

## Procalcitonin as A Predictor of Left Ventricular Systolic Dysfunction in Sepsis Patients

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### ABSTRACT

**Background:** Release of cytokines in sepsis can cause left heart failure and left ventricular systolic dysfunction (LVSD). In sepsis, there is a releasing of Procalcitonin (PCT) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) because of the stimulation of cytokine pro inflammation effected by activated NF- $\kappa$ B. This study aimed to examine PCT as a predictor of LVSD in sepsis, value of Area Under the curve (AUC), sensitivity, specificity, cut off point, and probability of PCT and TNF- $\alpha$  as a predictor of LVSD, and then to identify the best diagnostic predictor of LVSD.

**Subjects and Method:** This was a cross-sectional study. A sample of 71 sepsis patients aged >18 years old admitted to the intensive care unit (ICU) from November 2016 to March 2017 was selected for this study. The dependent variable was left ventricle ejection fraction (LVEF). The independent variables were PCT and TNF- $\alpha$ . LVEF and diastolic function were measured by transthoracic echocardiography. Data on PCT and TNF- $\alpha$  level were obtained from medical record.

**Results:** As many as 22 patients had mild sepsis (30.9%), 40 patients had severe sepsis (56.4%), and 9 patients had septic shock (12.7%). The AUC value of PCT level was 0.81 (95% CI 0.71-0.91;  $p < 0.001$ ). Optimal cut off point was  $\geq 7.88$  ng/mL and Diagnostic Odd Ratio (DOR) was 5.55. The AUC value of TNF- $\alpha$  level was 0.73 (95% CI 0.60-0.86;  $p = 0.002$ ). Optimal cut off point was  $\geq 7.36$  pg/ml and DOR was 5.03. Multivariate analysis was resulted that PCT was the best predictor of LVSD (AUC 0.70), and TNF- $\alpha$  (0.69). The combination of PCT + TNF $\alpha$  would increase diagnostic value with AUC 0.77.

**Conclusion:** PCT was a better predictor of LVSD than TNF- $\alpha$ . This finding is significant, since it will enable clinicians to easily diagnose LVSD by measuring PCT. The combination of PCT and TNF $\alpha$  was the best efficient diagnostic predictor of LVSD.

**Keywords:** Left Ventricular Systolic Dysfunction, predictor , PCT, TNF- $\alpha$

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### BACKGROUND

Sepsis still becomes health problem since this causes a high mortality and morbidity rate. Sepsis may be associated with concurrent left ventricular systolic dysfunction (LVSD) and patients who develop LVSD during sepsis may have a worse prognosis than patients who do not. The release of local cytokine products along with pro-inflammatory cytokines, excessive nitric oxide and cytosolic calcium, can lead to

mitochondrial dysfunction and cell apoptosis. In severe sepsis, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) elevated and will cause abnormalities in the heart and liver. In the heart, TNF- $\alpha$  will cause myocardial dysfunction. In the liver, TNF- $\alpha$  will stimulate procalcitonin (PCT) secretion, that is a microbial infection biomarker. These cellular events can lead to myocardial dysfunction characterized as LVSD (Flierl *et al*, 2008 and Fernandes, 2012). To diagnose LVSD, clini-

cians should use an echocardiogram. Unfortunately, not all hospitals in Indonesia have echocardiogram machines. The identification of predictors LVSD in septic patients is expected to decrease morbidity and mortality rates. Until now, no specific predictors have been available to detect LVSD. The diagnosis of LVSD may be possible in a peripheral hospital by simply measuring PCT or TNF- $\alpha$  levels in serum.

