

## Umbilical cord blood interleukin-6 level as a predictor of early-onset neonatal sepsis

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### Abstract

**Background** Neonatal sepsis is a global health problem contributing significantly to neonatal morbidity and mortality. It is difficult to clinically distinguish neonates with and without sepsis. Interleukin-6 (IL-6) concentration in neonates has high sensitivity and specificity to predict neonatal sepsis in infants at risk.

**Objective** To determine the utility of umbilical cord blood IL-6 as a predictor of early-onset neonatal sepsis.

**Methods** This prospective cohort study was conducted in neonates born to mothers with sepsis risk factors from December 2020 to January 2021. We measured IL-6 from umbilical cord blood taken after placental expulsion. IL-6  $\geq 16.4$  pg/mL was considered to be elevated. Subjects were monitored for signs of clinical sepsis until 72 hours after birth. We also recorded the presence of other maternal and infant risk factors of sepsis and assessed association between IL-6 and other risk factors with the occurrence of sepsis, expressed as relative risk (RR) with 95% confidence interval (95%CI).

**Results** During the study period, 40 neonates were born to mothers with sepsis risk factors; 13 (32.5%) developed clinical sepsis. Significantly more infants with elevated IL-6 developed neonatal sepsis (55.5%) than those with normal IL-6 (13.6%). After multivariate analysis incorporating other significant variables, the risk factors predictive of clinical early-onset neonatal sepsis were IL-6 [RR 5.54 (95%CI 1.68-18.25); P=0.016], prematurity [RR 4.92 (95%CI 1.66-14.59); P=0.014], and initial Apgar score [RR 3.38 (95%CI 1.34-3.38); P=0.046].

**Conclusion** In neonates with maternal risk factors, an IL-6 level of  $\geq 16.4$  pg/ml is associated with an increased risk of early-onset neonatal sepsis.

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**Keywords:** neonatal sepsis; IL-6; clinical sepsis

Neonatal sepsis is a global health problem that significantly contributes to neonatal morbidity and mortality. Up to 13% of neonatal deaths in Indonesia are caused by sepsis. Its mortality rate in Indonesia has reached 15 deaths per 1,000 live births.<sup>1</sup> The mortality rate of early-onset neonatal sepsis is 8%, while that of late-onset neonatal sepsis is 37.2%.<sup>2</sup> In Dr. Sardjito General Hospital, Yogyakarta, the prevalence of early-onset neonatal sepsis is 22.5%.

The risk factors for neonatal sepsis can be neonatal or maternal. Neonatal risk factors include preterm birth, low birth weight, and low Apgar score, whereas maternal risk factors comprise intrapartum fever, premature rupture of membranes (PROM) > 18 hours, chorioamnionitis, and urinary tract infection (UTI).<sup>3</sup>

The clinical manifestations of early-onset neonatal sepsis are often difficult to recognize.

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Therefore, neonates with suspected sepsis are given antibiotics empirically, which may increase the incidence of side effects.<sup>4</sup> Bacteremia can still occur in the absence of clinically manifested sepsis. There is no specific diagnostic test to determine neonates' need for empiric antibiotic therapy.<sup>5</sup>

Central nervous system and growth disorders, such as cerebral palsy, microcephaly, as well as hearing and visual impairment can occur due to neonatal sepsis.<sup>6</sup> In addition, seizures, post-infection encephalopathy, hydrocephalus, ventriculomegaly, cerebral infarctions, brain infections such as brain abscess, ventriculitis, and subdural empyema, may also occur as a result of neonatal sepsis.<sup>7</sup>

The outcome of neonatal sepsis depends on several factors, such as gestational age, onset of neonatal sepsis, economic condition, complications (meningitis), rapid diagnosis, and effective antibiotic treatment. The latter remains a challenge because of the non-specific clinical manifestations of neonatal sepsis, low blood culture positivity rates caused by maternal antibiotic therapy, and limited blood specimen volume.<sup>8</sup> Various biological markers, such as acute phase proteins, cytokines, and cell surface antigens have been studied as predictors of early-onset sepsis. However, results have been inconclusive due to small sample size, inconsistent definitions of sepsis across studies, and different ranges of reference values for biological markers. For example, C-reactive protein (CRP), the most widely used biological marker for the diagnosis of neonatal sepsis, increases 12-24 hours after antigen stimulation. Hence, the use of CRP as an early predictor for neonatal sepsis may be limited.<sup>9</sup>

Interleukin-6 (IL-6) is an inflammatory cytokine produced immediately after induction of inflammation, peaking 6 hours afterwards. IL-6 measurement results can be obtained within a few hours after umbilical cord blood sampling, so clinicians can make immediate treatment decisions. A study reported that IL-6 had better sensitivity and specificity than CRP, immature-to-total neutrophil ratio (IT ratio), micro-erythrocyte sedimentation rate (micro-ESR), and complete blood count. The sensitivity and specificity of IL-6 were 95.83% and 87.5%, respectively, while the positive predictive value (PPV) and negative predictive value (NPV) were 92% and 93.3%, respectively.<sup>11</sup> In this study, we aimed to examine the utility of IL-6 as a predictor of early-onset neonatal sepsis.

