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Original Article

Association Between Sepsis Risk Calculator and Infection Parameters for Neonates with Risk of Early-Onset Sepsis

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ABSTRACT

C-reactive protein (CRP) is an acute-phase reactant protein that is primarily induced by the IL-6 action during the acute phase of an inflammatory or infectious process. The bacterial infection is a potent stimulus, leading to a rapid elevation of CRP levels within hours while the CBC and symptom are often misleading and/or absent. American Academy of pediatrics (AAP) is recommended routine blood examination test Complete Blood Count (CBC), C-reactive protein (CRP), and blood culture along with empirical antibiotic in neonates with early onset sepsis risk (EOS) risk even asymptomatic. The previous study is showed there were no correlation of CRP and EOS risk. This study aims to evaluate the CRP and CBC profile in neonate with risk of EOS. Methods of this study are using the sepsis risk calculator (SRC) to calculate the probability of neonatal early onset sepsis (EOS) based on maternal risk and infant's clinical presentation. Neonates with ≥ 34 weeks of gestation who were started on antibiotic treatment after laboratory examination and blood culture were taken. EOS risk estimation were compared including CRP, leukocyte, and thrombocyte count. ANOVA applied to distinguished laboratory examination between stratified risk groups. The result is showed using 82 subjects who met the inclusion and exclusion criterias, The EOS risk level was stratified into green, yellow, and red group. The p-value of CRP level, platelets, white blood cells were 0.35, 0.54 and 0.48 where p-value was considered as significant if < 0.05 . The conclusion of this study is there were no correlation of CRP level and EOS risk

Keywords: Sepsis risk calculator, infection parameter, risk of early onset sepsis, C-reactive protein, Complete Blood Count

ABSTRAK

C-reactive protein (CRP) adalah suatu reaksi fase akut protein yang diinduksi oleh aktivasi dari IL-6 selama fase akut dari inflamasi atau proses infeksi. CRP adalah sebuah indikator yang penting pada pasien dengan risiko sepsis. Infeksi bakterial adalah suatu stimulus yang berpotensi meningkatkan kadar CRP dalam beberapa jam dimana darah lengkap dan klinis pasien seringkali tidak berubah secara signifikan. American Academy of paediatrics (AAP) merekomendasikan pemeriksaan darah rutin antara lain darah lengkap, CRP dan kultur darah bersamaan dengan pemberian antibiotik namun penelitian sebelumnya menemukan bahwa tidak didapatkan hubungan antara kadar CRP dengan risiko sepsis. Tujuan dari penelitian ini adalah untuk mengevaluasi kadar CRP dan darah lengkap pada bayi baru lahir dengan risiko sepsis awitan dini. Metode yang digunakan pada penelitian ini dengan menggunakan sepsis risk calculator (SRC) untuk menghitung probabilitas risiko sepsis awitan dini berdasarkan risiko ibu dan klinis pasien. Bayi baru lahir dengan risiko sepsis awitan dini dengan usia gestasi ≥ 34 minggu dilakukan pengambilan darah lengkap, kultur darah dan CRP sebelum pemberian antibiotik. Laboratorium yang dibandingkan diantara ketiga kelompok risiko sepsis termasuk CRP, leukosit, dan jumlah trombosit. ANOVA diterapkan untuk menilai perbedaan antara kelompok risiko. Hasil dari penelitian ini yang melibatkan 82 subjek yang memenuhi kriteria inklusi dan eksklusi, Kelompok berdasarkan rekomendasi SRC dikelompokkan menjadi kelompok hijau, kuning, dan merah. Nilai p dari CRP, trombosit, sel darah putih adalah

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0,35,0,54 dan 0,48 di mana nilai *p* dianggap signifikan jika $<0,05$. Kesimpulan dari penelitian ini adalah tidak didapatkan hubungan antara risiko sepsis awitan dini dan CRP.

