

DIAGNOSTIC VALUES OF IMMATURE GRANULOCYTES, EOSINOPENIA AND I/T RATIO IN DETECTION OF EARLY ONSET NEONATAL SEPSIS IN NEONATES WITH BACTERIAL INFECTION RISK

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ABSTRAK

Early Onset Neonatal Sepsis (EONS) adalah penyebab signifikan morbiditas dan mortalitas pada neonatus. Masih sangat sulit untuk dapat menetapkan diagnosis EONS dan standar emas pembentukan sepsis secara mikrobiologi. Sehingga, dengan menggunakan parameter hematologi seperti granulosit immatur, eosinopenia, dan rasio I/T dapat digunakan sebagai penanda diagnosa sepsis secara lebih efisien, cepat, dan murah. Tujuan dari penelitian ini adalah untuk mengevaluasi apakah granulosit immatur, eosinopenia, dan rasio I/T dapat digunakan sebagai deteksi EONS dengan infeksi bakteri. Sampel darah diambil dari vena 35 neonatus berusia 0-6 yang dirawat di Neonatal Intensive Care Unit (NICU) Rumah Sakit Dr Soetomo jam dengan risiko tinggi infeksi bakteri dan tidak menerima pengobatan antibiotik. Penelitian ini menggunakan pendekatan cross sectional. Granulosit immatur, eosinopenia, dan rasio I/T dianalisis secara terpisah menggunakan alat analisis otomatis. Seluruh darah diambil dari plasenta pasien sebagai kultur. Kemudian, nilai granulosit immatur, eosinopenia, dan rasio I/T dibandingkan dengan hasil kultur darah. Dari 35 sampel neonatus, granulosit immatur menunjukkan sensitivitas 38,1%, spesifisitas 64,3%, nilai prediktif positif (PPV) 61,5%, dan nilai-nilai prediktif negatif (NPV) 40,9 %. Eosinopenia menunjukkan sensitivitas 28,6%, spesifisitas 100%, PPV 100%, dan NPV 48,3%. Rasio I/T menunjukkan sensitivitas 23,8%, spesifisitas 85,7%, PPV 71,4%, dan NPV 42,7%. Kesimpulan yang diperoleh dari penelitian ini adalah IG menunjukkan nilai yang paling sensitif dan eosinopenia menunjukkan nilai spesifisitas yang paling tinggi untuk mendeteksi EONS.

Kata kunci: granulosit immatur, eosinopenia, Rasio I/T, early onset neonatal sepsis

ABSTRACT

Early Onset Neonatal Sepsis (EONS) is a significant cause of morbidity and mortality in neonates. It is difficult to establish the diagnosis of early onset neonatal sepsis and the gold standard of establishing sepsis is by microbiology method. Thus, using hematology parameters like immature granulocytes, eosinopenia, and I/T ratio can be used as markers to diagnose sepsis more efficient, faster and inexpensive. The aim of this study is to evaluate if immature granulocytes, eosinopenia, and I/T ratio could be used as markers to diagnose EONS in neonates with bacterial infection. Blood samples were taken from 35 neonates patients' veins. The patients admitted to the Neonatal Intensive Care Unit (NICU) of the Dr Soetomo Hospital were 0-6 hours old with a high risk of bacterial infection and received no antibiotic treatment, this study was cross sectional. Immature granulocytes, eosinopenia and I/T ratio were analyzed separately using automatic analyzer instrument. Whole blood was taken from patients' umbilical cord for culture. Then, value of immature granulocytes, eosinopenia and I/T ratio were compared to the blood culture results. From 35 neonatal samples, IG showed a sensitivity of 38.1 %, specificity 64.3 %, positif predictive values (PPV) 61.5%, and negative predictive values (NPV) 40.9 %. Eosinopenia showed a sensitivity of 28.6%, specificity 100%, PPV 100% and NPV 48.3%. I/T ratio showed a sensitivity of 23.8%, specificity 85.7%, PPV 71.4% and NPV 42.7%. IG showed the most sensitivity value and eosinopenia showed the most specificity value for detection of EONS.

