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Artikel Penelitian

TNF-α and Interleukin-6 Levels in Clinical Early Onset Neonatal Sepsis Toward Acute Liver Injury

Kadar TNF-α dan Interleukin-6 Neonatus dengan Klinis Sepsis Neonatorum Awitan Dini Terhadap Terjadinya *Acute Liver Injury*

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ABSTRACT

TNF-α and IL-6 level have an important role in acute liver injury during early onset neonatal sepsis. This study aims to investigate the correlation of TNF-α and IL-6 levels in neonates with clinical early onset neonatal sepsis toward the occurrence of acute liver injury 44 neonates with clinical early onset neonatal sepsis based on SIRS criteria and Rodwell hematologic scored ≥3 were included in this study. Acute liver injury is defined as elevated of AST, ALT, or AST: ALT ratio <1. TNF-α and IL-6 levels were measured using ELISA methods. This study showed that TNF-α correlated significantly with AST level (p<0,001, r=0,570), ALT level (p<0,001, r=0,554), and AST: ALT ratio (p<0,001, r=0,652). This study also showed that IL-6 correlated significantly with AST level (p<0,001, r=0,523), ALT level (p<0,001, r=0,482), AST: ALT ratio (p<0,001, r=0,603). Regression test using backward methods showed that TNF-α influence acute liver injury (indicated by AST, ALT, and AST: ALT ratio) more than IL-6. We concluded that TNF-α and IL-6 level in clinical early onset neonatal sepsis correlated with acute liver injury, whereas early onset neonatal sepsis was correlated with acute liver injury.

Keywords: *Acute liver injury, clinical early neonatal sepsis, IL-6 level, TNF-α level*

ABSTRAK

Kadar TNF-α dan IL-6 berperan penting dalam terjadinya *acute liver injury* selama terjadinya sepsis neonatorum awitan dini.Tujuan penelitian ini yaitu untuk mengetahui hubungan kadar TNF-α dan IL-6 neonatus dengan klinis sepsis neonatorum awitan dini terhadap terjadinya *acute liver injury*.Penelitian ini melibatkan 44 neonatus dengan klinis sepsis neonatorum awitan dini berdasarkan kriteria SIRS dan skor hematologi Rodwell dengan nilai ≥3. *Acute liver injury* didefinisikan meningkatnya AST, ALT, atau rasio AST:ALT <1. Kadar TNF-α dan IL-6 diukur dengan menggunakan metode ELISA. Hasil studi menunjukkan bahwa kadar TNF-α berkorelasi secara signifikan dengan nilai AST (p<0,001, r=0,570), nilai ALT (p<0,001, r=0,554), dan nilai rasio AST: ALT (p<0,001, r=0,652). Studi ini juga menunjukkan bahwan kadar IL-6 berkorelasi secara signifikan dengan nilai AST (p<0,001, r=0,523), nilai ALT (p<0,001, r=0,482), dan nilai rasio AST: ALT (p<0,001, r=0,603). Tes regresi analisis dengan metode *backward* menunjukkan bahwa pengaruh kadar TNF-α terhadap *acute liver injury* yang ditandai dengan AST, ALT, dan rasio AST: ALT lebih besar daripada kadar IL-6. Hasil penelitian menunjukkan adanya hubungan kadar TNF-α dan IL-6 neonatus dengan klinis sepsis neonatorum awitan dini terhadap terjadinya *acute liver injury*, dan didapatkan adanya hubungan neonatus dengan klinis sepsis neonatorum awitan dini terhadap terjadinya *acute liver injury.*

Kata Kunci: *Acute liver injury*, kadar IL-6, kadar TNF-α, sepsis neonatorum awitan dini

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INTRODUCTION

Neonatal sepsis is an important causal factor for mortality and morbidity in newborn. The prevalence of neonatal sepsis reached 1.4 million neonates annually, and its incidence was 1-5 per 1000 live births (1,2). Indonesian Basic Health Research in 2007 showed that neonatal sepsis was the major cause of mortality in newborn aged 7-28 days (3). Previous study also supported that neonatal sepsis was the most causal factor for neonatal hepatitis which in turn lead to acute liver injury (4). Acute liver injury was defined as functional impairment of liver that is caused by several factors including hypo-perfusion, bacterial infection, endotoxin, and inflammatory mediator (4). Acute liver injury indicated worse prognosis in neonatal sepsis (5-7).

