

Current-proven neonatal sepsis in Indonesian tertiary neonatal intensive care unit: a hematological and microbiological profile

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ABSTRACT

Background and Objectives: Neonatal sepsis is the third leading cause of neonatal death in the world. The patterns of pathogens causing neonatal sepsis varies in many countries. This study was aimed to identify hematological and microbiological profile of culture-proven neonatal sepsis in Indonesian tertiary neonatal intensive care unit (NICU).

Materials and Methods: Hospital based cross-sectional study was conducted in all inborn neonates that were suspected sepsis neonatal over a period of six months from April to September 2019. Complete blood count, c-reactive protein (CRP) and blood culture were examined before antibiotic administration. Statistical analysis were calculated based on Chi-Square's Test and Mann-Whitney U test and $p < 0.05$ considered significant.

Results: One hundred four inborn neonates admitted to NICU and diagnosed with suspected neonatal sepsis were recruited. Culture-proven neonatal sepsis were confirmed in 52 (50%) neonates, 13 (25%) in early-onset neonatal sepsis (EONS) and 39 (75%) in late-onset neonatal sepsis (LONS). The most common abnormal hematological profile were anemia and thrombocytopenia, with amount of 61.5% and 75%, respectively. High CRP only detected in 36.4% and only 18.5% experienced leukopenia. Gram negative bacteria responsible in 75% from total isolated pathogens. *Klebsiella pneumoniae* accounted for 48.1% followed by coagulase negative staphylococci (CONS) for 17.3% and *Enterobacter cloacae* for 11.5%.

Conclusion: Anemia and thrombocytopenia were the top two hematological profile of culture-proven neonatal sepsis. Most causes of culture-proven neonatal sepsis were Gram negative bacteria and the dominant pathogen was *K. pneumoniae*.

Keywords: Neonatal sepsis; Bacteremia; Blood culture; Gram negative bacteria; *Klebsiella*

INTRODUCTION

Neonatal sepsis is a clinical syndrome that occurs in infants in the first month that characterized by sys-

temic infection and bacteremia. It is classified into two types based on the age of onset of the findings, early-onset neonatal sepsis (EONS) occurring with ≤ 72 hours and late-onset neonatal sepsis (LONS) occurring after 72 hours (1-3). Neonatal sepsis is the third leading cause of neonatal death in the world (4). There has been a decrease in neonatal mortality between 1990 and 2017, from 36.6 (35.5-37.8) to 18 (17-19.9) deaths per 1000 live births. Nonetheless, the Sustainable Development Goals (SDGs) stipulate that all countries should aim to reduce the neona-

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tal mortality rate (NMR) to 12 deaths per 1000 live births or less by 2030 (5).

Blood culture is the gold standard examination for neonatal sepsis diagnosis (2). The presence of pathogen isolation on blood culture examination or positive PCR examination in neonates with clinically neonatal sepsis is categorized as a culture-proven neonatal sepsis (6). The patterns of pathogen causing neonatal sepsis differ in many countries according to the local microbial pattern and to the onset of neonatal sepsis (2, 7). Little information is known about the incidence and distribution of neonatal sepsis pathogens and profile in Indonesia, especially in Surabaya, so this study aims to provide an overview of the hematological and microbiological profile of culture-proven neonatal in tertiary neonatal intensive care unit in Indonesia.

MATERIALS AND METHODS

Study design and ethical clearance. This hospital based observational analytic cross-sectional study conducted for 6 months, between April and September 2019. An ethical clearance certificate was approved by Ethical Committee in Health Research of Dr. Soetomo General Academic Hospital Surabaya (ref. no. 1047/KEPK/III/2019).

Study population. All inborn neonates admitted to the NICU and with suspected sepsis neonatal were eligible in this study. For this study, diagnostic criteria for suspected neonatal sepsis was according to definitions of blood stream infection in the newborn by Haque (2005) (6). Suspected neonatal sepsis was the presence of clinically neonatal sepsis accompanied by increasing c-reactive protein/ CRP (>10 mg/dL or >2 SD above normal value) or at least 2 abnormal inflammatory laboratory results. Local Clinical Practice Guidelines are carried out by examining complete blood count, c-reactive protein (CRP) and blood culture before administered antibiotics. Neonatal sepsis was categorized into early-onset neonatal sepsis (EONS; ≤ 72 hours) and late-onset neonatal sepsis (LONS; >72 hours) based on the age of onset sepsis and also into culture-proven and suspected neonatal sepsis based on the positivity blood culture result. Neonatal characteristic consisting of gender, mode of delivery, birth weight, gestational age, and maternal risk factors (premature rupture of mem-

branes, preeclampsia/ eclampsia, and prenatal history of steroids) were reported in this study.

