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Correlation between neopterin levels in premature infants with sepsis and the signs and symptoms of neonatal sepsis using the Töllner sepsis score

Abstract: Neonatal sepsis in premature infants is difficult to diagnose, and available markers are still varied. Neopterin is a derivative of pyrazino-pyrimidine produced by macrophages when stimulated by interferon γ. This study aimed to measure and compare the concentration of serum neopterin between groups of neonates with and without sepsis, and its correlation with Töllner's sepsis score (TSS). This analytic observational study with a cross-sectional design included 46 premature infants with (n=23) and without neonatal sepsis (n=23). Data analysis using the Mann-Whitney U-test was performed to compare the levels of neopterin in premature infants with and without neonatal sepsis, and the Spearman rank correlation test to determine the correlation between neopterin levels and TSS. Neopterin levels in the sepsis group were higher than in the non-sepsis group (116 vs. 41.1 nmol/L; p<0.001). This study also showed a positive correlation between neopterin levels in premature infants with neonatal sepsis and TSS (r=0.776, p<0.001). Neopterin levels were higher in premature infants with neonatal sepsis than in those without it and positively correlated with TSS.

Keywords: neonatal sepsis; neopterin; premature; Töllner sepsis score.

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Introduction

Although mortality rates for children aged <5 years have improved in many countries worldwide, neonatal mortality rates (deaths in the first 28 days of life) have shown little improvements. Neonatal mortality rate now accounts for >42% of deaths in children aged <5 years, up from 37% in 2000 when the Millennium Development Goals (MDGs) were set [1]. Infections account for about 36% of these deaths. Neonatal sepsis is a clinical syndrome caused by a systemic response to infection during the first month of an infant's life [2]. One study showed that, compared with term infants (TI), premature infants (PI) have as much as 4.8 times higher risk of sepsis [3].

Early signs of sepsis are often non-specific, which results in inadequate treatments. To accurately identify neonates with sepsis, attempts have been made to use physiologic parameters, hematologic indices, and cytokine profiles. C-reactive protein (CRP) and procalcitonin levels have been widely used as markers to diagnose sepsis. However, in PI, the increase in CRP level often occurs at postnatal age >48 h [4, 5]. Procalcitonin level is a sensitive marker, but, like CRP, often gives false-negative results. The procalcitonin test is also more expensive to perform [5, 6].

Neopterin is a derivative of pyrazino-pyrimidine formed by guanosine triphosphate in the tetrahydrobiopterin synthesis pathway. The derivatives are produced by macrophages when stimulated by interferon γ (IFN-γ) produced by T lymphocytes [7]. Increased neopterin level has been shown to be a sensitive and specific marker in cellular immune activation in certain conditions such as allograft rejection, bacterial infections, and malignancy [8, 9]. Research on the role of neopterin in the diagnosis of sepsis in PI has not been conducted before in Indonesia.

The purpose of this study was to measure and compare the value of serum neopterin in PI with and without sepsis, and to determine the correlation between neopterin levels and the signs and symptoms of neonatal sepsis using the Töllner sepsis score (TSS) [10].

Materials and methods

This is an analytic observational study with a cross-sectional design. Inclusion criteria were appropriate-for-gestational age premature infants (30–36 weeks of gestation), with and without neonatal sepsis, and who were referred to or delivered at the Department of Child Health, Hasan Sadikin Hospital. TSS was calculated for each patient.

In developing countries, a different scoring system is often used to help diagnose neonatal sepsis, so we cannot depend exclusively on culture results. We used TSS because it included both laboratory parameters, such as leukocyte and thrombocyte counts, CRP, and immature/total neutrophil ratio, and clinical parameters, such as skin color, body temperature, muscle tone, breath rate, abdominal distension, and imperfect microcirculation [10, 11]. A point was given for each parameter (0, 1, 2, or 3), according to its severity (e.g., 0 for normal muscle tonus, 1 for hypotonia, and 2 for flaccid tonus; 0 for normal leukocyte count, 1 for leukocytosis, and 3 for leukopenia). Infants with sepsis were defined as having a TSS of ≥10. No infants should have a history of antibiotic use, be in a critical condition, or have congenital abnormalities.

An additional 1 mL of blood was taken from the tubes, then centrifuged at 3500 rpm for 10 min; subsequently, 0.5 mL of serum was taken from the blood samples and neopterin serum level was determined using the Neopterin ELISA Kit (IBL, Hamburg, Germany). These tests were species specific, and a level of >10 nmol/L during the first 48 h was considered as elevated. The study was conducted after obtaining approval from the Health Research Ethics committee of the Faculty of Medicine of Padjadjaran University, Hasan Sadikin Hospital.

