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Correlation of Nitrite Oxide with Severity and Survival Rate of Sepsis Patients

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ABSTRACT

The objective of this research was to determine the correlation between Nitric Oxide (NO) levels with the severity of sepsis, to describe the kinetics of NO levels, and to evaluate it in predicting mortality. This research was a longitudinal cohort observational analytical study. The variables were serum NO levels and SOFA scores, which were serially evaluated. The correlation test and difference test were used for statistical analysis. The survivor and the non-survivor group consisted of 14 (41.18%) and 20 (58.82%) patients, respectively. There was a correlation between serum NO levels and the SOFA score at the 24-hour observation (r=0.403; p=0.041). Non-parametric Mann-Whitney test showed that there was no kinetics of NO levels at 0, 24, 72, and 144-hour observation (p-values =0.897 and 0.703, respectively). NO levels > 111,16 µmol/L at the 24th hour could predict the risk of death with hazard ratio 4.7 compared to NO levels < 111,16 µmol/L. The survival rate of patients with serum NO levels <111,16 µmol/L and > 111,16 µmol/L was 83.3% and 37.5%, respectively. There was a correlation between serum NO levels and SOFA scores at the 24-hour observation. However, there was no kinetics of NO levels at serial evaluations. Nitric oxide levels with a cut-off of 111,16 µmol/L at 24 hours could predict the survival of septic patients. Utilization of serum NO level at 24th hour can be used to evaluate the severity of septic patients and aggressive management if there is an increase in serum NO levels > 111,16 µmol/L at 24 hours.

Keywords: Nitric oxide, SOFA score, survival

INTRODUCTION

Sepsis remains a serious health problem with high morbidity, mortality, and treatment cost.[1] The Lipopolysaccharide (LPS) of Gram-negative bacteria that enter the body will trigger an increase in serum Nitric Oxide (NO) levels. Increased serum NO levels have a very strong relationship with an increase in cysteine aspartate specific protease called caspase 3, an executor mediator of apoptosis.[2-4] Apoptosis has an important role in the event of organ failure.[5]

The severity of sepsis patients in intensive care unit is monitored with Sepsis-related Organ Failure Assessment (SOFA) scores (Table 1) and it is thought that serum NO levels can be used as a screening test to detect the presence of apoptosis, as the basis for organ failure in addition to other parameters such as routine blood test, procalcitonin, and CRP.[2,6] Serum NO levels can be measured using an affordable facility (ELISA method) and cost, it is widely available even in type C Hospital and provides accurate results.[2]

Current method used to evaluate the severity of sepsis is SOFA score consisting of: respiratory-PaO_2 FIO_2 mmHg(kPa), coagulation-platelet x10^3/µL, liver-bilirubin mg/dL (µmol/L), Cardiovascular-MAP, Dopamine, Neurologic -GCS: eye (score 4), speech (score 5) and motor (score 6), renal-creatinine mg/dL (µmol/L).[7] The SOFA score requires several clinical assessments and laboratory tests. The use of a proper parameter to estimate the severity and predict the survival of sepsis subjects at an early stage will accurately assist the management of septic patients, resulting in the improvement of patient survival. This parameter must be affordable and accessible even in type C hospitals. Serum NO levels can be used as a screening test to detect the presence of apoptosis, as the basis for organ failure.[2,6]

Haryanto found a decrease in NO levels at 12, 24, 36, and 48 hours after exposure to LPS.[2] The protocol for antibiotics administration to septic patients in the ICU was carried out for 3 days (0, 72, and 144-hour observation). The study carried out similar observations at 0, 24th, 72nd, and 144th hours or days 0, 1, 3, and 6.