Kata kunci: sepsis risk calculator, parameter infeksi, risiko sepsis awitan dini, C-reactive protein, darah lengkap

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INTRODUCTION

Early onset sepsis (EOS) can be related to microorganisms obtained from the mother where pathogenic colonization occurs in the perinatal period. With rupture of the amniotic membrane, microorganisms in the vaginal flora or other pathogenic bacteria can reach the amniotic fluid and fetus.¹ Increasing risk of early onset of sepsis is in line with increasing of maternal temperature ($\geq 37.5^{\circ}\text{C}$), rupture duration of the membranes (≥ 18 hours) along with gestational age (less than 34 weeks and more than 40 weeks of gestation) and also low birth weight.² American Academy of Pediatrics (AAP) recommends neonates from chorioamnionitis mother, to take laboratory examination and received antibiotic treatment even if the baby is asymptomatic.³ This CBC counts and C-reactive proteins (CRPs) recommendation can be used as guidance of antibiotic treatment decisions in well-appearing infants, and the potential utility of clinical examination to identify EOS in at-risk infants.⁴

The use of antibiotics may cause several complications, longer length of stay on NICU, several pain procedures, lower rate of breastfeeding, changes of intestinal microbes, necrotizing enterocolitis and antibiotic resistance.⁵

Sepsis Risk Calculator (SRC) is the interactive calculator produces the probability of Early Onset Sepsis per 1000 babies by entering values for the specified maternal risk factors along with the infant's clinical presentation.⁶ SRC can be calculated in an infant born ≥ 34 weeks gestation. After entering the clinical presentation (well-appearing, equivocal, and clinical illness), SRC recommendation were assessed and considered

in each group (green, yellow and red). The red group is the most vulnerable to suffer or have higher probability of EOS.

Sepsis Risk Calculator (SRC) is originally introduced by Kaiser Permanente, and a validated tool which has been used and studied in many countries in predicting EOS.⁷⁻⁸ Kerste *et al* on 2016 study the implementation of SRC, there were reduced of antibiotics used 50%.⁹ Even the SRC was promising tools, the comparison between each group has not been evaluated yet.

The aim of this study is to evaluate the result of SRC on Complete Blood count and CRP level in neonates with Early Onset of Sepsis.-

MATERIALS AND METHODS

The study was approved by the Ethical Committee in Health Research of Dr. Soetomo Academic-Teaching Hospital Surabaya (625/Panke.KKE/x/2017). This observational study with the cross-sectional design was conducted in NICU Dr. Soetomo Academic-Teaching Hospital from November 2017 until April 2018, on newborns with gestational age ≥ 34 weeks who had EOS risks and were born in this hospital within the study period. The subject was selected using a consecutive total sampling method and sample size was determined using a prospective-cohort calculation. Routine laboratory examination comprising of CBC and CRP was performed in all subjects. Blood culture was only obtained in 42 subjects. The inclusion criteria of this study were newborns who had gestational age ≥ 34 weeks, EOS risks, appropriate gestational age (AGA). Subject are excluded if any of major congenital abnormality.

Neonatal Sepsis Risk Calculator: SRC can be accessed through <https://neonatalsepsiscalculator.kaiserpermanente.org/> website or smartphone. The required information in SRC application are the incidence of EOS was set as at 0.5/1000 live births according to CDC national incidence. Group B streptococcus (GBS) status was set as unknown because GBS status was not routinely assessed in Dr. Soetomo Academic-Teaching Hospital Surabaya. The score will be shown as personal risk stratification of EOS for each newborn according to the clinical presentation (well-appearing, equivocal, and clinical illness) and EOS risk level (green, yellow, dan red). With the SRC method, the baby will be grouped based on three groups, namely the green, yellow and red groups. Where the green group is the group that does not need blood tests or antibiotics. In the yellow group, patients are recommended to do a blood culture examination without empirical antibiotics and it is recommended to monitor vital signs in the NICU. In patients who enter the red group, empirical antibiotics are recommended to be given immediately

Blood Culture: As blood culture is a gold standard of bacteremia we also observed the characteristic of the patient and the result of CBC and CRP between SRC group. The blood will be obtained through a peripheral vein (equal to 1 cc) as the gold standard diagnosis of EOS. BacT system was used as the microbial culture method and transferred into the Mullerhinton agar to check antimicrobial susceptibility (AST) in Vitex 2 Compact.

Abnormal leukocytes: Leukocyte abnormality values are less or more than normal values. Less if $<5,000 / \text{mm}^3$ and more if $> 34,000 / \text{mm}^3$ in infants aged 0 days - 1 week. Blood counts measurement is using CELLPACK DCL from Sysmex. Blood count were taken before antibiotic admission, in the first 12 hours of life.

C-Reactive protein (CRP) is expressed in units of mg / L. Normal CRP value is $<10 \text{ mg / L}$ and abnormal if more than 10 mg/L . Measurement of CRP using Flex® cartridge from Sysmex. CRP were taken before antibiotic admission, in the first 12 hours of life.