Keywords: immature granulocytes, eosinopenia, I/T ratio, early onset neonatal sepsis

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INTRODUCTION

Neonatal sepsis is a major cause of morbidity and mortality, especially in the first week of life. According to its occurrence, neonatal sepsis can be classified into two subtypes, thus are early onset sepsis (EOS) and late onset sepsis (LOS). Early-onset sepsis is a perinatal infection that occurs soon after birth (less than 72 hours

of life) and mostly caused by bacteria from the mother's vagina. EOS has a frequency of 3.5 cases per 1,000 live births, with a mortality rate of 15-50 % (Paolucci et al 2012).

Examination of the blood culture is the gold standard for sepsis diagnosis, but this examination has several limitations, such as require long time, the cost is

relatively expensive, and low positive rate (Saleh et al 2008). While the condition of the patient requires prompt and appropriate treatment, it is necessary for another diagnostic tool that not spent much time but accurate.

Rapid diagnostic test, which can distinguish infected infants from uninfected infants will have a significant impact on newborn infants care. still there is no single diagnostic test that can detect bacterial infections in newborn infants. Identification of newborns at risk for infection, especially EOS is generally only based on a combination of anamnesis, clinical features, and additional observation (Polin 2012).

Several studies have analyzed the subcomponents of the White Blood Cells (WBC) such as, immature granulocytes (IG), the ratio of immature neutrophils compared to total neutrophil (I/T ratio), and eosinopenia to identify infants with infection. However, there are limited studies on the diagnostic value in detecting EOS using WBC subcomponents. The purpose of this study is to provide information about the diagnostic values of IG, eosinopenia, and the ratio of I/T in EOS detection due to bacterial infection.

MATERIAL AND METHODS

This was an observational study with a cross-sectional study. Done in Neonatal Intensive Care Unit Emergency Room (NICU-IRD) Department of Pediatrics Dr. Soetomo hospital Surabaya, from February to April 2013. Newborns age 0-6 hours and have not received antibiotic therapy enrolled this study. Babies born with congenital anomalies and infants born outside the IRD Dr. Soetomo hospital Surabaya were excluded.

Blood samples for culture of blood taken from umbilical cord blood and stored in transport medium produced by BACTEC. Blood samples for complete blood examination taken from a vein and saved using EDTA anticoagulant and examined using hematoanalyzer in Clinical Pathology Laboratory Installation Dr. Soetomo hospital. IG, ratio of I/T, and eosinopenia examination calculated by Cell-Dyn Ruby (For Laboratory Use Only software version 2.0ML). IG consisted of promyelocyte, myelocyte, and metamyelocyte, and its cut-off value was 1% (Ansari-Lari et al 2003). I/T ratio was the ratio between immature neutrophil granulocytes against neutrofi total. Immature neutrophil granulocytes consisted of neutrophils rods, metamyelocyte, myelocyte and promyelocyte. I/T ratio >0.2 indicates the presence of suspicious abnormalities and infections. Eosinopenia was the total number of eosinophils and

based on previous research, eosinopenia's cut-off in this study is less than or equal to 50 cells/uL (Abidi et al 2008). Diagnostic value of immature granulocytes, ratio of I/T, and eosinopenia calculated from 2x2 tables. For additional analysis, researchers saw the area under the curve from ROC curve (Receiver Operating Characteristics) to find the best value of the cut-off immature granulocytes, ratio of I/T, and eosinopenia in this study. Statistical calculations performed with SPSS 17.0 version.

RESULTS

During research (February-April 2013), researchers obtained 35 samples.

