EOSINOPENIA AND NEUTROPHIL LYMPHOCYTE RATIO ON EARLY NEONATAL SEPSIS

DIAGNOSTIC VALUE OF EOSINOPENIA AND NEUTROPHIL TO LYMPHOCYTE RATIO ON EARLY ONSET NEONATAL SEPSIS

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Abstract

Purpose. To determine the diagnostic value of eosinopenia and neutrophil to lymphocyte ratio (NLR) for diagnosing Early Onset Neonatal Sepsis (EONS).

Methods. This is a cross sectional study that conducted in Neonatology Subdivision of Child Health Department of R.D. Kandou General Hospital Manado from July to October 2017. Samples were obtained from all neonates that were met the inclusion criteria as having EONS. Datas were encoded using logistic regression analysis, biserial point correlation, chi square, and ROC curve with p value <0.05 considered as significant.

Results. Of 120 neonates who met the inclusion criteria, 73 (60.8%) were males and 47 (39.2%) were females. Ninety (75%) subjects were included in sepsis group and 30 (25%) were in non sepsis group. Mean eosinophil count in EONS and non-EONS group were 169.8 ± 197.1 cells/mm³ and 405.7 ± 288.9 cells/mm³, respectively, considered as significant (p<0.001). Result from diagnostic test of eosinopenia from the EONS group (cut-off point 140 cells/mm³) obtained 60.0% sensitivity and 90.0% specificity. Mean NLR in EONS and non-EONS group were 2.82 ± 2.29 and 0.82 ± 0.32, respectively, also considered as significant (p<0.001). Result for diagnostic test of NLR in EONS group (cut-off point 1.24) obtained 83.3% sensitivity and 93.3% specificity.

Conclusion. Eosinopenia has a high specificity value as a diagnostic marker of EONS and an increased in NLR has a high sensitivity and specificity value as a marker to diagnose EONS.

Keywords: Eosinophil count, Neutrophil to lymphocyte ratio, Early onset neonatal sepsis
Introduction

Neonatal sepsis is a clinical syndrome arising from the invasion of microorganisms into the bloodstream that arises in the first month of life.\(^1\) Neonatal sepsis is still a major problem in neonatal care and still contributes significantly to disability and death. At least 1 million deaths occurring in the newborn period (0-28 days) per year are caused by infection, of which nearly 25\% is the cause of newborn mortality and accounting for 10\% of infant mortality worldwide.\(^2,3\) Based on the time of occurrence, neonatal sepsis is divided into two types: Early Onset Neonatal Sepsis (EONS) that occurred within the first 72 hours of life and Late Onset Neonatal Sepsis (LONS) that occurred after 72 hours of life.\(^1\)

According to Indonesian Demographic Health Survey data in 2012, there were 32 deaths per 1000 live birth.\(^4\) In R.D. Kandou General Hospital Manado, during the period of January-December 2013, the incidence of infant mortality due to sepsis were as many as 127 cases (30.1\%) of total 421 babies died.\(^5\)

The accurate and timely diagnosis of neonatal sepsis remains a challenging issue due to its nonspecific clinical presentation. The diagnosis criteria of EONS that we used in this study was based on at least two clinical (hyper- or hypothermia, apnea or bradycardia spells, increased oxygen requirement, feeding intolerance, abdominal distension, lethargy and hypotonia, hypotension, skin and subcutaneous lesions such as petechial rash, abscess, sclerema) and two laboratory criteria (WBC < 5,000, WBC > 20,000, ITR > 0.2, platelet count < 100,000, CRP > 10 mg/L).\(^6\) Evidence of infection from blood cultures itself often show insignificant results and takes too much time to obtain.\(^7\) Efforts have been made to diagnose EONS using an easy and cheap tools such as eosinophil count and neutrophil to lymphocyte ratio (NLR).\(^8,9\)

From the previous studies, eosinopenia has a good sensitivity and specificity in diagnosing sepsis on both EONS and LONS, but they were using adult’s sepsis cut off point.\(^8,9\) In this study, we try to use point of intersection from neonatal subjects. The diagnostic value of NLR in EONS alone has never been done in neonates. This study were purposed to provide information about diagnostic value of eosinophil count and NLR for detecting EONS.
Materials and methods

A cross-sectional study was conducted in Neonatology Ward of R.D. Kandou General Hospital Manado from July to October 2017. Study subjects were all neonates who met the inclusion criteria. We used consecutive sampling method with a sample size of 120 neonates. Study subjects then performed anamnesis, physical examination, and also laboratory examination (hematology profile, differential count, eosinophil count, and blood culture).

