

Delta Neutrophil Index and C-Reactive Protein as Predictors of Mortality in Early Onset Neonatal Sepsis at Dr. Moewardi Hospital

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ABSTRACT

Background: Neonatal sepsis is an infection disease in newborns that still be a major problem in developing countries such as Indonesia. Early onset sepsis has higher mortality and morbidity. A simple and applicable biomarkers are needed to predict mortality in early onset neonatal sepsis. This study aimed to investigate whether Delta Neutrophil Index (DNI) and C-Reactive Protein (CRP) can be used as predictors of mortality in early onset neonatal sepsis.

Subjects and Method: A cohort study that was conducted at neonatal HCU and NICU at Dr. Moewardi Hospital from March to June 2023. Total of 30 neonates aged 0-72 hours which met the inclusion criteria and became research subjects. The dependent variable was the mortality of neonatal sepsis. The independent variable were levels of DNI and CRP values. The instruments were flowcytometry to measure levels of DNI and Enzyme Linked Immunoassay (ELISA) for determining CRP values. Data were analyzed using SPSS 25.0. Bivariate analysis was using Chi square and Fisher exact followed by multivariate analysis using logistic regression with $p < 0.05$.

Results: Thirty early onset septic neonates were included in study, of whom 17 children died and 13 children survived. Both DNI and CRP had significant correlation with mortality of neonatal sepsis ($p < 0.001$). The DNI cut-off value of $\geq 5.4\%$ (OR = 10.83; 95% CI 1.96 to 59.63; $p = 0.004$) can predict mortality in early onset neonatal sepsis. The CRP cut-off values of ≥ 5.75 mg/L (OR = 15.56; 95%CI 2.59 to 93.57; $p = 0.001$) could predict early onset neonatal sepsis mortality. Other factors such as gender, gestational age, birth weight and blood culture couldn't be used as predictors of mortality in neonatal sepsis, with p value > 0.05 .

Conclusion: DNI and CRP could be used as predictors of mortality in early onset neonatal sepsis.

Keywords: delta neutrophil index, c- reactive protein, mortality predictor, neonatal sepsis.

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BACKGROUND

Neonatal sepsis is defined as a systemic

bacterial, viral, or fungal infection that is a potentially threatened to aterm and preterm

babies. Sepsis affects four to twenty-two newborns per 1000 live births globally (Glaser et al, 2021). The incidence rate at Dr. Moewardi Hospital were 572 neonatal sepsis patients in one year, and 339 (59.2%) of these patients died. Neonatal sepsis has a high mortality rate, therefore predictors are needed to identify the mortality rate so that it can guide prevention and therapy strategies (Singh et al, 2018).

C-Reactive Protein (CRP) is composed of a pentameric structure, contains 187 amino acids and is synthesized from hepatocytes, and is an acute phase protein. CRP synthesis is stimulated by IL-6, TNF- α , IL-1 and other proinflammatory cytokines. The half-life is between 24 to 48 hours. The lower limit of normal is considered to be 1 mg/dL in the neonatal period (Sreenivas et al, 2016). Acute phase reactants such as CRP have been investigated as potential biomarkers for neonatal sepsis (Ershad et al, 2019). Elevated CRP reflects inflammation, which may be a causal pathway of mortality predictors in neonatal sepsis (Singh et al., 2018).

Delta Neutrophil Index (DNI) is the differential of leukocytes measured in the MPO channel and that measured in the basophil cell nucleus lobe channel. This reflects the fraction of immature granulocytes in the blood circulation. In a study explained that DNI was a potentially useful diagnostic and predictive tool in sepsis. The role of DNI in predicting mortality is explained in Celik's study (2019) which found that DNI increased significantly in patients with high mortality. DNI has a sensitivity level of 75% and 65% specificity as a predictor of neonatal sepsis mortality (Celik et al, 2019).

Research regarding the level of DNI and CRP-value as predictors of sepsis mortality in the neonatal population is still very limited, therefore authors performed this study in order to further investigate whether DNI and CRP can be used as predictors of

mortality in early onset neonatal sepsis.

SUBJECTS AND METHOD

1. Study Design

This research is an analytic observational study with cohort prospective design. It was performed in Dr. Moewardi General hospital, Surakarta, Indonesia from March to June 2023.

2. Population and Sample

Neonatal patients (aged 0-28 days) diagnosed early onset sepsis who were treated in the NICU and neonatal HCU at Dr. Moewardi hospital Surakarta between March to June 2023. We used consecutive sampling technique. We excluded patients who had congenital anomalies, history of born to a mother with preeclampsia or eclampsia, severe asphyxia, meconium aspiration, polycythemia, intraventricular hemorrhage, pneumothorax and hemolysis (RH and ABO incompatibility).