Table 1. Patients Characteristic

Patients Characteristic	Amount
Total sample	35 (100%)
Male	16 (45.7%)
Female	19 (54.3%)
Mean weight (gram)	2737±695
Maternal risk factor	
Early fetal membrane rupture	9
Meconial	22
Labor sepsis	0
Fetal risk factor	
Premature newborn	12
Low baby birth weight	10
Labor	
Spontaneous	28
Cesarean dissection	5
Vacuum extraction	2
Blood culture result	
Steril	14
Gram positive bacteria	15
Gram negative bacteria	6
Rasio IT >0,2	7
0,2	28
Eosinophils <50/pul	6
50/pul	29
IG > 1%	13
1%	22

From 35 samples, 16 samples (45,7%) were male, and 19 samples (54,3%) were female. Common maternal risk factor on this research was meconial (22 samples), whereas common fetal risk factor was premature newborn (12 samples). Total positive culture were 21 samples (60%) and 14 samples (40%) were steril. Most bacteria which may caused sepsis was gram positive bacteria; 15 samples (table 1). The common bacteria that triggered to sepsis in this research was Staphylococcus haemolyticus (33.3 %) and Escherichia coli (19.1%) (table 2).

Table 2. Profile of Bacteria that triggered to EOS

Bacteria	Amount (%)
<i>Staphylococcus haemolyticus</i>	7 (33.3)
<i>Escherichia coli</i>	4 (19.1)
<i>Staphylococcus hominis</i>	2 (9.5)
<i>Klebsiella pneumonia</i>	1 (4.8)
<i>Staphylococcus xylosus</i>	1 (4.8)
<i>Micrococcus lylae</i>	1 (4.8)
<i>Enterobacter aerogenes</i>	1 (4.8)
<i>Staphylococcus warneri</i>	1 (4.8)
<i>Staphylococcus saprophyticus</i>	1 (4.8)
<i>Staphylococcus uberis</i>	1 (4.8)
<i>Streptococcus bovis</i>	1 (4.8)
<i>Staphylococcus aureus</i>	1 (4.8)

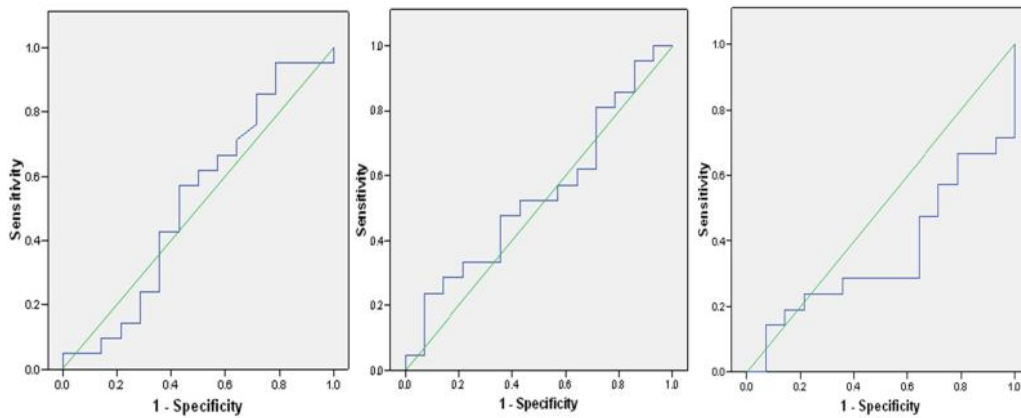


Figure 1. ROC curve, a. IG, b. Eosinopenia, c. I/T Ratio on EOS patients

Sensitivity and specificity value based on ROC curve and cut off score of previous research (IG>1%, Eosinopenia<50/ μ L dan I/T ratio>0) (picture 1). Diagnostic value of UG, I/T ratio, and eosinopenia ond EOS patients with cut off score from previous research or with modified (increased or decreased) cut off score from previous reasearch, showed on tables below (table 3, 4, and 5)

Table 3. Diagnostic value of IG on EOS patients

	Literature (1%)	AUC (2%)	AUC (3%)
Sensitivity (%)	38.1	5	5
Specificity (%)	64.2	93	100
PPV (%)	61.5	50	100
NPV (%)	40.9	56.5	41

Table 4. Diagnostic value of I/T ratio on EOS patients

	Literature (>0.2)	AUC (>0.1)	AUC (>0.07)
Sensitivity (%)	23.8	52.4	61.9
Specificity (%)	85.7	50.0	35.7
PPV (%)	76.2	61.1	59.1
NPV (%)	42.9	41.2	38.5

