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# Absolute Neutrophil Count as Predictor of Early Onset Sepsis

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**Abstract:** Background: Infant mortality rate (IMR) was found to increase in the newborn. The most frequent causes of death are infection, prematurity, low birth weight (LBW), neonatal asphyxia and birth trauma, respectively. Absolute neutrophil count (ANC) can be used as a marker of infection because of its faster, easier, simpler and cheaper nature. Objective: The study aims to identify the influence of an increase and decline in ANC on newborns from mothers with risk factors for early onset sepsis. Methods: This study was conducted as a prospective cohort study from December 2013 to July 2014. The population included 120 newborns whose mother has risk factors of early onset sepsis and admitted to Dr. Wahidin Sudirohusodo Hospital, and joined hospital. The subjects were divided into three groups, ANC <1800/mm<sup>3</sup>, ANC 1800-5399/mm<sup>3</sup> and ANC >5400/mm<sup>3</sup>. Results: Newborn from mother with risk factor of infection with ANC >5400/mm<sup>3</sup> and ANC 1800-5399/mm<sup>3</sup> shows a significant difference with p = 0.000 (p < 0.001); OR 8.143; IK 95% 2.440-27.173. Cut off point of 10.710-10890/mm<sup>3</sup> was found from ROC analyses in ANC >5400/mm<sup>3</sup> group with sensitivity and specificity 89.47% and 80.95% respectively; PPV (Positive predictive value) 80.95%; NPV (Negative predictive value) 89.47%; p=0.000; OR 36.125; IC 95% 5.820 – 224.224. Conclusions: Absolute neutrophil count >10.710/mm<sup>3</sup> in a term newborn from mother with infection risk factors can be used as predictor for early onset sepsis 36 fold higher than the ANC <10.710/mm<sup>3</sup>.

**Keywords:** Early Onset Sepsis, Absolute Neutrophil Count, Predictor Factor

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## 1. Introduction

Neonatal mortality is an indicator of a country's health status.<sup>1</sup> Data from the Indonesian Demographic Health Survey in 2007 shows there were 34 deaths per 1.000 births; in 2011 the number was 27.95 deaths per 1.000 live births.<sup>1</sup> In South Sulawesi neonatal mortality was reported to be 495 or 3.31 deaths per 1.000 live births, while Millenium Development Goals' (MDG's) target is 23 per 1.000 live births.<sup>2</sup>

Although infection can be caused by virus, yeast, and parasite, yet it is bacterial infection that play important role in neonatal sepsis. Exposure could occur in intrauterine period (in utero), during the delivery process, and right away after the birth. If the exposure occurs in utero or during delivery process, the sepsis is classified as early onset sepsis and if the exposure occurs after birth, it is classified as late onset sepsis. If the exposure continues and the microorganism enters the blood stream, the body will respond to remove the microorganism. A variety of systemic

response will appear as clinical manifestation, and in later stage will lead to changes in organ function. This, depends to the virulence, the course of disease and the body response.<sup>1, 3</sup> In response to bacterial infection, our body will release neutrophil from bone marrow into the circulation and later on will migrate to the site of infection. As the consequences, neutrophil count in circulation will increase to ensure the availability of neutrophil to perform phagocytosis. However, studies on animal showed that bone marrow reserves on neonates are very low. This resulted in neutrophil depletion in neonatal sepsis, infact, immature neutrophil can be seen in peripheral blood.<sup>3</sup> Monroe et al. have reported neutropenia and bone marrow granulocyte depletion in neonate with Streptococcus group B infection, both in human and animals.<sup>4</sup> However, research conducted by Bhandari et al. indicates that the ANC is higher in newborn who have sepsis than those without sepsis.<sup>5</sup> therefore research on the extent to which the role of ANC as a predictor of neonatal sepsis is

important to perform.

Currently, blood culture is the gold standard in diagnosing neonatal sepsis. However, result from blood culture generally comes out after 3–5 days and its accuracy is questionable because either positive or negative results can be found in common bacterial infection or from contamination. On the other hand, delay in diagnosis poses threat to baby’s survival. It has been reported that C–reactive protein (CRP) increase in 50 -90% patients with neonatal sepsis but this protein also found to elevate in a variety of non infectious organ damage with specificity and sensitivity of 84% and 23%, respectively. This examination also require advance medical technology and it is expensive.<sup>6</sup> Moreover, there was also IT ratio test, which had previously reported to increase >90% in infants with sepsis, but the condition can also be found in non-infectious respiratory disease.<sup>7</sup> Therefore, study for a faster, easier, simpler and cheaper inflammatory marker is needed, among the markers is ANC in its association to early onset neonatal sepsis.