## Methods

This was an observational prospective cohort study. Subjects were neonates hospitalized at Dr. Sardjito General Hospital. The primary data collected included risk factors of sepsis, IL-6 concentration, and clinical manifestations of sepsis from birth to 72 hours of age. Clinical sepsis was defined as the occurrence of at least one symptom or sign within 72 hours after birth in at least four symptom groups. Inclusion criteria were neonates born at RSUP Dr. Sardjito General hospital from mothers with risk factors for early-onset neonatal sepsis (intrapartum fever, premature rupture of membranes PROM > 18 hours, chorioamnionitis, and UTI) who obtained parental informed consent to participate in this study, whereas exclusion criteria were respiratory distress syndrome and meconium aspiration syndrome. Our study protocol had been approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada/Dr. Sardjito General Hospital.

We classified neonates into two groups, one with normal and another with elevated IL-6 levels. The minimum required sample size was 20 subjects in each group. An IL-6 level of  $\geq 16.4$  pg/mL was considered elevated. Using consecutive sampling, eligible neonates were included until the sample size was met. Shortly after placental expulsion, 2 mL of umbilical cord blood was drawn and stored in a vacutainer tube. After all samples were collected, IL-6 concentration was measured using the ELISA method (*BioLegend*, San Diego, California). This assay was available at the laboratory of Dr. Sardjito General Hospital. Subjects who had clinical sepsis also underwent blood cultures.

The maternal risk factors assessed were peripartum fever, PROM, emergency caesarean section, clinical chorioamnionitis, UTI, CED, overweight, age, parity, and HIV status, while the neonatal risk factors examined were low birth weight, prematurity, and low Apgar score. Bimanual examination results were not assessed because such data was not available for referred mothers. Bivariate analysis of the associations between IL-6 level and other risk factors with clinical neonatal sepsis was done using the chi-square or Fishers' exact test. Multivariate analysis by logistic regression with backward method was done to determine the

dominant factor influencing the occurrence of early-onset neonatal sepsis. Risk factors with a P value of  $<0.25$  in the bivariate analysis were entered in the multivariate analysis.

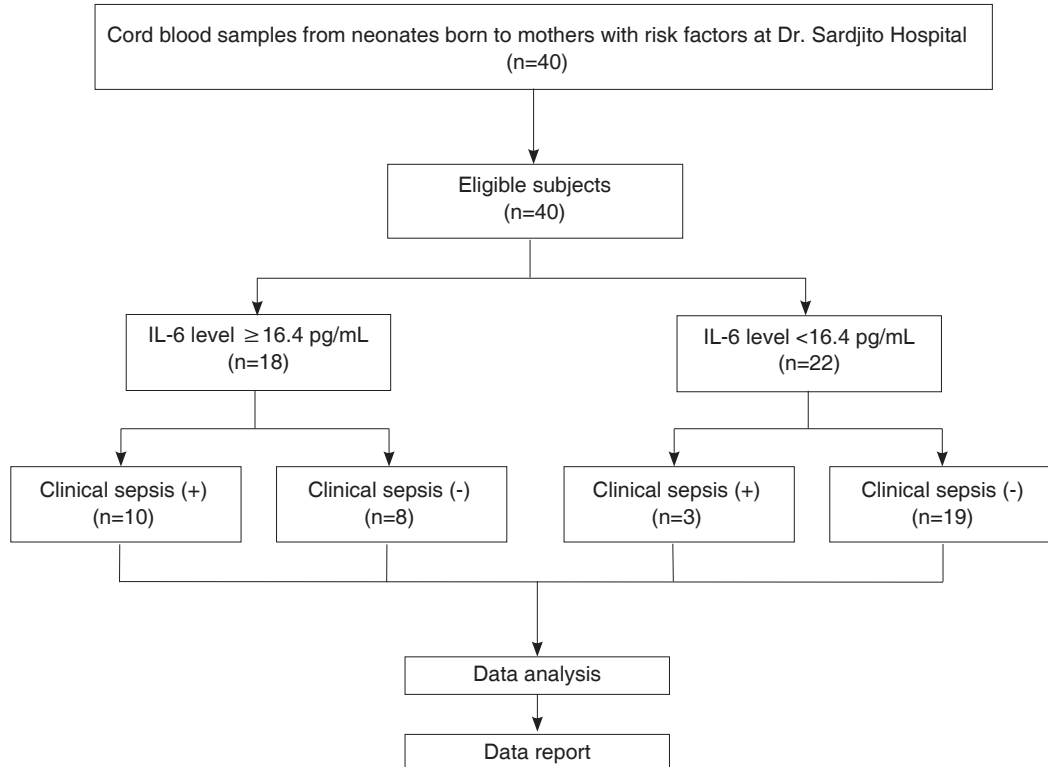
Data were processed and analyzed using SPSS version 23 (IBM, Armonk, New York). Bivariate regression analysis was performed to determine the relative risk of each variable. Multivariate analysis was performed to identify variables independently associated with neonatal sepsis when other influencing factors were considered.

