

Review Form 1.6

Journal Name:	Journal of Complementary and Alternative Medical Research
Manuscript Number:	Ms_JOCAMR_88640
Title of the Manuscript:	Procalcitonin as a Marker of Sepsis Due to Bacterial Infection
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Compulsory REVISION comments	Their meanings of abbreviated words such as: PCT, SIRS, ROC, MOD, MORF, PCR, TNF, and CRP should be noted. Write the scientific names in italics. Mention in which country the research was developed and highlight in bold the components of the format such as: type of research, population, sample, data collection instruments, statistical analysis and procedures.	
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Are there ethical issues in this manuscript?	<i>(If yes, Kindly please write down the ethical issues here in details)</i>	

Reviewer Details:

Name:	Dante Daniel Cruz Nieto
Department, University & Country	Universidad Nacional José Faustino Sánchez, Peru

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Compulsory REVISION comments		
	See The Attachment	
Minor REVISION comments		
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PART 2:

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
Are there ethical issues in this manuscript?	<i>(If yes, Kindly please write down the ethical issues here in details)</i>	

Reviewer Details:

Name:	Ozjoifor Ugochukwu Okechukwu
Department, University & Country	Nigerian Defence Academy, Nigeria

Procalcitonin as a Marker of Sepsis Due to Bacterial Infection

ABSTRACT

Sepsis is one of the world's health problems that commonly cause mortality. The incidence of sepsis keeps on increasing each year. Many diagnostic parameters for sepsis caused by bacterial infection have been used but sometimes are not specific and misleading. Seeing the high number of sepsis cases needed a prompt and precise diagnostic. This study aims to evaluate the sensitivity and specificity of PCT in sepsis patients caused by bacterial infection. A cross-sectional study on 54 adult patients with SIRS (12 – 75 years old) in Mitra Keluarga Bekasi Timur Hospital, used patients' medical records from October to December 2016. The diagnostic test was analyzed using the ROC curve. In this study, there were 37 samples, 28 were sepsis patients, and 9 were non-sepsis samples. The test result of sensitivity and specificity of PCT was 88.5% and 81.8%, cut off value ≥ 0.8 , AUC 0.90 with p-value < 0.05 . The conclusion is that PCT can be a prompt, ideal, and efficient diagnostic marker for sepsis caused by bacterial infection with high sensitivity and specificity tests.

Keywords: *Bacterial Infection, Procalcitonin, Sepsis.*

Introduction

Until now, sepsis is still a health problem globally and often causes death. Delay in diagnosis and misdiagnosis in patients with sepsis are still common so it can threaten the patient's life. Sepsis is an acute systemic inflammatory response in the body to infection. If not appropriately treated, sepsis can lead to severe sepsis and septic shock

Comment [u1]: State the full meaning of PCT

Comment [u2]: State the full meaning of SIRS

[1]. Sepsis often occurs in hospitals, such as in post-operative patients, patients on ventilators in the ICU (Intensive Care Unit), or catheters in geriatric patients [2].

Over the past two decades, sepsis has been one of the top ten causes of death in the United States. The incidence of sepsis in the United States is about 750,000 cases per year, of which 225,000 are fatal [3]. Between 1979 – 2000, the sepsis incidence in the United States increased from 82.7 to 240.4 patients per 100,000 populations, whereas the incidence of severe sepsis ranged from 51 to 95 patients per 100,000 populations [4]. In Indonesia, the mortality rate for sepsis is still high, namely 56.83% (Yogyakarta) and 54.17% (Palembang). Even in 2004 in Solo, 83.1% of patients with sepsis died [5]. Several studies have stated that the mortality rate for severe sepsis has decreased, but in reality, it is still high at around 25% - 45% [6]. Seeing that there are still many cases of sepsis, an ideal sepsis marker is needed to detect sepsis as early as possible, namely attributes that are specific and sensitive, easy to use, fast, and directly proportional to the emergency [7].