This study aimed to evaluate the correlation of

Table 1. SOFA score[7]

System	0	1	2	3	4
Respiratory PaO_2 / FIO_2 mmHg(kPa)	≥400 (53.3)	<400 (53.3)	<300 (40)	<220 (26.7)	<100 (13.3)
SaO_2/FIO_2		221-301	142-220	67-141	<67
Coagulation Platelet, $x10^3/\mu L$	≥150	<150	<100	<50	<20
Liver Bilirubin, mg/dL (µmol/L)	<1.2 (20)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	>12.0 (204)
Cardiovascular Hypotension	MAP ≥70 mmHg	MAP <70 mmHg	Dopamine < 5/ dobutamine (µg/kg/min)	Dopamine 5.1-1.5/ epinephrine ≤ 0.1/ norepinephrine ≤ 0.1 (µg/kg/min)	Dopamine 5.1-1.5/ epinephrine > 0.1/ norepinephrine > 0.1 (µg/kg/min)
Neurologic Glasgow coma score	15	13-14	10-12	6-9	<6
Renal Creatinine, mg/dL (µmol/L)	<1.2 (110)	1,2-1,9 (110-170)	2.0-3.4 (171-299)	3.5-4.9 (300-440)	>5.0 (440)

NO levels with the severity of sepsis, describe the kinetics of NO levels and evaluate the role of NO in predicting mortality and survival through survival analysis.

METHODS

This study was analytic observational cohort research. Research ethics were obtained from the Ethics Committee of Gadjah Mada University with the number KE/FK/1476/EC/2019. The subjects of this study were septic patients diagnosed with sepsis by the doctor in charge of patient, based on signs of infection and organ failure assessed by SOFA score > 2.[8] Subjects were treated in the intensive care unit at Raden Mattaher Hospital, Jambi from December 2019 to April 2020 and were monitored at the 0, 24th, 72nd, and 144th hours. The study variables were serum NO levels and SOFA scores evaluated at 0, 24th, 72nd, and 144th hours.

The outcomes were classified into a survivor and non-survivor groups. Inclusion criteria were patients diagnosed with sepsis and patients or their families who agreed to participate in the study. Exclusion criteria were patients with hematological malignancies. Research consent was obtained from the patient or family. Venous blood samples were taken. Serum NO levels were measured with the Nitric Oxide Assay Kit Invitrogen Thermo Fisher Scientific ® lot number 208879007.

The SOFA score is a combined 0-24 score of SaO2/FIO2; Platelets; Bilirubin; Mean Arterial Pressure (MAP), use of dobutamine, epinephrine, norepinephrine; Glasgow Coma Scale (GCS) score; Serum creatinine, urine output. The observation carried out in this study was at the 0th hour (at the time of diagnosis) and at the time indicated later, such as the 24th, 72nd, and 144th hours. The minimum sample size is 32 samples. Data analysis was carried out using SPSS 23 statistics with correlation test, difference test, and survival analysis.[9,10]

RESULTS AND DISCUSSIONS

Subjects in this study were 34 people with sepsis in the ICU, consisting of 20 (58.82%) males and 14 (41.18%) females. The survivor group consisted of 14 (41.18%) patients and the non-survivor group consisted of 20 (58.82%) patients. There was a correlation between NO levels and SOFA scores at the 24-hour observation (r=0.403; p=0.041), but no correlation was found at 0, 72, and 144-hour observation (r-values were 0.157; 0.111; -0.055, respectively); p>0.05).

There was no kinetics of NO levels at 0, 24, 72, and 144-hour observation with p-value of 0.871; 0.583; 0.631 and 1.00, respectively.

Nitric oxide levels > 111,16 mol/L at 24 hours can predict the risk of death with hazard ratio 4.7 compared to NO levels < 111,16 mol/L. The survival rate of patients with serum NO levels < 111,16 mol/L and > 111,16 mol/L was 83.3% and 37.5%, respectively. Clinical and laboratory characteristics of patients at intensive care unit, there were 34 subjects with 20 (58.82%) males and 14 (41.18%) females. A female patient was one of inclusion and exclusion in this study. This was following the research of Widodo *et al*.., which found sepsis in 22 (52.4%) males and 20

(47.6%) females in the Cipto Mangunkusomo Hospital, Jakarta.[11]

There were 14 (41.18%) patients in the survivor group 20 (58.82%) patients in the non-survivor group. This was different from the research of Kadir *et al.*, which reported good outcomes in 40 (70.18%) cases and poor outcomes in 17 (29.82%) cases.[4] Previous research also found the high mortality (65.7%) of sepsis in intensive care unit data of Prof. Dr. R. D. Kandau Hospital in Manado.[1] However, research by Kadir *et al.* at Hasan Sadikin Hospital, Bandung found low mortality in 17 (29.82%) sepsis cases among neonatal subjects, in contrast to this study, which involved adult subjects.[4]