All data obtained were recorded and tabulated, then the Mann-Whitney U-test was used to compare the levels of neopterin in PI with and without sepsis, whereas the Spearman rank correlation test was used to determine any correlation between neopterin levels and TSS. Data analysis was performed using SPSS for Windows version 17.0. A p-value of ≤0.05 was considered to indicate significance.

Results

The study was conducted from May to July 2013. During the first 3 months of the study, there were 46 PI, divided equally into the sepsis group and the non-sepsis group.

Table 1 shows that sepsis is more common in male infants, with a mean premenstrual age of 35 weeks (range, 30–36 weeks), mainly vaginally delivered, and with a mean birth weight of 1912 g (range, 1250–2400 g). Characteristics data of both the sepsis and the non-sepsis group were not significantly different (p<0.05), indicating a homogeneous distribution of infants in both groups.

Table 2 shows that neontal sepsis was predisposed by several maternal risk factors, with premature rupture of membrane (PROM) occurring in eight neonates (34.8%), meconeal amniotic fluid occurring in seven infants (30.4%), and maternal fever during delivery in two infants (8.7%). Only in meconeal amniotic fluid levels did the two groups differ significantly (p=0.004).

Blood cultures were performed in all infants with sepsis. A positive culture result was only obtained from seven subjects (30.4%). The most common species were *Klebsiella peneumoniae* in four subjects (57% positive cultures), whereas in the other three subjects, *Staphylococcus hemolyticus*, *Serratia marcesens*, and *Alcaligus faecalis* were identified by culture.

The serum concentration level of neopterin in the sepsis group had a mean of 116±49.9 nmol/L (range, between 51.3 and 177 nmol/L; median, 129 nmol/L), whereas in the non-sepsis group the mean was 41.1±9.04 nmol/L (range, between 21.1 and 66.4 nmol/L; median, 41 nmol/L). The level of serum neopterin was significantly higher in the sepsis than in non-sepsis group (p<0.001).

Figure 1 shows a positive correlation between levels of neopterin in PI with sepsis and TSS (r=0.776, p<0.001), indicating that the higher the TSS, the higher the neopterin levels.

Table 1 General characteristics of neonatal subjects.

Characteristics	Sepsis group (n=23)	Non-sepsis group (n=23)	p-Value
Gender			0.376
Male	13	10	
Female	10	13	
Gestational age, weeks			0.793
Mean	35 (1.8)	34 (1.48)	
Median	35	34	
Range	30–36	32–36	
Delivery			0.599
Vaginal birth	20	19	
Non-vaginal birth	3	4	
Birth weight, kg			0.823
Mean	1912 (25.7)	1893 (33.5)	
Median	1900	2000	
Range	1250–2400	1300–2400	

Discussion

In this study, sepsis was more prevalent in male infants with low birth weight, who were born spontaneously, and who have a history of maternal meconeal amniotic fluid, premature rupture of membranes after >24 h, and

Table 2 Maternal risk factor for sepsis.

Characteristics	Sepsis group (n=23)	Non-sepsis group (n=23)	p-Value
PROM >24 h	8	3	0.084
Amniotic meconeal fluid	7	0	0.040
Maternal fever	2	0	0.148
Hypertension	1	4	0.155
Polyhydramnios	0	0	[a]
Diabetic mothers	0	0	[a]

PROM, premature rupture of membrane. [a]The test was not conducted.

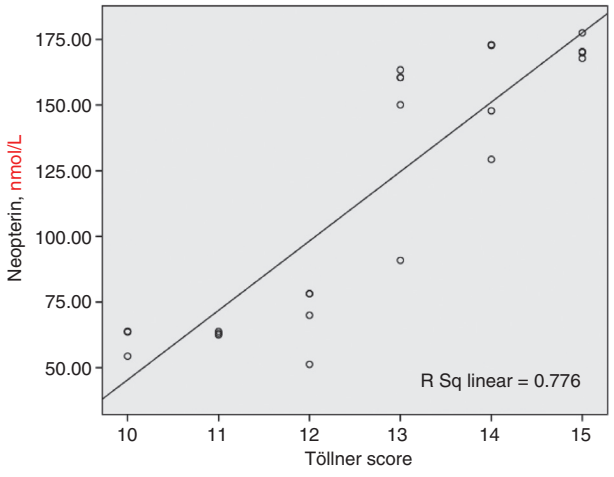

Figure 1 Correlation between neopterin levels and Töllner sepsis score.

maternal fever before labor. However, only maternal meconeal amniotic fluid was significantly different ($p<0.05$) in the non-sepsis group. Utomo [12] also reported that sepsis mainly occurs in low-birth-weight male infants with meconeal amnitotic fluid history. Shah et al. [13] reported that sepsis was more common in infants with low birth weight, a history of birth asphyxia, and maternal meconeal amniotic fluid. A wider scale research is needed to analyze the characteristics of neonatal sepsis in Indonesia.

