

RESEARCH ARTICLE

Lipopolysachharide Binding Protein, Soluble-Intercellular Adhesion Molecule-1, Procalcitonin, and Protein C Activity and Clinical Outcome in Systemic Inflammatory Response Syndrome (SIRS) or Sepsis Patients

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BACKGROUND: Biochemical markers may be used in diagnosis, prognostic and monitoring treatment and therapy for sepsis patients. In this study we used Lipopolysachharide Binding Protein (LBP), serum-Intercellular Adhesion Molecule-1 (ICAM-1), Procalcitonin (PCT) and protein C activity. LBP is related to lipopolysachharide or gram-negative bacterial endotoxin which bound to LBP and induced inflammatory response. ICAM-1 is associated with endothelial dysfunction in response to systemic inflammatory and septic condition. PCT increased in bacterial infection and in severe systemic inflammatory. Role of Protein C is protecting the intravascular system to systemic inflammation, sepsis and the concomitant intravascular coagulopathy. The aim of this study was to examine the associations between levels of serum LBP, sICAM-1, PCT, and protein C activity with the clinical outcome of SIRS or sepsis patients

METHODS: We included 19 post surgery patients with SIRS criteria from intensive care unit (ICU) and evaluated the level of LBP serum with Chemiluminescent Enzyme Immunoassay (Diagnostic Product Co.), ICAM-1 with ELISA (R&D System), PCT with immunochromatography (BRAHMS), protein C activity with chromogenic method (Dade Behring). We performed the samples serially at the first admission of patients and after 72 hours. Data were analysed by non-parametric with Wilcoxon test and Mann-Whitney test. Correlation study between biomarkers calculated by Kendall's tau and Spearman's rho.

RESULTS: Of 19 patients, 9 (47,4%) died and 10 (52,6%) surviving. The level of LBP serum decreased after 72 hours in surviving-sepsis patients, and increased in non-surviving sepsis patients with significant different levels at 72 hours examination ($P < 0.05$). The level of soluble-ICAM-1 which was high in the first admission showed in non-surviving sepsis patients, but the difference levels was not significant between surviving and non-surviving patients ($P > 0.05$). In all patients were found high level of PCT serum since the first admission examination, decreasing levels were occurred significantly in surviving patients after 72 hours ($P < 0.05$) where high PCT levels were found in non-surviving patients after 72 hours. The median level of plasma protein C activity was low at the first admission especially in non-surviving sepsis patients, the decreasing level was not significantly different after 72 hours ($P > 0.05$) both in surviving and non-surviving patients.

CONCLUSIONS: Increasing level of LBP and PCT in sepsis patients showed that those biomarkers useful for predict the clinical outcome in sepsis patients. Decreasing protein C activity level was not a good predictor in worsening clinical outcomes. Soluble ICAM-1 level was not a good marker for predict risk of sepsis severity. LBP and PCT tests were more useful in serially testing from the first admission of sepsis patients, those tests are more faster than bacterial culture.

KEYWORDS: Sepsis, SIRS, Lipopolysachharide Binding Protein, soluble- Intercellular Adhesion Molecule-1, Procalcitonin, Protein C

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Sepsis or Systemic Inflammatory Response Syndrome (SIRS) is still a big problem in Hospital with high mortality in intensive care unit. Sepsis incidence and its mortality has been increasing year to year. The increasing number of sepsis incidence is 8.7% annually (1). The source of infection in sepsis patients could be caused by gram-negative or gram-positive bacteria, virus and fungi. Lipopolysachharide (LPS) or endotoxin on the outer membrane of gram-negative bacteria with its protein binding (LPS binding protein/ LBP) activated host immune system and will activate complement cascade, coagulation and kinin-kalikrein system. In cellular immune system activate endothelial cell, macrofag, monocyte, lymphocyte and neutrophil to release proinflammatory cytokines such as TNF- α , IL-1, IL-6, IL-8 and others (2,3,4,5).

TNF- α , and IL-6 induced liver to release acute phase protein serum amyloid A (SAA), C-reactive protein (CRP) and Procalcitonin (PCT). Sekresi PCT secretion is earlier and much more than SAA dan CRP secretion (6). The half life of TNF- α which 17 minutes is difficult to be detected in the first sepsis progression (3). Furthermore IL-6 has low sensitivity and specificity in adults sepsis patients (2).

Uncontrollable secretion of proinflammatory cytokines rapidly in abnormal high levels could obtained pathological condition. This pathological effect caused by endothelial activation which release adhesion molecules such as E-selectin, ICAM-1, etc (7) and plasminogen activator inhibitor-1 (PAI-1) (8). PAI-1 is strong inhibitor to tissue plasminogen activator which may lyse fibrin clot. In this condition activated Protein C induced fibrinolysis and inhibit trombosis as an inflammatory response (9). Ultimately over reactive response to infection obtained endovascular endothelial damage, microvascular thrombosis, organ ischemic, multiorgan dysfunction and death (4).