Multiple liver biomarkers such as bilirubin, albumin, alkali phosphatase, AST, ALT, LDH, or combination of each biomarker could not predict mortality rate more than 50% (8). Several cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), IL-1β, transforming growth factor- β (TGF-β), IL-18, Interferon-γ (IFN-γ), and IL-10, which are produced by Kupfer cells indicates hepatocellular function impairment responding to septic condition. Therefore these cytokines could be used as biomarker for hepatic dysfunction in septic condition (4). TNF-α is a major cytokine involved in systemic inflammatory response syndrome (SIRS) and stimulates hepatocyte to produce IL-6. In combination, TNF-α and IL-6 are involved in acute phase protein production (4).

There are limited research about TNF-α and IL-6 and their role in acute liver injury in neonatal sepsis. Previous study reported that TNF-α and IL-6 were associated with worse neurodevelopmental outcome in neonatal sepsis. In vivo study showed that TNF-α, IL-6, and LPS (lipopolisacharides) induce hepatic hypermetabolism and decrease hepatic function (9). TNF-α played an important role as biomarker for acute liver injury that was caused by liver abscess (*Entamoeba hystolytica*) in rats (10). This study aims to investigate the correlation of TNF-α and IL-6 levels in clinical early onset neonatal sepsis with acute liver injury.

METHOD

Design

This study used a cross sectional design to investigate the association of TNF-α and IL-6 levels in clinical early onset neonatal sepsis toward acute liver injury. The study was conducted in Neonatal ward Saiful Anwar General Hospital. Subject was consecutively selected based on the following inclusion criteria, i.e. newborn with clinical early onset neonatal sepsis, mode of delivery either per-vaginal or section caesarean, and parents agreement. Neonates with congenital disorders were excluded from the sample.

Early onset neonatal sepsis was defined as sepsis that occurred in first 3 days after birth and characterized by SIRS and Rodwell hematologic score ≥3 (11,12). Briefly, SIRS is defined as heart rate more than 180 beat/minute or below 100 beat/minute, respiratory rate higher than 60 time/minute with retraction or oxygen desaturation, temperature >38,5°C or <36°C, leukocytosis ≥25000 in newborn, 30000 in 12-24 hours after birth, 21000 in second days; or leukopenia <5000. The diagnosis of SIRS is confirmed when at least 2 from the above 4 criteria were fulfilled, in which one of the fulfilled criteria should be included is either abnormal temperature or abnormal leukocyte count.

Rodwell hematologic scored as ≥3, if it meets leukopenia ≤5000 or leukocytosis ≥25000 in newborn or ≥30000 12-24 hours after birth, thrombocytopenia ≤15000/uL, increasing ratio of immature neutrophil:total neutrophil >0,12, immature neutrophil: mature neutrophil >0,3 , degenerative changes of polymorphonuclear cells (PMN) (toxic granulation or vacuole cytoplasmic), increased PMN:neutrophil or increasing or decreasing number of PMN or increasing immature PMN (13-15).

Acute liver injury is defined as elevated serum amino transferase more than 2 times above normal (AST 10-40 U/L ALT 1,2-23,1 U/L), or AST:ALT ratio <1. The ALT is a specific enzyme to detect hepatocyte necrosis in hepatocyte cytoplasm, while AST is an enzyme located in mitochondrial and cytoplasm of liver, skeletal, heart, muscle, renal, and brain. The elevation of those enzymes and cytoplasm release described cell destruction. Cell destruction happens when these enzyme elevate and release cytoplasm. The value of AST:ALT ratio less than 1 indicate that the elevation of both serum amino transferase is mostly due to liver injury than other organs (16,17).

