

LEUCOCYTE, NEUTROPHILS COUNTS AND PROCALCITONIN LEVELS IN *SALMONELLA* AND GRAM-NEGATIVE BACTEREMIAS

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ABSTRACT

Background: The laboratory marker of leucocytes, neutrophils and procalcitonin (PCT) are elevated in Gram-negative-infected patients. *Salmonella* species, a cause of typhoid fever, are also a type of Gram-negative bacteria. We investigated the laboratory marker of bacterial infection levels in *Salmonella* and Gram-negative bacteremias.

Methods: This retrospective study was conducted in Jakarta, Indonesia. Sixty-one patients with positive blood cultures of *Salmonella* or Gram-negative bacteria who were admitted to the hospital from April 2014 through May 2017 were included. Twenty-seven patients (44,3%) had *Salmonella*, and 34 patients (55,7%) had Gram-negative bacteremias. The following laboratory parameters were recorded: leucocyte count, neutrophil count, and PCT levels. Bivariate analysis was used to analyze the differences in the laboratory marker between *Salmonella* and Gram-negative bacteremias.

Results: Gram-negative bacteremia was significantly associated with an elevated leucocyte count ($p < 0.001$), neutrophil count ($p < 0,001$) and PCT levels ($p < 0,001$). The leucocyte count cut-off of $\geq 10.5 \times 10^3 / \mu\text{L}$, a neutrophil count cut-off of $\geq 80,9\%$ and a PCT level cut-off of $\geq 1,18 \text{ ng/ml}$ were significantly higher in the Gram-negative bacteremia group compared with the *Salmonella* group ($p < 0,001$ for each variable).

Conclusion: Leucocyte, neutrophil counts, and PCT levels in Gram-negative bacteremia were higher than in *Salmonella* bacteremia.

Keywords: Gram negative bacteremia, leucocyte, neutrophils cells counts, procalcitonin, *Salmonella* bacteremia.

ABSTRAK

Latar belakang: Penanda laboratorium leukosit, neutrofil dan prokalsitonin (PCT) meningkat pada pasien dengan infeksi Gram negatif. Spesies *Salmonella* penyebab penyakit demam tifoid juga merupakan bakteri Gram negatif. Penelitian ini dilakukan untuk menganalisis penanda laboratorium infeksi bakterial pada penderita dengan bakteremia *Salmonella* dan bakteremia Gram negatif.

Metode: Penelitian retrospektif dilakukan di Jakarta, Indonesia. Sebanyak 61 pasien dengan *Salmonella* atau bakteremia Gram negatif yang dirawat di rumah sakit sejak bulan April 2014 sampai Mei 2017 diikutsertakan dalam penelitian ini. Sebanyak 27 pasien (44,3%) menderita bakteremia *Salmonella*, dan 34 pasien (55,7%) dengan bakteremia Gram negatif.

Hasil laboratorium yang dicatat adalah jumlah leukosit, neutrofil, dan kadar PCT. Analisis bivariat digunakan untuk menganalisis perbedaan penanda laboratorium infeksi bakterial antara bakteremia *Salmonella* dengan bakteremia Gram negatif.

Hasil: Penderita dengan bakteremia Gram negatif memiliki jumlah leukosit ($p < 0,001$), jumlah neutrofil ($p < 0,001$) dan PCT ($p < 0,001$) meningkat secara bermakna dibandingkan dengan penderita *Salmonella* bakteremia. Jumlah leukosit dengan nilai titik potong $\geq 10,5 \times 10^3 / \mu\text{L}$ ($p < 0,001$), jumlah neutrofil dengan nilai titik potong $\geq 80,9\%$ ($p < 0,001$) dan kadar PCT dengan nilai titik potong $\geq 1,18 \text{ ng / ml}$ ($p < 0,001$) secara bermakna lebih tinggi pada kelompok bakteremia Gram negatif dibandingkan dengan kelompok *Salmonella*.

Kesimpulan : Jumlah leukosit, netrofil, dan kadar PCT pada penderita bakteremia Gram negatif lebih tinggi dibandingkan dengan penderita bakteremia *Salmonella*.

Kata kunci: Bakteremia Gram negatif, lekosit, jumlah netrofil, prokalsitonin, bakteremia *Salmonella*.