We investigate patients who have SIRS or sepsis criteria. The treatment of patients used Early Goal-Directed Therapy (EGDT) protocol guidelines (10). Blood draw was performed serially at the first admission and after 72 hours treatment for LBP, ICAM-1, PCT and protein C activity testing, furthermore blood culture also performed at the the first admission, and other examination for monitoring patient's condition.

Materials & Methods

SUBJECT AND SAMPLE

The sample consist of men and women (n=19) who admitted into intensive care unit (ICU) Wahidin Sudirohusodo Hospital, with infection suspected and clinical signs of SIRS, medical recor was collected up to 72 hours (cohort observasional). Subjects were after surgery patients who have risk for SIRS or sepsis.

The patients treatment in this study used Early Goal-Directed Therapy (EGDT) protocol guideline (10). With this protocol the central venous oxygen saturation (ScvO₂), mean arterial pressure (MAP), central venous oxygen saturation dan hematokrit was maintained for increasing haemodynamic parameter (Oxygenous supply in SIRS or sepsis patients).

Blood sample were collected at the first admission and after 72 hours for LBP, ICAM-1, PCT and protein C activity testing and for blood culture at the first admission. Other laboratory testing was used for monitoring the result of treatment. Blood culture result was used for directing appropriate antimicrobial therapy.

The study protocol was approved by Faculty of Medicine Hasanuddin University Ethics Committee. Written informed consent was obtained from each subject.

SIRS/SEPSIS CRITERIA

Clinical signs of SIRS are two or more of the following:

- Temperature $\geq 38^{\circ}\text{C}$ or $\leq 36^{\circ}\text{C}$
- HR ≥ 90 beats/min
- Respirations ≥ 20 /min
- WBC count $> 12.000/\text{mm}^3$ or $\leq 4.000/\text{mm}^3$ or $> 10\%$ immature neutrophils

Sepsis: SIRS with a presumed or confirmed infectious process. **Severe sepsis:** Sepsis with organ dysfunction, hipoperfusion abnormality or hypotension, supported by urine output ($< 0,5$ ml/kg body weight/hour) and other laboratory examination (creatinin, urea, trombositis, albumin, SGOT, SGPT, bilirubin). (11)

LABORATORY EXAMINATION

Blood samples were collected into the plain tube for LBP, ICAM-1 and PCT examination, blood was centrifuged to obtain the sera, protein C activity sample was collected from plasma citrate. The samples were performed in Prodia Clinical Laboratory, Jakarta. Hematology testing with EDTA-blood sample was performed in Clinical Pathology Department, Wahidin Sudirohusodo Hospital, Makassar. LBP serum was performed in Immulite System (chemiluminescent immunometric assay, DPC, USA). ICAM-1 serum was performed with Elisa method (R&D Systems, USA). PCT

serum was performed with immunochromatography method (the rapid assay platform, BRAHMS, Germany) in semiquantitative results with 30 minutes incubation time. Protein C activity plasma was performed with chromogenic substrate method. Protein C in patient's sample was activated by specific venom activator.

STATISTICAL ANALYSIS

A 2-sided P value of <0,05 was considered significant for evaluating mean and median differences between the first admission groups and after 72 hours groups, using SPSS for window version 11.5.

Results

BASELINE CHARACTERISTICS

Baseline characteristics of subjects are shown in table 1. The subjects are post surgery patients who have clinical sign of SIRS and risk of sepsis.

Table 1. Baseline Characteristics of the Patients

Variable	(N = 19)
Age (%)	
9 - 18 years	31,6
20 - 36 years	42,1
53 - 68 years	26,3
Sex (%)	
Men	47,4
Women	52,6
Clinical and laboratory criteria for SIRS	
Temperature ($^{\circ}\text{C}$)	$37,7 \pm 0,6$
Heart rate (beats/min)	106 ± 20
Systolic blood pressure (mm Hg)	$113 \pm 18,5$
Respiratory rate (breaths/min)	$23,7 \pm 4,6$
Partial carbon dioxide pressure (mm Hg)	$43,7 \pm 6,5$
Leukocyte (per mm^3)	15.930 ± 10.800
SIRS/sepsis classificatio when admission to the ICU (%)	
SIRS	68,4
Suspected Sepsis	31,6
Positive blood culture	63,2
BP levels more than referece values	100,0
Outcomes (%)	
Survive	52,6
Non-survive	47,4

LBP SERUM LEVELS AND RISK OF CLINICAL OUTCOME

The LBP levels decreased in surviving patients after 72 hours whereas its increased in non-surviving patients. Mann-Whitney test showed a significantly different between the means of LBP levels in surviving patients and non-surviving patients at 72 hours blood examination ($p < 0.005$). While the LBP levels at the first admission into the ICU were not significantly different in surviving patients and non-surviving patients (Table 2.)