There was a positive correlation between NO levels and SOFA scores at the 24-hour observation (r=0.403; p=0.041), but no correlation was found at 0, 72 and 144-hour observation (r-values were 0.157; 0.111; - 0.055; p>0.05 (see Table 2). The research of Chandra *et al.* also found a correlation between serum NO levels and the outcome of sepsis patients.[12]

There was no significance of serum NO levels of patients in the survivor and non-survivor groups. This was different from previous research by Chandra *et al.*, which found that the median serum NO levels of non-survivor patients (36.50 mol/L) was significantly higher than that of survivors (18.60 mol/L) (p=0.016).[12] In addition, Haryanto *et al.* found increased serum NO levels in the LPS-induced sepsis group compared to the control group.[2]

There was no significant difference in serum NO levels at the 0, 24, 72, and 144-hour observations in survivor (p=0.897) and non-survivor (p=0.703) group (see Table 3 and Figure 1). Research by Haryanto *et al.* using experimental animals Balb/C mice with LPS-induced sepsis found decreased NO levels as the kinetics of LPS-induced sepsis at 12, 24, 36, and 48-hour observation after LPS exposure.[2] This was due to a decrease in apoptotic cell scores and caspase-3 expression, which has a very strong relationship with NO levels.

Table 2. Correlation between NO and SOFA score

	n	Serum NO levels (µmol/L)	SOFA score	r	p
0th hour	34	94.32±85.41*	6.29±2.13*	0.157	0.374a
24th hour	26	74.00 (11.47-344,07)**	6.62±2.45*	0.403	0.041b
72nd hour	18	92.76±84.64*	5.78±2.26*	0.111	0.662a
144th hour	11	42.70 (12.28-388,15)**	5.27±2.65*	-0.055	0.872b

a Pearson correlation test b Spearman correlation test *Parametric data: mean±SD **Non-parametric data: median (range)

Table 3. Analysis of difference of serum NO on 0, 24th, 72nd, and 144th hour

Subject Group	Serum NO Levels During Observation (µmol/L)				p
	0	24	72	144	
Survivor	64.51 (5.36-311,08)	70.40 (24.56-344,07)	78.95 (12.28-167,90)	68.78 (24.45-144,32)	0.897a
Non-survivor	95.23 (13.05-318,35)	94.10 (11.47-304,93)	63.85 (20.02-388,13)		0.703a

a Non-parametric Mann-Whitney test

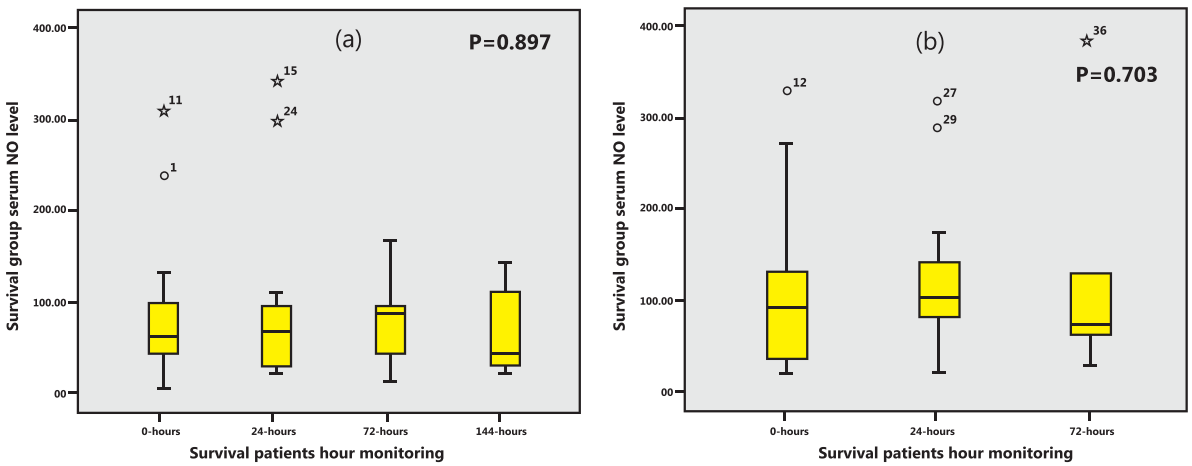

Figure 1. Box plot diagram of serum NO levels of sepsis patients at 0, 24, 72, and 144-hour observation based on clinical outcome (a) survivor group (b) non-survivor group

