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INTERLEUKIN-18 AND PROCALCITONIN FACTORS AS PREDICTORS OF EARLY-ONSET NEONATAL SEPSIS

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ABSTRACT
Neonatal sepsis is a leading cause of morbidity and mortality worldwide, and one of the most common causes of neonatal death (15%). Early-onset neonatal sepsis (ESNAD) has a higher case-fatality rate than late-onset neonatal sepsis (SNAL). The gold standard for neonatal sepsis diagnosis is bacterial detection through blood culture, but this test has limitations such as being time-consuming and not being available in all health facilities. This study aims to determine the relationship between perinatal risk factors, hematological parameters in infants, procalcitonin, IL-18 with the incidence of SNAD. The population of this study were all babies who had risk factors for SNAD according to the PP Idai UKK Neonatology Clinical Practice Guidelines. The research sample was infants from an accessible population who met the inclusion and exclusion criteria. To fulfill statistical rules and fulfill the requirements for data analysis, in this study a minimum of 30 of each will be taken. The use of umbilical cord blood is a promising diagnostic tool in the diagnosis of SNAD. The IT and CRP ratios are elevated in umbilical cord blood from birth and are good indicators for detecting SNAD in infants at risk of infection. Umbilical blood procalcitonin is elevated in infants born >32 weeks with proven sepsis and low in infants with risk factors but without proven sepsis. Therefore, it is concluded that umbilical blood procalcitonin is a promising biomarker for detecting SNAD. IL-18 serves as a risk variable for neonatal sepsis. IL-18 has good discriminatory ability in the diagnosis of neonatal sepsis.

Keywords: hematological parameters; interleukin-18; neonatal sepsis; procalcitonin; SNAD

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INTRODUCTION

Neonatal sepsis is a leading cause of morbidity and mortality worldwide, and one of the most common causes of neonatal death (15%) of all neonatal deaths (Choudhary et al., 2017). The World Health Organization (WHO) estimates that there are 2.9 million deaths worldwide due to sepsis each year (44% of which are in children under 5 years of age), and a quarter of these are due to neonatal sepsis (Fleischmann et al., 2021). The incidence of neonatal sepsis in developing countries ranges from 7.1 to 38 per 1,000 live births, while in developed countries it ranges from 1 to 5 per 1,000 live births (Kamalakannan, 2018). The Indonesian Demographic and Health Survey (SDKI) reported that the Neonatal Mortality Rate (NAR) in 2017 was 15 per 1,000 births, while the Sustainable Development Goals (SDGs) target this to be 12 per 1,000 births by 2030. One important cause contributing to the high mortality rate and severity of neonatal illness is neonatal sepsis (Kartika et al., 2022).

Early-Onset Neonatal Sepsis (NAS) has a higher case-fatality rate than late-onset neonatal sepsis (NAS) (Camacho-Gonzalez et al., 2013; Hayun, 2015). The case-fatality rate for early-onset sepsis ranges from 16.7% to 19.4%, with bacteria being the most common cause (Polin et al., 2012). In

addition to high mortality, neonatal sepsis also causes significant morbidity, both short-term and long-term, including neurodevelopmental impairment (Zea-Vera and Ochoa, 2015). Delayed diagnosis contributes to the high incidence and mortality of neonatal sepsis. This is due to the difficulty in identifying classic signs of sepsis in newborns. Over 90% of cases of ADSN present with at least one symptom, but asymptomatic bacteremia also occurs. Furthermore, many newborns are infected by their mothers but do not develop symptoms immediately after birth. Conversely, not all infants with mothers with risk factors develop neonatal sepsis (Polin et al., 2012; Brady and Polin, 2013).

The gold standard for diagnostic neonatal sepsis is the detection of bacteria from blood cultures, but this test has limitations: it is time-consuming and not all healthcare facilities are capable of performing it (Shirazi et al., 2010; Narasimha and Harendra Kumar, 2011). Negative cultures are often obtained due to insufficient blood sample volume and a high risk of contamination with commensal bacteria on the skin (M Paolucci et al., 2012; Shah and Padbury, 2014; van Herk et al., 2016). One diagnostic tool for neonatal sepsis is the ratio of immature neutrophils to total neutrophils (IT ratio). An IT ratio, leukopenia, and neutropenia suggest bacterial infection in ADSN. Meanwhile, leukocytosis, neutrophilia, and an increased IT ratio are associated with ADSN. The commonly used reference value for the IT ratio is >0.2. The neutrophil-lymphocyte ratio (NLR) is a risk factor for sepsis that is gaining increasing attention. Research by Sumitro et al. concluded that the NLR can be used as an alternative marker for neonatal sepsis that is easy and relatively inexpensive, especially in developing countries (Sumitro et al., 2021).