Considering the limited supportive facilities in most area in country; a simple laboratory parameter which can predict sepsis thus helping the physician in diagnosis and treating neonatal sepsis. Study on absolute neutrophil count as predictor for early onset sepsis had never been performed in South Sulawesi. This study aims to identify the influence of ANC increase and decrease in newborn to the possibility of early onset sepsis.

## 2. Methods

This is a prospective cohort study, conducted in Pediatric Department of Dr. Wahidin Sudirohusodo Hospital and other networking hospitals in Makassar from December 2013 until July 2014. Study population and sample are newborn appropriate for gestational age from mother with risk factor of infection.

A total of 149 newborns met the inclusion criteria, of which 29 newborns were excluded because they refuse to participate, received resuscitation and invasive procedure. Inclusion

criteria in this study were: newborn (spontaneous delivery, appropriate for gestational age, without category A nor B), single birth, blood sample were successfully collected and were stored immediately after birth, born from mothers with  $\geq 2$  risk factors (fever  $>38^{\circ}\text{C}$ , white blood cells  $>15.000/\text{mm}^3$ , meconium – stained amniotic fluid and/ or the color is not clear, premature rupture of membrane  $>18$  hours). Newborn who were diagnosed with highly suspected sepsis and received antibiotic treatment before the samples were collected, had neonatal asphyxia, congenital anomalies, received resuscitation or any invasive procedures before sampling, born from mothers with co-morbid disease other than risk factor for sepsis, received antibiotic treatment for other indication, were not included in the study. Newborn with early onset sepsis were given treatment according to the guidelines in pediatric medical service standard.<sup>5</sup>

Before starting the study, informed consent were obtained from the parents and asking for permission to for blood test from the baby. This study had been approved by the Ethics and Industry Research Committee of the hospital, and the Faculty of Medical, Hasanuddin University, Makassar.

Early onset sepsis (EOS) is a clinical syndrome appeared within the first 72 hours after birth, caused by microorganism invasion into the bloodstream. Neonatal sepsis diagnosis was established if the newborn have  $\geq 2$  category A or  $\geq 3$  category B.<sup>6</sup> The ANC is the neutrophil percentage multiplied by the number of leukocytes; in units per  $\text{mm}^3$ , and considered as increase when  $\geq 5.400/\text{mm}^3$  and considered as decrease when  $<1.800/\text{mm}^3$ . The number of leukocytes is the level of leukocyte as measured with a blood analyzer (Sysmex); in units per  $\text{mm}^3$ . Initial blood sample is a blood sample taken in the umbilical vein from the baby shortly after birth.

Data was analyzed with SPSS 17. Univariate and bivariate analyses were performed: *Mann-Whitney test*, *Fisher Exact test*, sensitivity and specificity, positive predictive value and negative predictive value and also *Receiver Operator Curve* (ROC). It is not significant, when  $p>0.05$ , significant if  $p\leq 0.05$ , and very significant if  $p<0.01$ .

**Table 1.** Clinical signs and symptoms that support the direction of neonatal sepsis.

Category A	Category B
1. Respiratory Distress (such as apnea, respiratory rate $>60/\text{minute}$ , chest indrawing, expiratory grunting, central cyanosis)	1. Tremor
2. Seizure	2. Lethargy or weak
3. Unconscious	3. Drowsiness or decreased activity
4. Temperature instability (supported to sepsis)	4. Irritable
5. Delivery in an unhygienic environment (supported to sepsis)	5. Vomiting (supported to sepsis)
6. The condition deteriorate rapidly and drastically (supported to sepsis)	6. Distended abdomen (supported to sepsis)
	7. Signs appear after 4 days of age (supported to sepsis)
	8. Meconial amniotic fluid
	9. Not drinking well, previously well (supported to sepsis)

## 3. Result

Total sample participated in the study were 120 term;

appropriate for gestational age newborn. The number then was grouped into 3; newborn with  $\text{ANC} \geq 5.400/\text{mm}^3$ ,  $\text{ANC} 1.800\text{-}5.399/\text{mm}^3$  and  $\text{ANC} \leq 1.800/\text{mm}^3$ .

**Table 2.** Sample Characteristics.

Sample Characteristics	Outcome		Total	P
	EOS n (%)	Not EOS n (%)		
Sex				
Male	19 (15.8)	50 (41.7)	69 (57.5)	0.455
Female	11 (9.2)	40 (33.3)	51 (42.5)	
Maternal temperature				
Maternal fever	6 (13.3)	46 (38.3)	52 (51.7)	0.833
Non maternal fever	14 (11.7)	44 (36.7)	58 (48.3)	
Maternal Leukocyte				
Leucocytosis	23 (19.2)	65 (54.2)	88 (73.3)	0.634
Normal	7 (5.8)	25 (20.8)	32 (26.7)	
Premature Rupture of Membrane (PROM)				
PROM	15 (12.5)	46 (38.3)	61 (50.8)	0.916
No PROM	15 (12.5)	44 (36.7)	59 (49.2)	
Amniotic color				
Not Clear	15 (12.5)	39 (32.5)	54 (45)	0.525
Clear	15 (12.5)	51 (42.5)	66 (55)	

In table 3, shows a statistically significant difference in the mean value of high ANC to the outcome.