Bacterial culture is the gold standard for examining sepsis, but it takes a long time [7]. Over the last few years, several markers of infection and sepsis have been tested, but none of the attributes can accurately differentiate between bacterial and non-bacterial infections [8]. In the early 1990s, procalcitonin (PCT) was first described as a marker of bacterial infection with reasonable specificity and sensitivity. PCT levels are elevated during systemic inflammation, mainly when a bacterial infection causes it. Several studies have shown that PCT has better sensitivity and specificity for diagnosing bacterial infections than C-reactive protein, IL-6, and IL-8 in various clinical situations [8].

O'Connor et al. studied patients admitted to the intensive care unit and found that

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PCT levels were significantly increased in patients with sepsis, severe sepsis, and septic shock compared with patients without SIRS or infection. PCT is the most appropriate laboratory test variable for diagnosing infection, with a sensitivity of 89% and specificity of 94%. In death cases, the serum PCT level was never <1.1 ng/ml [8]. Based on the above data, we wanted to test whether PCT can be used to diagnose sepsis.

Based on the description of the background of the problem above, the problem can be formulated as follows "Can PCT be a marker of sepsis due to bacterial infection?" The aim of the study namely to test PCT as a marker of sepsis due to bacterial infection.

Literature Review

Inflammation is the body's protective response that aims to eliminate the cause of tissue damage. Inflammation protects by weakening, destroying, or neutralizing agents that damage the body (microbes, toxins). Besides being able to heal, inflammation can also repair damaged tissue. Blood vessels, plasma proteins, blood cells, and components of the surrounding connective tissue have an essential role in the inflammatory process [9]. Infection is an inflammatory response due to the presence of microorganisms or the entry of microorganisms into tissues that should be sterile [10].

SIRS is a systemic inflammatory response due to various causes such as infection, pancreatitis, ischemia, multiple trauma, inflammatory process, hemorrhagic shock, or a combination of these symptoms. Systemic inflammation responses syndrome is not always associated with infection and occurs in very complex pathogenesis involving many cells and stimulating the secretion of various hormones. Systemic inflammation responses syndrome can be declared if two or more of the following manifestations are

found: a) Temperature $> 38^{\circ}\text{C}$ or $< 36^{\circ}\text{C}$; b) Heart rate > 90 beats/minute; c) Respiration > 20 breaths/minute or $\text{PaCO}_2 < 32$ mmHg; d) Leukocyte count $> 12,000/\text{mm}^2$, $< 4,000/\text{mm}^2$ or $> 10\%$ immature cell (band) [10]. Sepsis is a SIRS to the infection of a specific organ based on a positive culture result at that site or with clinical suspicion of infection [10; 11].

The most common complications of sepsis are severe sepsis and septic shock. Severe sepsis is sepsis accompanied by organ dysfunction (MODS/MOF), hypoperfusion, or hypotension (systolic pressure < 90 mmHg or a decrease of > 40 mmHg from the previous state without other causes) and is sometimes accompanied by ketoacidosis, oliguria and decreased consciousness. Septic shock is part of severe sepsis characterized by impaired tissue perfusion and persistent hypotension despite adequate fluid resuscitation [11].

A bacterial infection usually causes sepsis (though viruses and fungi can also cause it). The most significant cause of sepsis is Gram-negative bacteria with a percentage of 60% to 70% of cases, which produce various products that can stimulate immune cells. These cells will be stimulated to release inflammatory mediators. The product that plays an essential role in sepsis is LPS or endotoxin glycoprotein complex, which is the main component of the outer membrane of Gram-negative bacteria. Lipopolysaccharides (LPS) stimulates tissue inflammation, fever, and shock in infected patients. The structure of lipid A in LPS is responsible for reactions in the patient's body. Until the late 1990s, Gram-negative bacteria such as *E. coli*, *Enterococcus* species, *Klebsiella*, and *Pseudomonas* were the most common bacteria found in septic patients due to nosocomial infections [12]. Gram-positive bacteria rarely cause sepsis, with an incidence of 20% to 40% of all cases [3; 13]. Gram-positive bacteria are easier

to trigger phagocytosis by leukocytes, and the peptidoglycan in their cell walls has less potential to trigger pro-inflammatory cytokines than endotoxins from Gram-negative bacteria [14].