A study by Khalthia et al., showed that the use of umbilical blood is a promising diagnostic tool in the diagnosis of ADSN (Kalathia et al., 2013). Umbilical blood cultures detect organisms comparable to peripheral venous blood cultures (Mulay et al., 2022). Many studies have measured inflammatory markers of sepsis from peripheral venous blood, usually 12-24 hours after birth, in predicting ADSN, but very few studies have tested CBC parameters, IT ratio, CRP in newborns with premature rupture of membranes and other infection risk factors using umbilical blood samples at birth in predicting ADSN. A study conducted by Shakhwat Alam et al., found that the IT ratio and CRP were increased in umbilical blood from birth and were good indicators in detecting ADSN in infants at risk of infection (Shakhawat Alam et al., 2021). Umbilical blood procalcitonin is increased in infants born >32 weeks with proven sepsis and is low in infants with risk factors but no proven sepsis. Therefore, it is concluded that umbilical blood procalcitonin is a promising biomarker for detecting ADSN (Dongen et al., 2021) (Akbarian-rad et al., 2021). IL-18 is a member of the IL-1 superfamily and has an important role in the inflammatory process (Kaplanski, 2018). IL-18 functions as a risk variable for neonatal sepsis. ROC curve analysis confirmed that IL-18 has good discriminatory ability in the diagnosis of neonatal sepsis (Li et al., 2022). The relationship between neonatal sepsis and umbilical blood IL-18 levels has not been studied to date. This study aims to determine the relationship between perinatal risk factors, hematological parameters in infants, procalcitonin, IL-18 with the incidence of SNAD.

METHOD

This research is an observational study with a case control study design. The sample was selected using non-probability sampling using consecutive techniques. The population of this study were all babies who had risk factors for SNAD according to the PP Idai UKK Neonatology Clinical Practice Guidelines. The research sample was infants from an accessible population who met the inclusion and exclusion criteria. To fulfill statistical rules and fulfill the requirements for data analysis, in this study a minimum of 30 of each will be taken. Data analysis was conducted in three stages, namely univariate analysis, bivariate analysis and analysis of the scoring system determination. Variables that will be included in the statistical analysis with logistic regression, with variables that can be assessed if they have a p value <0.25.

RESULT

Table 1.
Laboratory Findings in Neonatal Sepsis

Examination	Values Indicating Sepsis
CBC	
Trombosite	< 150.000/µL
Absolute neutrophil count	<1500/µL (mild) <1000/µL (moderate) <500/µL (severe)
IT Ratio	>0,2
IT^2	>0,02
CSF Examination	
Leukocytes	>20 mm^3
Protein	Ter, > 100 mg/dL Preterm > 290 mg/dL < 70-80% from serum levels
Glucose Biomarker	
CRP	>1 mg/dL
Prolactin	>1 mg/mL
Presepsin	>850 ng/mL

Abbreviations: IT, immature to total neutrophil ratio; CRP, c-reactive protein

Source : (Glazer et al., 2020)

Neonatal Sepsis Diagnosis

Table 2.
Hematology scoring system by Rodwell et al., for the diagnosis of SNAD

Criteria	Abnormality	Score
Leukocytes	≤5,000/µl	1
	≥25,000 at birth	1
	≥30,000 at 12-24 hours	1
	≥21,000 at ≥ 48 hours	1
PMN (polymorphonuclear neutrophils)	No found mature PMNs	2
	Increase/decrease	1
	1800-5400	0
Immature PMN count	Increase	1
	600	0
MN I:T ratio (Immature: Total)	Increase	1
	≤0,120	0
PMN I:M ratio (Immature: Mature)	≥0,3	1
	≤0,3	0
Degenerative changes in PMNs	Toxic granular/cytoplasmic vacuoles	1
Trombosite	≤150,000/µl	1

Source : (Rodwell et al., 1988)

DISCUSSION

Supporting Tests

Diagnosing neonatal sepsis remains a challenge for clinicians due to the wide variety of symptoms. A rapid and accurate diagnostic test that can be used as a definitive guideline for establishing a neonatal sepsis diagnosis has yet to be developed. Blood culture is the gold standard for diagnosing sepsis, but because results take a long time to obtain, additional tests are needed to provide prompt empirical therapy. Other tests include a complete blood cell count (CBC), polymerase chain reaction (PCR), CRP (c-reactive protein), procalcitonin, and inflammatory markers such as IL-6, IL-8, and tumor necrosis factor alpha (TNF-alpha). PCR testing is a sensitive and rapid technique, making it increasingly used in the examination of various body fluid specimens, without the need to wait for culture results (Hammad and Ms, 2018; Oeser et al., 2020).