Pattern recognition receptors (PRRs) consisting of a toll-like receptor (TLR) and CD14 on the surface of monocytes can recognize pathogen associated molecular patterns (PAMPs) that enter the body. The interaction between PAMPs and PRRs on the surface of monocytes results in the activation of pro-inflammatory cytokines (TNF- $\alpha$ , interleukin [IL]-1 $\beta$  and IL-6) and anti-inflammatory cytokines (IL-4, IL-5, IL-6, and IL-10). Lipopolysaccharide (LPS) binds with the CD14 receptor and TLR-4 on the monocyte surface and activates intracellular signal transduction involving various adaptor proteins such as: myeloid differentiation protein 88 (MyD88), TNF receptor associated factor (TRAF), and kinase enzyme-like inhibitor kappa b kinase (IKK). These cellular conditions activate a transcription protein called nuclear factor kappa B (NF- $\kappa$ B). NF- $\kappa$ B is a transcription protein in the cytoplasm activated by binding fragmentation within inhibitor kappa b (IKB), which enters the nucleus of monocytes and initiates RNA transcription of pro- and anti-inflammatory cytokine molecules (Guntur, 2001; Kibe *et al*, 2011; Celes *et al*, 2013).

Under normal circumstances, TNF- $\alpha$  levels are almost undetectable in the blood, and peak concentrations rise within 2h if there is sepsis. Since TNF- $\alpha$  has a very short half-life (approximately 18.2 min) and is unstable, it has rarely been used as a sepsis biomarker (Oliver *et al*, 1993).

This study aimed to prove PCT as a predictor of LVSD in sepsis, to search a value of Area Under the Curve (AUC), sensitivity, specificity, cut off point and probability PCT and TNF- $\alpha$  as a predictor of LVSD in sepsis patients, to search the best diagnostic predictor of LVSD in sepsis patients.

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## SUBJECTS AND METHOD

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This was a cross-sectional study carried out at the tertiary intensive care unit (ICU) in Dr. Moewardi Hospital, Surakarta, Central Java, Indonesia, from November 2016 to March 2017. A sample of 71 patients aged over 18 years old was selected for this study by simple random sampling.

We chose patients with sepsis as defined by the American College of Chest Physicians/Society of Critical Care Medicine consensus criteria (Delinger *et al*, 2008). The exclusion criteria were as follows: all sepsis cases with ischemic heart disease, heart failure, chronic kidney disease or valvular heart disease. This study was approved by the Ethical Review Committee from Universitas Sebelas Maret /Dr. Moewardi Hospital and informed consent was obtained from the patients or their legal representatives.

Using a formula based on diagnostic research and the area under the curve (AUC) output, the sample size for this study was at least 68 and we analyzed 71 patients. The variables evaluated by the researchers included: LVEF, PCT, and TNF- $\alpha$ . Serum samples taken to measure PCT and TNF- $\alpha$  were centrifuged within 45 min after collection. The resulting plasma was frozen at -20°C until it was ready to be analyzed in a blinded fashion. PCT was analyzed with electrochemiluminescence using an enzyme-linked fluorescent assay method (VIDAS equipment and Brahms reagents). The normal value of PCT was <0.05 ng/mL.

TNF- $\alpha$  was measured by applying a quantitative and which enzyme immuno-assay with human TNF- $\alpha$ /TNFSF1AHS (R&D systemsInc., Minneapolis, MN, USA); the reagent kit used for human TNF- $\alpha$  was also from R&D Systems. The immune-assay technique using human TNF- $\alpha$  was conducted with a Microplate Reader 680 series device. The normal value of TNF- $\alpha$  was 0.5 pg/mL Transthoracic echocardiography performed by 2 cardiologists to determine left ventricular systolic function (LVSF) and LVEF using the Simpson method (De Backer, 2011 and Griffey et al, 2010).

Diastolic function was assessed by measuring E and A peak velocities using a spectral Doppler to measure mitral in flow. LVSD was defined as LVEF  $\leq$ 45% (De Backer, 2011). Diastolic dysfunction was defined as an E/A ratio  $<$ 1 with a deceleration time (DcT)  $>$ 240ms or E/A ratio  $>$ 2 with DcT  $<$ 160ms (Griffey et al, 2010). Data were analyzed with a 2 x 2 table and a

receiver operating characteristic (ROC) curve using SPSS 22 for Windows. Analysis with 2 x 2 tables was used to determine the sensitivity, specificity, and probability of PCT and TNF- $\alpha$  as LVSD predictors in septic patients. ROC curve analysis to determine the AUC and optimal cut-off points for PCT and TNF levels were determined in septic patients who developed LVSD.