Inclusion criteria were include all neonates with EONS suspects, born either vaginally or sectio caesaria and obtained parental consent to join this study by signing the informed consent form. Study was conducted under the approval of the ethics committee of R.D. Kandou General Hospital Manado with the number 100/EC-KEPK/VII/2017. Post operative neonates due to congenital abnormalities were excluded from the study.

Neonates were suspected sepsis if they have fulfilling two major criteria or one major criteria plus two or more minor criterias. Septic risk factors include major risk factors of premature rupture of membranes (PROM) >18 hours, intrapartum fever (>38°C), chorioamnionitis, greenish and foul smelling amniotic fluid, fetal heart rate >160x/minute. The minor risk factor consisted of premature rupture of membranes >12 hours, intrapartum fever (> 37,5ºC), low apgar score (min 1 score <5 and min 5 score <7), very low birth weight (<1500 grams), vaginal discharge untreated, and the mother is suspected of having a urinary tract infection.10-12

Neonates were suspected of having EONS if there were 4 or more clinical symptoms and 2 or more hematologic profile abnormalities (with or without positive blood cultures). Clinical symptoms of neonates suspected sepsis such as lethargy, decreased of suction reflex, wheezing, irritable, seizures, bradycardia, apnea, tachypnea, oxygen saturation measured with pulse oxymeter <85%, pale, decreased perfusion, hypothermia, hyperthermia, hypotonia, vomiting, diarrhea, ileus, bloating, feeding intolerance, elongated gastric emptying times, anemia, jaundice, petechiae and purpura. Hematologic profile abnormalities such as IT ratio ≥ 0.2, leukopenia (<5,000 cells/mm³), leukocytosis (>25,000 cells/mm³) or thrombocytopenia (<100,000 cells/mm³).13

Christensen et al13 in a retrospective studies that were conducted in the United States from January 1st 2002 to May 31st 2009 with a sample size of 63,000 and the study sample was a healthy neonate born at 22 to 42 weeks of gestation obtained the normal range of eosinophils count was 140 – 1300 cells/mm³ with average value of 550 cells/mm³.
The data were processed with statistical product and services solutions (SPSS) version 23. Descriptive analysis were used to illustrate the characteristic of the data, ROC analysis to assess the cut off point of eosinophils count and neutrophil to lymphocyte ratio, Chi square to test the diagnostic value of eosinophils count and neutrophil to lymphocyte ratio, by calculating the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).
Results
There were 120 neonates suspected with sepsis that fulfill the inclusion criteria (Figure 1). Table 1 showed the characteristic of our samples. Incidence of EONS were mostly found in males, 53 (59%), compared with females, 37 (41%). Blood culture were made and resulting in 18 positive growth, consist of *Klebsiella pneumonia* (9), *Enterobacter aerogenes* (3), *Escherichia coli* (1), *Staphylococcus hominis* (2), *Staphylococcus saprophyticus* (1), and *Staphylococcus aureus* (2).

Mean eosinophil count from the EONS group was 169.8 ± 197.1 cells/mm³, meanwhile from non-EONS group was 405.7 ± 288.9 cells/mm³ with p<0.001 considered as significant. We also tallied their NLR and found that mean NLR from EONS and non-EONS group was 2.82 ± 2.29 and 0.82 ± 0.32, respectively, with p<0.001 considered as significant also. Figure 2A describes data distribution of eosinophil count in EONS and non-EONS group, while figures 2B describes data distribution of NLR in both groups.