Table 2. LBP, ICAM-1 and Protein C levels at the first admitted into the ICU and 72 hours

Markers (means)	First Admission levels			72 hours		
	surviving	Non-Surviving	P#	Surviving	Non-surviving	P#
LBP (ug/ml)	133,6	161,2	0,624	102,8	184,4	0,014
PCT (ng/ml)	≥ 10	≥ 10	0,923	$\geq 2 - < 10$	≥ 10	0,042
ICAM-1 (ng/ml)	554,0	697,5	0,567	604,4	656,2	1,000
Protein C (%)	50,4	37,9	0,064	50,7	31,6	0,120

Mann-Whitney Test

PROCALCITONIN SERUM LEVELS AND RISK OF CLINICAL OUTCOME

PCT serum level significantly decreased (table 2) after 72 hours in surviving patients (Wilcoxon test, $p < 0,05$), while in non-surviving patients there were no significantly increasing level between first admission and after 72 hours ($p > 0,05$) (table 3). Mann-Whitney test showed no significantly different between mean PCT level between surviving sepsis patients and non-surviving sepsis patients at the first admission ($p > 0,05$) (table 2), whereas there were significantly different mean level after 72 hours ($p = 0,042$).

ICAM-1 SERUM LEVELS AND RISK OF CLINICAL OUTCOME

The median of ICAM-1 levels decreased not significantly ($p > 0,05$) in surviving and non-surviving patients (Table 3.), and no significantly different at first and 72 hours levels between surviving patients and non-surviving patients ($p > 0,05$). There were increasing means of ICAM-1 levels after 72 hours in surviving patients while its decreased in non-surviving patients (Table 2.)

Table 3. LBP, ICAM-1 and Protein C levels in surviving and non-surviving patients

Markers (median)	SIRS/Sepsis ' Survive			SIRS/Sepsis ' Non-survive			Ref. values
	First admission	72 hours	P*	First admission	72 hours	P*	
LBP (ug/ml)	154,0	103,0	0,093	170,0	200,0	0,715	2,0 – 15,2
PCT (ng/ml)	≥ 10	$\geq 2 - < 10$	0,00 □	≥ 10	≥ 10	1,000	<0,5
ICAM-1 (ng/ml)	560,3	431,8	0,721	772,2	431,4	0,345	115 - 306
Protein C (%)	46,6	43,0	0,959	25,8	25,0	0,109	70 - 140

* Wilcoxon Test

PROTEIN C ACTIVITY PLASMA LEVELS AND RISK OF CLINICAL OUTCOME

There were no significantly different levels of protein C activity at first and 72 hours (Table 2. and Table 3.). But the decreasing of protein C activity means levels were showed in non-surviving patients after 72 hours (Fig.3.). The means levels of protein C activity at the first admission were lower in non-surviving patients than surviving patients.

Discussion

Serum LBP level was higher in non-surviving sepsis patients than in surviving patients with good clinical outcome (12), while Opal et al also did not find any such correlation, but they reported significantly lower serum LBP levels in nonsurvivors than in survivors within 24 hours of onset of sepsis. They hypothesized that synthesis of LBP fails in the presence of rapidly progressive septic shock (13). In surveillance study from hundreds patients has done by Carroll et al showed many diseases or condition which may increase serum LBP levels such as sepsis, meningococemia, abdominal infection and inflammatory bowel disease. Increasing serum LBP levels ($>46\mu\text{g/ml}$) found in sepsis patients with suspected gram negative infections and related to high mortality number, showed a strong relationship between serum LBP level and severity of disease (12). Severity of disease may be occurred in conjunction with increased endotoxin in the systemic circulation. Endotoxemia may originate from regional hypoperfusion and mucosal ischemia, and promote translocation of endotoxin to the systemic circulation (14). In this study we did not perform endotoxin examination. Blood culture were performed at the first patient's admission into the ICU, bacterial identification was not known until this study was reported and so we are unable to assess its correlation with serum LBP levels. Suspected infection status was reported from clinical signs before received the culture results.

LBP testing at the first admission was not significantly different between survivors and non-survivors, showed that initial LBP testing is not effective for predict clinical outcome of sepsis, it was suggested that all sepsis patients have synthesized LBP progressively since initial systemic infection occurred. Whereas serum LBP levels decreased after 72 hours in survivors and increased in non-survivors, the difference serum LBP levels were also significant in survivors and non-survivors after 72 hours examination ($p = 0,014$, table 2). Serum LBP levels

after 72 hours had positive correlation with patient's clinical outcome ($r = 0,560$, Kendall's tau test), showed that serum LBP levels more useful in serial testing for predict clinical outcome of sepsis.

