest was 0.2 ng/mL. In the

Table 1. qSOFA and SIRS criteria

qSOFA (quick SOFA) criteria
Respiratory rate of ≥ 22 x/minute
Altered mental status (Glasgow Coma Scale ≤ 13)
Systolic blood pressure ≤ 100 mmHg
SIRS criteria
Body temperature >38°C or < 36°C
Heart rate > 90 x/minute
Respiratory rate ≥ 20x/minute or PaCO2 < 32 mmHg (4.3 kPa)
Leukocyte count > 12,000/mm^3 or < 4,000/mm^3 or > 10% immature neutrophil

Table 2. Baseline characteristic of subjects

Characteristics	Sepsis group	Healthy group	p
Number of subjects	71	30	
Male	37	12	
Female	34	18	
ICU	11	-	
ED	42	-	
RR	18	-	
Age (years)[a]	46.5 (± 14.3)	31.3 (± 2.8)	
Systolic blood pressure (mmHg)[a]	99 (± 14.5)	118 (± 6)	< 0.0001
Leukocyte count (x $10^3/mm^3$)[a]	18.2 (± 10.8)	5.5 (± 1.4)	< 0.0001
Respiratory rate (x/minute)[a]	25 (± 2.5)	18 (± 2)	< 0.0001
Duration of treatment (days)[a]	3.4 (± 2.1)	-	

healthy group using CLIA methods, the highest PCT level was 0.72 ng/mL, and the lowest was 0.01ng/mL. In quantitative ICT method, the highest PCT level was 1.22 ng/mL, and the lowest was 0.2 ng/mL. There was a significant difference in PCT level between CLIA and quantitative ICT method in the sepsis group (p=0.009) and healthy group (p=0.002). The PCT level using CLIA method and quantitative ICT method between the sepsis group and the healthy group was significantly different. The mean, standard deviation of PCT level between two methods in sepsis and the healthy group, could be seen in Table 3 and 4.

In the sepsis group, 47 (66.2%) samples had higher PCT levels using quantitative ICT method, and 24 (33.8%) samples had a higher PCT level using CLIA method. Procalcitonin levels in positive microbial culture between blood specimens and other specimens were significantly different when using CLIA method (p < 0.001) and quantitative ICT (p < 0.001). The PCT levels between blood specimen and another specimen could be seen in Table 5. This study showed that PCT levels using CLIA method had a better Area Under Curve (AUC) than quantitative ICT method, as seen in Figure 1 and Table 6.

Procalcitonin levels in 101 samples using CLIA method with a cut-off value ≥ 0.27 ng/mL had a sensitivity as good as the quantitative ICT method but had a better specificity, positive predictive value, and negative predictive value than quantitative ICT method. The sensitivity, specificity, positive predictive value, and negative predictive value between the two methods could be seen in Table 7.

Based on sex, there were 37 males and 34 females in the sepsis group. This finding was due to that males had a higher inflammatory mediator (such as TNF-, IL-1, and IL-6) than females. Higher inflammatory mediator in the males was because of

Table 3. Mean and SD of PCT levels between two methods in sepsis and healthy group

Group	Method	n	Mean	SD	p
Sepsis	CLIA	71	16.13	40.91	
	Quantitative ICT	71	17.56	37.11	0.009
Healthy	CLIA	30	0.205	0.187	
	Quantitative ICT	30	0.342	0.374	0.002

Table 4. Median PCT level between two groups using CLIA and quantitative ICT method

Method	Group	n	Median (min – max)	p
CLIA	Sepsis	71	1.78 (0.01 – 265.16)	< 0.0001
	Healthy	30	0.165 (0.01 – 0.72)	
Quantitative ICT	Sepsis	71	3.66 (0.1 – 200)	< 0.0001
	Healthy	30	0.21 (0.1 – 1.4)	

Table 5. Mean of PCT levels between blood and, urine and sputum specimens

Method	Positive culture (blood specimen)		Positive culture (urine and sputum specimen)		p
	Mean	SD	Mean	SD	
CLIA (ng/mL)	23.7	62.3	8.74	15.4	< 0.001
Quantitative ICT (ng/mL)	26.8	49.9	8.8	13.1	< 0.001

Table 6. The area under curve of PCT levels using CLIA and quantitative ICT method

			Asymptotic 95% CI	
Method	AUC	p	Lower bound	Upper bound
CLIA	.839	.000	.764	.914
Quantitative ICT	.786	.000	.699	.872

Table 7. Diagnostic value of PCT levels using CLIA and quantitative ICT method

	CLIA	Quantitative ICT
Cut-off (ng/mL)	≥ 0.27	≥ 0.27
Sensitivity (%)	74.6	74.6
Specificity (%)	86.7	66.7
Positive predictive value (%)	93.0	84.1
Negative predictive value (%)	59.1	52.6

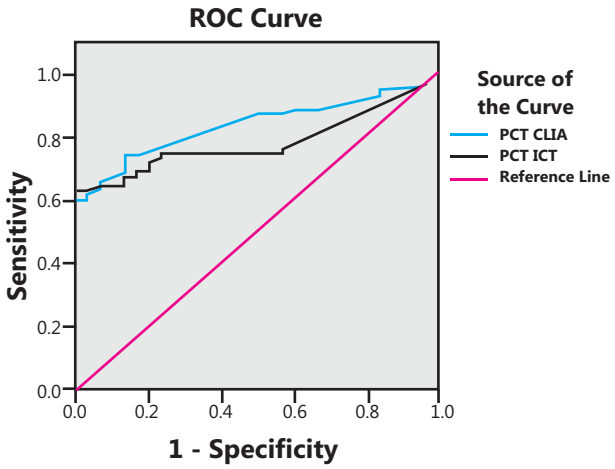

Figure 1. ROC curve of PCT levels with CLIA and quantitative ICT method

estradiol in a female can increase immune function.[8] The incidence rate of sepsis from the Dr. Soetomo General Hospital in 2013 and 2014 also found that males had a higher incidence of sepsis than females. The mortality rate of sepsis in the Dr. Soetomo General Hospital was also higher in males than females.[8,9]