## RESULTS

This study was conducted from November 2016 to march 2017 in tertiary ICU at Moewardi Hospital Surakarta Indonesia. Research subjects consisted of 71 patients divided into 22 mild sepsis (30.9%), 40 severe sepsis (56.4%) and 9 septic shock (12.7%). There were 69 patients having systolic dysfunction (97.2%), meanwhile there were 48 patients having left ventricular systolic dysfunction (67.6%).

**Table 1. Basic characteristic of the study participants**

Variabel	LVSD (n= 48)	Non LVSD (n= 23)	P
Gender:			
Male	18 (37.5%)	8 (34.8%)	0.520
Female	30 (62.5%)	15 (65.2%)	
Age (Years)	57.2 $\pm$ 15.3	51.5 $\pm$ 16.7	0.160
Elderly:			
Non-elderly	27 (56.3%)	15 (65.2%)	0.324
Elderly	21 (43.8%)	8 (34.8%)	
Sepsis Severity:			
Mild Sepsis	0 (0.0%)	22 (95.7%)	$<$ 0.001
Severe Sepsis	39 (81.3%)	1 (4.3%)	
Septic shock	9 (18.8%)	0 (0.0%)	

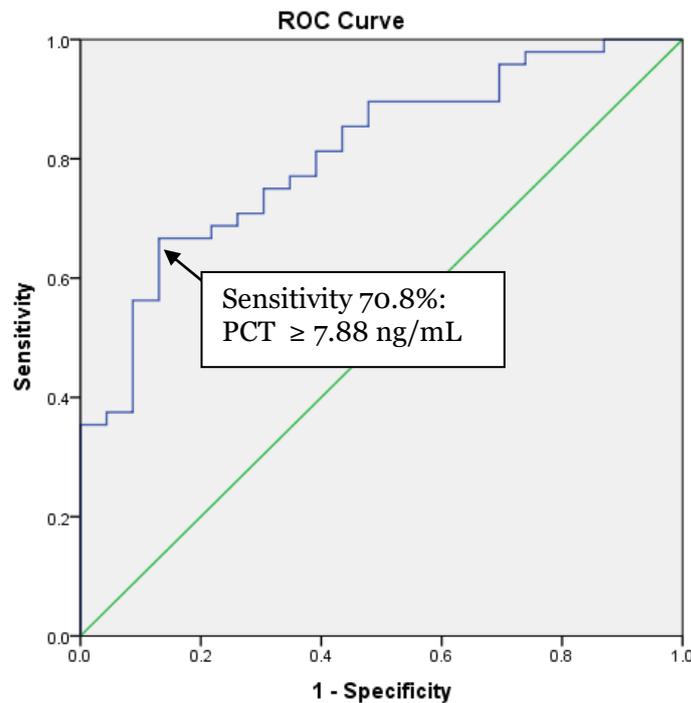
Abbreviations- LVSD: left ventricular systolic dysfunction

The sepsis were grouped based on LVSD. This characteristic showed that there were not differences in gender ( $p=0.520$ ), age ( $p=0.160$ ) in both elderly ( $>$ 60years old)

and non-elderly ( $p=0.324$ ). First, there was a different characteristic for patients of LVSD compared to non LVSD based on sepsis degree ( $p<001$ ).

The AUC value of PCT level was 0.81 (95% CI 0.71-0.91),  $p < 0.001$ . Optimal cut off point was  $\geq 7.88$  ng/mL with sensitivity 70.8% and specificity 69.6%, and Diag-

nostic Odd Ratio (DOR) was 5.55 (95% CI 1.88-16.42) with PCT probability  $\geq 7.88$  ng/mL. Next, the occurrence of left ventricular systolic dysfunction was 84.7%.



