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Serum Nitric Oxide as Early Predictor of Poor Outcome in Neonatal Sepsis

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Abstract **Objective** This study examines the serum nitric oxide (NO) as an indicator of poor outcome in neonatal sepsis. **Study Design** This prospective observational study was conducted in Dr. Hasan Sadikin General Hospital, Bandung, from September to November 2014. All subjects fulfilled inclusion criteria were tested serum NO metabolite at admission, then were followed up to determine the final outcome, grouped as Group I-good outcome, Group II-poor outcome. Logistic regression analysis was performed to determine independent variables associated with poor outcome, estimated as the odds ratio (OR) and the 95% confidence interval (95% CI). **Results** Fifty seven neonates were enrolled in this study. There was a good relation between NO level and poor outcome in neonatal sepsis ($p<0.01$). The level of NO metabolite was a significant independent factor of the poor outcome in neonatal sepsis in the multivariate regression logistic analysis (OR 25.975, p=0.000, 95% CI 4.354–154.952). It showed good discrimination with AUC 0.815 (95% CI 0.676 to 0.955), and good calibration (p=0.192). **Conclusion** A high serum NO level is independently associated with poor outcome in neonatal sepsis.

Keywords: *nitrit oxide, neonatal sepsis, outcome*

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1. Introduction

Neonatal sepsis is still important causes of morbidity and mortality among poor and developing countries.[1] Estimated 4 million newborns died caused by severe infection. The neonatal sepsis rate in Asia ranging from 7,1−38/1,000 while developed countries such as USA and Netherland estimated 1,5−3,5/1,000 cases [2].

Early recognition of neonates who are at high risk for poor outcome or even mortality allows for timely therapy and improves the overall outcomes [3]. Prior studies have identified a lot of risk factors associated with sepsis such as prematurity, low birth weight, premature membrane rupture, maternal factor such as pyrexia, poor intra- and postpartum hygiene, invasive medical procedures, and place of birth. Likewise, in our study, sepsis-associated factors we measured were onset sepsis, different body weight, maternal and neonatal risk factor [4,5]. In this study, we input nitric oxide (NO) metabolite as early predictor of neonatal sepsis outcome. Nitric oxide plays a significant role in the pathogenesis of severe conditions of sepsis. Over production of nitric oxide induced vasodilatation, changed in cardiovascular function, and decreased myocardium contractility [6]. Previous studies done by Shi et al. [7] showed NO level in sepsis higher than healthy control, but none showed NO as early risk factor to predict poor outcome. This study was conducted to investigate NO as an indicator of poor outcome in neonatal sepsis.

2. Methods

2.1. Setting

This prospective observational study was conducted in Dr. Hasan Sadikin General Hospital, Bandung, from September to November 2014. Subjects were selected by consecutive sampling.

2.2. Inclusion and Exclusion Criteria

Newborns who fulfilled two or more SIRS criteria with a proven bacterial infection or probable infection such as pneumonia, necrotizing enterocolitis (NEC) or chorioamnionitis were enrolled in this study. Neonates with major congenital abnormalities were excluded.

2.3. Data Collection

Neonates that fulfilled inclsion criteria were run for blood testing after parent's approval. The complete blood count was tested in Hasan Sadikin Hospital. Serum NO levels were taken by the time of patient's admission, and processed to its stable metabolites ; nitrate (NO_3) and nitrite (NO_2) using the Griess reaction. Two ml blood was drawn, centrifuged with 1,500 rpm for 15 minutes, kept

under -20°C before sent to *Laboratory Research and Esoteric Testing* Prodia Bandung.

The subjects were followed prospectively and then divided in two groups according to the outcome: Group I-good outcome and Group II-poor outcome. Poor outcome defined as subjects suffered two or more organ dysfunction, or septic shock or died during hospitalization, while good outcome grouped based on clinical and laboratory improvement, discharged from hospitalization in good condition.

2.4. Statistical Analysis

All risk factors were analyzed by using Chi-square test for categorical data and t-test or Mann-Whitney test for numeric data. Bivariate analysis were then subsequently subjected to a stepwise multivariate logistic regression analysis to determine the independent contribution of poor outcome due to neonatal sepsis. Receiver operating characteristic (ROC) curve analysis was used to calculate area under the curve (AUC), determine the cut off point, as well as calculate the sensitivity, specificity, positive predictive value, negative predictive value, and positive likelihood ratio. Each variable to the risk of sepsis outcome was expressed as the OR with a 95% CI. Discrimination was determined using the receiver operating characteristic (ROC) analysis and calibration was analyzed using the Hosmer-Lemeshow goodness-of-fit test. All analysis was processed using SPSS software for Windows version 17.

The study has been approved by the Research and Ethics Committee of the Universitas Padjadjaran Medical School, Indonesia, and written parental consent was obtained.

3. Results

3.1. Study Population

Since September to November 2014, 68 neonates were eligible to assess. Eleven neonates were excluded and 57 were fulfilled the inclusion criteria. All subjects then continuosly follow up to determine outcome as shown in Figure 1.