**Table 3.** Mean of high ANC on the Outcome.

High ANC ( $\geq 5.400/\text{mm}^3$ )	Outcome	
	EOS	Not EOS
Mean	14503	8726
SD	4046	3312
Range	6670-23600	5400-18700

Mann-Whitney = 48.000 p = 0.000

Table 4, shows a very significant difference on the occurrence of EOS between high ANC group and normal ANC group, with p=0.000 (p<0.01); OR 8.143; IC 95% 2.440–7.173 which shows that group with ANC  $\geq 5.400/\text{mm}^3$  has 8.1 times greater risk of having EOS compared to ANC

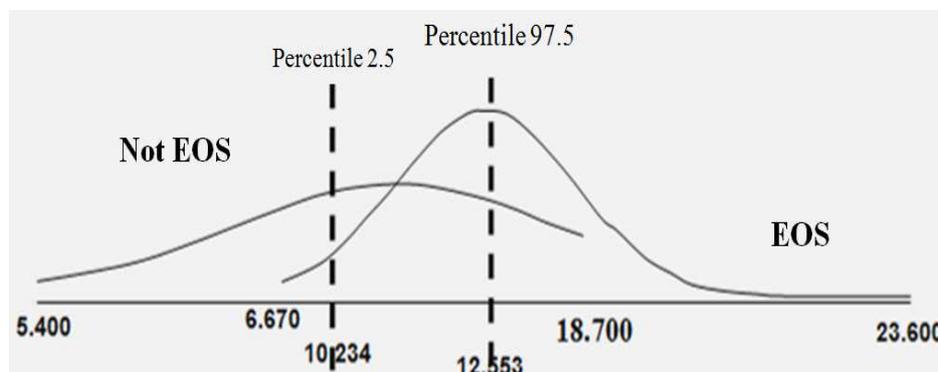
1.800–5.399/ $\text{mm}^3$ .

**Table 4.** Association between high ANC and normal ANC on the outcome.

ANC group	Outcome		Total n (%)
	EOS n (%)	Not EOS n (%)	
High ANC ( $\geq 5.400/\text{mm}^3$ )	19 (47.5)	21 (52.5)	40 (100)
ANC Normal (1.800-5.399/ $\text{mm}^3$ )	4 (10.0)	36 (90.0)	40 (100)
Total	23 (28.8)	57 (71.2)	80 (100)

Fisher's Exact p=0.000 OR=8.143 IC 95% = 2.440-27.173

Receiver operator curve (ROC) analyses of ANC  $\geq 5.400/\text{mm}^3$  group found the lowest cut off point at 2.5 percentile was on not EOS group, with ANC of 10.234/ $\text{mm}^3$  while the highest cut off point was on 97.5 percentile in the EOS group with ANC 12.553/ $\text{mm}^3$  as shown in figure 1.



**Figure 1.** Cut off point ANC increased between EOS group and not EOS group.

Table 5 shows statistical analyses on the accuracy of each cut off point for high ANC, where ANC 10.710–10.890/ $\text{mm}^3$

has the biggest Area Under Curve (AUC) by 0.852; sensitivity and specificity of 89.47% and 80.95%, respectively; positive

predictive value of 80.95%; negative predictive value of 89.47%; and  $p=0.000$  ( $p<0.01$ ).

**Table 5.** Sensitivity, specificity, positive predictive value, and negative predictive value of every cut off point of ANC.

ANC (/mm <sup>3</sup> )	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	AUC	P
10.230–10.430	89.47	71.42	73.91	88.23	0.805	0.001
10.440–10.700	89.47	76.19	77.27	88.89	0.828	0.000
10.710–10.890	89.47	80.95	80.95	89.47	0.852	0.000
10.900–11.200	84.21	80.95	80.00	85.00	0.826	0.000
11.210–11.230	78.94	80.95	78.94	80.95	0.799	0.001
11.330–11.930	73.68	80.95	77.78	77.27	0.773	0.003
12.030–12.430	73.68	85.71	82.35	78.26	0.797	0.001
12.530–12.630	68.42	90.47	86.67	76.00	0.794	0.001

PPV: Positive predictive value NPV: Negative predictive value

Table 6, shows a very significant statistical analyses between cut off point ANC  $\geq 10.710/\text{mm}^3$  and the occurrence of EOS with  $p=0.000$  ( $p<0.01$ ); OR 36.125; IC 95% 5.820-224.224.