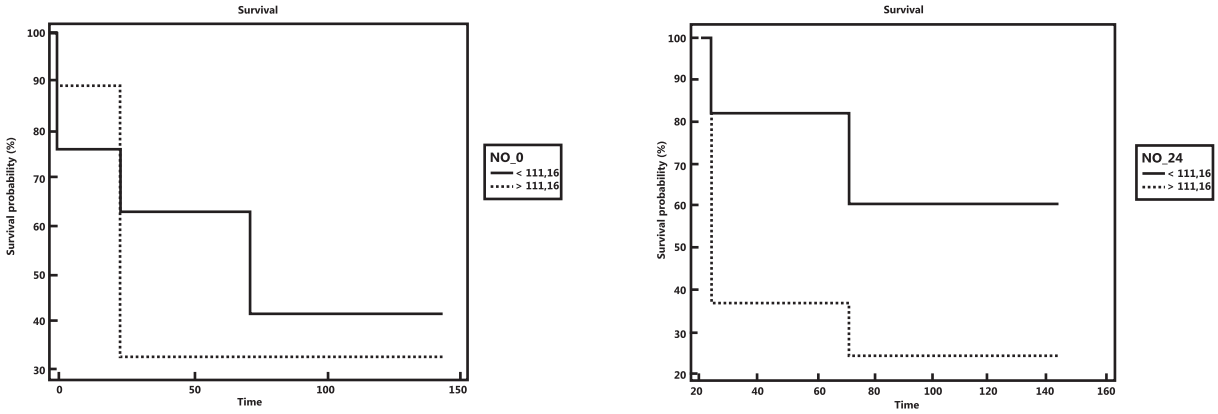

Figure 2. The survival rate based on cut-off point of serum NO levels of 111,16 µmol/L at (a) 0 and (b) 24-hour observation

The cut-off value of the ROC curve for the measurement of serum NO levels on mortality in sepsis patients was 111,16. Serum NO levels at 24-hour observation with values < 111,16 mol/L and > 111,16 mol/L were significantly different with a p log-rank of 0.041 ($p < 0.05$). Hazard ratio of 4.659 indicated that subjects with serum NO levels > 111,16 mol/L were likely to show poor mortality 4.6 times higher compared to subjects with serum NO levels < 111,16 mol/L. Serum NO level < 111,16 mol/L at 24-hour observation showed the survival rate of 83.3%, whereas serum NO level > 111,16 mol/L at 24-hour observation showed the survival rate of 37.5% (see Figure 2).

Research by Haryanto *et al.* suggested that serum NO levels with a cut-off of 104,85 mol/L could be used as a screening test to detect the presence of caspase-3 expression (apoptosis). The survival rate of sepsis patients based on the cut-off point of serum NO levels of 104,85 µmol/L in this study was not statistically significant ($p=0.749$).[2,9,10]

The odds ratio value of serum NO level >111,16 mol/L compared to serum NO level <111,16 mol/L at 0, 24 and 72-hour observation was not significant (OR values were 1.57; 4.71, respectively); 3.67; $p>0.05$. The risk ratio value of serum NO level >111,16 mol/L compared to serum NO level <111,16 mol/L at 0, 24 and 72-hour observation was not significant (odds ratio values were 1.32; 2.44, respectively); 1.57; $p > 0.05$.

CONCLUSIONS AND SUGGESTIONS

There was a correlation between serum NO levels and SOFA scores at the 24-hour observation. There was no kinetics of NO levels at 0, 24, 72, and 144-hour observation. Nitric oxide levels with a cut-off of 111,16 mol/L at 24 hours could predict the survival of sepsis patients.

The use of NO levels in the management of sepsis: NO examination at 24 hours to evaluate the patients severity of sepsis and aggressive management if there is an increase in serum NO levels > 111,16 mol/L at 24 hours.

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