Original Article
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Comparison Between Serum Procalcitonin Measurement Using Rapid Test Fluorescent Immunoassay (FIA) Method and Electrochemiluminescence Immunoassay (ECLIA) Method In Sepsis Detection

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Objective: Procalcitonin (PCT) is widely used as an indicator in detecting bacterial infection in sepsis, but it requires a moderately high cost and a big analyzer with a large sample capacity. It is more favorable for a laboratory in which revenue depends heavily on the national health insurance to have a small-sized analyzer, with small throughput, which can conduct a mono-test at an affordable price. We aim to compare procalcitonin values between two analyzers in sepsis cases.

Material & methods: A cross-sectional study was conducted in Saiful Anwar General Hospital centralized laboratory in 2018. The samples were measured for PCT with two different methods: fluorescent immunoassay (FIA) and electrochemiluminescence immunoassay (ECLIA). The results of the two methods were compared. We also examine the correlation of the results with Sequential Organ Failure Assessment or Pediatric Logistic Organ Dysfunction-2 scores.

Results: As many as 99 patients, consisted of 69 adults and 30 children, were diagnosed with sepsis (2 days to 85-year old). There was no significant mean difference in PCT measures between the two methods ($p > 0.05$). A strong correlation was found between both methods ($r = 0.941$, $p < 0.0001$). There was no significant difference between the two methods for detecting sepsis in adult patients.

Conclusion: PCT measurement using the FIA method (FREND™ PCT) can be used for detecting sepsis in an adult patient since it has a good correlation and no significant difference with the ECLIA method (Cobas e411 Roche). Pediatric patients need special attention and further research.

Keywords: Procalcitonin, sepsis, FIA, ECLIA

Introduction

Sepsis is a life-threatening critical condition in patients leading to multiple organ failure, caused by an inadequate host response to an infection [1]. Despite modern life aids and antibiotic, sepsis patient remains major problem with mortality rate around 30%–60% worldwide. It is estimated that 30 million people experienced sepsis, and in countries with lower middle income, more than 6 million neonates and children died from sepsis every year [2]. Delays in diagnosing bacterial infections and sepsis are the most decisive factors associated with poor outcomes, where confirmation of sepsis is an important element in the early management of patients with suspected infection [3]. The latest diagnostic criteria of sepsis (Sepsis-3) are still difficult to apply because the initial identification still uses clinical signs and symptoms, such as fever, leukocytosis or leukopenia, tachycardia, and dyspnea [4,5].

Sepsis is also one of the leading causes of infant and child morbidity and mortality worldwide. The most common causes of sepsis infections in children include respiratory infections, followed by non-specific infections, bacteremia, urinary tract infections, gastrointestinal infections, central nervous system infections, as well as surgical and soft tissue. In Indonesia, the source of infection mainly comes from respiratory infections, which is 36%–42% with a higher incidence

of sepsis in the neonatal and infant groups <1 year compared with ages 1–18 years (9.7: 0.23 cases per 1,000 children). The Indonesian Pediatric Society published national guidelines for the diagnosis and management of pediatric sepsis in 2016. The diagnosis of sepsis is based on the presence of infection, including predisposing factors for infection, signs or evidence of ongoing infection, and response of inflammation; and signs of organ dysfunction or failure, which is established by pediatric logistic organ dysfunction-2 (PELOD-2) score [6]

PCT is a biomarker for an infection, has been widely used as an early stratification in patients with suspected sepsis. PCT is very helpful in antibiotic management and also has a diagnostic value on the severity of an infectious disease [7]. The prompt treatment is expected to have an impact on reducing morbidity and mortality due to sepsis and also reducing the bacterial development of antibiotic resistance [1] Clinically, PCT greater than 2 ng/ml is associated with a high risk of sepsis and PCT less than 0.5 ng/ml is associated with a low risk of developing sepsis. All PCT measurements use standardized immunoassay techniques for Brahms PCT Luminescence Immunoassay. The automated procalcitonin test used in hospitals mostly uses different methods in detecting antibody-PCT-antibody complexes and the characteristics of each examination which are all standardized by the BRAHMS LIA measurement. Semi-quantitative PCT testing with point-of-care testing (POCT) also requires a 200 µl sample serum or plasma with the duration within 1 hour. Brahms PCT-Q uses lateral flow immunochromatography that causes reddish/brown bands that can be classified and compared with color cards [7–9]. The main obstacle for PCT measurement is that it requires a big analyzer, which is relatively expensive, with a large sample capacity. Along with technological development, currently available analyzers with small size and small throughput, mono-test, and more economical costs. It is more favorable to get results faster in simpler health facilities, especially in peripheral areas, in the era of Indonesian national health insurance [10].