## Results

From 13 December 2020 to 31 January 2021, 40 neonates were born to mothers with risk factors for early-onset neonatal sepsis. None of the subjects showed signs of neonatal sepsis immediately after birth. During the monitoring period, neither meconium aspiration syndrome nor respiratory distress syndrome were found, thus no infant was excluded.

During the observation period, 13 infants (32.5%) experienced clinical sepsis. Elevated IL-6 was found in 18 subjects, ten of whom had sepsis. Out of the 22 subjects with normal IL-6, three had sepsis. We performed blood culture in 12/13 subjects with clinical sepsis; none were positive. The study flowchart is shown in **Figure 1**.

We obtained an equal number of male and female subjects. No subjects were born to mothers under 20 years of age or mothers who had peripartum fever or chorioamnionitis. Nine subjects (22.5%) were born to mothers with PROM and 22 (55%) were born to mothers with UTI. Eleven subjects (27.5%) were born to nulliparous mothers and 14 (35%) were born by emergency caesarean section. One subject (2.5%) was born to a mother with HIV and 11 (27.5%) were born with Apgar scores lower than 7. Twenty-two (55%) neonates had overweight mothers and four (10%) had mothers with chronic energy deficiency (CED). There were 15 preterm newborns (37.5%) and 19 low birth weight neonates (47.5%). None of the subjects had major congenital anomalies, hyaline



**Figure 1.** Subject flow chart

membrane disease, or meconium aspiration syndrome. Characteristics of subjects are shown in **Table 1**.

On bivariate analysis, we found that IL-6 level, prematurity, low birth weight and initial Apgar score had P values of <0.25 (**Table 2**). These variables were subsequently entered into the multivariate analysis.

Multivariate analysis revealed that elevated IL-6, prematurity, and Apgar score <7 were significantly associated with sepsis (**Table 3**), but low birth weight was not. Neonates with elevated IL-6 levels were 5.54 times more likely to develop sepsis than those with normal IL-6 levels [RR 5.54 (95%CI 1.68 to 18.25); P=0.016].

**Table 1.** Basic characteristics of subjects

Characteristics	(N=40)
Sex, n (%)	
Male	20 (50.0)
Female	20 (50.0)
Gestational age, n (%)	
≥ 37 weeks	25 (62.5)
< 37 weeks	15 (37.5)
Maternal age, n (%)	
< 20 years	0
≥ 20 years	40 (100)
Parity, n (%)	
Nullipara	11 (27.5)
Multipara	29 (72.5)

**Table 2.** Bivariate analysis of clinical sepsis and risk factors

Variables	Clinical sepsis		RR	95%CI	P value
	Yes (n=13)	No (n=27)			
IL-6 level, n (%)			4.07	1.32 to 12.61	0.005
≥ 16.4	10	8			
< 16.4	3	19			
Maternal UTI, n (%)			1.31	0.52 to 3.31	0.564
Yes	8	14			
No	5	13			
PROM, n (%)			1.53	0.61 to 3.82	0.437 <sup>§</sup>
Yes	4	5			
No	9	22			
Prematurity, n (%)			3.75	1.39 to 10.08	0.006* <sup>§</sup>
Yes	9	6			
No	4	21			
Low birth weight, n (%)			2.48	0.91 to 6.77	0.056
Yes	9	10			
No	4	17			
APGAR score, n (%)			3.08	1.33 to 7.13	0.020* <sup>§</sup>
<7	7	4			
≥7	6	23			
Emergency C-section, n (%)			1.59	0.66 to 3.82	0.480 <sup>§</sup>
Yes	6	8			
No	7	19			
Maternal overweight, n (%)			1.31	0.52 to 3.31	0.564
Yes	8	14			
No	5	13			
CED, n (%)			0.75	0.13 to 4.36	1.000 <sup>§</sup>
Yes	1	3			
No	12	24			