When the body is infected, there will be a reaction from the immune system. The immune system is divided into the natural/innate/non-specific/innate immune system, and the acquired/specific/received immune system [15]. The innate immune system acts as the body's first line of defense in signaling when there is an infection and rapid activation to initiate an inflammatory reaction when pathogens enter through the body's physical, mechanical and chemical protection. The activated cellular innate immune system consists of monocytes, macrophages, neutrophils, eosinophils, and NK cells. The humoral natural immune system is activated in the form of soluble proteins such as complement, CRP, and cytokines. The acquired immune system is a specific immunity against pathogens and has an immunologic memory to prevent re-infection and assists the innate immune system through the activity of lymphocyte cells. T lymphocytes are cellular, and B lymphocytes are humoral. An inappropriate immune reaction will occur if the immune system fails to overcome the infection [11; 16].

The pathophysiology of sepsis is caused by complex interactions between microbial marker molecules, leukocytes, humoral factors, and vascular endothelium. The molecular components of the microbial cell wall are called PAMPs. When microbes enter the body, PAMPs bind to PRRs, namely TLRs, on the surface of APCs (macrophages and monocytes) [17]. Examples include Gram-negative LPS binding to TLR-4 and the LBP-mediated CD14 complex or Gram-positive bacterial peptidoglycan binding to TLR-2. Once bound to the TLR, signal transduction activates NF-KB [15]. In addition, this antigen carries a specific polypeptide load originating from MHC class II

and will bind to CD4+ on T lymphocytes through TCR [3]. The release of proinflammatory mediators occurs that can trigger a cascade of immune responses, both in the innate immune system and the acquired immune system, which can cause symptoms of septicemia [18].

T lymphocytes, especially Th1 and Th2 cells, play an essential role as immunomodulators in the body's effort to react to sepsis. T helper 1 produces IFN- γ , IL-2, and GM-CSF. IFN- γ stimulates macrophages to secrete proinflammatory cytokines IL-1 β and TNF- α . The reaction of these proinflammatory cytokines manifest systemically as SIRS, characterized by hypercytokinemia. Excessive increased immune response turns out to be wrong, patients can experience a shock phase, and MOD can end up in MOF and death [11]. Therefore, Th2 secretes anti-inflammatory cytokines such as IL-1ra, IL-4, and IL-10, which are in charge of modulating and suppressing an exaggerated immune response [3].

Proinflammatory cytokines also affect the vascular endothelium [3]. One of them is triggering the release of glycoproteins P and E-selectin by the endothelium and L-selectin on neutrophils which causes neutrophils to roll along the vessel wall and form weak bonds with the endothelium [16]. This soft binding was strengthened by stimulating these inflammatory cytokines' PG-E2 formation and ICAM-1 expression. The presence of ICAM-1 causes neutrophils that GM-CSF has sensitized to adhere to [3] easily. After that, neutrophils activated by IL-8 leave the blood vessels at the site of infection due to the production of nitric oxide (NO), which can induce local vasodilation and allow migration [18].

Upon entering the host's body, the bacteria will be opsonized. That is, antibodies and complement fragments will envelop them. After neutrophils are at the site of

infection, PRRs on the neutrophil surface will recognize the opsonized protein on the bacterial surface, and a phagocytic process occurs. Bacteria in neutrophils will be killed by the presence of lysozyme released by neutrophils. This lysozyme can also cause endothelial wall lysis. Neutrophils can also kill bacteria by forming super oxidants and free radicals that will affect respiration in the mitochondria of cells [16; 19]. As a result of this process, the endothelium becomes necrotic, resulting in damage to the endothelium of blood vessels. This damage turns out to be causing vascular disorders (vascular leak), causing multiple organ damage. In addition, MOF is also caused by thrombosis and coagulation in small blood vessels resulting in septic shock, which ends in death [3].