Hematological parameters, such as the ratio of immature (I) to total (T) PMNs (PMN I:T ratio), the ratio of immature (I) to mature (M) PMNs (PMN I:M ratio), and degenerative changes in PMNs, along with the PMN I:M ratio, are the most reliable tests for the early diagnosis of sepsis and can be assessed through a peripheral blood smear. Red cell distribution width (RDW) can increase in conditions of ineffective red blood cell production or increased red blood cell destruction, which commonly occurs during inflammation or infection. In sepsis, inflammatory mediators such as IL-6, TNFα, and proinflammatory cytokines cause erythrocyte hemolysis, suppressing erythropoiesis and erythrocyte maturation in erythroid tissue. An increase in RDW levels can reflect the degree of ongoing inflammation and oxidative stress and provide prognostic information regarding the risk of death (Lakhey and Shakya, 2017; Sukewanti et al., 2019). Other biomarkers have been extensively investigated, particularly to support the diagnosis when culture results are negative. The most widely investigated biomarkers are CRP and procalcitonin. Procalcitonin has a higher sensitivity in the early stages of sepsis than CRP because it is detected 3 hours after exposure and peaks at 6 hours. C-reactive protein begins to increase 10 to 12 hours after pathogen exposure and peaks at 48 to 72 hours (Hedegaard et al., 2015; Iroh Tam and Bendel, 2017; Shane et al., 2017). IL-18 is a cytokine that stimulates various cell types and has pleiotropic functions. IL-18 was initially discovered as a factor that increases IFN-γ production from anti-CD-3-stimulated Th1 cells, particularly in the presence of IL-12. After stimulation with IL-12, naive T cells develop into IL-18 receptor (IL-18R) expressing Th1 cells, which increase IFN-γ production in response to IL-18 stimulation (Simonsen et al., 2014).

Hematology and culture tests using neonatal umbilical blood samples are currently being developed for the diagnosis of ADSN. The parameters tested include CBC, IT ratio, CRP, and blood culture. Shakhawat Alam et al. (2021) conducted a study on umbilical blood tests for ADSN screening in 147 neonates at risk of infection. Of the several parameters studied, CRP and IT ratio from umbilical blood were found to be statistically significantly increased in neonates at risk of infection, with significantly better accuracy than venous blood samples. CRP in umbilical blood had a sensitivity, specificity, PPV, and NPV of 100%, 89.3%, 43.9%, and 100%, respectively. While the sensitivity, specificity, PPV, and NPV of CRP from venous blood samples were 100%, 77.1%, 27.3%, and 100%, respectively. Other parameters such as procalcitonin can also be examined through umbilical blood samples, umbilical blood procalcitonin examination can be useful to differentiate infected and healthy newborns with or without risk factors for sepsis.

The diagnosis of sepsis cannot rely solely on culture results from either venous or umbilical blood samples, as false-negative results can still occur (Oeser et al., 2020). A diagnosis cannot be made solely based on clinical presentation or other hematological tests. Therefore, several researchers have developed a system that integrates all data, including clinical presentation, hematological tests, cultures, and other predictors, to diagnose neonatal sepsis more accurately and quickly. Observing clinical symptoms in the first hour after birth is a safe option and can reduce the need for in-hospital diagnostic tests and unnecessary antibiotic use (Montaner Ramón et al., 2023).
Assessment of each hematological parameter using the hematological scoring system proposed by Rodwell et al. can be used for the first time to establish an early diagnosis of sepsis in neonates (Rodwell et al., 1988; Saleem et al., 2014; Deleon et al., 2015; Iskandar et al., 2016).

CONCLUSION

Therefore, it is concluded that umbilical blood procalcitonin is a promising biomarker for detecting SNAD. IL-18 serves as a risk variable for neonatal sepsis. IL-18 has good discriminatory ability in the diagnosis of neonatal sepsis.

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