Hematological and microbiological profile. Complete blood count performed by automated hematology analyzer and include white blood count (WBC) differential as evaluation of the WBC based on light scattering characteristics. Anemia defined hemoglobin level <14 g/dL. Leukopenia defined total leukocyte count (TLC) $<4000/\text{mm}^3$ while leukocytosis defined TLC $>34000/\text{mm}^3$. Thrombocytopenia defined platelet count $<150000/\text{mm}^3$ and classified into mild thrombocytopenia ($100000-150000/\text{mm}^3$), moderate thrombocytopenia ($50000-99000/\text{mm}^3$) and severe thrombocytopenia ($<50000/\text{mm}^3$). C-reactive protein (CRP) performed with particle enhanced turbidimetric immunoassay (PETIA) principal. High CRP defined > 10 mg/dL. Blood culture was obtained from venous blood with a minimum volume of 1 ml and directly inoculated into Bactec® bottle containing liquid blood culture media. The specimens were sent to the Clinical Microbiology Laboratory of Dr. Soetomo General Academic Hospital and incubated at $35-37^\circ\text{C}$ for five days or until indicator of bacterial growth to be positive. Medium that showed bacterial growth was subcultured into solid culture media (blood agar plate, MacConkey agar plate and chocolate agar plate) and incubated for 18-24 hours in aerobic condition. Identification and susceptibility test were carried out using the BD Phoenix semi-automated system, and interpreted as extended spectrum beta lactamase (ESBL) based on Clinical and Laboratory Standards Institute (CLSI) 2015 (8).

Data and statistical analysis. Data was listed by number (percentage) and by median (interquartile range) methods. SPSS was used for data and statistical analyzing. Categorical data were analyzed using Chi-square's test or Fisher's exact test. Numerical data were analyzed using Mann-Whitney U test with significance defined as p-value <0.05 .

RESULTS

Culture-proven neonatal sepsis basic characteristic. During the study period, a total of 492 inborn neonates were admitted to the NICU and 104 neonates fulfill the diagnostic criteria for suspected neonatal sepsis. Identified pathogen from blood

culture were confirmed in 52/104 (50%) suspected neonatal sepsis. Incidence rate of culture-proven neonatal sepsis in this study was 10.6% from the total NICU admissions. Culture-proven LONS were reported 39/52 (75%) from all positive blood culture. The median birth weight and gestational age were 1500 (1250-1800) grams and 33 (31.5-34) weeks, respectively. Table 1 listed the basic characteristics of

culture-proven neonatal sepsis.

Hematological profiles of culture-proven neonatal sepsis. Anemia and thrombocytopenia were the two most common abnormal hematological profile in this study. Thirty two (61.5%) neonates had anemia. Anemia more experienced in LONS than EONS. Neonates with culture-proven LONS significantly had