**Figure 1. ROC curve diagnostic accuracy of PCT level to diagnose LVSD in sepsis patients. Width of ROC curve 0.81 (0.71-0.91)**

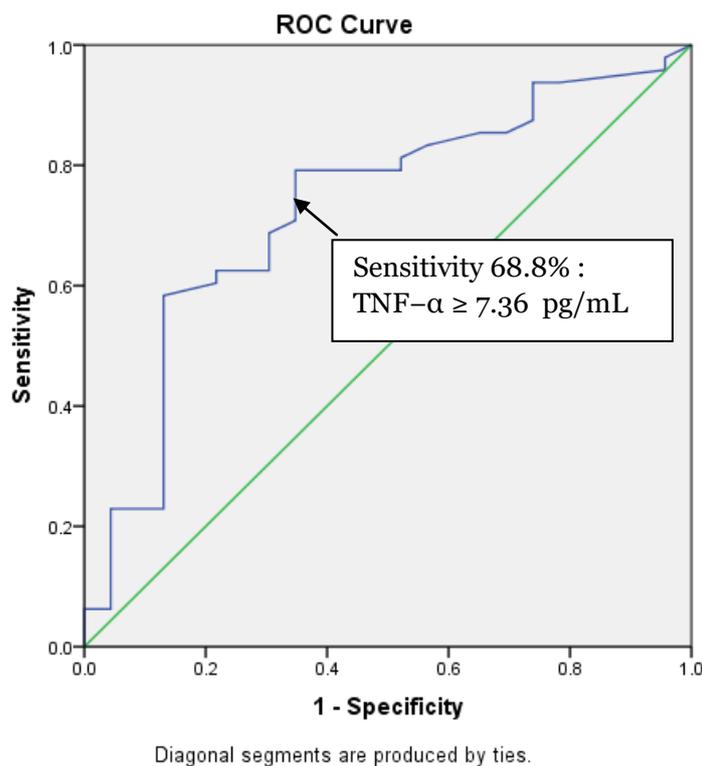
**Table 2. Comparison of area under the curve in PCT and TNF- $\alpha$  for LVSD**

Cut off variable	Area under the curve (AUC)	P	95% Confidence Interval	
			Lower limit	Upper limit
PCT	0.81	<0.001	0.71	0.91
TNF- $\alpha$	0.73	0.002	0.60	0.86

The AUC value of TNF- $\alpha$  level was 0.73 (95% CI 0.60 to 0.86;  $p = 0.002$ ). Optimal cut off point was  $\geq 7.36$  pg/ml with sensitivity 68.8%, and specificity 69.6%. Diagnostic Odd Ratio (DOR) was 5.03 with TNF- $\alpha$  probability  $\geq 7.36$  pg/ml, and left ventricular systolic dysfunction occurrence was 83.4%.

Bivariate analysis of LVSD and factors such as age, severity of sepsis, levels of PCT and TNF- $\alpha$  showed that PCT and

TNF- $\alpha$  had a value of  $p < 0.25$  and these variables were included in multivariate analysis. Multivariate analysis indicated that PCT was good with an AUC of 0.70, sensitivity of 70.8% and specificity of 69.6%. The predicted value of TNF- $\alpha$  was quite good with an AUC of 0.69, sensitivity of 68.8% and specificity of 69.6%. The combination of PCT + TNF- $\alpha$  would increase diagnostic value with AUC 0.77 (see Table 3).



**Figure 2. ROC curve diagnostic accuracy of TNF- $\alpha$  level to diagnose LVSD in sepsis patients. Width of ROC curve 0.73(0.60-0.86)**

**Table 3. Multivariate analysis in both the comparison among AUC of PCT, TNF- $\alpha$  and the combination of PCT+ TNF- $\alpha$  as a predictor of LVSD in sepsis patients**

Cut off variable	AUC	95% CI		p
		Lower limit	Upper limit	
PCT	0.70	0.57	0.83	0.006
TNF- $\alpha$	0.69	0.56	0.83	0.009
PCT+TNF- $\alpha$	0.77	0.64	0.90	<0.001