There is also a FREND™ PCT test with quantitative PCT measurements in serum & plasma heparin, citrate, EDTA with a fluorescent immunoassay reader system. The principle of the method is a rapid test with an immunoassay sandwich with fluorescent nanoparticles. This study aims to compare the serum procalcitonin values detected with a big analyzer using the ECLIA method and the FIA rapid test (POC) method. The objective is to investigate whether the rapid test can reliably be used in detecting sepsis.

Material & Methods

This research is cross-sectional study conducted at the Central Laboratory of Saiful Anwar General Hospital, Malang, Indonesia. Participating subjects were recruited and consented during July to December 2018. The inclusion criteria in this study were patients admitted in Saiful Anwar General Hospital, Malang, who was diagnosed with sepsis who underwent procalcitonin laboratory tests and agree to participate. Blood was drawn in a tube with separator gel, then the serum was collected and stored in −80°C refrigerator within 6 months. The exclusion criteria were patients with highly jaundice, hemolysis, or lipemic serum. We used a consecutive sampling technique to obtain the samples.

The serum was examined for PCT levels in parallel with the FIA rapid test method using FREND™ PCT and the ECLIA method using Elecsys BRAHMS PCT Cobas e411 Roche. Diagnosis of sepsis is established by SOFA scores for adult patients and PELOD-2 scores for pediatric patients. The examination of PCT levels by the ECLIA method uses the sandwich principle, with a total inspection duration of 18 minutes and has a detection threshold of 0.02–100 ng/ml. The FIA method rapid test is a rapid quantitative measurement of immunoassay based on the principle of procalcitonin sandwiches on serum or plasma samples (Lithium-heparin, Citrate, and EDTA) using fluorescently conjugated nanoparticle antibodies, which form immune complexes, with procalcitonin found in patient samples. The detection threshold of the instrument is 0.07–32.00 ng/ml. The total time needed for procalcitonin examination with FREND™ PCT is 3 minutes. Prior to an inspection, the tool has passed quality control testing through a QC cartridge that contains several controls, including checking optical parts, laser strength, alignment, and mechanical integrity of the tool. Data were analyzed using SPSS for Windows version 25.0 and Medcalc For Windows version 14.8.1.0. p-value of <0.05 is considered as significant. A categorical comparative test of McNemar and correlation analysis of Spearman correlation test, Passing Bablok, and Bland–Altman Plot was conducted when deemed appropriate.

Results

As many as 99 blood samples of patients newly diagnosed with sepsis were obtained. The subjects were 69 adult samples and 30 samples of children whose procalcitonin levels were examined by the ECLIA and FIA methods with the characteristics listed in Table 1. The subjects age ranges from 2 days to 85 years.

Table 1. Characteristics of research subjects.

Parameter	PCT ECLIA (n = 99)	PCT FIA (n = 99)
Gender		
Male	51	51
Female	48	48
PCT > 2 ng/ml (sample of pediatric patients)	15	15
PELOD-2 score > 11	2	2
PCT > 2 ng/ml (sample of adult patients)	54	54
SOFA score ≥ 2	48	48
PCT (Mean ± SD)	13.58 ± 13.37	13.82 ± 12.68
PCT (CV)	0.98	0.91

We tested all samples for data normality using the Kolmogorov–Smirnov test and obtained not normally distributed. Therefore, it continued with the Wilcoxon paired numerical comparative test, between the two methods, and the results showed no significant difference between the procalcitonin results with the ECLIA and FIA methods which can be seen in Table 2. Then, data continue analyzed with Spearman correlation test showed r = 0.941 (p-value < 0.0001) between two methods, with plot diagram, is shown in Figure 1. The agreement between the procalcitonin results with the ECLIA and FIA methods analyzed with Passing and Bablok Regression can be seen in Figure 2 and using the Bland–Altmann Plot of agreement that can be seen in Figure 3.

Table 2. Pair t-test (Wilcoxon) test of procalcitonin by ECLIA and FIA methods.

Parameter	PCT FIA (n = 99)
Z	−1.099[a]
Asymp. Sig. (2-tailed)	0.272[b]

[a]Based on negative ranks.

[b]Wilcoxon Signed Ranks Test

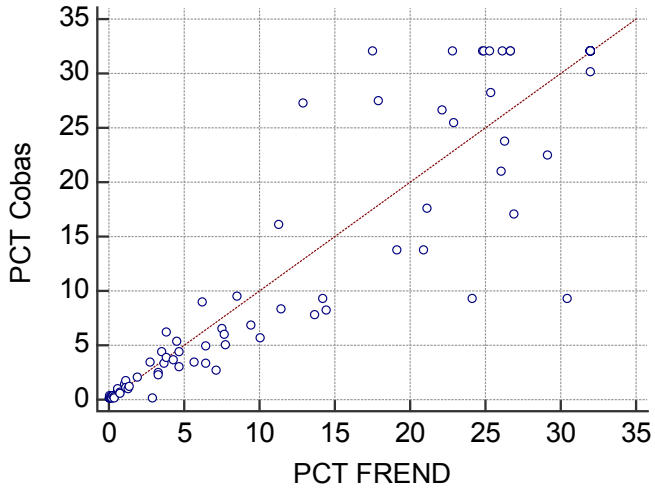

Figure 1. Spearman correlation test for the ECLIA and FIA methods on PCT examination.
Plot diagram of PCT measured using Cobas ECLIA method compared to FREND™ FIA method based on spearman's correlation test.