Table 1. Proven neonatal sepsis basic characteristics and hematological profiles

Characteristics	Total n (%)	EONS n (%)	LONS n (%)	p
Neonatal Risk Factors				
Gender				0.331*
Male	30 (57.7)	9 (69.2)	21 (53.8)	
Female	22 (42.3)	4 (30.8)	18 (46.2)	
Mode of delivery				0.017**
Vaginal delivery	16 (30.7)	8 (61.5)	9 (23.1)	
<i>Sectio Caesaria</i>	35 (67.3)	5 (38.5)	30 (61.5)	
Birth Weight (grams)	1500 (1250-1800) [§]	1500 (1000-1900) [§]	1500 (1250-1750) [§]	0.589 [#]
< 1000	2 (3.8)	2 (15.4)	0 (0)	0.564**
1000 – <1500	20 (38.5)	4 (30.8)	16 (41.0)	
1500 – <2500	27 (51.9)	7 (53.8)	20 (51.3)	
≥ 2500	3 (5.8)	0 (0)	3 (7.7)	
Gestational Age (weeks)	33 (31.5-34) [§]	33 (31-34) [§]	33 (32-34) [§]	0.414 [#]
< 28	5 (9.6)	2 (15.4)	3 (7.7)	1.000**
28 – <32	8 (15.4)	3 (23.1)	5 (12.8)	
32 – <37	33 (63.5)	7 (53.8)	26 (66.7)	
≥ 37	6 (11.5)	1 (7.7)	5 (12.8)	
Maternal Risk Factors				
Premature rupture of membrane	15 (28.8)	4 (30.8)	11 (28.2)	1.000*
Preeclampsia/ Eclampsia	21 (40.4)	4 (30.8)	17 (43.6)	0.415*
Prenatal Steroid	12 (23.1)	3 (23.1)	9 (23.1)	1.000*
Hematological Profiles				
Hemoglobin level (g/dL)	13.15 (12.45-15.45) [§]	15.5 (13.0-17.7) [§]	12.9 (11.85-14.3) [§]	0.012 [#]
< 14	32 (61.5)	4 (30.8)	28 (71.8)	0.008*
Total leucocyte count (TLC, /mm ³)	11700 (6405-17590) [§]	11690 (3980-14530) [§]	11710 (6955-18785) [§]	0.315 [#]
<4000	8 (15.4)	4 (30.8)	4 (10.3)	0.096**
Absolute neutrophil count (ANC, /mm ³)	7310 (4920-11580) [§]	5620 (2620-11070) [§]	7400 (4555-12130) [§]	0.492 [#]
	1840 (1120-3145) [§]	1690 (1050-2090) [§]	2080 (1190-3245) [§]	0.286 [#]
Absolute lymphocyte count (/mm ³)	35500 (9000-152500) [§]	117000 (11000-231000) [§]	29000 (9000-128000) [§]	0.375 [#]
Platelet count (/mm ³)	5 (9.6)	2 (15.4)	3 (7.7)	0.512*
100000-150000	6 (11.5)	1 (7.7)	5 (12.8)	
50000-99000	28 (53.8)	5 (38.5)	23 (59)	
<50000	6.4 (3.0-13.05) [§]	6.49 (2.8-10.9) [§]	6.4 (3.2-13.2) [§]	0.575 [#]
CRP (mg/dL)	20 (38.5)	4 (30.8)	16 (41)	0.541*
High CRP (>10)				

[§]median (inter-quartile range) *Chi-Square's test **Fisher's exact test [#]Mann Whitney U-test

lower hemoglobin level than culture-proven EONS. Only 15.4% (8/52) culture-proven neonatal sepsis had an abnormal leukocyte count. All of these neonates had leukopenia and none had leukocytosis. Thrombocytopenia and high CRP were observed in 75% (39/52) and 38.5% (20/52) culture-proven neonatal sepsis, respectively. The hematological profiles of culture-proven neonatal sepsis are listed in Table 1.

Microbiological profile of culture-proven neonatal sepsis. Fifty two neonates identified pathogen isolated from the blood. According to the Gram staining, the Gram negative bacteria were responsible for 75% of culture-proven neonatal sepsis (76.9% EONS and 74.4% LONS). Coagulase negative staphylococci (CoNS) dominated Gram positive bacteria, whilst *Klebsiella* spp. dominated Gram negative bacteria as a cause of culture-proven neonatal sepsis in this study. More than half of the Gram negative pathogen isolated and identified in this study were *Klebsiella* spp. and nearly 90% of them were *Klebsiella pneumoniae*. There was only one *K. pneumoniae* bacteria which did not produce extended spectrum beta lactamase (ESBL). The three common etiology of culture-proven neonatal sepsis in this study were *K. pneumoniae*, CoNS and *Enterobacter cloacae*. Three neonates isolated more than one identified bacteria from the blood culture (mix pathogen), with *Aeromonas hydrophila* – *Enterococcus cloacae* in EONS and *K. pneumoniae* – *Enterococcus faecalis* and *K. pneumoniae* ESBL(+) – *E. coli* ESBL(+) in LONS. The other of microbiological profiles in culture-proven neonatal sepsis are listed in Table 2.