\*significant P<0.05 Chi-square test; § Fisher's exact test

**Table 3.** Multivariate analysis

Variables	RR	95%CI	P value
IL-6 level ≥ 16.4 <16.4	5.54	1.68 to 18.25	0.016
Prematurity Yes No	4.92	1.66 to 14.59	0.014
APGAR score <7 ≥7	3.38	1.34 to 3.38	0.046
Low birth weight Yes No	2.89	0.97 to 8.63	0.085

## Discussion

Neonatal sepsis is one of the major causes of neonatal morbidity and mortality. Early diagnosis and management are necessary to obtain better outcomes. In our study, IL-6 level in cord blood was significantly associated with clinical early-onset neonatal sepsis. A previous study found that IL-6 level increased in infection.<sup>12</sup> Prashant *et al.*<sup>13</sup> reported the sensitivity and specificity of IL-6 to be 54% and 96%, respectively. Moreover, Schultz *et al.*<sup>14</sup> found that premature infants could adequately produce IL-6, suggesting that IL-6 examination can be used in both term and preterm neonates to predict early-onset neonatal sepsis. In our study, the confidence intervals were wide, which may have been caused by the small sample size or the imbalance between the number of subjects with and without clinical sepsis.

Subjects who experienced clinical sepsis underwent blood cultures. None of the 12 cultures had bacterial growth. The lack of bacterial growth on blood culture may have been caused by insufficient volume of blood specimens or maternal antibiotic therapy before delivery. This condition is known as culture-negative early-onset neonatal sepsis. Early-onset neonatal sepsis confirmed by culture is referred to as culture-proven early-onset neonatal sepsis.<sup>15</sup> The ratio between culture-proven and culture-negative early-onset neonatal sepsis has been reported to be approximately 1 to 16.<sup>16</sup> Therefore, the lack of bacterial growth on blood culture does not exclude neonatal sepsis.<sup>5</sup>

We performed IL-6 examination using the ELISA method at the end of the study, after all samples

had been collected. Cord blood specimens were centrifuged immediately after delivery, then serum was separated and frozen until all specimens could be tested at once.<sup>17</sup>

In our study, prematurity was significantly associated with early-onset neonatal sepsis. Preterm infants were more likely to experience neonatal sepsis than term infants, possibly because they receive less transplacental immunoglobulin than term counterparts, since passive transfer of transplacental immunoglobulin occurs in the last trimester of pregnancy.<sup>18</sup>

PROM was not significantly associated with early-onset neonatal sepsis in our study. Maternal antibiotic therapy for PROM and preterm PROM (PPROM) may reduce the incidence of early-onset neonatal sepsis.<sup>19</sup> Although on bivariate analysis we obtained an RR of 1.53 for the association between PROM and clinical sepsis, this result was not statistically significant.

Maternal UTI also had no significant association with early-onset neonatal sepsis. All mothers with UTI in our study received antibiotic therapy before delivery. Reed *et al.*<sup>20</sup> found that neonatal exposure to antibiotics before birth can be a protective factor against necrotizing enterocolitis (NEC) and neonatal mortality.

We found that emergency cesarean section was not significantly associated with early-onset neonatal sepsis. In contrast, a previous study noted that early-onset neonatal sepsis was four times more common in neonates born through emergency, compared to elective, cesarean section.<sup>21</sup> Emergency cesarean section is more likely to cause neonatal laceration (OR=1.7), which may serve as a port of entry for infections.<sup>22</sup> None of our subjects who underwent emergency cesarean section experienced laceration, which may explain the lack of association with neonatal sepsis.

Our results support early IL-6 examination for the rapid detection of neonatal sepsis, especially in neonates born to mothers with risk factors. In a previous study, IL-6 was not predictive of neonatal sepsis in neonates without risk factors.<sup>23</sup> A limitation of our study was that sepsis was diagnosed by clinical assessment only, since none of the blood cultures showed growth. In addition, IL-6 examination was carried out simultaneously for all samples at the end



of the study, so that there was a considerable time lag from umbilical cord blood sampling at birth until the results of the IL-6 examination were known. Moreover, IL-6 examinations can presently only be done in tertiary hospitals. Another limitation is the lack of precision of our findings, indicated by the wide confidence intervals for RR, which may be due to the small number of subjects.

The strength of our study was the prospective cohort design, which showed the temporal relationship between predictor factors and clinical sepsis. Our use of cord blood specimens also reduced the possibility of unwanted consequences of blood sampling in neonates.

We conclude that neonates with IL-6 levels of  $\geq 16.4$  pg/mL had a higher risk of developing clinical early-onset neonatal sepsis than those with IL-6 below those levels. IL-6 can be used as a quick early test to predict clinical early-onset neonatal sepsis. Further studies with a larger number of subjects are needed to confirm our findings.

## Conflict of interest

None declared.

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