An ROC curve to analyze the ability of eosinophil count to diagnose EONS (Fig.3A) demonstrates that with a cut-off of 140 cells/mm³, there is a 60.0% sensitivity and a 90.0% specificity. A similar analysis with NLR showed that the best specificity and sensitivity for EONS was at NLR 1.245 with sensitivity of 83.3% and specificity 93.3% (Fig.3B).
Discussion

In our study, neonates with sepsis were found more in male than female. In EONS group, there were 90 (75%) neonates, in whom 53 (59%) are males. This result shows that male neonates are more susceptible to sepsis than female neonates. These findings are in accordance with a study in Denpasar which showed that neonatal sepsis incidence is found higher in males (56.8%) than females (43.2%). Kardana also obtained neonatal sepsis incidence higher in males than in females.

In this study, out of 90 neonates with EONS, there are only 18 positive bacterial growth found in blood cultures, 5 of which are gram positive bacteria: *Staphylococcus hominis* (2), *Staphylococcus saprophyticus* (1), *Staphylococcus aureus* (2) and 13 are gram negative bacteria, which are *Klebsiella pneumoniae* (9), *E. coli* (1) and *Enterobacter aerogenes* (3). The most common type of bacteria found was *Klebsiella pneumonia*. Results obtained concerning the cause of EONS in a previous research conducted by Bagus et al in Dr. Soetomo Surabaya Hospital are *E.coli* 19.1%, followed by *Staphylococcus hominis* 9.5%, *Klebsiella pneumonia 4.8*, *Enterobacter aerogenes 4.8*., and *Staphylococcus saprophyticus 4.8*.. The type of bacteria were found different with the previous study because there were different bacterial pattern variation present in their study location.

Traditionally, the definition of sepsis has included isolation of a pathogen from a normally sterile body fluid such as blood or cerebrospinal fluid (CSF). The clinical features of sepsis can be induced by potent pro-inflammatory cytokines, the term systemic inflammatory response syndrome (SIRS) has also been used when describing neonatal sepsis. For blood cultures, a minimum of 0.5-1 ml of blood should be obtained from two different venipunctures from two separate sites. Commonly used non-culture based diagnostic test include total and differential WBC count, absolute and immature neutrophil counts, and the ratio of immature to total neutrophils. WBC count has limitations in terms of sensitivity. ITR > 0.2 suggestive of a bacterial infection and found to be predictive when used in combination with CBC obtained at more than 4 hours of age. The main benefit of WBC count is its negative predictive value since normal serial values make it unlikely that a blood culture will be positive. WBC values are dynamic, so serial measurements over 24 hours might be more informative. CRP, procalcitonin, haptoglobin, fibrinogen, inflammatory cytokines were also diagnostic tests that measure an inflammatory response.
Current evidence shows no one factor can be used to diagnose sepsis, however, promising results have been seen when two or more of these factors are combined. Due to the lack of consistent evidence in this area, no such list has yet been developed. The best methods involved in the investigation of EONS is using the combination of maternal risk factors, clinical sign and symptoms, and various laboratory markers that are available. The gold standard for a definitive diagnosis is a blood culture. However, blood cultures can take as long as 48-72 hours making them an unreliable tool in determining if treatment is needed in critical hours once the disease has begun. They also lack a high positive predictive value with less than 50% of the cases being positive. Blood cultures should taken before antibiotic therapy initiated. A negative result of blood culture doesn’t exclude sepsis diagnosis as about 26% of all neonatal sepsis could be due to anaerobes. Furthermore, the etiological agent may not be isolated by media used in our study such as viral (e.g. rubella, cytomegalovirus), protozoal (e.g. Toxoplasma gondii), and treponemal (e.g. Treponema pallidum) pathogens. Earlier studies said that positive blood culture only found in 30-40% sepsis cases.