## DISCUSSION

This study aimed to demonstrate the efficacy of PCT and TNF- $\alpha$  as a predictors of LVSD in sepsis, and to identify an AUC, sensitivity, specificity, optimal cut-off point and probability for PCT and TNF- $\alpha$  as predictors of LVSD in sepsis. Furthermore, our goal was to identify for the best diagnostic predictor of LVSD in sepsis. To identify complications of LVSD (left heart failure) in sepsis, cardiologists should use echocardiography, which requires special skills and devices, and is not always avail-

able in health-care facilities, especially in peripheral hospitals. In addition, it is sometimes very difficult to perform echocardiography in patients who are not "echogenic", including those who are obese, those with chronic obstructive pulmonary disease (COPD), or patients in the ICU who are attached to various life support devices (Griffie et al, 2010). The results of this study are expected to make it easier to diagnose septic patients with LVSD, simply by checking the serum levels of PCT or TNF- $\alpha$  (without using an echocardiogram).

This study will have clinical applicability in peripheral hospitals (hospital types C or D in Indonesia). Those with severe sepsis, exhibit hemodynamic changes and hypoperfusion in some organs. With worsening sepsis, the ability of cardiac muscle to contract will decrease, leading to cardiac dilatation and decreased stroke volume, ejection fraction and systemic vascular resistance; vasodilation of blood vessel and hypotension will worsen until septic shock occurs. Echocardiography has an advantage over invasive examinations because it can be done at the bedside and can easily be done repeatedly without causing the patient harm (Griffiee et al, 2010 and De Backer, 2011).

In severe sepsis, there was haemodynamic changing and hypoperfusion of tissue in some organs. Then, multiple organ dysfunction syndrome (MODS) and septic shock would occur. Septic shock in adults refers to a state of acute circulatory failure characterized by persistent arterial hypotension unexplained by other causes. The chronicle of MODS consisted of respiratory failure, liver and intestinal failure, and renal failure. Haematologic and myocardial failure was the last manifestation of MODS, whereas SSP failure could occur in the beginning or in the last of disease step (Pinsky, 2012).

The recent issues mentioned that sepsis complication into heart was signed by myocardial depression and heart index reduction. Myocardial depression in the beginning of sepsis was still reversible in 7-10 days, however, if sepsis was getting serious, the worse condition would change into irreversible because of septic shock and patients could die (Cavazzoni et al, 2010 and Jozwiak et al, 2011).

This was the first diagnostic study to search a main predictor taken from variables of PCT, and TNF- $\alpha$  in sepsis patients

to diagnose LVSD. There were 97.2% of sepsis patients having diastolic dysfunction, and this meant that severe sepsis occurred. Therefore, aggressive liquid resuscitation had to be given to those patients. This research proved that PCT could be used as a predictor of LVSD in severe sepsis patients. PCT level was  $\geq 7.88$  ng/mL and this could be used to diagnose sepsis patients having LVSD with sensitivity 70.8% and specificity 69.6%. DOR was 5.55 with probability of sepsis patients having LVSD and increasing PCT level occurred 84.7%.

The results of this study are in line with Liu et al. (2013), which indicated that when PCT levels were  $>2$  ng/mL, a patient may progress toward severe sepsis or septic shock. In addition, it is important to monitor patients with a PCT  $>2$  ng/mL, and antibiotics should be considered. If PCT levels are very high ( $>10$  ng/mL) the risk of death is higher, as high PCT levels are significantly associated with septic shock, multiple organ dysfunction syndrome and poor outcomes.

In addition, TNF- $\alpha$  level was  $\geq 7.36$  pg/ml, and the patients were diagnosed as sepsis patients who had LVSD with sensitivity 68.8% and specificity 69.6%.

Multivariate analysis used ROC curve method and found the use of single predictor as LVSD predictor with AUC value of PCT (0.70). This result was better compared to TNF- $\alpha$  (0.69) with sensitivity 70.8% and specificity 69.6%. The use of double biomarker as a predictor of LVSD showed that AUC value of PCT + TNF- $\alpha$  (0.77) was better compared to PCT alone (0.70) with sensitivity 70.8% and specificity 69.6%.

Based on the results of this study, it can conclude that PCT and TNF- $\alpha$  could be used as LVSD predictor for sepsis patients, with AUC 0.81 and 0.73, respectively.

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