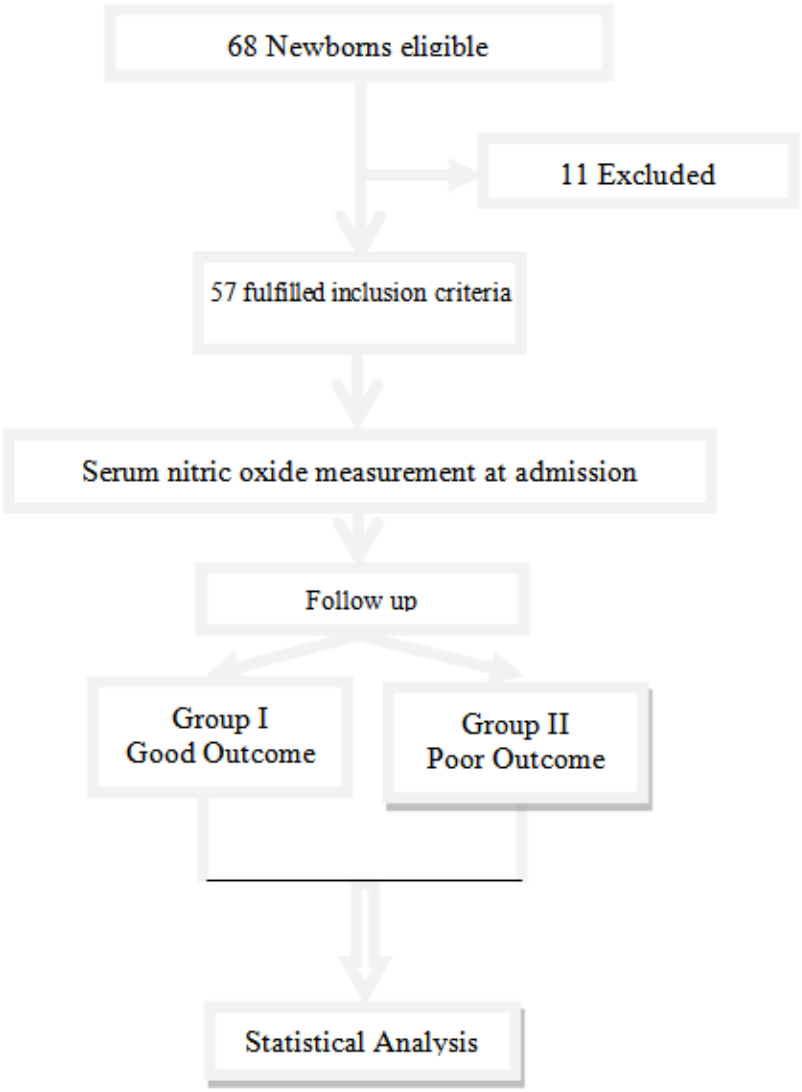

Figure 1. Participant's Flow Diagram

It was shown that subjects consist of 37 males and 20 females. Forty were in Group I and other 17 were matched in Group II. Difference characteristics of both groups were shown in Table 1.

Table 1. Characteristics of subject and outcome of neonatal sepsis

Characteristics	Outcome	
	Group I Good Outcome (n=40)	Group II Poor Outcome (n=17)
Gender		
Female	13	7
Male	27	10
Mode of delivery		
Vaginal	33	16
Caesarian section	7	1
Birth Weight (grams)		
< 2500	11	8
≥ 2500	28	10
Gestational age		
Preterm	8	5
Term	32	12
Place of birth		
Hospital	25	10
Outside the hospital	15	7
Temperature		
Mean (SD)	37.33 (0.9529)	36.3 (0.98)
Median	37.35	36
Range	(36 – 38.9)	(35.1 – 38.5)
Tachypnea		
Present	16	13
Absent	24	4
Tachycardia		
Present	7	14
Absent	33	3
WBC (/mm^3)		
Mean (SD)	21,685 (9,714.7)	25,517 (14,300.1)
Median	22.750	30.200
Range	(3,600 – 39,800)	(3,400 – 45,000)
Positive blood culture Probable infection	4	4
Pneumonia	21	5
NEC	10	3
Chorioamnionitis	9	9

3.2. Level of Nitric Oxide

Cut off value of serum NO taken from ROC curve analysis recorded in this study was >13.7 µM/L, with AUC 0.815. It showed 70.5% sensitivity, 92.5% specificity, 80% positive predictive value (PPV), 88% negative predictive value, 9.41 time greater in predicting poor outcome of neonatal sepsis, as plotted in Figure 2.