Sample Preparation

Blood sample was taken from eligible subject in no more than 3 days after birth, approximately 2,5 cc (peripheral venous) as the specimen for measuring AST and ALT level. The laboratory examination was performed in Clinical Pathology Laboratory, Saiful Anwar General Hospital.

Measurements of TNF-α and/or IL-6

TNF-α and/or IL-6 were measured by ELISA methods using Human TNF-α and/or IL-6 (R&D System, catalogue number HSTAOOC) according to manufacture instructions. Briefly, 50 µL assay diluents were filled into each well. After added 200 µL standard solution or sample, microplate was closed and then incubated for 3 hours at room temperature. After incubation process, each well was washed from remaining solution and dried before added with 400 µL buffer solution. Next, buffer solution were removed. This washing process were repeated 3 times. Washing process was followed by adding 200 µL TNF-α and/or IL-6 conjugate in each well, closed, and then incubated for 2 hours at room temperature. Incubation process was then followed by washing process using buffer solution. Subsequently, each well was added by 50 µL substrate, closed, and then incubated for 1 hour at room temperature and in dark room. Each well was then added by 50 µL stop solution. Optical density should be measured using microplate reader (Biorad 520, wavelengths 450 nM) in 30 minutes.

Statistical Analysis

Statistical analysis used in this study include normality test using Kolmogorov-Smirnov, correlation test using Pearson test, and regression linier using backward methods. In this study we used regression analysis to examine the influence of TNF-α and IL-6 toward AST, ALT, and AST:ALT ratio. All statistical analysis were conducted using software SPSS for Windows 21.

RESULTS

Subjects Characteristics

Table 1 shows baseline characteristics of 44 subjects

consist of newborn clinical early onset neonatal sepsis in neonatology ward, Saiful Anwar General Hospital (March-April 2015). The characteristics of subjects were divided into neonatal history (gestational week, and birth weight), mother background (age, education, job, and history of infection), clinical, and laboratory findings. Based on the subject characteristics, our study identified that all of the subjecst were exposed to the risk factors of infection as showed by neonatal and mother characteristics, and clinical and laboratory findings. From the neonatal and mother characteristics we identified the following risks, i.e premature rupture of membrane (amniotic) more than 18 hours, greenness color and smelly amniotic fluid, fever, maternal tachycardia, fetal tachycardia, prematurity, birth weight less than 2000 grams, first minute APGAR score 0-6. In contrary, we did not found the higher proportion of history of urinary tract infection, leucorrhea, and tachycardi in mother. The clinical and laboratory findings suggested higher proportion of these following conditions such as: tachycardy, leucocytosis, thrombocytopenia which indicated an infection. Overall, based on the laboratory finding, the proportion of hematologic score 3-4 was higher than 5-6 indicating a probable septic condition, and microbiological assessment using blood culture method showed several etiological factor for neonatal sepsis but 12 (20%) of the subjects did not show any positive bacteria.

Table 1. Characteristics of subject

Characteristic	Frequency (n=44)
Neonatal history	
Sex	
Male	24(24/44)
Female	20(20/44)
Gestational week	
<28 weeks	7(7/44)
28-32 weeks	6(6/44)
32-34 weeks	9(9/44)
34-36 weeks	22(22/44)
37-<42 weeks	-
≥42 weeks	-
Birth Weight	
<1000 grams	6(6/44)
1000-1500 grams	16(16/44)
1500-2500 grams	22(22/44)
≥2500 grams	-
Fetal tachycardia	
Yes	13(13/44)
No	8(8/44)
Amniotic fluid	
Clear + reddish	30(30/44)
Green (cloudy, meconeal)	14(14/44)
Duration of amniotic membrane disruption	
<18 hours	24(24/44)
>18 hours	20(20/44)
Mode of delivery	
Abdominal delivery (section cesaria)	33(33/44)
Vaginal delivery	11(11/44)
APGAR score at first minute	
<5	17(17/44)
5-6	15(15/44)
≥7	12(12/44)
Mother backgrund	
Age	
<17 years old	1(1/44)
17-35 years old	38(38/44)
>35 years old	5(5/44)