3. Study Variables

The independent variable of this study was levels of DNI and CRP values, while the dependent variable in this study was the mortality of neonatal sepsis.

4. Operational Definition of Variables

Early onset neonatal sepsis was defined when neonate has symptoms in two or more systems (respiratory, cardiovascular symptoms, metabolic disorders, temperature irregularity, neurological abnormality) and two or more laboratory abnormalities (Leukocytes $<5,000$ or $>20,000/\text{mm}^3$, Absolute Neutrophil Count (ANC) <1000 or $>17,000/\text{mm}^3$, immature per total neutrophil ratio >0.2 , platelets $<100,000/\text{mm}^3$) until 72 hours of age.

Mortality was determined as death occurring during hospitalization.

DNI taken from the blood sample obtained from the difference between the number of neutrophil fractions and eosinophil fractions measured in the MPO channel, minus

the number of PMN fractions measured in the cell nucleus lobularity channel by scatter light using flowcytometry on the Automated Hematology Analyzer tool.

CRP taken from the blood sample where plasma levels were measured using the CRP method using the particle enhanced turbidimetric immunoassay (PETIA) technique with an Enzyme Linked Immunoassay (ELISA) measuring instrument.

Gestational Age was determined from calculation of pregnancy time from the first day of last menstruation (very preterm, moderately preterm, late preterm).

Birth weight was defined the subject's weight at birth was measured using the GEA weight measuring instrument (very low birthweight (VLBW), low birthweight (LBW), normal birthweight (NBW), high birthweight (HBW)).

5. Study Instruments

The demographic data were obtained by caregiver's interview. The patient's medical status data were taken from medical record of patient. The DNI levels and CRP values were examined in clinical pathology laboratory of Dr. Moewardi Hospital, Surakarta, Indonesia.

6. Data analysis

We analyzed all the data statistically with SPSS-based statistical software (version 25).

Chi-square test was applied for bivariate analysis, and $p < 0.05$ was considered statistically significant.

7. Ethical Clearance

This study was approved by the Ethics Committee of Dr. Moewardi General Hospital, Surakarta, Indonesia.

RESULTS

1. Characteristic Sample

This study involved 30 neonates diagnosed early onset neonatal sepsis according to clinical criteria. The description of the characteristics of research subjects based on mortality events in this study includes sex, gestational age, birth weight, type of delivery and blood culture. Mortality of early-onset neonatal sepsis was 56%. The highest gestational age of patients who died was in the 28-33 weeks age group (52.9%). The most common deaths occurred in LBW group (47%) compared to VLBW and NBW group. Mortality in caesarean section group (70.6%) was higher than spontaneous (29.4%) delivery group. Nine patients (52.9%) who died had growth of pathogenic germs in the blood cultures examination. In the characteristic table, the p-value showed $p > 0.05$, where there was no significant difference in mortality in neonatal sepsis patients (Table 1).

Table 1. Characteristic of subjects

Variable	Mortality		Survived		p
	N = 17	%	N = 13	%	
Sex					0.691
Male	11	64.7	10	76.9	
Female	6	35.3	3	23.1	
Gestational age					0.305
28-33 weeks	9	52.9	5	38.5	0.271
34-37 weeks	3	17.6	1	7.7	
≥ 37 weeks	5	29.4	7	53.8	
Birth weight					0.487
VLBW	2	11.8	1	7.7	
LBW	8	47.1	5	38.5	
NBW	7	41.2	7	53.8	
Delivery					0.176

Variable	Mortality		Survived		p
	N = 17	%	N = 13	%	
Caesarian	12	70.6	6	46.2	0.713
Spontaneous	5	29.4	7	53.8	
Blood culture					
Organism growth	9	52.9	6	46.2	
No organism growth	8	47.1	7	53.8	