One of the important aspects of acute infection is the decline in eosinophil count that spreads through the blood circulation quickly and persistently. Pathophysiology of eosinopenia during infection may be caused by the combination of the increased peripheral eosinophil sequestration due to localization of site infection which can be caused by the increased drainage through lymphatic flow, chemotaxis process in inflammation, increased in peripheral eosinophil sequestration, pressure towards eosinophil releasing process from the bone marrow, and pressure towards eosinophil formation in bone marrow.

During bacterial sepsis, the bacteria’s endotoxin and lipopolysaccharide activate macrophage, neutrophil, and dendritic cells to release proinflammatory cytokines such as Interleukin (IL)-1, IL-6, and TNF-α which will then activate the hypothalamus pituitary adrenal (HPA) axis. Paraventricular nucleus in anterior hypothalamus will release corticotropin releasing hormone (CRH) which stimulates the anterior hypophysis to release adrenocorticotropin hormone (ACTH) into circulation. ACTH will stimulate the synthesis and release of glucocorticoid from the adrenal gland. Glucocorticoid will inhibit eosinophil release from the bone marrow, thus detaining eosinophil adhesion, migration, and chemotaxis process through the inhibition of IL-3, IL-5, granulocyte-
macrophage colony-stimulating factor (GM-CSF), chemokine and integrin, so that the amount of eosinophil will be reduced. This explains why the sepsis group has lesser eosinophil count than the non-sepsis group.\textsuperscript{22,23}

Neutrophil and lymphocyte are important components of the immune system which initially fights off infection.\textsuperscript{24} During sepsis, a strong adhesion between neutrophil and endothelium was made, causing the failure of neutrophil to migrate to the infection site.\textsuperscript{25} Moreover, bacterial products and cytokines released during sepsis also delay neutrophil apoptosis, which contributes to the degree of sepsis severity.\textsuperscript{26} Neutrophil itself has a short life-span, only about 24 hours. So, sepsis patient who has trouble in apoptosis process will have a prolong neutrophil life in the blood. This is allegedly caused by NFkB activation and the decrease of level 3 caspase.\textsuperscript{25,27}

Lymphocytopenia happens due to deployed lymphocyte into inflammation or infection site. In addition, lymphocyte apoptosis is significant in patients with sepsis. Lymphocytopenia as a sign of lymphocyte apoptosis is a part of the host’s normal immune response to stop and control an exaggerated immune response with the aim to stop further tissue damage. Within the first 24 hours of sepsis, lymphocytopenia happens because it was deployed from the blood circulation to the site of infection which then leads to depletion of T cells CD4+ and CD8+ in the blood.\textsuperscript{28,29,30}

In this study, mean eosinophil count was 169.8 ± 197.1 cells/mm\textsuperscript{3} from the EONS group and as for non-EONS group it was 405.7 ± 288.9 cells/mm\textsuperscript{3}. Based on the logistic regression analysis, a very significant correlation was found between eosinophil count and sepsis occurrence with p<0.001, where the lower the eosinophil count is, the higher the chance of sepsis to take place. Furthermore, a very significant correlation was found between eosinophil count with EONS incidence in the point biserial result, with r\textsubscript{pb} = - 0.419 and p <0.01. The ROC analysis showed AUC 83.5% with an eosinophil cut-off point of 140 cells/mm\textsuperscript{3} and sensitivity = 60.0%, specificity = 90.0%, positive predictive value = 94.7%, and negative predictive value = 42.9%. This cut-off point has an OR of 13.5 (CI 95%: 3.8 – 47.8).

This study also looking for NLR corellation with EONS, and we found that mean NLR was 2.82 ± 2.29 from the EONS group and 0.82 ± 0.32 for non-EONS group. Based on the logistic regression analysis, a very significant correlation also found between NLR and sepsis incidence, where the higher the ratio, the higher the incidence of EONS as well with p value <0.0001. Based on the point biserial test, a very significant correlation between NLR and EONS incidence with r\textsubscript{pb} =
0.419 and p <0.0001. Based on the ROC curve with a 1.245 cut-off point, the result shows 83.3% sensitivity, 93.3% specificity, 94.7% positive predictive value, and 65.2% negative predictive value, with an OR=70.0 (CI 95%: 15.0-325.8).