Sepsis, defined as a systemic inflammatory response, is not wholly an inflammatory response. Several previous studies found no evidence of a more dominant role for proinflammatory reactions in sepsis, so a new concept that could occur in sepsis was proposed, namely CARS and MARS [11]. It is well known that after proinflammatory mediators are released, the body will release anti-inflammatory mediators to restore homeostasis by regulating and modulating the effects of these proinflammatory mediators [10]. These processes include SIRS (proinflammatory), CARS (anti-inflammatory), and MARS (a mixture of SIRS and CARS) [20]. It can lead to homeostasis (balance of SIRS and CARS), apoptosis (death with minimal inflammation), organ dysfunction (predominant SIR phase), and organ system suppression (dominant CARS phase). Other studies have found that sepsis is an immunosuppressive condition. Based on the evidence obtained, there is a loss of ability in delayed-type hypersensitivity reactions in sepsis and the ability to eliminate infection [8].

Several sepsis biomarkers, such as inflammatory cytokines (both pro- and anti-inflammatory), PCT, and CRP, have been stated as diagnostic parameters. However, some routine laboratory tests in sepsis, such as CRP or leukocyte count, are non-specific and sometimes misleading. Examples such as TNF and IL-6 are not specific for certain types of inflammation. Procalcitonin levels selectively increase in inflammation due to bacterial infection [21]. Seeing the high mortality rate in sepsis often caused by delayed diagnosis and proper management, proper examination of sepsis due to condition is needed [22].

In recent years PCT has been declared one of the most accurate markers in differentiating sepsis from other non-infectious causes of SIRS. An increase in PCT levels is a marker of an inflammatory process in the immune system [7]. In 1993 Assicot et al. stated that procalcitonin (PCT) levels were elevated in patients with bacterial infections and were associated with the severity of infection [8; 23]. PCT levels < 0.5 ng/ml were found in SIRS patients without infection. A serum PCT level of $0.5 - 2.0$ ng/ml is indicated by abnormal conditions or local infection and requires a supportive diagnosis of sepsis. Meanwhile, levels > 2 ng/ml were found in patients with sepsis or uncontrolled systemic bacterial infection [24]. Measurement of PCT levels can be used to diagnose and monitor the course of the disease and follow-up therapy in severe bacterial infections and sepsis [7; 19].

Procalcitonin is a 116 amino acid peptide with a molecular weight of 13 kDa protein encoded by the CALC-1 gene on the short arm of chromosome and produced in C cells of the thyroid gland as the prohormone calcitonin. Normally all PCT is cleaved in the thyroid into calcitonin so that under physiological conditions, PCT levels are so low that they cannot be detected [11, 21]. The CALC-1 gene consists of 6 exons, of

which exons 1 to 4 are encoded to produce pre PCT, a peptide chain consisting of 141 amino acids having 25 hydrophobic signaling amino acids. Pre PCT is responsible for producing PCT, especially during inflammation. A series of signals at the N terminal with hydrophobic properties of these amino acids can induce attachment to the endoplasmic reticulum, wherein the amino acids are cleaved by endopeptidase and produce PCT. In thyroid C cells, PCT will be re-divided to form N-terminal fragments, namely aminoprocaltitonin (57 amino acids), calcitonin (32 amino acids) located at the center of the peptide, and CCP-1 or katalcain (21 amino acids) at the carboxyl-terminal end. [8; 21].

There is another transcriptional pathway in the CALC-1 gene that produces CGRP, in which CGRP is expressed mainly in the central nervous system and acts as a potent vasodilator. As with PCT, CGRP synthesis also increases in sepsis, although at lower concentrations. 25 PCT detected in plasma during inflammation is not produced in thyroid gland C cells [21]. When a bacterial infection attacks the body, CALC-1 gene expression will occur in all body tissues and stimulate all tissues to release PCT, increasing circulating PCT [7; 22]. In a study using the quantitative calculation of CT-mRNA expression from Taq-Man PCR, it was found that during sepsis, PCT was evenly produced by almost all body tissues (liver, lung, kidney, fat tissue, and muscle) compared to cytokines. PCT release in the inflammatory process can be caused by direct induction by microbial toxins (e.g., endotoxin) or hormonal or cell-mediated responses (e.g., IL-1 β , TNF-, IL-6). It occurs because the cytokines inhibit the proteolysis of PCT into calcitonin in the endoplasmic reticulum. However, PCT induction is also weakened by cytokines released during viral infection (e.g., IFN- γ) [21]. Usually, PCT levels in viral infections are always < 1 ng/ml [8].

