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Original Article

A comparison of neutrophil gelatinase-associated lipocalin and immature to total neutrophil ratio for diagnosing early-onset neonatal sepsis

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Abstract

Background Neonatal sepsis is a clinical syndrome caused by the invasion of microorganisms into the bloodstream. Early diagnosis of early-onset neonatal sepsis (EONS) is difficult. Laboratory tests with high sensitivity and specificity are needed in order to make early diagnoses in newborns.

Objective To compare the sensitivity and specificity of neutrophil gelatinase-associated lipocalin (NGAL) and immature to total (IT) neutrophil ratio for the diagnosis of early-onset neonatal sepsis.

Methods This observational study with cross-sectional design was conducted in the Neonatology Division, Prof. R. D. Kandou General Hospital from November 2012 to April 2014. Consecutive sampling was applied. There were 103 newborns with suspected EONS who fulfilled the inclusion criteria. Complete blood counts, blood cultures, as well as NGAL and IT ratio measurements were performed.

Results NGAL was not significantly more sensitive than IT ratio [80.4% vs. 67.3%, respectively; (P=0.058)]. However, NGAL had lower specificity than IT ratio (27.7% vs. 50.0%, respectively; P=0.016). The positive predictive values (57.0% *vs.* 64.9%, respectively; P=0.176), and negative predictive values (54.2% *vs.* 52.6%, respectively; P=0.451) were similar in both diagnostic tests.

Conclusion Immature to total neutrophil (IT) ratio has higher specificity compared to NGAL for early diagnosis of EONS. However, the difference in sensitivity between the two test is not statistically significant. **[Paediatr Indones. 2016;56:107-10.]**

Keywords: *EONS, NGAL, IT ratio, newborn*

Neonatal sepsis is a clinical syndrome characterized by systemic symptoms and bacteremia, occurring in the first month of life. Infection in this early life is associated with a high mortality rate of 13 to 15%.[1] The incidence of neonatal sepsis in developing countries remains higher (1.8-18/1000 live births) than in developed countries (4-5/1000 live births).[2]

Development of medical technology, the use of new potent antibiotic medication, and advances in neonatal care have decreased mortality due to neonatal sepsis, but a high incidence of sepsis persists.[3,4] The diagnosis of neonatal sepsis is difficult because of non-specific signs and symptoms in newborns. In addition, blood cultures take several days to yield results. As such, laboratory examinations with high sensitivity and specificity are needed to confirm a diagnosis of sepsis in neonates prior to confirmation from blood cultures several days later. Early diagnosis and early treatment significantly decrease morbidity and mortality rates.[5]

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Neutrophil gelatinase-associated lipocalin (NGAL) of the specific/secondary granules is released abruptly upon neutrophil stimulation and reaches a plateau within 30 minutes.[6-8] Morbidity and mortality due to neonatal sepsis is high because of high incidence, difficulty in identifying causative microorganisms, non-specific clinical signs, and a lack of predictors for early diagnosis. To our knowledge, there have been no published studies that compared the use of NGAL to IT ratio for the diagnosis of neonatal sepsis. This study was conducted to compare the sensitivity and specificity of NGAL to IT ratio in full term neonates with early-onset neonatal sepsis.

Methods

We conducted a cross-sectional, observational analytic study at the Neonatalogy Division of Prof. R. D. Kandou General Hospital from November 2012 until April 2014. Study subjects were all neonates who fulfilled the inclusion criteria. We used a consecutive sampling method. Study subjects underwent clinical and laboratory exams (hematological profile, IT ratio, blood culture, and NGAL).

Inclusion criteria were full term newborns, born spontaneously by vaginal or caesarian section delivery, who were suspected to have neonatal sepsis, and whose parents provided written informed consent. Exclusion criteria were congenital anomalies, multiple births, or asphyxia. Suspected sepsis was defined as the presence of septic risk factors (1 major risk factor with 2 or more minor risk factors/ 2 or more major risk factors). Major risk factors were premature rupture of the membranes >18 hours, maternal intrapartum fever (>38°C), chorioamnionitis, persistent fetal tachycardia (>160 bpm), and foul-smelling amniotic fluid. Minor risk factors were premature rupture of the membranes >12 hours, maternal intrapartum body temperature of >37.5°C, low Apgar score (minute 1 < 5, minute 5 < 7), very low birth weight (<1,500 grams), and untreated maternal urinary tract infection.[9,10]

Neonatal sepsis is suspected sepsis with 4 or more clinical symptoms and the presence of 2 or more abnormalities in the hematological profile (with either positive or negative blood culture results). Clinical symptoms suggesting neonatal sepsis include tachypnea, apneic spells, severe apnea, bradypnea, less than 85% oxygen saturation with a pulse oxymeter, bradycardia, pallor, decreased perfusion, hypothermia, hyperthermia, lethargy, hypotonia, decreased activity, seizures, vomiting, diarrhea, abdominal distention, ileus, decreased food intake, feeding intolerance, anemia, jaundice, as well as petechiae or purpura. Abnormal hematological profile was defined as leukocytosis (>25,000/mm^3), leukopenia (<5,000/mm^3), or thrombocytopenia <150,000/mm^3). The data obtained were analyzed by Chi-square and Z-tests using *SPSS software version 21*. This study was approved by the Ethics Committee of the University of Sam Ratulangi Medical School, Manado.

Results

In this study, 103 neonates were suspected to have EONS, 56 infants of whom were subsequently diagnosed with EONS. The 56 infants with EONS consisted of 39 males and 17 females.