DISCUSSION

Fifty two neonates (10.6%) were identified as culture-proven neonatal sepsis from 492 inborn neonates during this study. This incidence is comparable to incidences reported in other countries as Ethiopia (9.8%) (9), Nigeria (10.6%) (10) and Iran (12.17%) (11). Among all of the culture-proven neonatal sepsis, 57.7% were male, 94.2% were weighing less than 2500 grams and 88.5% were born <37 weeks of gestational age. Higher rate of culture-proven neonatal sepsis in male may be related with X-linked immunoregulatory genes and haploid of X chromosome in males (12). Animal studies also have shown a predominance of the Th-1 type immune response,

Table 2. Microbiological profile of proven neonatal sepsis

Blood Culture Results	Total n (%)	EONS n (%)	LONS n (%)
Gram positive	11 (21.2)	2 (15.4)	9 (22.5)
<i>Staphylococcus aureus</i>	1 (1.9)	0 (0)	1 (2.6)
Coagulase negative staphylococci (CONS)	9 (17.3)	2 (15.4)	7 (17.9)
<i>Bacillus cereus</i>	1 (1.9)	0 (0)	1 (2.6)
Gram negative	38 (73.0)	10 (76.9)	29 (74.4)
<i>Achromobacter</i> spp.	1 (1.9)	1 (7.7)	0 (0)
<i>Acinetobacter baumannii</i>	1 (1.9)	0 (0)	1 (2.6)
<i>Enterobacter cloacae</i>	6 (11.5)	2 (15.4)	4 (10.3)
<i>Klebsiella</i> spp.			
<i>Klebsiella oxytoca</i>	2 (3.8)	1 (7.7)	1 (2.6)
<i>Klebsiella ozaenae</i>	1 (1.9)	0 (0)	1 (2.6)
<i>Klebsiella pneumoniae</i>	25 (48.1)	6 (46.2)	19 (48.7)
<i>Proteus mirabilis</i>	1 (1.9)	0 (0)	1 (2.6)
<i>Serratia marcescens</i>	1 (1.9)	0 (0)	1 (2.6)
Mix pathogens (>1 isolated bacteria)	3 (5.8)	1 (7.7)	2 (5.1)

higher production of pro-inflammatory cytokines (IL-2 and TNF- α) in males rats after LPS administration, and vice versa in female rats (13). The risk of neonatal sepsis increases in proportion to the decrease in birth weight and gestational age (2). Meta-analysis study in Ethiopia reported low birth weight and preterm were 1.42 and 3.36 times more likely to develop neonatal sepsis compared to normal birth weight and term neonates (14). Culture-proven neonatal sepsis dominated in male, preterm and low birth weight also reported by several previous study in India, Saudi Arabia, South Africa, and China (9, 15-18).

More neonates born by *Section caesaria* in this study and significantly higher in the culture-proven LONS compared to culture-proven EONS (Table 1). However, study from Saudi Arabia, China and Canada found that birth route had no impact on culture-proven LONS (15, 16, 19). *Section caesaria* may altered colonization with normal human commensals compared to term infants born vaginally, who will have a variety of colonization beneficial microbiota from the maternal vagina, intestinal and skin microbiota immediately after birth, and responsible for the subsequent alteration of the immune system (19, 20). Intestinal microbiota dysbiosis may causing bacterial translocation across the intestinal into the bloodstream and increases the risk of LONS (21).

Systemic bacterial infection in an immature neonatal hematopoietic system may lead to malfunctions of the hematopoietic system (18). Anemia, leukopenia and thrombocytopenia were observed in 32/52 (61.5%), 8/52 (15.4%), and 39/52 (75%), respectively, in our study. Anemia was observed 12.5% in EONS and 87.5% in LONS. Significantly lower Hemoglobin (Hb) level was detected in LONS than EONS. A study in Iran agreed with this study, anemia was detected in 32.7% and was higher in culture-proven LONS (60%) than in EONS (27%). Mean hemoglobin level also reported significantly lower in LONS than EONS (13.5 ± 1.8 and 15 ± 2 , $p=0.002$) (22). Different definition of anemia was used in a six years retrospective study in China with hemoglobin level < 9 d/dL. Anemia experienced 25% of the neonatal sepsis (18). The development of anemia in septic patient related with alteration in red blood cells (RBCs). Binding of RBCs membrane and the endotoxin (lipopolysaccharide, LPS) altered the RBCs morphology and rheology and increased the clearance of affected RBCs from circulation (23, 24).