The results of the categorical comparative hypothesis test with the McNemar test on both methods are listed in Table 3.

Table 3. McNemar test for procalcitonin examination by ECLIA and FIA methods in adult patients.

Parameter	Value	Exact Sig. (2-sided)
McNemar test		1.000
N of valid cases	99	

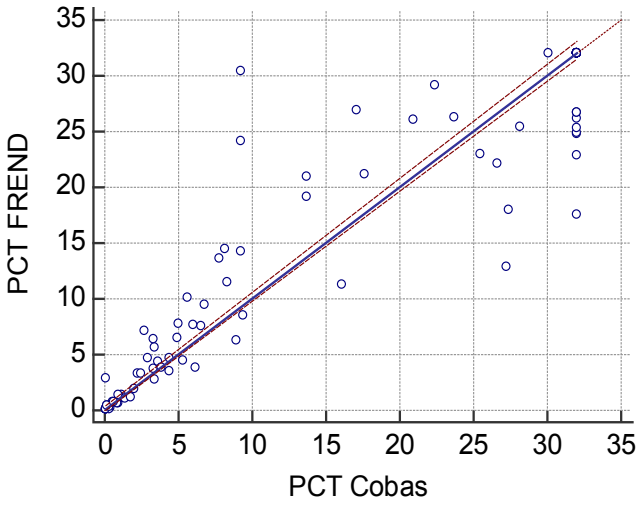

Figure 2. Passing and Bablok Regression ECLIA and FIA methods on PCT examination.
Passing and Bablok Regression graph of procalcitonin results in ECLIA method with Cobas and FIA method with FREND™.

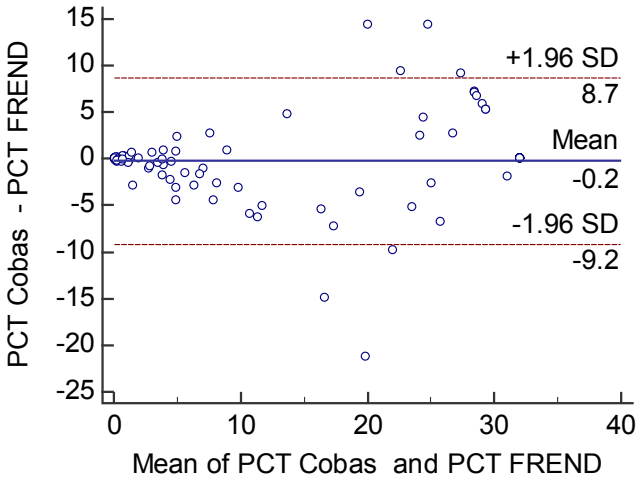

Figure 3. Bland-Altman plot of the ECLIA and FIA methods on PCT examination
The Bland-Altmann plot agreement between the two methods for procalcitonin measurement. Axis X illustrates the mean value of procalcitonin measurements by both methods. The Y-axis illustrates the difference between the ECLIA and Cobas methods and the FIA and FREND™ methods.

Discussion

PCT is a prehormone of calcitonin produced by thyroid parafollicular C cells that plays a role in the process of calcium homeostasis. However, PCT is also mostly produced from non-thyroid tissue as an acute phase reactant which is a response to inflammatory stimuli, especially endotoxins and lipopolysaccharides of bacteria, and several inflammatory mediators. The

role of PCT in the inflammatory response to infection is still not fully understood, but it is thought to contribute to local and systemic immune responses by modulating both immune and vascular function [7,8,10].

PCT is a calcitonin precursor polypeptide consisting of 166 amino acids with a molecular weight of 13 kDa. In healthy individuals, procalcitonin is produced by C cells of the thyroid gland and is released in the bloodstream at levels <0.05 ng/l, through transcription and translation of the PCT gene, CALC-1, which is found on chromosome 11. The transcription process goes fast and is separated into three products, such as katacalcin, calcitonin, and terminal fragments N [7,9].