The results of NGAL as a diagnostic test for neonatal sepsis, with a cut-off point of ≥155 ug/L, showed sensitivity of 80.4%, specificity 27.7%, positive predictive value 57.0%, and negative predictive value 54.2% (OR=1.6). Chi-square test revealed that X^2 = 0.919 with P <0.169 (**Table 1**). The results of IT ratio as a diagnostic test, with cut-off point of 0.2, had sensitivity of 67.3%, specificity 50.0%, positive predictive value 64.9%, and negative predictive value 52.6% (OR=2.1). Chi-square test revealed that X^2 = 2.879 with P=0.045 (**Table 1**).

Table 1. Comparison of the diagnostic value of NGAL and IT ratio for the diagnosis of EONS

Variables	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	OR	X^2 test
NGAL	80.4	27.7	57.0	54.2	1.6	P=0.169
IT ratio	67.3	50.0	64.9	52.6	2.1	P=0.045
Z-test	P=0.058	P=0.016	P=0.176	P=0.451		

PPV=positive predictive value, NPV=negative predictive value

A comparison of NGAL and IT ratio by Z-test revealed that sensitivity and specificity had P values of P=0.058 and P=0.016, respectively, while positive predictive and negative predictive values were P=0.176 and P=0.451, respectively (**Table 1**).

Discussion

Sepsis is the leading cause of neonatal mortality. The diagnosis is easy to miss because of non-specific signs and symptoms accompanying sepsis. However, if sepsis is left untreated it may cause abrupt clinical deterioration, or even death. Moreover, the use of broad-spectrum antibiotics poses the potential problem of bacterial resistance to multiple antibiotics.[11] Based on the child's age at the time of disease onset, neonatal sepsis can be classified into two forms: early-onset neonatal sepsis (EONS) which occurs within the first 72 hours of life and late-onset neonatal sepsis (LONS) which occurs after the first 72 hours of life.[12] The clinical features of neonatal sepsis vary greatly. Therefore, diagnostic criteria should include routine laboratory investigations and other special examinations. The clinical features of neonatal sepsis, in the form of signs and symptoms, are not specific. Early diagnosis of neonatal sepsis is important in the management and prognosis of patients. Classic symptoms of sepsis that usually occur in older children are rarely seen in neonates, but the impact of delayed diagnosis can be fatal.[12-14]

A previous study in Manado found that neonatal sepsis was more common in male (60.5%) than in female infants (39.5%).[15] Likewise, a Denpasar study reported male predominance in neonatal sepsis (56.8% male *vs.* 43.2% female).[16] We, too, observed a male predominance in the incidence of neonatal sepsis. This male predominance may be due to sex-linked factors in host susceptibility to infection. The X chromosome may have genes affecting thymus gland function and immunoglobulin synthesis.[17] As males have only one X chromosome, male neonates may be more susceptible to infection than female neonates.[5,18]

The IT ratio is the ratio of immature neutrophils to total neutrophils (mature and immature neutrophils) calculated from peripheral blood smear preparations. An IT ratio equal to or greater than 0.2 is considered to be abnormal. In neonates, neutrophils respond to bacterial infections 3-4 hours after exposure.[19] The peripheral blood smear examination to calculate IT ratio is a simple and inexpensive examination, but it requires competent personnel and may be somewhat subjective.[20] In our study, IT ratio with a cut-off point of ≥0.2 yielded a sensitivity of 67.3%, specificity of 50.0%, positive predictive value 64.9%, and negative predictive value of 52.6% (OR=2.1). Chi-square test revealed a significant association between IT ratio and a diagnosis of sepsis (P=0.045). In contrast, Makkar *et al*. showed that IT ratio > 0.2 had a sensitivity of 93.75% and a specificity of 94.44% in neonates with EONS.[21] This difference was probably due to the differing inclusion criteria of the studies. The previous study defined EONS as a perinatal infection that occurs in the immediate postnatal period within ≤7 days of the postnatal period.[21] The inclusion criteria in our study were newborns within 3 days of life and blood sampling was done shortly after birth.

In our study, NGAL as a diagnostic test with a cut-off point ≥ 155 ug/L had a sensitivity of 80.4%, specificity 27.7%, positive predictive value 57.0%, and negative predictive value 54.2% (OR=1.6). Chi-square test showed that there was no significant association between NGAL examination and a diagnosis of sepsis (P <0.169). In contrast, a previous study reported sensitivity of 96% and specificity 56% for serum NGAL cut-off levels > 155 ug/L for children with a median age of 26 months (range 1 mo-13 years). Their subjects, hospitalized with symptoms and signs of acute infections, were categorized into five groups: bacterial infection, suspected bacterial infection, viral infection, suspected viral infection, and other.[22] Again, the different results in the two studies are likely due to different inclusion criteria. Our study inclusion criteria were full-term neonates with suspected neonatal sepsis. In neonates with bacterial infection, NGAL levels reach peak levels within <24 hours,[18] and begin to decline on the second and third days.[22] NGAL levels do not increase in healthy neonates unless an infection occurs.[23] In this study, we found increased NGAL levels in neonates with EONS. This observation is consistent with a report that NGAL level increased rapidly, within 30 minutes after infection.[22] The expression of NGAL in trophoblast cells was significantly increased in pregnant women with intrauterine infection.[24]

This study is the first of its kind to be conducted in Manado and in Indonesia to compare the utility of

NGAL and IT ratio tests in full term neonates with suspected EONS. The cross-sectional study design has its limitations. Therefore, further studies with serial examinations are needed to validate the results in helping diagnose neonatal sepsis.

In conclusion, IT ratio has a higher specificity than NGAL for the diagnosis of early-onset neonatal sepsis. The sensitivity of NGAL is higher than that of IT ratio for diagnosing EONS, but the difference is not statistically significant.

Conflict of interest

None declared.

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