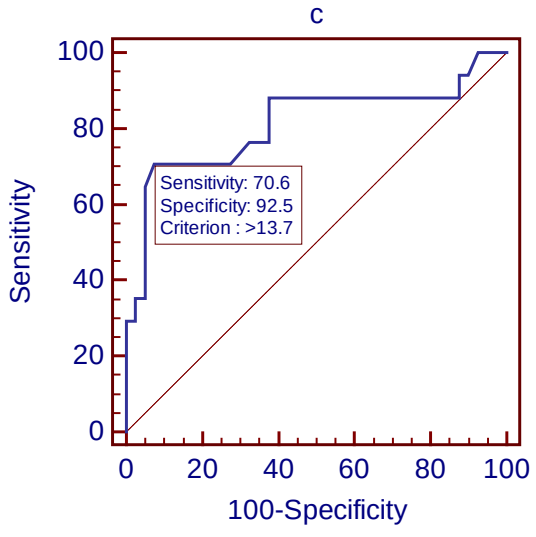

Figure 2. ROC curve analysis of nitric oxide

Bivariate logistic regression analysis compared body weight, risk factors at birth, onset of sepsis, and NO with poor outcome in neonatal sepsis as shown in Table 2. There was statistically significant difference between two groups for birth weight and NO level (p <0.05).

Table 2. Factors associated with the outcome of neonatal sepsis

Variable	Outcome		P value
	Group I Good Outcome (n=40)	Group II Poor Outcome (n=17)	(95% CI)
Birth weight (grams)			0.047*
< 2,500	11	8	
≥ 2,500	28	10	
Risk factors at birth			0.077*
Presence	18	12	
No presence	22	3	
Onset			0.206*
Early onset	21	12	
Late onset	19	5	
NO level (µM/L)			
≤ 13.7	37	5	< 0.01**
>13.7	3	12	

*Chi-square test; ** Exact Fischer

Multivariate logistic regression modelling was then performed using statistically significant bivariate variables. Only NO level was shown to have significant association with poor outcome (OR, 95%CI) (Table 3).

Table 3. Multivariate logistic regression model associated with poor outcome

Variable	Koef (B)	P value	OR (95% CI)
Birth weight	-.281	.746	0.755 (0.137–4.147)
NO	3.257	.000	25.975 (4.354–154.952)
Constant	-1.753	.000	.173

The NO level >13.7 µM/L was significant independent factor for predicting poor outcome in neonatal sepsis. The risk to become poor outcome 25.975 times greater for newboen with elevated NO level. This final model showed good discrimination with AUC 0.815 (95% CI 0.676 to 0.955), and good calibration (p=0.192).

4. Discussion

Neonatal sepsis can progress to severe sepsis and septic shock, expand to multiple organ failure depending on the degree of severity. Early prediction of neonatal sepsis outcome has been challenging in reducing mortality in newborns and plays an important role in clinician's assessment.

In this study, it was shown than NO as a significant independent risk factor of poor outcome in neonatal sepsis (p=0.00). Overproduction of NO induced by infection and inflammation can cause cytotoxic to host cells and play an important role in complex immuno-inflammatory response. Bacterial toxins caused infection with different ways. Gram negative bacteria induced i-NOS production directly. Therefore, gram positive bacteria produced superantigens (SAgs) that bind to the variable portion of β-chain (Vβ) of the T-cell receptor, induced i-NOS production and elevated serum NO level, so more severe the infection was, the higher NO level was [8,9].

Nitric oxide was heterogeneously distributed in the vascular system, tissue perfusion, decreased cardiac output, kidney failure, molecularly linked to tissue hypoxia, and eventually leads to severe form of sepsis to multiple organ dysfunction syndrome [10]. Nitric oxide concentrations are associated with some indices of severity, development of perfusion failure, and the detrimental consequences [11,12]. Tissue hypoxia related to the dysfunction of mitochondria and energy failure in neonatal sepsis, where leads to ATP depletion, later role in the course of neonatal sepsis development [13, 14].By determine the NO level, it was hoped that clinician could differ severity of sepsis earlier, adjust prompt treatment timely, reduce morbidity and mortality.

Other study performed by Shi et al. [7] showed that serum NO level in sepsis group higher compare to control. The study conducted by Duke et al. [15] compared that serum NO level lower in survived group. Other studies done by Mahmoud et al. [16] showed the cut off value ranged from 10.1 to 60.4 μmol/L, with the median level of 35.2 μmol/L. The different value of NO level might be caused by different equipment used in all study, different methods and analysis, other factors that supposed to contribute was the different facility available, climate, and geographic area.

The other factors such as body weight, onset sepsis and risk factors at birth were not associated with poor outcome in neonatal sepsis after multivariate analysis. This result is contrary to the previous study by Leal et al [4]. that stated all these factors contributed to the sepsis outcome. As shown in Ibraheem [17] percentage of mortality in early onset sepsis was higher than in late onset sepsis. Predicted this contrast result because of different sample size.

To our knowledge, this study is the first one in our department and has not been used routinely in our centre. Our setting has several limitations due to lack capacity of neonatal intensive care unit, medications, ventilators, monitors.

There were several limitations in this study. First, there was no control group. Second, double infection by viral and fungal pathogens can not be excluded. Third, the NO serum only measured one time. Further study is still needed to be done in multicentre study with larger sample size.

5. Conclusion

Serum nitric oxide is associated with neonatal sepsis severity. It can be a promosing biomarker needed to predict poor outcome in neonatal sepsis earlier and adjust prompt treatment.

Conflict of Interest Statement

No competing interest to declare.

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