Plasma procalcitonin concentration increases with stimulation by bacterial endotoxins within 2–3 hours after bacterial invasion, because procalcitonin production is activated in all parenchymal tissues. Procalcitonin levels are stable after 6–12 hours, remain high for up to 48 hours, and can last for up to 7 days. The half-life of procalcitonin is about 20–35 hours after the loss of inflammatory signals, such as bacterial endotoxin. Procalcitonin is degraded by proteolysis and a small portion is excreted through the kidneys [7,9]. The biological function of PCT itself is still unclear; it has been reported that PCT has pro and anti-inflammatory properties. The immunological role of PCT may be a cofactor that can modulate the impact of shock from endotoxins. PCT regulates in response to pro-inflammatory mediators, such as IL-1β, TNF-α, and IL-6. Some studies show that serum procalcitonin decreases against cytokines (such as interferon-gamma) released during viral infections [9]

PCT was first discovered as an additional biomarker that was useful for guiding sepsis in 2006 where PCT acted as a good diagnostic marker for sepsis, severe sepsis, and septic shock. In an analysis of 30 studies, Wacker et al. [11] found that high PCT levels did indeed indicate sepsis, and low PCT levels had to prevent the initiation of antibiotics. However, they cannot recommend specific cut off, and PCT levels must be interpreted in a clinical context [11]. A more recent meta-analysis defines a certain threshold level of 0.5 mg/dl, and finds that it is most useful and accurate in diagnosing sepsis in ICU patients, but worst in immunocompromised patients. They recommend that low PCT levels should help to exclude bacteremia [12].

PCT levels can increase in certain situations without infection. For example, in conditions of prolonged or severe cardiogenic shock, anaphylactic, prolonged and prolonged organ perfusion anomalies, small cell lung cancer or thyroid medullary C-cell carcinoma, early after major trauma, major surgical intervention, severe burns, and in neonates (<48 hours after birth). PCT may also fail in the initial phase of infection [13]. It was recently reported that the diagnostic accuracy of PCT in patients with acute kidney injury is lower than in patients without acute renal failure (ARF) because PCT is eliminated through the kidneys and/or liver. PCT levels were found to be higher in patients with infection with ARF, but an increase in PCT levels in ARF patients was (at least partially) related to the severity of infection and kidney dysfunction. The diagnostic accuracy of PCT for infections in patients with ARF is not lower than in patients without ARF. PCT is a useful marker of bacterial infection in patients with AKI, but a different cut-off must be applied [14]. A study conducted by Svaldi et al. [15] on 475 research samples, states that the condition of hematological diseases that have severe leukopenia and immunocompromise can affect PCT levels, with positive predictive value (cut-off 2 ng/ml) and negative predictive value (cut-off) 0.5 ng/ml) PCT for sepsis is 93% and 90% in patients with leukocyte counts > 1,000/mm3, 66% and 63% in leukopenia conditions.

PCT is measured with immunoassay technique and all tests were standardized using the original Brahms PCT luminescence immunoassay. Currently, PCT rapid test measurement with Brahms PCT-Q has also been developed using lateral flow immunochromatography which produces reddish or brown color bands, classified into four PCT levels (<0.5; 0.5–2.0; 2.0–10; > 10 ng/ml) [8,16].

Our study compared two PCT measurement methods. The first is the FREND™ PCT FIA method and the second is the Elecsys BRAHMS PCT Cobas e411 Roche autoanalyzer ECLIA method. We used serum samples from 99 newly diagnosed sepsis patients. The Wilcoxon test shows that there is no significant difference in PCT concentration measured between the two methods. There is a strong correlation (Spearman's $r = 0.941$, $p < 0.0001$) between the results of procalcitonin in both methods. In addition, a categorical comparative test with McNemar's test on the sample is also performed, the results obtained in the form of numbers into the sepsis category or not.

However, the comparative regression line between the two methods shows a difference, this is seen at higher PCT concentrations, but not at the lower end, where the medical cut-off is located. Current literature supports that PCT thresholds ≥ 0.5 and ≥ 2.0 ng/ml to detect the possibility of sepsis, and PCT ≥10 ng/ml indicate a high likelihood of severe sepsis or septic shock [8,16]. Furthermore, in the PCT diagnostic test, the FIA method using the ROC curve showed an AUC result of 0.62 ($p = 0.0075$), with a sensitivity of 82.35% and a specificity of 41.67%. Whereas in the PCT diagnostic test the ECLIA method using the ROC curve showed almost the same results, AUC of 0.61 ($p = 0.01$), with a sensitivity of 80.39% and specificity of 41.67%. There are some discrepancies between procalcitonin results in comparison to the SOFA scores (36%) and PELOD-2 scores (40%) in both methods. This might be due to data limitations that affect the calculation of the PELOD-2 score.

Conclusion and Limitations

The conclusion of this study is the examination of procalcitonin by the FIA (FREND™ PCT) method has many advantages, which include portability, more affordable cost, comfort, and relatively fast results so that it can be used in health facilities in remote areas that do not have an analyzer for sepsis detection. This method has a strong correlation and is not significantly different from the ECLIA method (Cobas e411), especially in adult patients. Its usage in pediatric patients requires further research.

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