INTRODUCTION

Procalcitonin (PCT) is a protein composed of 116 amino acid with a molecular mass of 13 kDa.¹ The definite source of serum PCT is uncertain, but it has been speculated that PCT is produced by liver cells, monocytes cells, and macrophage cells in response to infection.² Serum PCT levels increase rapidly

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during various bacterial infection, especially Gram-negative bacterial infections.³ The outer membrane component of Gram-negative bacteria (i.e. endotoxin or lipopolysaccharides) has been shown to be a strong inducer of PCT during bacterial infection. These bacteria cause the host to produce pro-inflammatory cytokines, which leads to increased PCT production.^{3,4} Elevated cytokines levels also cause the host to increase production of leucocyte and neutrophils cells. The lipopolysaccharides component plays a large role in the severity of Gram-negative infections. In clinical settings, PCT together with leucocyte and neutrophil counts are commonly used as markers of infection.⁵

Salmonella species, a cause of typhoid fever, are also Gram-negative bacteria that contain endotoxin on their cell surface. Binding of *salmonella* endotoxin to CD14/Toll-like receptor (TLR)4 on macrophage cells activates nuclear factor kappa B (NFκB) to produce pro-inflammatory cytokine and increase inflammatory cytokines, resulting in elevated PCT levels.^{6,8} In clinical practice, leucocyte and neutrophil counts can be used as a marker of bacterial infection.⁹ In addition, several studies have reported that serum PCT levels are useful in distinguishing Gram-negative bacteremia from Gram-positive bacteremia.^{3,10} However, there have been no studies comparing laboratory markers of bacterial infection in Gram-negative and *Salmonella* bacteremias. Therefore, we conduct this study to investigate the differences in leucocyte and neutrophil cell counts and PCT levels among *Salmonella* and Gram-negative bacteremias.

METHODS

This retrospective study was conducted at a private hospital in Jakarta, Indonesia from April 2014 through May 2017. Patients older than 17 with Gram-negative or *Salmonella* bacteremias were included in this study. Clinical characteristics, leucocyte and neutrophil counts and PCT levels were recorded for each subject. The PCT measurement was performed by immunoluminometric assay (B.R.A.H.M.S. Diagnostica AG, Germany). Automated blood culture (BD BACTEC™ Blood Culture System, Becton Dickinson, USA) was used to determine the subjects with Gram-negative or *Salmonella* bacteremias.

STATISTICAL ANALYSIS

The rule of thumb formula with ≥ 20 subjects for each variable was used to calculate the minimum sample size.¹¹ In this study, the total number of variables was 3. Therefore, the sample size required was 60 subjects. The Mann-Whitney U test was used to analyze the nonparametric data. Area under the curve (AUC) analysis was used to obtain the cut-off of leucocyte and neutrophil counts and PCT levels between the two groups. The chi-square test was used to analyze the categorical data. SPSS version 20 (IBM SPSS Statistics Corp., Armonk, NY, USA) was used for the statistical analysis.

RESULTS

Sixty-one patients were included in this study: 27 patients (44,3%) had *Salmonella* and 34 patients (55,7%) had Gram-negative bacteremias. The clinical characteristics and laboratory parameters of both groups of patients are presented in Table 1.

Table 1. Comparison clinical characteristics and laboratory parameters: *Salmonella* and Gram-negative bacteremias group

Variable	<i>Salmonella</i> bacteremia group	Gram-negative bacteremia group
Sex, no, female/male	13/14	22/12
Age, y, median (IQR)	32 (28-53)	32 (19-42,5)
Temperature, °C, median (IQR)	39,2 (38,5-40)	38,8 (38,2-39,7)
Hemoglobin, g, median (IQR)	12,7 (12,4-13,3)	12,5 (11,6-13,2)
Hematocrit, %, median (IQR)	38,4 (37,2-41,4)	38,3 (36,9-40,2)
Leucocyte x1000/μL, median (IQR)	6,17 (5,24-8,36)	13,61 (7,76-17,4)*
Neutrophils counts, %, median (IQR)	69,9 (62-75,5)	86,2 (78,8-90,4)*
Platelet count x1,000/μL, median (IQR)	150 (13,3-211)	163 (96,5-275)
PCT levels, ng/ml, median (IQR)	0,6 (0,3-0,9)	7,9 (2,1-32,8)*

Abbreviations: PCT, procalcitonin; IQR, interquartile range.