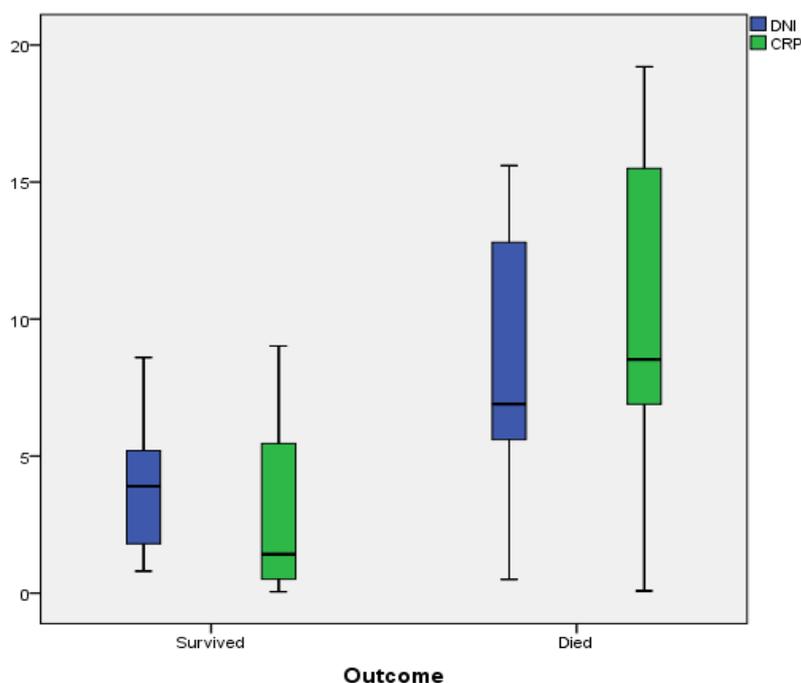


Figure 1. Comparison of mean between DNI and CRP groups in dead and survived patients

Based on the independent t test, the DNI levels in the group of patients who died (Mean= 8.08; SD= 4.39) was higher compared to the group of patients who survived (Mean= 3.84; SD= 2.23) (See figure 1). The

statistical test results obtained a value of $p=0.002$, there was a significant difference in the results of the level of DNI between patients who died and survived (Table 2).

Table 2. Comparison of mean between DNI and CRP groups in dead and survived patients

Variable	Total		Died		Survived		p
	Mean	SD	Mean	SD	Mean	SD	
DNI	6.24	4.15	8.08	4.39	3.84	2.23	0.002
CRP	6.68	5.77	9.67	5.56	2.77	3.15	0.001

CRP values in the group of patients who died (Mean= 9.67; SD= 5.56) had a higher value compared to the group of patients who survived (Mean= 2.77; SD= 3.15) as indicated by the p value = 0.001

which means that there was a significant difference in the results of the CRP values between the group of patients who died and the group of patients who survived. (Table 2)

This study showed that 17 patient

subjects died and 13 patient subjects survived. The best cut-off levels for DNI and CRP values were levels of DNI $\geq 5.40\%$ and CRP levels ≥ 5.75 mg/L which had sensitivity and specificity $> 76\%$.

The effect of high levels of DNI and CRP on the risk of mortality in neonatal sepsis was analysed using Odd Ratio (OR) value and then tested for significance using the Chi square test (Table 3).

DNI levels in neonatal sepsis $\geq 5.40\%$ had a mortality 76.5%, while neonatal sepsis patients with level of DNI $< 5.4\%$ had a mortality percentage of 23.5%. The OR of DNI levels as a predictor of early-onset neonatal sepsis mortality was 10.83 (95% CI 1.96 to 59.83; $p=0.004$). This showed that level of DNI $\geq 5.40\%$ had risk of mortality 10.83 times greater than DNI $< 5.4\%$ (Table 3).

CRP values in the group of patients who died with CRP values ≥ 5.75 mg/L was 82.4%. Patients who survived with CRP values < 5.75 mg/L are 76.9%. The OR for CRP was 15.56 (95% CI 2.59 to 93.57; $p =$

0.001) which mean that neonatal sepsis patients with CRP ≥ 5.75 mg/L had a mortality risk 15.56 times greater than patients with CRP < 5.75 mg/L with a value of $p=0.001$ ($p<0.05$). We concluded that the CRP values had a significant effect on the risk of death in neonatal sepsis (Table 3).

Furthermore, the factors that were assessed as predictors of mortality in neonatal sepsis were in the caesarean birth and the gestational age group which had bivariate analysis results with $p < 0.25$ followed by multivariate analysis using logistic regression. The multivariate analysis showed those levels of DNI $\geq 5.40\%$ and CRP values ≥ 5.75 mg/L with OR results respectively 15.91 (95% CI 1.20 to 211.09; $p = 0.036$), 53.62 (95% CI 2.23 to 1287.66; $p = 0.014$) were the factors that could be use as predictors of neonatal sepsis mortality (Table 4).

Other factors such as gender, gestational age, birth weight and blood culture couldn't be used as predictors of mortality in neonatal sepsis, with $p > 0.05$.

