Diagnostic Accuracy of Procalcitonin as a Marker of Gram-Negative Bacteremia on Sepsis and Septic Shock Patients in Intensive Care Unit (ICU)

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Abstract: Background: Prediction of pathogen's species among sepsis and septic shock patients within hours would be helpful in accelerating proper treatment. A biomarker like PCT could bea potential method to decrease the time of identification & prevent antibiotic resistency.

Objectives: To assess the usefulness of procalcitonin (PCT) to predict blood culture (BC) results.

Methods: The authors retrospectively analyzed data of sepsis and septic shock patients in ICU Dr.Wahidin Sudirohusodo Hospital from January 2015 to June 2016 with BC and PCT draw simultaneously in ≤ 24 hours. Kruskal-Wallis analysis was used for multi group comparisons. The diagnostic performance of PCT to predict gram-negative bacteremia was tested using a ROC curve.

Results: A total of 90 diagnostic episodes met the inclusion criteria. A significantly higher value of PCT was found in gram-negative BC (77.3 ng/mL, 0.43–200.01) than that in negative BC (50.15 ng/ml, 0.45-200.01)gram-positive BC (28.9 ng/mL, 0.75–200.01) or fungal BC (31.5 ng/mL, 30.33–32.62).

For gram-negative bacteremia identification in sepsiscut-off value ≥ 6.8ng/mL for PCT yielded sensitivity 77.3 %, specificity58.6 %,PPV 58.6 %, NPV 77.2 % andAUC 0.655. In septic shock cut off value ≥50,82 ng/ml, Sensitivity 82,4 %, Specificity 63,6 % , PPV 63,6%, NPV 82,3%, AUC 0,713.

Conclusions:The diagnostic accuracy of PCT to predict gram negative bacteremia is sufficient to good, it may be useful for differentiating gram-negative from gram-positive and fungal bloodstream infection with a significantly higher PCT level indicating gram-negative bacteremia. The high NPV may represent a useful tool to exclude the presence of gram negative bacteremia to guide the empirical antimicrobial therapy

Key Words: Procalcitonin; Sepsis; Septic shock; Blood Culture; Gram-negative bacteremia.

I. INTRODUCTION

Sepsis is one of the most common causes of death in hospitalized patients. Blood culture has been considered a gold standard for the detection of bacterial infections and is one of the procedures in sepsis bundle (3 hour bundle). Conventional microbiological culture is time consuming, beyond its specificity and accuracy. Estimates of delayed results from blood cultures are between 48-72 hours. Meanwhile, the presence of biochemical tests that can rapidly predict the probability of blood culture results may help empirical antibiotic therapy in septic patients and prevent antibiotic resistance.[1,2]

Procalcitonin is a calcitonin prohormone and is synthesized by C cells in the thyroid gland, produced in response to endotoxin or to mediators released in response to bacterial infections. Procalcitonin is synthesized by a large number of tissues and organs in response to invasion of bacterial pathogens, fungi, and parasites. Procalcitonin serum is a peptide of 116 amino acids, and increases in levels are associated with systemic bacterial infections. Procalcitonin levels increase (up to 5000-fold) between the first 2 to 4 hours on the severe form of systemic inflammation or in bacterial infections reaching peak levels within 6 hours, and persist for 24 hours after the healthy trial animals are injected with gram negative bacterial endotoxin. Its biological half-life is 22-26 hours, which is advantageous when compared with CRP or other acute phase markers.[3,4,5,6]

PCT is produced in response to bacterial endotoxin and host inflammatory cytokines, and can differentiate bacterial infections from viral infections. It is well known that gram-positive or gram-negative bacteria or fungi activate different Toll-like receptor (TLR) pathways, resulting in the production of different proinflammatory cytokines that stimulate PCT release. This illustrates that different types of pathogens can produce different levels of procalcitonin production as well. This can be relevant in bloodstream infections, in which PCT can guide the clinician in the provision of appropriate initial therapy which is essential for patient outcomes.[7,8]

II. MATERIALS AND METHODS

Patients and samples

This was a retrospective observational analytic study and was approved by the Ethics Committee of dr. Wahidin Sudirohusodo Hospital. The authors reviewed the clinical records of all patients with a diagnosis of sepsis or septic shock from January 2015 to Juni 2016 and registered

those in agreement with the diagnostic protocol consisting of a blood culture and measurement of serum PCT. The authors excluded the diagnostic episodes done on patients with missing tests and those not completed with an 24-hour time interval. Clinical and microbiologic data were obtained from the comprehensive electronic medical

Procalcitonin Assay

Plasma procalcitonin concentration was measured using automated immunofluorescent assays of procalcitonin in human plasma (EDTA, heparin) samples (Brahms PCT sensitive KRYPTOR kit for Brahms KRYPTOR, Hennigsdorf, Germany) according to the supplier's protocol. The normal procalcitonin concentration was defined as <0.05 ng/mL according to supplier reference values.