In sepsis, bacteremia begins with the colonization of bacteria that most commonly occurs in the airway mucosa, intestinal tract, and urogenital tract. After the colonization, the bacteria then penetrate the epithelial cells (transcellular, paracelullar, or intracellular). This condition then leads to the activation of granulocytes or mononuclear cells to detect the bacteria by the phagocytosis process. This process is then followed by the introduction of pathogens through pathogen-associated molecular patterns (PAMPs) that will be recognized by toll-like receptors (TLRs) [14].

The introduction of pathogens will further activate the immune process. The immune system will initiate the inflammatory response by secreting cytokines and chemokines that will induce molecules to attract other immune cells to the site of infection and trigger an adaptive immune response. T lymphocytes will recognize the antigen, inducing them to secrete lymphokines [14, 15].

IFN-γ is the central stimulus for the activation of GTP-cyclohydrolase. Once GTP-cyclohydrolase I is activated, fibroblasts or the endothelial cells will then produce tetrahydrobiopterin. However, because of the relative deficiency of 6-pyruvoyl-tetrahydropterin synthase in humans and primates, the activation of GTP-cyclohydrolase leads to the accumulation of 7,8-dihydroneopterin triphosphate, which is converted by phosphatases to neopterin and 7,8-dihydroneopterin [16].

Our study subjects had somewhat higher levels of neopterin than subjects in previous studies. Boseila et al. [9] reported a cut-off point of 32 nmol/L with a mean level of 66.5±24 nmol/L in the sepsis group and 12.8±9.7 nmol/L in the non-sepsis group. Another study conducted by Radunovic et al. [17] showed that the neopterin level in PI with no sepsis was 7.2±2.1 nmol/L for infants at 30–35 weeks of gestation and was 9.2±2.2 nmol/L for infants at 35–37 weeks of gestation. This result may have been caused by the different patterns of environmental condition, hygiene, and microorganisms involved in neonatal sepsis between developed and developing countries.

Extremely high concentrations of neopterin in the serum and urine were observed during acute viral infection compared to during acute bacterial infection. Neopterin alone, or even better in combination with CRP, is a very useful marker for supporting the differential diagnosis of viral vs. bacterial infections [18, 19]. Our study did not determine viruses as the etiology of sepsis.

About 78.2% of infants with sepsis in our study had maternal risk factors such as a history of fever and of meconeal amniotic fluid. This condition may have heightened the neopterin levels since the intrauterine period. Ip et al. [20] showed that meconeal amniotic fluid or premature rupture of membranes could lead to elevated levels of neopterin. High neopterin levels are also probably due to the differences in genetic factors between developed and developing countries. The response of interferon gene expression is influenced by several genes, e.g., the interferon-β1 *(IFN-β1)* gene, the 2′-5′-oligoadenylate synthetase *(OAS1)* gene, and the 2′-5′-oligoadenylate synthetase *(OAS2)* gene. Carthagena et al. [21] found that the expression of genes influences interferon response and that the expression of these genes may differ between individuals. High levels of neopterin can also be explained by the hygiene hypothesis. It is probable that the presence of infection by bacteria or viruses in a sterile condition such as in the intrauterine period will activate the immune system, which could increase the levels of regulatory T cells, resulting in higher neopterin levels [22].

Limitations of this study include the following: viral culture was not conducted; viral infection is probably one factor that influences the high levels of neopterin. This study did not carry out either any long-term monitoring of infants with sepsis to assess the prognosis of this infection. Another limitation is that we did not determine the cut-off point of neopterin levels owing to the small sample size, nor did we compare the possible superiority of neopterin

to CRP or procalcitonin as a marker for infection. However, this study could be used as an initial or baseline research for further, larger research on neopterin levels in PI, especially in developing countries like Indonesia.

In conclusion, neopterin levels are higher in PI with sepsis than in those without. Neopterin levels positively correlated with the signs and symptoms of neonatal sepsis, as indicated by TSS. The higher levels of neopterin in the sepsis group with intrauterine risk factors could probably activate cellular immunity even during the intrauterine period.

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