Table 5. Diagnostic value of Eosinopenia on EOS patients

	Literature (<0.050)	AUC (<0.115)	AUC (<0.129)
Sensitivity (%)	28.5	47.6	52.4
Specificity (%)	100	71.4	64.3
PPV (%)	100	71.4	68.8
NPV (%)	48.3	47.6	47.4

DISCUSSION

Neonatal sepsis is a clinical syndrome bacteremia with systemic signs and symptoms of infection accompanied by evidence of the bacteria growth in blood cultures, urine or cerebrospinal fluid, in the first four weeks of life (Hornik et al 2012, Paolucci et al 2012). According to The International Pediatric Sepsis Consensus Conference in 2005, sepsis is a clinical syndrome caused by presence of systemic inflammatory response syndrome (SIRS) with the presence of infection (either suspected or proven infection). According to the time of occurrence, neonatal sepsis can be classified into two subtypes, early onset sepsis (EOS) and late onset sepsis (LOS). Less blood volume (<0.5 ml) for examination of

blood cultures is a frequent problem encountered in newborns. Recent research has shown that the blood volume of 0.5 ml were unable to detect low levels of bacteremia (< 4 colony forming units (CFU)/ml). Culture of blood taken from the umbilical artery and vein is an alternative way of taking culture apart from a peripheral vein. Besides can get a lot of blood, it is also not invasive and traumatic.

This study found the most common bacteria culture that caused sepsis was gram positive bacteria. In developed countries, the most common bacteria found were Group B Streptococcus (GBS) ($>40\%$ of cases), *Escherichia coli*, *Haemophilus influenzae*, and *Listeria monocytogenes*, while in developing countries such as Indonesia, the most common bacteria was gram positive bacteria. Morbidity and mortality in gram positive bacteria was lower than gram negative bacteria because gram positive bacteria released peptidoglycan cell wall fragments that stimulate the immune system and works as an exotoxin (super innerantigen) that enrolled the inflammatory process.

There are 2 types of neutrophils, immature neutrophils (1 lobus) and mature neutrophils (2-5 lobule). IG consisted of promyelocyte, myelocyte, and metamyelocyte cells. Normal IG located in the bone marrow and is rarely found in the peripheral blood cells. Studies at Johns Hopkins Hospital emergency room (Ansari-Lari et al 2003) using ROC analysis showed that the IG gives better performance than the WBC and ANC in predicting infection; IG has a low sensitivity (33%) and good specificity (88%).

I/T ratio is the ratio between immature neutrophil granulocytes to total neutrophils. Neutrophils are cells that served as one of the body's defense mechanism. The number of neutrophils varies in the first week of life. The lowest limit of the total number of normal neutrophils in newborns is 1800/mm³, then increased to 7200/mm³ at 12 hours of life. The infection will cause the release of neutrophils into the blood stream, thereby increasing the number of white blood cells. Immature neutrophils commonly found in infections and sepsis (Hiew et al 1992, Frey et al 2000). Eosinopenia expressed in acute inflammation. Bass (1975) namely the disappearance or decrease in the number of circulating eosinophils is due to tissues localized by intravascular diffuse margination or eosinophils destruction; suppression the production of eosinophils. The result of eosinophils diagnostic value in patients with EOS in this study with the eosinophils cut-off number was $\pm 50/uL$ were as follows: sensitivity 28.5%, specificity 100%, PPV 100% and NPV of 48.3%. According to Handojo (1988), the sensitivity of the results in this study (28.5%) were categorized as very

low values, whereas the specificity (100%) is very high. The results of this study do not support previous research conducted by Abidi et al (2008) and Gil et al (2003) because the population and sample were different.

CONCLUSION

Of the three parameters in the detection of EOS, the highest sensitivity is IG, then I/T ratio and eosinopenia. The highest specificity was eosinopenia, then I/T ratio and IG.

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