Table 1. Characteristics of subject

Characteristic	Frequency (n=44)
Education	
Elementary School	1(1/44)
Junior High School	12(12/44)
Senior High School	28(28/44)
Bachelor Degree	3(3/44)
Occupation	
Housewife	35(35/44)
Health professionals	2(2/44)
Others	7(7/44)
History of fever	
Yes	26(26/44)
No	18(18/44)
History of leucorrhea	
Yes	23(23/44)
No	21(21/44)
History of urinary tract infection	
Yes	13(13/44)
No	31(31/44)
Leukocyte count	
<15000	12(12/44)
>15000	26(26/44)
Tachycardia	
Yes	21(21/44)
No	23(23/44)
Administration antibiotics (mother)	
Yes	31(31/44)
No	13(13/44)
Clinical findings	
Pulse rate> 180 times/minute or < 100 times/minute	
Yes	29(29/44)
No	15(15/44
Respiratory rate> 60 times/minute with retraction or oxygen desaturation	
Yes	36(36/44)
No	8(8/44)
Unstable body temperature	
Yes	32(32//44)
No	12(12/44)
Capillary refill time> 3 second	
Yes	29(29/44)
No	15(15/44)
Laboratory findings	7(7/44)
Leukocyte count	17.672,84±9438,41
Thrombocyte count	147295,45±103582,93
Elevated I/T ratio	0,116±0,113
Elevated I/M ratio	0,1498±0,15063
C-Reactive protein	3,383±5,285
Elevated total PMN count	70,58±13,56
Elevated immature PMN	5,5±3,04
Hematologic score system	
3	21
4	13
5	6
6	4
Klebsiella pneumonia	6
Staphylococcus coagulase negative	2
Serratia liquifeciens	6
Steril	12

Note: *Subject that were 44 neonates with clinical early onset neonatal sepsis have risks factor for infection including premature rupture of membrane (amniotic) more than 18 hours, greenness color and smelly amniotic fluid, fever, elevated leukocyte count, urinary tract infection, leucorrhea, maternal tachycardia, fetal tachycardia, prematurity, birth weight less than 2000 grams, first minute APGAR score 0-6.*

The Role of TNF-α and *IL-6 in Predicting AST, ALT, and AST:ALT Ratio as the Indicator for Liver Injury*

The correlation analysis reveal a statistically significant and

moderate correlation between TNF- α and IL-6 with the three indicators for acute liver injury (AST, ALT levels and AST:ALT ratio) with slightly higher strength in AST:ALT ratio. Meaning that both of the marker TNF- α and IL-6 are associated with the condition of acute liver injury.

Table 2. The correlation between TNF-α and *IL-6* with AST, ALT level and AST:ALT ratio

Independent Variables	Dependent Variables		
	AST	ALT	AST:ALT rasio
TNF-α	r=0,570; p<0,001	r=0,554; p<0,001	r=0,652, p<0,001
IL-6	R=0,523, p<0,001	R=0,482, p<0,001	R=0,603; p<0,001

The regression using three outcome indicators for acute liver injury shows that TNF α & il-6 simultaneously have significant and higher explaining variance when using AST:ALT ratio as the indicator for acute liver injury compared to AST or ALT level. Simultaneously the level of TNF and IL-6 explain 42,5% variance of AST:ALT ratio, and 30,7% variance of ALT, and 32,5% of AST. Partially, in all regression model with three different outcome indicator, TNF has higher and significant coefficient impact in explaining acute liver injury. The coefficient impact of TNF is higher when the IL-6 was removed from the model, without significant changes in explaining variance. This indicating that TNF is a better marker compared to IL-6 in predicting the acute liver injury as measured by AST, ALT level and its ratio. Furthermore the use of AST:ALT level has a better explaining variance for indicating acute liver injury.