Table 3. Bivariate analysis of the effect of DNI and CRP on the risk of mortality in neonatal sepsis

Variable	Mortality		Survived		OR	95% CI		p
	N	%	N	%		Lower	Upper	
DNI								
>5.40	13	76.5	3	23.1	10.83	1.96	59.83	0.004
<5.40	4	23.5	10	76.9	Ref.			
CRP								
>5.75	14	82.4	3	23.1	15.56	2.59	93.57	0.001
<5.75	3	17.6	10	76.9	Ref.			
Sex								
Male	11	64.7	10	76.9	0.50	0.11	2.81	0.691
Female	6	35.3	3	23.1				
Gestational Age								
< 37 weeks	12	70.6	6	46.2	2.80	0.62	12.66	0.176
≥ 37 weeks	5	29.4	7	53.8				
Birth weight								
< 2500 kg	10	58.8	6	46.2	1.68	0.39	7.15	0.491
≥ 2500 kg	7	41.2	7	53.8				
Delivery								
Caesarian	12	70.6	6	46.2	2,80	0.62	12.66	0.176
Spontaneous	5	29.4	7	53.8				

Variable	Mortality		Survived		OR	95% CI		p
	N	%	N	%		Lower	Upper	
Organism								
Growth	9	52.9	6	46.2	1.31	0.31	5.58	0.713
No Growth	8	47.1	7	53.8				

Table 4. Multivariate analysis of the effect of DNI and CRP on the risk of mortality in neonatal sepsis

Variable	Mortality		Survived		OR	95% CI		p
	N	%	N	%		Lower	Upper	
DNI								
>5.40	13	76.5	3	23.1	15.91	1.20	211.09	0.036
<5.40	4	23.5	10	76.9	Ref.			
CRP								
>5.75	14	82.4	3	23.1	53.62	2.23	1287.66	0.014
<5.75	3	17.6	10	76.9	Ref.			
Gestational age								
<37 weeks	12	70.6	6	46.2	9.04	0.45	183.53	0.152
≥37 weeks	5	29.4	7	53.8	Ref.			
Delivery								
Caesarian	12	70.6	6	46.2	0.69	0.064	7.38	0.758
Spontaneous	5	29.4	7	53.8	Ref.			

DISCUSSION

This study assessed 30 patients with neonatal sepsis at Dr. Moewardi hospital consist of 17 patients died and 13 subjects were alive. Sex, gestational age, birth weight, method of delivery and blood culture were assessed in both groups. In the two groups there were no significant differences in basic characteristics.

In our study, there was a significant difference in the mean of DNI and CRP values in the death group, where the mean DNI and mean CRP respectively were (Mean= 8.08; SD= 4.39%) with $p = 0.002$ and 9.67 ± 5.56 mg/L with $p = 0.001$. This is in line with Lee et al's research even though the average CRP in their study was higher. Lee et al found that the DNI and CRP levels at 72 hours in neonatal sepsis patients who died had values of (Mean= 8.4; SD= 6.8%) and (Mean= 67.5; SD= 25.1) mg/L (Lee et al., 2013).

Early diagnosis and therapy are needed to avoid morbidity and mortality in neonatal sepsis. Currently, there are no specific

markers to predict mortality in neonatal sepsis. SNAPPE-II is a scoring system that can be used to detect mortality in neonatal sepsis. Scoring results of >37 is associated with a higher mortality rate in neonatal sepsis (Harsha et al., 2015). Easier and cheaper markers are needed to predict mortality in early onset neonatal sepsis. In the results of the bivariate test, significant differences were found in the levels of DNI ($p = 0.002$) and CRP values ($p = 0.001$), where in the group of patients who died, the levels were increased in both.

Our study evaluated the role of DNI levels for predicting mortality in early-onset neonatal sepsis. Our research found that level of DNI was a predictor of mortality in sepsis with OR 10.83 (95% CI= 1.96 to 59.83; $p = 0.004$.) This showed that neonatal sepsis patients with DNI levels $\geq 5.40\%$ were 10 times risk of mortality greater compared to patients with DNI values $< 5.40\%$. This result is due to research by Lee et al (2013) who also found that DNI at 72 hours had a Relative Risk of 1.47 (95% CI= 1.10 to 5.62)

even though they used a higher cut off value of 12.48%. The distinction could be caused by differences in the number of samples, mean gestational age, and mortality rates in our study and previous studies. DNI has been reported in previous studies to correlate with the incidence of DIC which will worsen the condition of sepsis and increase the risk of mortality in sepsis patients. (Nahm et al., 2008).