Significant difference from the *Salmonella* bacteremia group (P < 0,05)

Compare with *Salmonella* bacteremia, Gram-negative bacteremia was significantly associated with an elevated leucocyte count ($p < 0,001$), neutrophil count ($p < 0,001$) and PCT levels ($p < 0,001$). The cut-off points of the three variables based on the AUC analysis are listed in Table 2. We found a leucocyte count cut-off of $\geq 10,5 \times 10^3/\mu\text{L}$, a neutrophil count cut-off of $\geq 80,9\%$ and PCT levels cut-off of $\geq 1,18$ ng/ml were significantly higher in the Gram-negative bacteremia group compared with the *Salmonella* group ($p < 0,001$ for each variable). (Table 3).

DISCUSSION

We found that the leucocyte count, neutrophil count and PCT levels in the Gram-negative bacteremia group were significantly higher than those in the *Salmonella* group. Our findings regarding laboratory markers of bacterial infection in Gram-negative bacteremia are consistent with previous studies that demonstrated high levels of leucocyte and neutrophils and PCT levels in patients with bloodstream infections caused by Gram-negative bacteria.^{12,13} During bacterial infection, bone marrow increases the production of white blood cells (WBCs) and neutrophils. Neutrophils are an important type of WBC constituting 50–70% of all circulating WBCs and have the ability to kill most bacteria.^{14,15} The neutrophil cells in

blood circulation migrate and reach infected tissue sites via the vascular endothelium.^{9,15}

A study of pediatric patients revealed that WBC and neutrophil measurements could be used to discriminate bacterial and viral infections.¹⁶ A meta-analysis study showed that PCT is fairly accurate at diagnosing bacteremia and low serum PCT levels are useful for ruling out bacteremia-infected patients.¹⁷ High serum PCT levels in patients with suspected infections are associated with Gram-negative bacteremia.¹⁸ However, we found that PCT levels in the *Salmonella* group were minimally elevated. A previous study revealed that only 10.7% of septic patients suffering from *Salmonella* bacteremia had high PCT levels.¹⁹ The PCT levels in the majority of patients with *Salmonella* infection are less than 0.5 ng/ml.¹⁹ Based on previous findings and our new results, we suggest that low PCT levels in *Salmonella* infection are due to less systemic inflammation response compared with infections of Gram-negative bacteremia. *Salmonella* is known to be intracellular bacteria pathogen. Unlike Gram-negative-infected patients, patients suffering from *Salmonella* do not manifest leukocytosis or increased neutrophil count.^{20,21} In addition, tumor necrosis factor alpha and Interleukin 6 in *Salmonella* infection are not elevated significantly compared with patients suffering from sepsis caused by Gram-negative bacteria.^(19,20)

Table 2. Area under the curve and cut-off point leucocyte, neutrophils counts and PCT levels between *Salmonella* and Gram-negative bacteremias.

Variable	AUC (95 % CI)	Cut-off	Sensitivity (%)	Specificity (%)
Leucocyte x1000/ μL	0,80 (0,68-0,92)	$\geq 10,5 \times 10^3/\mu\text{L}$	60,6	99,17
Neutrophils,%	0,87 (0,78-0,97)	$\geq 80,9\%$	72,7	99,1
PCT levels, ng/ml	0,86 (0,76-0,96)	$\geq 1,18$ ng/ml	84,8	79,2

Abbreviations: AUC, Area under the curve; CI, confidence interval, PCT, procalcitonin.

Table 3. Proportion of leucocyte, neutrophils counts and PCT levels: comparison between *Salmonella* and Gram-negative bacteremias.

Variable	<i>Salmonella</i> bacteremia group	Gram-negative bacteremia group	p
Leucocyte			
<10,5x10 ³ / μL	25 (92,6)	13 (38,2)	<0,001
$\geq 10,5 \times 10^3/\mu\text{L}$	2 (7,4)	21 (61,8%)	
Neutrophils			
<80,9%	25 (92,6)	9 (26,5)	<0,001
$\geq 80,9\%$	2 (7,4)	25 (73,5)	
PCT levels			
<1,18 ng/ml	22 (81,5)	6 (17,6)	<0,001
$\geq 1,18$ ng/ml	5 (18,5)	28 (82,4)	

Chi-square tests were used to analyze between two groups.

CONCLUSION

Patients infected by Gram-negative bacteremia exhibit higher leucocyte, neutrophil counts, and PCT levels than patients afflicted with *Salmonella* bacteremia.

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