Blood Culture

For each sample, an 5 to 10mL whole blood was inoculated into BACTEC aerobic and anaerobic bottles (Becton Dickinson, Sparks, MD). BACTECThe bottleswere incubated in a BACTEC FX automated blood culture system (Becton Dickinson). All bottles flagged positive were removed from the instrument and was taken for Gram-stain and culture on solid media for subsequent analysis. Identification of microorganisms was performed with conventional methods.

Statistical Analysis

The comparison of serum procalcitonin values across groups was performed using the Kruskal-Wallis test.A p value 0.05 was considered statistically significant.The diagnostic performance of PCT was evaluated using a receiver operating characteristic curve, with AUC (Area Under the Curve) as the indicator of diagnostic accuracy The cutoff value of PCT to predict gram-negative bacteremia was selected considering the sum of the highest sensitivity and specificity.Statistical analysis was performed using SPSS for Windows (version 11.5; SPSS Inc, Chicago, IL).

III. RESULTS

Characteristics of the study population and distributions of causative pathogen

Over 18 months, a total of 90 diagnostic episodes met the inclusion criteria, 51 sepsis and 39 septic shock. Overall, 55 (61,1 %) men and 35 (38,9 %) women were included. The mean age of the patients was 42,96 years. There are 36 diagnostic episodes of negative BC and 54 of positive BC which consists of 39 gram negative bacteremia, 13 gram positive bacteremia, and 2 fungemia were included in this study.

Measurements of Procalcitonin

Serum PCT concentrations in patients with Gram-negative BC were significantly greater than in patients with negative BC, gram-positive BC, or fungal (77.25 ng/ mL 50.15 ng/ml, 28,87 ng/mand 31.47 ng/mL, respectively, $P < 0.05$) [Table 1].

Table 1. Comparison of PCT value according to BC results

Variable	BC results	N	Mean	SD	Median	Min	Max
Procalsitonin	Negative BC	3	50,15	67,91	21,70	0,45	200,01
	Gram negative BC	6	77,25	77,73	38,30	0,43	200,01
	Gram positive BC	3	28,87	55,77	8,07	0,75	200,01
	Fungal	9	31,47	1,61	31,47	30,33	31,47
	Total	9	58,40	71,69	27,39	0,43	200,01

Kruskal-Wallis (p < 0,05)

Diagnostic accuracy of procalcitonin

Receiver operating characteristic (ROC) analysis was performed to reveal the diagnostic accuracy of PCT concentrations to distinguish Gram-negative BC from negative BC, gram positive BC and fungal BC in shock and septic shock.In sepsis, an optimal PCT cut-off value of 6.8 ng/mL resulted in an AUC value of 0.655 (95% confidence interval [CI], 0.498–0.812, $p > 0.05$), sensitivity of 77.2%, specificity of 58.6 %, PPV of 58.7 %, and NPV of 77.3%. [Figure 1]

In shock septican optimal PCT cut-off value of 50.8 ng/mL resulted in an AUC value of 0.713 (95% confidence interval [CI], 0.546–0.879, $p < 0.05$), sensitivity of 82.4 %, specificity of 63.6%, PPV of 63.7 %, and NPV of 82.4%. [Figure 2]

Table 2. Comparison of ROC value base on PCT Best cut off

(ROC curve) PCT best cut off	(a) 6.8 ng/ml	(b) 50.8 ng/ml
Sensitivity	**77.2 %**	**82.4 %**
Specificity	**58.6 %**	**63.6 %**
PPV	**58.7 %**	**63.7 %**
NPV	**77.3 %**	**82.4 %**

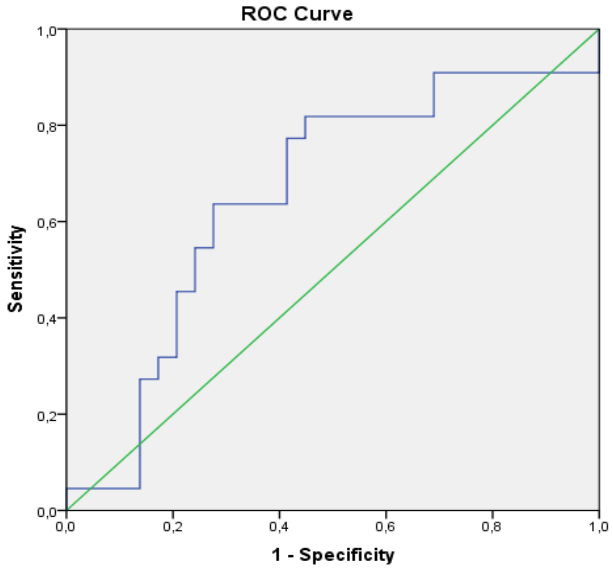

Figure 1. Receiver operating characteristic curve

(a)