Significantly decreasing serum PCT levels in severe sepsis or septic shock patients who may have good clinical outcome is consistent with Harbarth's report (15). Serum PCT levels significantly increased after 2-6 hours after LPS/endotoxin injection and maintaining a plateau through 8 and 24 h (16), showed in this study with a positive correlation between LBP and PCT at the first admission examination ($r = 0,551$, Kendall's tau test). PCT was induced in the liver by proinflammatory cytokine IL-6 (6). PCT testing with rapid platform assay is more useful, shorter turn around time compare to blood culture. This testing is effective for early prediction of sepsis clinical outcome, and may be used in conjunction with antimicrobial therapy. Antimicrobial therapy has important influence on serum PCT levels (15) so the dynamic levels of serum PCT in antimicrobial therapy may be useful for monitoring the effectivity of treatment. Serum PCT levels after 72 hours have positive correlation with clinical outcome of sepsis ($r = 0,521$, Kendall's tau test). It showed that initial examination is not useful to predict the clinical outcome, it was needed serial examination to found the risk progression of sepsis.

Serum ICAM-1 levels were high in all patients and was not significantly changed after 72 hours (table 2 and table 3), it was suggested in this study the endothelial dysfunction was occurred both in surviving or non-surviving sepsis patients, this adhesion molecule was induced in activated endothelial cells and the become dysfunction (7). This study also showed positive correlation between serum LBP levels and serum ICAM-1 levels at the first admission ($r = 0,630$, Spearman's rho test), suggested that endothelial dysfunction was induced by LPS/endotoxin. In non-survivors multiple organ failure have been occurred due to endothelial damage, serum ICAM-1

levels slight decreased in this patients. However the dynamic levels of ICAM-1 in this study did not show a significantly different ($p>0.05$) between survivors and non-survivors. So ICAM-1 testing was not suggested for predicting clinical outcome of sepsis patients.

Systemic inflammatory process and sepsis impair the endogenous fibrinolytic potential by inflammatory cytokines so may diffuse endovascular injury, microvascular thrombosis, multiorgan dysfunction, and death. The conversion of protein C to protein C activity may be impaired during sepsis as a result of down-regulation of thrombomodulin from endothelial cell by inflammatory cytokines (8). In our study was showed from negative correlation between protein C activity and ICAM-1 ($r = -0.76$ with Spearman's rho) which may suggested endothelial dysfunction with high serum ICAM-1 level correlated with low plasma protein C activity level. The low level of plasma protein C activity found in sepsis patients and increasing risk of death (17, 18, 19, 20). This study could not give a good evidence for protein C activity testing as a predictor marker for clinical outcome from the first admission into the ICU or for monitoring in serial testing.

It was difficult to differentiate the etiological sepsis or systemic inflammatory cases from infection or from other sources due to the lack of specificity for identifying systemic inflammatory. C-reactive protein (CRP) has been used for predicting sepsis patient (21, 22). In this years several markers have developed as sepsis markers such as LBP and procalcitonin (23, 24). Inflammation was associated with endothelial activation which expressed adhesion molecule (7). Proinflammatory cytokines (TNF- α , IL-1 β) and IL-6 are capable of activating coagulation and inhibiting fibrinolysis, while procoagulant thrombin may stimulate multiple inflammatory pathways.

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Of 10 survivors, 6 patients have been surviving from severe sepsis and have recovered, in this patients serum PCT levels were > 10 ng/ml and serum LBP levels were high (177 – 205 ug/ml) and after recovered the level of serum LBP decreased in the range 104 – 158 ug/ml consistent with decreasing serum PCT levels (<10 ng/ml).

Serum LBP, PCT and ICAM-1 levels of sepsis patients in this study increased. Whereas plasma protein C activity levels decreased, especially in severe sepsis patients who were shock and death. This showed that LPS binding protein (LBP) induced systemic inflammatory and its progression to sepsis, and associated to positive corelation between serum LBP levels and serum PCT levels. Systemic inflammatory and sepsis then activate endothelial dysfunction which were associated with positive correlation between serum LBP levels and serum ICAM-1 levels. Endothelial dysfunction may promote hypercoagulation which were related to the low level of plasma protein C activity in sepsis patients with high level of serum ICAM-1.

In summary, The initial levels of serum LBP and serum PCT could not used for predicting sepsis clinical outcome. Increasing serum LBP and PCT levels in severe sepsis patients who shock and death showed that these markers were useful for predicting the clinical outcome in sepsis patients when performed in serial testing. LBP testing may performed within 1 hour and PCT testing within 30 minutes. Those testing are more effective than blood culture which need 3 days incubation time. High levels of serum ICAM-1 at the first admission or in serial testing could not suggested for predicting the risk of sepsis because its levels did not change significantly, neither for plasma Protein C activity.

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