Statistics

Data were analyzed using SPSS (Statistical Package for the Social Sciences). The value was presented as the mean \pm standard deviation (SD). Normality test was tested using Kolmogorov-Smirnov test. If the data distribution was normal, T-paired test would be used and Wilcoxon test would be performed if the distribution was not normal. Chi-Square test was utilized to assess the homogeneity of the subjects according to the demographic characteristic and laboratory examination.

RESULTS AND DISCUSSION

The population of this study is infants who had the risk of early onset sepsis (born to mothers who had a history of premature rupture of membranes for more than 18 hours, mothers with chorioamnionitis and had indications for intrapartum prophylactic antibiotics but inadequate). There were 82 patients were included in this study but only 42 patients that have blood culture results. Characteristics of the subject that have blood culture were described in Table 1. An inadequate intrapartum antibiotic is the most cause of risk of Early Onset of Sepsis.

Inadequate intrapartum antibiotics are the higher percentage of EOS risk in this study population. Gestational age, maternal highest temperature, and PROM have a nonlinear correlation with EOS risk.¹⁰ Previous study is

Table 1. Characteristics of the Subject

Characteristics (n=42)	
Maternal	
Chorioamnionitis n (%)	4 (9.5%)
Rupture of the membrane \geq 18 Hours n (%)	22 (53%)
Inadequate intrapartum antibiotic n (%)	26 (62%)
Infant	
Mean gestational age, (week)	36.7 \pm 2.2
Mean Body weight, gram	2523 \pm 566.3
Mean heart rate (time/minute)	150 \pm 155
Mean Respiratory Rate (time/minute)	46 \pm 47.6
Oxygen Support (Mechanical ventilation)	4

* Data are in number and percentage. This is the characteristic of 42 patients, the most EOS risk in this study was an inadequate intrapartum antibiotic. Four patients with needed oxygen support more than room oxygen.

showed that an adequate antibiotic which used by mothers with premature rupture of membranes will reduce the risk of infection in neonates with *Relative Risk* [RR]=0.67, 95% CI 0.52–0.85)¹¹ An inadequate antibiotic in patient with PROM will increase the risk of EOS with OR 37.0 (95% CI 9.7–140.9). The mean of gestational age on the population are below than 37 weeks, this event increases the incident of Early Onset Of Sepsis with incidence 3.0 cases per 1000 birth life.¹²

Sepsis risk calculator recommends the management of the patient with EOS risk according to clinical presentation such as vital sign (tachycardia, tachypnea, and abnormality of body temperature), usage of mechanical ventilation used and vasoactive drugs. In this study, vital signs on the red group had abnormal mean Heart Rate (166.4(6.2)) and Respiratory Rate 64.3(4.38).

CBC and CRP Analysis between SRC Groups were described in Table 2. The laboratories were Complete Blood Count (CBC) values and CRP in 82 patients, where all blood samples were taken 8 hours after birth 1 time and repeated if the clinical deterioration has occurred. In this study, there were no significant differences as a statistic between the three groups of both CBC and CRP values with mean values still in the normal range. Similar to the previous study, Acthen et al 2017 found EOS risk was not correlated with changes in infection parameters. They found negative correlations between both EOS risk, CRP level and leukocyte count within 6 h of the start of antibiotics, as well as CRP level between 6 and 24 h after start of treatment.¹³ CRP production is a non-specific response to disease and cannot be

used alone as a diagnostic test for septicaemia. The sensitivity and specificity of CRP (at 72 hours of admission) in diagnosis of acute neonatal sepsis were 76.92% and 53.49% respectively while it had a positive predictive value of 80% and negative predictive value of 48.94%. Over all the diagnostic accuracy of CRP in diagnosis of neonatal sepsis was 70.07%.¹⁴

Patient with positive blood culture's characteristics, and laboratory results were described in Table 3. This study is found that two patients with positive blood culture have a normal level of CRP and one patient on the green group have abnormal CRP level. Contradiction with this result, A study in India (2016) have found the abnormality of CRP in 92.95% of positive culture cases. There is also a statistically significant relationship between positive blood cultures and CRP. The CRP test is positive at 64.34% of early onset sepsis and 35.66% of late onset sepsis.¹⁵

In the study by Carola et al 2017, the management recommendations based on the EOS calculator after clinical evaluation are presented including the 5 neonates with culture-proven sepsis and 142 neonates with culture-negative sepsis who were treated with antibiotics for ≥ 7 days. Empiric antibiotics would have been recommended in 23.5% of the neonates in the Cohort. Blood culture only was recommended for 8.9% of the neonates. No empiric antibiotics or laboratory evaluations were recommended for the remaining 66.7%. In that cohort, 142 neonates were treated with prolonged antibiotics (7 days or more) for suspected culture-negative sepsis.