Our study also evaluated the role of CRP levels as a predictor of mortality in early-onset neonatal sepsis. We found that CRP was a predictor of mortality in sepsis with an OR was 15.56 (95% CI 2.59 to 93.57; $p = 0.001$). This showed that neonatal sepsis patients with CRP levels ≥ 5.75 mg/L have a 15 times greater risk of mortality compared to patients with CRP values < 5.75 mg/L. This is in equal with Lee et al. (2008) research. CRP at 72 hours in Lee et al's study had a relative risk of 2.05 (95% CI= 0.69 to 5.15) (Lee et al, 2013). The distinction could be caused by differences in sample size, average gestational age, and mortality rate of this study compared to previous studies.

CRP is an acute phase reactant which is more often used to diagnose neonatal sepsis. CRP begins to increase 4-12 hours after infection and has a peak value 24-60 hours and levels will decrease when the infection improves (Hofer et al., 2012). Hashen et al. (2020) researched that hsCRP can be used as a predictor of mortality with an hsCRP values of 24 and $p=0.018$ (Hashen et al., 2020). According to Turhan et al, in a 2012 study regarding mortality factors in neonatal sepsis stated that CRP was not related to the prognosis of neonatal sepsis (Turhan et al., 2015). This difference in results could be caused by the time of blood sampling in research subjects where we studied neonates aged 24-72 hours. Elevated CRP is related to the immune system and induces inflammation so this is associated

with organ damage and mortality in neonatal sepsis (Rasyida et al., 2023).

CRP values (OR= 53.63; $p=0.014$) compared to the DNI levels (OR= 15.91; $p=0.036$) in our multivariate analysis showed that a significant influence on mortality. The OR and p-values in the CRP group were better than those in the DNI group, thus it concluded that CRP is more dominant as a predictor of mortality in early-onset neonatal sepsis. This is different from research conducted by Celik in 2019 where the results showed that DNI (cut off 16.1%, sensitivity 75%, specificity 65%) was better as a predictor of neonatal sepsis mortality compared to CRP (Celik et al., 2019).

Other variables that could be predictors of mortality examined in this study are gestational age, birth weight, method of delivery and blood cultures. In our study, the gestational age variable had $p < 0.250$, then multivariate analysis was carried out using logistic regression but no significant differences were found. This is in line with research conducted at RSUD Dr. Moewardi previously by Hafidh et al. (2007) where no significant differences were found in gender and culture growth (Hafidh, 2007). In Rasyida et al. (2023) research, significant differences were found where gram-negative bacteria were a predictor of mortality and had an OR of 4.821 (95% CI= 10.18 to 22.842) (Rasyida et al., 2023). In our study, there was no distinction between gram-negative and gram-positive germ cultures.

In this study the highest gestational age in the group of patients who died was 28-33 weeks of gestation (52.9%). LBW was the group with the highest mortality with 8 patients (47.1%). The caesarean section method of delivery resulted in 12 deaths (70.6%). In research by I Made Kardana, it was stated that LBW and gestational age increasing the risk of mortality in neonatal sepsis (Kardana et al., 2011). Birth weight

influences the mortality of neonatal sepsis. LBW neonates usually occur in premature babies. The condition of the immune system is immature, the ability to eat is not good, the body has less fat layers, causing temperature instability, low glucose stores, making it more susceptible to hypoglycaemia. In LBW neonates defence mechanisms against bacterial infections are weakened. These factors include a lack of neutrophils, impaired chemotaxis of neutrophils and monocytes, complement deficiency, and decreased neutrophil bactericidal activity. The conditions that have been mentioned are mechanisms that explain the condition of low birth weight which will aggravate infections and increase the mortality of neonatal sepsis (Belachew et al., 2020). According to Celik, there was no significant difference in birth weight and prematurity for the sepsis group, both clinical sepsis and bacteriologically proven sepsis (Celik et al., 2019). In our study also showed that there were no significant differences in birth weight, gestational age and method of delivery in the two groups.

Our study did not examine influencing factors such as blood transfusions, the used of total parenteral nutrition and the use of mechanical ventilators which can be the limitation of this study.

In conclusion, there was a relationship between increasing DNI and CRP on mortality in early-onset neonatal sepsis. DNI levels $\geq 5.4\%$ (OR = 10.83) and CRP values ≥ 5.75 mg/L (OR = 15.56) could be used to predict mortality in early onset neonatal sepsis.

AUTHOR CONTRIBUTION

Almira Muthia Deaneva is the lead author who conducted the research, conducted data analysis, and wrote the manuscript. Rustam Siregar examined the background and discussion of the research. Sri Martuti

formulated the research framework.

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CONFLICT OF INTEREST

In this study there was no conflict of interest.

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