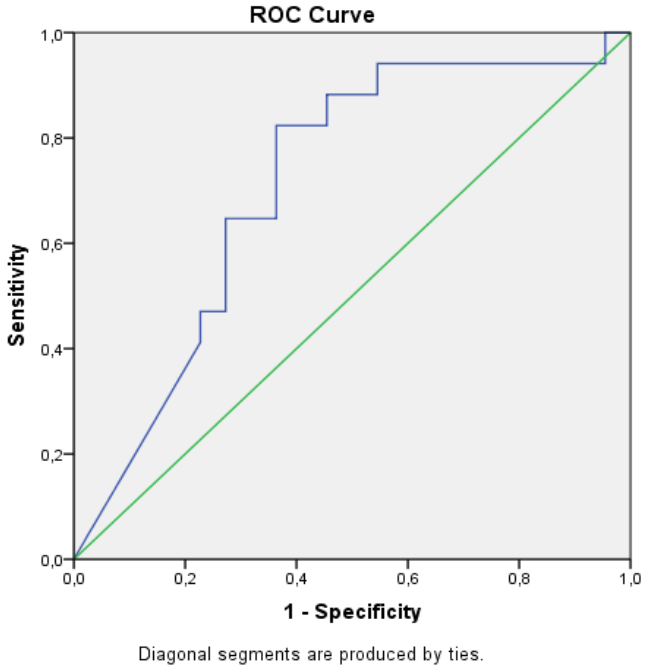

Figure 1. Receiver operating characteristic curve

(b)

Figure 1. Receiver operating characteristic (ROC) curves of different cut-offs of PCT as a marker of gram negative bacteremia in: (a) Sepsis patients (AUC 0.655, 95% CI 0.498–0.812; *P*> 0.05); (b) Septic shock patients (AUC 0.713, 95% CI 0.546– 0.879, *P*< 0.05). Sensitivity, Specificity, Positive Predictive Value (PPV) ,Negative Predictive Value (NPV) are reported for the best cut-off values found in each ROC.

IV. DISCUSSION

In this study we found that there were 54 positive cultures (60%) and 36 negative cultures (40%), this is not in accordance with the literature which states that the result of positive blood culture is 30% of the total blood culture. 37 of 54 positive blood cultures found 39 gram negative bacteria, 13 gram positive, and 2 fungus, this is in accordance with the literature which states that gram-negative bacteria is the most common type of bacteria that causes sepsis.[7,8]

In this study found significantly higher procalcinonin pro- tees in gram-negative cultures, compared with negative culture results, gram-positive bacteria, or fungi. This is due to differences in gram-negative, gram-positive, and fungal inflammatory cascade initiation, where the lipopolysaccharide (LPS) pattern in gram-negative can activate neutrophils through Toll like receptor 4 (TLR-4), and lipoteikoic acid in gram bacteria positive work through TLR-2. Toll like receptors activate the inflammatory cascade triggers that lead to the synthesis of proinflammatory cytokines and acute phase proteins. Gram-negative bacteria increase the production of tumor necrosis factor α (TNF-α) rather than a gram-positive infection. Differences were also found in plasma levels of interleukin (IL) -1, IL-6, IL-10, and IL-8. This causes gram-negative bacteremia to cause a greater inflammatory response than a gram-positive bacteria.[8-10]

In this study, the ROC curve obtained procalcinonin had a sensitivity of 77.2%, specificity 58.6%, 58.7% positive predictive value, negative predictive value 77.3%, with cut off value ≥ 6.8 ng / ml at under the curve (AUC) area of 0.655, which means procalcinonin has sufficient diagnostic accuracy (0.6-0.7) as a marker of gram-negative bacteria on the blood cultures of sepsis patients. The Cut off value of PCT in this study is similar to that obtained in a cohort study conducted by Shu Yuan Guo[10] who obtained a 6.47 ng / ml cut off value of PCT as a marker of gram negative bacteremia in septic patients but with sensitivity, specificity and value higher negative predictions (74%, 81%, 82%)

With the ROC curve obtained 82.4% Sensitivity, 63.6% specificity, 63.7% positive predictive value, negative predictive value 82.3% with AUC 0.713 which means PCT has a good diagnostic accuracy value (0.7-0, 8) as a marker of gram negative bacteremia in patients with septic shock. The research that examines the accuracy of diagnostic procalcitonin as a marker of gram negative bacteremia in patients with sepsis shock has never been done before. The high predictor prophylactitin (NDN) values for the detection of gram-negative bacteria in blood cultures can be useful tools for exclusion of gram-negative bacteria and guide empiric antimicrobial therapy regimens in septic patients and septic shock to reduce treatment costs and optimize therapy[3,7-12]

The disadvantage of this study is that cross-sectional retrospective research designs require validation

from longitudinal prospective studies, small sample size, exclusion of other types of culture, lack of population of fungi, and gram negative populations twice as many as gram-positive the occurrence of selection bias.

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