Table 2. CBC and CRP Analysis between SRC Groups

Laboratory results	Groups			p
	Green	Yellow	Red	
Complete Blood Count (mean \pm SD)				
Haemoglobin	16.7 (2.28)	15.8 (2.37)	15.49 (1.85)	0.19
White Blood Cell	18747 (6472)	15646 (4712)	14817 (7331)	0.54
Platelet	242043 (59622.7)	252875 (70656)	250909 (87464)	0.48
C-Reactive protein (mg/L)	2.73(8.6)	0.45(0.56)	8.36 (31.75)	0.35

* Data are in number and P values are the results of ANOVA. Patients on the red group had higher CRP level than green and yellow group.

Table 3. Patient with Positive Blood Culture Characteristics and Laboratory Results

Initial/Culture result	SRC Groups	BW/GA	Hb	WBC	PLT	CRP
N.S/ <i>Micrococcus Luteus</i>	Green	2600/37	21.5	24370	360000	0.66
M/ <i>Acinetobacter lwoffii</i>	Green	2600/38	16	11680	190000	13.68
N.F/ <i>Aerococcus viridans</i>	Red	3600/41	16.2	23030	296000	2.56

* BW = Body weight, GA = Gestational Age, Hb = haemoglobin, WBC = white blood cell, PLT = platelet, CRP = C-reactive protein.

All 5 neonates with positive blood cultures had abnormal CBC and CRP values.⁸

The sensitivity and the specificity of each CRP was 92.96% and 50.39%.¹⁶ C-reactive protein has the best predictive value when measured within 24 to 48 hours after infection. In healthy individuals, the CRP level is generally below 2 mg/L but can be up to 10 mg/L. There may be slight variations with age, sex, and race. It has a half-life of approximately 19 hours, begins to rise after 12–24 hours, and peaks within 2–3 days. Normal CRP values at two examinations (8 to 24 hours after birth and 24 hours later) were shown to have 99.7% negative predictive values and negative likelihood ratios of 0.15 which were proven to be sepsis.¹⁷

In the diagnosis of early-onset sepsis, previous studies are reported on widely differing sensitivities and specificities of CRP ranging from 29 to 100% and from 6 to 100%, respectively. The delayed induction of the hepatic synthesis of CRP during the inflammatory response to infection lowers its sensitivity during the early phases of sepsis.¹⁸

From the results of Complete Blood Count results, there were no significant differences between the three groups and in patients with positive blood cultures only one in three patients had a positive CRP score. Total white blood cells have a low Positive Predictive Value (PPV) for sepsis while platelet counts are insensitive or specific for the diagnosis of sepsis and are not very helpful for monitoring response to therapy.¹⁹

The blood culture results of patients belong to green group, positive culture was found in 2 patients (*Micrococcus Luteus* and *Acinetobacter lwoffii*), while in red group, 1 patient had positive blood culture (Multistrain resistant *Aerococcus viridans*). All patients with positive blood culture,

had risk factor of meconial amniotic fluid with inadequate antibiotics treatment. Meconial amniotic fluid could be sign of chorioamnionitis, which may enhanced the growth of bacteria in amniotic fluid and caused both maternal and neonatal infections.²⁰

Among 42 patients there were 3 patients with positive blood cultures (7.5%). The results of blood cultures obtained were *Micrococcus luteus* (1), *Acinetobacter lwoffii* (1), *Aerococcus viridans* (1) which had more than one class of antibiotic resistance. Blood culture is the gold standard for the diagnosis of sepsis, and when the adequate volume is obtained, culture has excellent sensitivity even when the baby has a very low level of bacteremia. However, many culture results were found to be negative especially when the baby appeared ill or antibiotics were received before culture was obtained. Based on the recommendation at least 1 mL of blood, either in 1 or divided into 2 0.5 mL cultures, obtained from infants with suspected sepsis before initiation of antibiotic therapy. However, sampling is limited by blood volume, especially in very low birth weight babies, who are at the highest risk for sepsis but have the lowest total blood volume. However, the sensitivity of blood culture decreased by 10% to 40% when 0.5 mL was inoculated compared to 1 mL. Therefore, adequate volume for culture must be ensured.²¹ The sensitivity of blood culture is almost 100% when 1 mL is inoculated and the baby has bacteremia concentration of at least 4 colony-forming units (CFU) per milliliter. The optimal time for culture taking in bacteremic conditions is as soon as possible in fever episodes based on heat followed by bacteremia or endotoxaemia in one or two hours. In newborns often have a shorter threshold for the commencement of antibiotics,

which results in low opportunities for isolated organisms in blood culture. This coincides with the low specificity of signs of sepsis in newborns compared to children and adults that contribute to a low positive rate in blood culture.²²⁻²³