Our results are similar to those obtained by Bagus et al who conducted a study to compare the diagnostic values of immature granulocytes, eosinopenia and IT ratios in detecting EONS in neonates (0-6 hours) with the risk of bacterial infection, whereas eosinopenia showed the highest specificity of detection to detect EONS.\(^{17}\) Study by Yefta et al\(^{31}\) also found that eosinophil percentage has a higher specificity than the sensitivity value in detecting neonatal sepsis.

Previous studies that have been done were used cut-off point from studies that conducted on adults. Study with cut-off point from neonatal samples itself have not been done anywhere yet. This study was performed with a 140 cells/mm\(^3\) as a cut-off point on eosinophil count for diagnosing EONS. NLR itself has never been performed to help diagnose EONS before. In this study, we used a cut-off point of 1.245.

This was the first study looking for any association between eosinophil count and NLR with incidence of EONS using cut-off points, sensitivity, and specificity with the subject of neonates. Limitations of this study is there was no follow-up examination in patients with higher levels of eosinophil counts and low NLR. To determine the risk of mortality in neonates with neonatal sepsis, serial examination of eosinophil and NLR is required.
References


5. R.D. Kandou General Hospital. Profile of Prof. Dr. R.D. Kandou General Hospital Manado 2013.


Tables

Table 1. Characteristics of study subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n=120), number (%)</th>
</tr>
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<tbody>
<tr>
<td><strong>Neonatal Data</strong></td>
<td></td>
</tr>
<tr>
<td>Age, days</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>54 (45)</td>
</tr>
<tr>
<td>1</td>
<td>40 (33.3)</td>
</tr>
<tr>
<td>2</td>
<td>26 (21.7)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
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<tr>
<td>Male</td>
<td>73 (60.8)</td>
</tr>
<tr>
<td>Female</td>
<td>27 (39.2)</td>
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<tr>
<td>Birth Weight, grams (SD)</td>
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<tr>
<td>Minimum</td>
<td>1450</td>
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<tr>
<td>Maximum</td>
<td>4100</td>
</tr>
<tr>
<td><strong>Maternal Data</strong></td>
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<tr>
<td>Gestational age, weeks</td>
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<tr>
<td>28-33 weeks (Preterm)</td>
<td>9 (7.5)</td>
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<tr>
<td>34-36 weeks (Late Preterm)</td>
<td>15 (12.5)</td>
</tr>
<tr>
<td>37-42 weeks (Term)</td>
<td>96 (80)</td>
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<tr>
<td>Sepsis Risk Factors</td>
<td></td>
</tr>
<tr>
<td>Major</td>
<td></td>
</tr>
<tr>
<td>Rupture of Membrane &gt; 18 hours, n (%)</td>
<td>57 (47.5%)</td>
</tr>
<tr>
<td>Maternal fever &gt; 38 °C, n (%)</td>
<td>15 (12.5%)</td>
</tr>
<tr>
<td>Chorioamnionitis, n (%)</td>
<td>8 (6.66%)</td>
</tr>
<tr>
<td>Foul smelling liquor, n (%)</td>
<td>36 (30%)</td>
</tr>
<tr>
<td>Sustained fetal heart rate &gt; 160x/min, n (%)</td>
<td>36 (30%)</td>
</tr>
<tr>
<td>Minor</td>
<td></td>
</tr>
<tr>
<td>Rupture of Membrane &gt; 12 hours, n (%)</td>
<td>13 (10.8%)</td>
</tr>
<tr>
<td>Maternal fever &gt; 37.5 °C, n (%)</td>
<td>5 (4.16%)</td>
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<tr>
<td>Low Apgar &lt; 5 at 1 min, &lt; 7 at 5 min, n (%)</td>
<td>17 (14.1%)</td>
</tr>
<tr>
<td>Very Low Birth Weight, n (%)</td>
<td>3 (2.5%)</td>
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<tr>
<td>Prematurity, n (%)</td>
<td>18 (15%)</td>
</tr>
<tr>
<td>Multiple gestation, n (%)</td>
<td>4 (3.33%)</td>
</tr>
<tr>
<td>Untreated Foul Vaginal Discharge, n (%)</td>
<td>52 (43.3%)</td>
</tr>
<tr>
<td>Untreated Urinary Tract Infection, n (%)</td>
<td>34 (28.3%)</td>
</tr>
</tbody>
</table>
Figures