In 1994 Dandona et al. experimented with the injection of endotoxin E. coli. PCT concentrations in subjects increase within 4 hours, peak in 12 to 48 hours, then slowly decrease over 48 to 72 hours. The decrease in PCT levels at the end inflammatory response acute phase is influenced by its long half-life of 25 to 35 hours. Compared with other inflammatory markers such as TNF- and IL-6, PCT takes a longer time to peak but is faster when compared to CRP. TNF- levels will peak in 90 minutes, followed by IL-6 in 180 minutes. However, both cytokines return to normal levels after 6 and 8 hours, by which time they have a narrow screening window [5; 21]. It shows that PCT is a better marker than the others. PCT was measured in serum using immunoluminometric assays. Specimens used are serum or plasma. PCT molecules are stable in both in vivo and in vitro conditions. In vitro stability: Samples should be inspected immediately before six hours if stored at room temperature and undergo 10% decomposition after 24 hours. At minus 20 0C the PCT will be stable for one month. Freezing and thawing cycles did not affect PCT concentration. The in vivo half-life is about 24 hours. PCT may be elevated in thyroid C cell carcinoma and small cell carcinoma [5].

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Research Method

This research type of research is a diagnostic test study, and there is no specific treatment on the sample. The sample was observed cross-sectionally. Research and data collection was conducted in the Medical Records of Mitra Keluarga Hospital, East Bekasi, and the study was conducted from October to December 2016. This study was conducted on adult SIRS and sepsis patients who met the criteria for sample acceptance and were treated in the ICU and in-patients at Mitra Keluarga Hospital, East Bekasi.

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The number of sepsis patients and SIRS suspects at Mitra Keluarga Hospital, East Bekasi, in 2014 was 54 people. The sample in this study was medical record data for SIRS and sepsis patients. The data needed are vital signs (pulse, temperature, respiratory rate), culture, and laboratory (leukocytes and PCT). Data recorded in medical records were taken from the blood of patients suspected of SIRS or sepsis, which were intended for complete blood count and PCT. Blood, sputum, pus, and urine are used for culture examination. In this study, there were 54 samples with the following details; 37 data met the study criteria (inclusion data), 17 data did not meet the requirements (exclusion data), and nine harmful control data (non-septic patients with low PCT levels). Calculation of the sample size in this study using the total sampling method or all cases in the ICU and in-patient at Mitra Keluarga Hospital, East Bekasi in October – December 2016. Researchers came to the medical records section of Mitra Keluarga Hospital, East Bekasi, to collect patient data through a permit given by the medical faculty of the Christian University of Indonesia. Researchers looked at data on SIRS patients or suspected sepsis in medical records treated in the intensive care unit (ICU) and in-patients. The data that has been collected will be processed through the stages of editing, coding, data entry, and cleaning. The diagnostic value of the PCT test on culture as the gold standard is determined by the sensitivity and specificity parameters of the PCT test in patients with sepsis which can be determined in table 2 x 2. In this study, the researchers used the ROC curve found in the IBM SPSS Statistic 23 application to determine the cut-off point of the test. Diagnostics in the form of graphs that describe the level of sensitivity and specificity.

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Result and Discussion

From October 2016 to December 2016, there were 54 suspects with SIRS and sepsis. Of the 54 samples studied, only 37 patients with SIRS (68.5%) could be included in the sample acceptance criteria. In this inclusion sample, 21 patients (56.8%) were male, and 16 patients (43.2%) were female.