Two patients had gram-positive blood cultures and one patient with gram-negative on this study has normal blood count and have an inadequate antibiotic as a risk factor. Newborns with mothers who received Intrapartum Antibiotic Prophylaxis (IAP) due to colonization of group B streptococci or chorioamnionitis, had a lower risk for Early Onset Sepsis than infants with mothers who did not receive an adequate IAP.²⁴ The classic study focusing on *Escherichia coli* infection, newborns were found to have bacteremia with high colonies. However, more recent studies include pathogens other than *Escherichia coli* in infants. A newborn with a risk of sepsis found that 68% of septic infants had bacteremia with a low colonization rate (≤ 10 Colony-forming units (CFU) / ml) and 42% had a 1 CFU / ml colony count. Calculation of low bacterial colonies will cause as much as 60% of culture to be false negative with a sample volume of 0.5 ml. Many blood cultures can help improve these test results, but studies in the newborn period have shown conflicting results.²⁵

On this study patient with red group, there were only 1 patient who had positive culture, These results differ from those of other researchers where a clinical evaluation of sepsis compared with blood cultures in patients diagnosed with sepsis which is showed sensitivity (62.5% [95% confidence interval (CI): 35.43- 84.80%]), specificity [63% (95% CI: 47.55-76.79%)], positive predictive value [37% (95% CI: 19.40-57.63%)] and value negative predictive [82.8% (95% CI: 66.35 -93.44%)]. There were statistically significant differences between blood culture results and clinical sepsis (p 0.014).²⁶

One patient with clinically ill appearance had the results of an *Aerococcus viridans* culture that had multi-resistance to antibiotics also have a normal range of blood count and CRP. Patients with *Aerococcus viridans* culture results in this study had risk factors for meconal amniotic fluid and inadequate antibiotic administration.

The organisms most commonly involved in early-onset sepsis in term infants and fewer term infants are GBS and *Escherichia coli*, which account for around 70%. Additional pathogens are other streptococci (viridans group *streptococci*, *Streptococcus pneumoniae*).²⁷ *Aerococcus*, *abiotrophia* which is a gram-positive-coccus bacteria - catalase negative is a group of rarely isolated bacteria as opportunistic agents of infection, although this organism can become a pathogen in immunocompromised patients. *Aerococcus* is an environmental isolate that can also be found in human skin. These bacteria have low virulence and only become opportunistic pathogens in immunocompromised hosts. Infection that occurs is often in the form of tissue damage (for example a heart valve) or may be nosocomial and is associated with prolonged hospitalization, antibiotic therapy, invasive procedures and the presence of foreign objects. The association of infection with *Aerococcus Viridans* in humans found an almost significant value in those with rupture of membranes during childbirth (P 0.073), prolonged rupture of membranes (P 0.058), those receiving Intrapartum Antibiotic Prophylaxis (IAP) (P 0.059) and women who smoked during pregnancy (P 0.062)²⁸ There were several limitations of this study. First, the number of samples was relatively small. Second, the lack of GBS status data of the subject's mother, since this test is not a routine in Indonesia. Third, due to financial restraints, blood culture test were only performed in half of the subjects.

In this study, two neonates with green recommendation had positive blood culture. The SRC tools incidence input in this study follows CDC recommendation (0.5 %). The result is similar to retrospective cohort study of Carola et al, in which, from 1159 infants born to mothers with clinical chorioamnionitis, the calculator would have missed 2 of 5 infants with culture-proven, early-onset sepsis. The SRC tools has been updated to enable the possibility of EOS incidence, as high as 4%. This update would enable to capture the two missed sepsis infants into the right risk and management category.

CONCLUSIONS

The results of Complete Blood Count and CRP levels between each group of SRC recommendation shown no significant differences. The analysis indicate that CRP level is uncorrelated with EOS risk, thus clinical judgement is necessary to accompany laboratory examination.

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CONFLICT OF INTEREST

There is no conflict of interest of this paper.

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