Leukopenia was the only abnormal leukocyte count in this study and detected in only 15.4%. Leukopenia only observed 30.8% in EONS and even less 10.3% in LONS. Retrospective study in Saudi Arabia in line with this study, only 9.4% leukopenia observed and developed 25.3% and 9.4% in EONS and LONS (15). About 35% of neonatal sepsis in China also experienced leukopenia, but with the higher limit of leukopenia ($<7500/\text{mm}^3$) (18). In contrast, leukocytosis only reported by 17.7% in Saudi Arabia, even less by 4% in China and none of neonates experienced leukocytosis in this study. The definition of leukocytosis differs from that used in this study. The total leukocyte count (TLC) is a very unreliable indicator of neonatal infection. A normal TLC does not rule out sepsis because as many as 50% of culture-proven neonatal sepsis were within normal limit of TLC (25). The TLC was associated with a higher likelihood ratio of neonatal sepsis only in leukopenia (26).

Three-quarter of culture-proven neonatal sepsis in this study experienced thrombocytopenia. As many as 72% of them classified as severe thrombocytopenia. In a study conducted by Guo et al. (18), thrombocytopenia detected in 29% and 43% of them had a severe thrombocytopenia. Thrombocytopenia also found in 26.5% neonatal sepsis in Saudi Arabia, even with a different limit (15). The pathogenesis of thrombocytopenia in neonatal sepsis is not fully un-

derstood. A direct pathophysiological mechanism of endotoxins produced by Gram negative bacteria may contribute (27). Neonatal sepsis caused by *K. pneumoniae* and *Candida* spp. encountered more anemia, more leukopenia and more thrombocytopenia than caused by other pathogens (18). *K. pneumoniae* was the predominant pathogen in this study and justified for the many cases of anemia, leukopenia and thrombocytopenia, although we did not evaluate fungi as the cause of the culture-proven neonatal sepsis.

High CRP (>10 mg/dL) only took place in 38.5% culture-proven neonatal sepsis in this study. High CRP was positive in only 30.8% and 41% of the EONS and LONS, respectively. Lesser positive CRP, even with lesser cut-off (>5 g/dL), was reported only 17.3% from total positive blood culture sample in Iran (14.4% EONS and 30% LONS) (22). Not concordance with this study, 58% neonates in Saudi Arabia had a high CRP and significantly more common in LONS than EONS (76.4% and 33.3%, $p<0.001$). Although the study did not explained the meaning of high CRP used (15). C-reactive protein (CRP) is an acute phase reactant which most frequently laboratory tests used in the diagnosis of neonatal sepsis (28). However, neonates with fetal hypoxia, respiratory distress syndrome (RDS), meconium aspiration, after trauma/ surgery, and after immunizations also had an elevated CRP. A false-positive rate of 8% has been found in healthy neonates (2). A meta-analysis from 31 study reported the sensitivity and specificity of CRP in diagnosing neonatal sepsis were 69% (95% CI 66- 71%) and 77% (95% CI 76-78%), respectively, with area under curve (AUC) 0.8458 (29).

In all culture-proven neonatal sepsis and by the age of neonatal sepsis (EONS and LONS), Gram negative bacteria dominate the isolated pathogens in this study over Gram positive bacteria. The most common identified pathogens were *K. pneumoniae*, and followed by coagulase negative staphylococci (CoNS) and *Enterobacter cloacae*, respectively. *K. pneumoniae* as the main pathogen in culture-proven neonatal sepsis also reported by Hasibuan et al. in Medan (30) and Wilar et al. in Manado (31) in other Indonesian tertiary NICU. These mostly isolated Gram negative bacteria were also reported in previous study in developing countries by Arowosegbea et al. (10) and Hematyar et al. (22) in 2017, Gao et al. in 2019 (32) and also in developed country by Al-Matary et al. in 2019 (15).