Figure 1. Characteristics of the Gender of the Research Sample

Of these 37 samples, 28 patients with sepsis (75.7%) and nine patients with SIRS (24.3%), and Nine samples (24.3%) of this inclusion sample were negative controls, namely patients with SIRS or non-bacterial sepsis with low PCT levels. Several causes of SIRS in this study include appendicitis, pancreatitis, or traffic accidents. The exclusion samples in this study amounted to 17 (31.5%) of 54 samples because they did not meet the SIRS criteria, bacterial culture was not examined, and PCT was not performed.

The age group suffering from SIRS or suspected sepsis in this study was 36-45 years, with 24.3%. The second largest age group is 46-55 years, with a percentage of 21.6%.

Figure 2. Age Characteristics of the Research Sample Patients

Of the 37 samples included in this study, there were 26 patients (70.3%) with positive bacterial culture results and 11 patients (29.7%) with negative culture, but the clinical diagnosis supported sepsis. The most common causative bacteria were *Klebsiella pneumoniae* (30.6%), *Escherichia coli* (16.7%), *Pseudomonas aeruginosa* (16.7%), and *Enterococcus faecalis* (11.1%), and *Acinetobacter baumannii* (8.3%).

Figure 3. Percentage of Germs that Cause Sepsis

The diagnostic value of the PCT test on culture as the gold standard is determined by a 2 x 2 table and has the following results:

Table 1. Assessment of PCT Diagnostic Test

PCT	Culture	Positive	Negative
	Positive	a	b

Negative	c	d
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$$\begin{aligned}
 \text{Sensitivity} &= a: (a+c) \\
 &= 24 \\
 & \quad 24 + 2 \\
 &= 24 \\
 & \quad 26 \\
 &= 0,92 \sim 92 \%
 \end{aligned}$$

$$\begin{aligned}
 \text{Specificity} &= d: (b+d) \\
 &= 9 \\
 & \quad 2 + 9 \\
 &= 9 \\
 & \quad 11 \\
 &= 0,82 \sim 82 \%
 \end{aligned}$$

Comment [u13]:

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The sensitivity and specificity of PCT in patients with sepsis due to bacterial infection were calculated based on the ROC curve. The best results obtained from the ROC curve in this study were sensitivity of 88.5%, specificity of 81.8%, AUC (Area Under Curve) of 0.90, and cut-off the number of PCT 0.8 with a p-value <0.05.

<i>Cut off PCT (ng/mL)</i>	<i>Sensitivity (%)</i>	<i>Specificity (%)</i>
≥ 2,0	76,9	81,8
≥ 1,5	80,8	81,8

Figure 4.
ROC Curve

Curve in Sepsis Patients

$\geq 1,0$	84,6	81,8
$\geq 0,8$	88,5	81,8
$\geq 0,6$	92,3	72,7

Table 2. PCT Sensitivity and Specificity Test in Sepsis Patients

Sepsis is the body's systemic inflammatory response to a severe infection.

Until now, sepsis is still a problem in the medical world because of the high mortality rate in patients with sepsis, so appropriate and fast parameters are needed for the early diagnosis of sepsis. It can be seen in the results of this study that the number of patients with SIRS and suspected sepsis was more in men than women (Figure 1), namely 21 male patients (56.8%) and 16 female patients (43.2%). It is following several previous studies that said that sepsis was more common in men than women [26].

In this study, the highest prevalence of SIRS or suspected sepsis occurred at the age of 36-45 years with 24.3% (Figure 2). However, in the older age group, the prevalence of sepsis decreased. It is not following previous research and theories. Chivite et al. studied septic patients aged over 18 years and found that most sepsis occurred in patients over 60 years [27]. According to epidemiological data on severe sepsis in several countries such as America and Europe, the mean age of patients with sepsis is 65 years [28]. Similarly, the incidence of sepsis in the United States in 2011 mainly occurred at 60 and continued to increase sharply until the age of 85 years and over [29].

According to the theory, elderly patients have a higher risk of contracting sepsis than younger patients. There has been a change in immune function in the elderly, so they tend to be easily infected and develop into sepsis. In addition, clinical signs and symptoms in elderly patients often do not appear, making it difficult to diagnose [30]. The difference in the age range prevalence in this study was due to differences in the sample characteristics.