The age range in the sepsis group was between 23-77 years old. Data from Artero *et al*. and Nasa *et al.* found that older age was an independent factor of sepsis. This old age would increase the risk of bacterial colonization, and the risk will be increased if there was comorbidity, malnutrition, and endocrine disease (hypoadrenalism, hypothyroidism, and hypogonadism) due to impairment in the immune response to sepsis.[10,11] Artero *et al*. reported that the age above 65 years old had a relative risk of sepsis 13 times higher.[10]

Vital signs in the sepsis group were significantly different from the healthy group. Mean leukocyte counts in the sepsis group were significantly higher than the healthy group. The increase of leukocytes, especially neutrophils and macrophages, was needed to eliminate microorganism through the phagocytosis mechanism. Neutrophils contain granules that have antimicrobial effects (-defensins, lysozyme, lactoferrin, metalloprotease).[12]

The mean respiratory rate in the sepsis group was significantly higher from the healthy group. Higher respiratory rate was due to inflammatory mediators increased activation of the respiratory center or as a response from metabolic acidosis. Mean of systolic blood pressure in the sepsis group was statistically different from the healthy group. The causes were endothelial impairment because of the pro-inflammatory cytokines, the increase of leukocyte adhesion, vasodilatation, lost of endothelial barrier function, and edema, would decrease cardiac output and lower the systolic blood pressure.[13]

The microbial culture from 112 specimens only had 40% positive results. This result could be caused by non-bacterial sepsis, and most of the subjects from this study had taken antibiotic therapy before the culture examination. Lever *et al*. and Prost *et al*. reported that only 50% of microbial culture examination had a positive culture, this was due to prior antibiotic therapy, technical errors (in specimen collection, inadequate specimen volume, and specimen transport procedure), and fastidious microorganism.[14,15] A study from Phua *et al.* in Singapore reported that microbial culture was less sensitive to identify all microorganism species, Polymerase Chain Reaction (PCR) might improve detection rates, many patients with clinical signs of sepsis save a positive PCR result even when the microbial culture was negative.[16]

This study found that there was a significant difference in mean PCT levels between CLIA and quantitative ICT method in the sepsis group. This finding was because the detection limit between those methods was different. CLIA method had a detection limit of 0.05–75 ng/mL, and quantitative ICT method had a detection limit of 0.20–200 ng/mL. With quantitative ICT method, there were 18 samples with PCT levels 0.20 ng/mL, so the PCT levels were noted as 0.20 ng/mL. This result could cause the difference of PCT levels between CLIA method and quantitative ICT method in the sepsis group and healthy group.

This study found that the Standard Deviation of PCT levels in sepsis group using CLIA and quantitative ICT method was higher than mean PCT levels; the

result could be caused by antibiotic therapy given before sample collection. Non-bacterial sepsis could also be the other cause since non-bacterial sepsis would not increase the production of PCT because PCT was more specific for bacterial infection. Those would affect the mean PCT levels and will cause a higher SD than the mean. The mean of PCT levels between the sepsis group and the healthy group was significantly different. This study showed the same results with a study from Schuetz *et al*. who reported that PCT level in healthy people was low (< 0.01 ng/mL), but in sepsis patient, PCT level increased until hundreds of times. Schuetz *et al*. also reported that the PCT level > 0.5 ng/mL was more likely due to bacterial infection and PCT level < 0.1 ng/mL was less likely due to bacterial infection.[17]

This study also found that the diagnostic value of PCT levels with a cut-off value 0.27 ng/mL gave a sensitivity of 74.6% using CLIA method and quantitative ICT method. CLIA method gave a better specificity, PPV, and NPV than the quantitative ICT method. Kalem et al. reported that PCT was an essential marker for bacterial infection, but history data of illness, physical examination, and other laboratory examination were needed for diagnosis of sepsis.[18]

The mean of PCT levels in the sepsis group between positive and negative culture results using CLIA method and quantitative ICT method was not different. The cause were PCT was produced from two alternative pathway, direct pathways which were induced by lipopolysaccharide (LPS) or other toxic metabolites from microorganisms and indirect pathway that was induced by inflammatory mediator such as IL-6, IL-1, TNF-, etc. These pathways may increase PCT levels in sepsis patient caused by bacterial and non-bacterial infection.[19]

These studies showed that PCT examinations using both methods could differentiate between sepsis and non-sepsis patients and both methods gave the same sensitivity, but CLIA method gave a better specificity, PPV, and NPV than the quantitative ICT method.

Limitation of this study was that there was no classification based on the severity of sepsis from subjects in the sepsis group and also the use of antibiotic therapy that could affect the results of the microbial culture.

CONCLUSION AND SUGGESTION

A difference of mean PCT levels was found between the sepsis group and healthy group using CLIA method and quantitative ICT method. With a cut-off value of 0.27 ng/mL, the CLIA method had the same sensitivity but a better specificity, PPV, and NPV rather than the quantitative ICT method. Further studies are needed to examine the PCT level using CLIA and quantitative ICT method with study subjects divided based on the cause of sepsis and PCT examination before antibiotic therapy.

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