Arowosegbea et al. provided Gram negative bacteria

for 78.9% of all isolated bacteria in 85 culture-proven neonatal sepsis with 26% case-fatality rate. *Klebsiella* spp. (31.6%), *Enterobacter* spp. (21.1%) and CoNS (15.8%) were the predominantly isolated pathogen reported in Nigeria, identically with isolated pathogen in this study. Gram negative bacteria were the only recognized pathogens in EONS and dominated with *Klebsiella* spp. However, Gram positive bacteria mainly CoNS dominated the isolated pathogens in LONS (10). Study in Iran reported 72.7% organism isolated from blood culture were Gram negative bacteria (*Escherichia coli* and *Klebsiella* spp.) (22). Gram negative bacteria (59.8%) dominate over Gram positive bacteria also reported in total isolates of neonatal sepsis in China. The four most predominant bacteria were *K. pneumoniae* (21.9%), *E. coli* (21.9%), group B *Streptococcus* (GBS, 13.2%), and *S. aureus* (6.8%). In EONS, GBS (30.0%) and *E. coli* (20.0%) were dominant, whereas in LONS, *K. pneumoniae* (25.6%) and *E. coli* (22.4%) were dominant (32). Culture-proven neonatal sepsis in Saudi Arabia also confirmed 61.1% of Gram negative bacteria in blood culture and was the leading cause of mortality in EONS and LONS. However, the most frequently isolated pathogen based on onset of neonatal sepsis was Gram positive bacteria, group B *Streptococcus* in EONS and *Klebsiella* spp. in LONS (15).

In contrast, two years retrospective cohort study in India reported the majority of total isolates pathogens were Gram positive bacteria. However, the most common isolated microorganism were *Klebsiella* spp. (31.1%), *S. aureus* (24.5%) and CoNS (22.9%). Gram positive bacteria had a higher isolation rate in EONS and LONS, whereas the most frequent microorganism was *Klebsiella* spp. in EONS and *S. aureus* in LONS (17). Different result also reported from culture-proven neonatal sepsis in South Africa with Gram positive bacteria constituted 53.4% of all positive blood cultures, mainly CoNS (25%), *E. coli* (20.3%) and *S. aureus* (18.2%). In EONS, Gram positive bacteria (mostly CoNS) was dominated even though Gram negative bacteria (mostly *E. coli* and *Klebsiella*) was dominated in LONS (9). The causative pathogens of culture-proven neonatal sepsis vary according to geographical differences and countries (28). In 2015, *K. pneumoniae* was reported as the predominant cause of bacteremia in preterm neonates admitted to NICU in Dr. Soetomo General Academic Hospital (33). High incidence of ESBL producer strain among *K. pneumoniae* in Dr. Soeto-

mo General Academic Hospital was also reported as 50.28% in 2011 (34). The high incidence of nosocomial infections is the reason for the high infection of *K. pneumoniae* and especially the ESBL strains in this study.

Bacillus cereus is a rare pathogenic bacterium, but in premature neonates it can be potentially serious infection of the bloodstream, lungs, and central nervous system (35). In our study, *B. cereus* was isolated in one preterm neonates (34 weeks of gestational age and 1900 grams) as a cause of neonatal sepsis. A previous study in 2013 reported the first report of severe *B. cereus* infections in premature neonates possibly originating from pooled breast milk (PBM). However, the origin of the *B. cereus* strains in the breast milk remain unknown (36). In our study, we did not perform environmental sample evaluation and bacteriological analysis from PBM or from breast milk itself. Meanwhile, breast milk is the main choice for nutrition in premature neonates used in our NICU.

CONCLUSION

Culture-proven neonatal sepsis mostly occurred in preterm and low birth weight neonates. Our study showed that late-onset neonatal sepsis (LONS) were significantly more in infant born by *Sectio caesaria* and significantly more anemia experienced than EONS. Anemia and thrombocytopenia were the most common hematological abnormalities. Severe thrombocytopenia was observed in 72% thrombocytopenia neonates. Isolated pathogens were dominated by Gram negative bacteria over Gram positive bacteria, in all culture-proven neonatal sepsis, in EONS and in LONS. *Klebsiella pneumoniae* was the most pathogen identified as cause of neonatal sepsis in this study, followed by CoNS and *Enterobacter cloacae*. The causative pathogens causing neonatal sepsis was very much dependent on the local microbial pattern.

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