There were patients with sepsis with positive culture results, as many as 26 patients (70.3%), and negative culture results in as many as 11 patients (29.7%). These data suggest that the etiology of sepsis is generally due to bacterial infection. Figure 3

shows the germs that cause sepsis, such as *Klebsiella pneumoniae* (30.6%), *Escherichia coli* (16.7%), *Pseudomonas aeruginosa* (16.7%), *Enterococcus faecalis* (11.1%), and *Acinetobacter baumannii* (8,3%). All of these bacteria are Gram-negative except *Enterococcus faecalis*. In addition, there are other Gram-negative bacteria, namely *Enterobacter cloacae*, *Providencia stuartii*, *Proteus mirabilis*, and *Morganella morganii*, with a percentage of 2.8% each.

In the Textbook of Internal Medicine Volume III, A. Guntur H stated that Gram-negative bacteria caused 60-70% of sepsis cases. Gram-negative bacteria have endotoxin lipopolysaccharide, which can stimulate the release of proinflammatory mediators that can cause symptoms of septicemia [3].

The results of the PCT diagnostic test in patients with sepsis on the results of bacterial culture have been analyzed using a 2x2 table and ROC curve (Figure 4). In table 2x2, sensitivity is 92%, specificity is 82%, while the ROC curve shows the best cut-off value at PCT levels of 0.8 ng/ml, AUC 0.90 with the sensitivity of 88.5%, specificity of 81.8% (Table 2). The sensitivity and specificity test results in this study had a high value. There are differences in sensitivity and specificity values between the 2x2 table and the ROC. It is due to the difference in the methods used in the analysis, so this difference is not considered significant.

The results of this diagnostic test support the results of previous studies in 30 meta-analytical studies (2013) with an average sensitivity of 77.0% and specificity of 79.0% [31]. In the Khoshdel study (2008) also reported similar results, but the sensitivity value was slightly lower than this study, namely sensitivity of 87.5% and higher specificity of 87.4% [32].

Metz has classified the AUC value into five different parts, namely 0.5 – 0.6 (very weak accuracy), 0.6 – 0.7 (weak accuracy), 0.7 – 0.8 (medium accuracy), 0.8 – 1 (high accuracy) [33]. The AUC value in this study was 0.90, which indicates that this study is very accurate. The PCT cut-off value resulting from this study is 0.8 ng/ml and is higher than the research conducted by Chan (2003) and Lee (2014). In Chan et al.'s study, the cut-off was 0.6 ng/ml, and in Lee et al.'s study, 0.75 ng/ml [23; 34]. The cut-off value in this study is thought to be due to differences in the sample characteristics, the sample number, and the research location. This PCT cut-off value of 0.8 ng/ml supports the theory that serum PCT levels of 0.5 – 2.0 ng/ml are usually found in local infections, and PCT levels > 2 ng/ml represent sepsis or uncontrolled systemic bacterial infection. [24]. This increase in PCT levels occurs due to stimulation by bacterial endotoxins or the body's inflammatory cell response to increasing PCT secretion [21]. Therefore, the cut-off results in this study are good and can indicate an increase in PCT levels in patients with sepsis bacterial infection.

Comment [u15]: the heading should be DISCUSSION

Conclusion

The best sensitivity and specificity of the PCT diagnostic test in patients with sepsis due to bacterial infection is 88.5% and 81.8% at the cut-off value of PCT levels 0.8 ng/ml. Based on the information above, it can be concluded that PCT can be relied upon to be a fast, ideal, and efficient marker for the diagnosis of sepsis in patients with sepsis due to bacterial infection. This study only included representatives of adults, so further research is needed on PCT as a marker of sepsis due to bacterial infection in children, infants, and neonates.

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Editor's Details:

Dr. Francisco Cruz-Sosa
Metropolitan Autonomous University, Iztapalapa Campus Av. San Rafael Atlixco, Mexico.