**Table 6.** Evaluation of cut off point high ANC  $\geq 10.710/\text{mm}^3$  on the outcome.

ANC (/mm <sup>3</sup> )	EOS n (%)	Not EOS n (%)
$\geq 10.710$	17 (89.5)	4 (19)
$< 10.710$	2 (10.5)	17 (71)
Total	19 (100)	21 (100)

Fisher's Exact  $p = 0.000$  OR = 36.125 IC 95% 5.820-224.224

## 4. Discussion

Several studies have shown an association between ANC levels and early onset sepsis, but its role as a prognostic parameter in determining the occurrence of early onset sepsis is not widely known. There were 120 patients in this study, maternal's body temperature was recorded as 62 patients had fever (51.7%) and 58 patients (48.3%) were not, with  $p=0.833$  ( $p>0.05$ ). Mother with leukocytosis were noted on 88 samples (73.3%), and normal leucocyte were found in 32 samples (26.7%), with  $p=0.634$  ( $p>0.05$ ). There were 54 patients (45%) whose amniotic fluid was greenish, while 66 patients (55%) had it clear, with  $p=0.525$  ( $p>0.05$ ). Table 1 summarizes a homogenic characteristic between EOS and not EOS.

In group with higher ANC  $> 5.400/\text{mm}^3$  the occurrence of EOS is 8.1 times fold greater than the group with ANC 1.800–5.399/ $\text{mm}^3$  with  $p=0.000$  ( $p<0.01$ ). Results from this study is consistent with study conducted by Bhandari *et al.* which suggested that ANC is higher in newborn with EOS compared to not EOS. High ANC in EOS group has median of 14.433/ $\text{mm}^3$  and range 6.670–23.600/ $\text{mm}^3$ . While in the not EOS group the median is 7.380/ $\text{mm}^3$ , and range 5.400-18.700/ $\text{mm}^3$ . Statistical analyses between the two groups shows significant differences, with  $p=0.000$ . This is consistent with study conducted by Bhandari *et al.* which shows that ANC median from EOS baby was significantly higher compared to not EOS baby, with  $p<0.05$ . The results also similar with studies by Schlapbach *et al.* Frakking *et al.*

Dzwonek *et al.* and Mohamed *et al.* which also found that high ANC is associated with the EOS with statistically significant results.5, 9-12

The results above shows that there are also no EOS occurred at high ANC group, so decision to determine EOS based on ANC  $> 5.400/\text{mm}^3$  was heterogeneous. Therefore, it is necessary to reassess homogenous ANC against EOS occurrence. ROC analyses was performed to find a cut off point of high ANC in order to predict the EOS, the result between 10.234/ $\text{mm}^3$  to 12.553/ $\text{mm}^3$ . The accuracy measurement of ANC in each cut off point shows that a range of 10.710-10.890/ $\text{mm}^3$  has the biggest Area Under Curve (AUC) 0.852; sensitivity 89.47%, specificity 80.95%, positive predictive value 80.95%, negative predictive value 89.47%, and  $p=0.000$ . Therefore, ANC  $> 10.710/\text{mm}^3$  was chosen as the cut off point because it was the lowest value of the highest AUC, and after statistical analyses was performed, a high OR was obtained in group ANC  $> 10.710/\text{mm}^3$ . This result suggests that ANC  $> 10.710/\text{mm}^3$  can be used as predictor for EOS with 36 times fold higher risk to suffer from sepsis than ANC  $< 10.710/\text{mm}^3$ . This result supports previous theories which implied that newborn from mother with infection risk factor and had high ANC are at high risk of neonatal sepsis. In newborn infant, non specific immunity plays crucial role in eliminating pathogens, thus, if the newborn have high ANC it can be inferred that there's some severe infection going on. This prediction is more likely in newborn from mother with infection risk factors. 10-12

This study can be used as a predictor factors for the occurrence of early onset sepsis in newborn from mother with infection risk factors and as an innovation in newborn management (based current evidence). The result from this study can be used as the ground for routine ANC examination in newborn from mother with infection risk factors. Therefore, if the result of ANC is high (ANC  $> 10.710/\text{mm}^3$ ) the baby then should be close monitored. If there are any clinical signs of sepsis, prompt treatment should be given. This can prevent delay in treatments in order to decrease morbidity and mortality caused by sepsis in newborn.

The strength of this study is design, cohort prospective,

which is the best methods in determining the course of disease or the effect observed in the study in order to explain the relationship between risk factors and the outcome effect. Therefore, the final result of this study can be used as a reference in re-evaluating diagnosis criteria for early onset sepsis and the management of newborn from mother with infection risk factors in order to suppress morbidity and mortality rate caused by sepsis.

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