All Neonates during study period (July-October 2017), n = 772

23 Intra Uterine Fetal Death (IUFD) were excluded

Inclusion criteria:
- Age 0-72 hours
- Sepsis risk factors: 2 major or 1 major and 2 or more minor
  Major include: PROM > 18 hours, intrapartum fever >38 C, chorioamnionitis, greenish and foul smelling amniotic fluid, and fetal heart rate>160 bpm.
  Minor include: PROM > 12 hours, intrapartum fever > 37.5 C, low apgar score, very low birth weight, vaginal discharge untreated, and untreated UTI.
- Sepsis clinical presentation

Total sample, n = 120
- Signing informed consent
- Draw blood for Septic Work Up Examination, positive if ≥ 2
  - Complete blood count (leucopenia or leucocytosis, thrombocytopenia)
  - ITR > 0.2
  - CRP > 6 mg/L
  - With positive or negative blood culture

90 EONS:
- (+) risk factors
- (+) sepsis clinical presentation
- (+) septic work up

30 Non-EONS:
- (+) risk factors
- (+) sepsis clinical presentation
- (-) septic work up

Data analysis:
- Eosinophil count
- Neutrophil to lymphocyte ratio (NLR)

Excluded:
- 267 healthy infants (term or preterm)
- 138 hyperbilirubinemia
- Respiratory distress:
  - 49 Hyaline Membrane Disease
  - 62 pneumonia neonatal
  - 51 Trancient Tachypnea of the Newborn
  - 42 congenital heart disease (sianotic or asianotic)
  - 6 congenital anomaly
  - 5 hipoxic ischemic encephalopathy

9 neonates were drop out:
- 4 underwent some operation procedure
- 5 die because of severe condition

Neonates suspected as having early onset neonatal sepsis (EONS), n = 129

9 neonates were drop out:
- 4 underwent some operation procedure
- 5 die because of severe condition

Non-EONS:
- (+) risk factors
- (+) sepsis clinical presentation
- (-) septic work up

Data analysis:
- Eosinophil count
- Neutrophil to lymphocyte ratio (NLR)
**Fig 1. Enrollment of the study** There were 772 neonates admitted in the study period. A hundred and twenty nine subjects fulfill EONS inclusion criteria and suspected as having EONS. Nine subjects were drop out, leaving a total of 120 neonates. They were done septic work up examination and results said that 90 were in EONS group and 30 were not. Then, analysis were done to look after the relationship between eosinophil count and also NLR with EONS.

**Fig 2A. Distribution of eosinophil count across EONS\(^a\) and non-EONS group** Mean eosinophil count from the EONS group was 169.8 ± 197.1 cells/mm\(^3\), meanwhile from non-EONS group was 405.7 ± 288.9 cells/mm\(^3\) with \(p<0.0001\).

**Fig 2B. Distribution of NLR\(^b\) across EONS and non-EONS group** Mean NLR from EONS and non-EONS group was 2.82 ± 2.29 and 0.82 ± 0.32, respectively, \(p<0.0001\).

EONS\(^a\) denotes Early Onset Neonatal Sepsis

NLR\(^b\) denotes Neutrophil to Lymphocyte Ratio
Fig 3A. ROC curve predicting the sensitivity and specificity of eosinophil count in detecting EONS. ROC curve for eosinophil count demonstrates that with a cut-off of 140 cells/mm$^3$, the sensitivity about 60.0% and specificity 90.0%. **Fig 3B. ROC curve predicting the sensitivity and specificity of NLR in detecting EONS.** ROC curve for NLR showed that the best specificity and sensitivity for EONS was at the cut-off of 1.245, with 83.3% sensitivity and 93.3% specificity.