Table 3. The regression model of TNF-α and IL-6 levels toward AST, ALT, AST:ALT ratio

Y	Regression equation model	*p-value*	R^2
AST	Model 1:		
	$\hat{y}_1$ =-17.613+ 18.025 x_1 + 0,775 x_2	0,000<α	0,357
	(p=0,139) (p=0,026) (p=0,163)		
	Model 2:		
	$\hat{y}_1$ = -19.995 + 25.725 x_1	0,000<α	0,325
	(p=0,095) (p=0,000)		
ALT	Model 1:		
	$\hat{y}_2$ =-18.277+ 24.292 x_1 + 0,754 x_2	0,000<α	0,326
	(p=0,235) (p=0,022) (p=0,293)		
	Model 2:		
	$\hat{y}_2$ = -20.594 + 31.784 x_1	0,000<α	0,307
	(p=0,178) (p=0,000)		
AST:ALT	Model 1:		
	$\hat{y}_3$ =-0,009+ 0,263 x_1 + 0,012 x_2	0,000<α	0,469
	(p=0,949) (p=0,007) (p=0,072)		
	Model 2:		
	$\hat{y}_3$ = -0,045 + 0,380 x_1	0,000<α	0,425
	(p=0,748) (p=0,000)		

Explanation: $\hat{y}_2$ = ALT, x_1 = TNF-α level, x_2 = IL-6 level, R^2 = determination coefficient

DISCUSSION

The baseline characteristics of our study showed that subject are mostly aterm, with low birth weight (2500 grams). Salem and colleagues reported that gestational age at 28-32 weeks is an independent risk factor for early onset neonatal sepsis (18). Another study showed that preterm birth caused neonatal sepsis in 30-40% neonates (19). Simonsen and colleagues reported that early onset neonatal sepsis occurred mostly in premature or low birth weight newborn (20). Conversely, Fida and colleagues reported that in aterm neonates with neonatal sepsis, there were no significant differences in sex, birth weight, mode of delivery, APGAR score in the first 5 minutes as compared to control group.

Risk factors for neonatal sepsis identified in this study are fever, leucorrhea, urinary tract infection, elevated leukocyte count, maternal and fetal tachycardia, prolonged premature rupture of membranes, dystocia, and low APGAR score. These findings was concurred with previous study reported that prenatal factor features such as chorioamnionitis, premature rupture of membrane (PROM), intrapartum fever, and prematurity were associated with increased risk of early onset neonatal sepsis (19). Simonsen and colleagues had showed that early onset neonatal sepsis increased 1% in PROM case (duration of PROM incidence and delivery was more than 18 hours) (20). Early onset neonatal sepsis increased more (about 1-4%) in mother with chorioamnionitis(20).

Early onset neonatal sepsis was defined as SIRS that was caused by infection in neonates before 72 hours after birth (12,22,23). Baseline characteristics focused on clinical sign of neonatal sepsis showed pulse rate >180 times/minute or <100 times/minute, respiratory distress, unstable body temperature, capillary refill time more than 3 seconds. Clinical signs of neonatal sepsis were marked by respiratory sign (tachypnea, grunting, retraction, respiratory failure), cardiovascular sign (tachycardia, bradycardia, hypotension, prolonged capillary refill time), and impaired of temperature regulation (hyperthermia or hypothermia) (24). Based on hematologic characteristics, this study showed that score 3-4 (probable sepsis) were more prevalent than score 5-6 (high probable sepsis). Theoretically, leukocyte count, CRP, PMN/neutrophil count, and I/T ratio were indicators that highly used to support diagnosis of sepsis. Total PMN count is more accurate as a diagnosis criteria for neonatal sepsis when compared to total leukocyte count. I/T ratio measurement was sensitive neonatal sepsis predictor, in which score ≥ 0,2 was good indicator for infection (20). Hematologic score could be used as early screening for neonatal sepsis which higher score indicated highly probable sepsis (13-15). Leukocyte count abnormality and total PMN count abnormality could be caused by immune system immaturity in neonates (21). The immune system in neonates adapts with intrauterine environment by decreasing Th1 cytokines production and increasing Th2 cytokines production. This condition had beneficial effect in lowering allo-immune rejection, thus decreasing the risk of prematurity. In early neonates, this condition could lead to a low immune response, both natural and adaptive (20).

Blood culture taken from 44 subjects with neonatal sepsis showed positive result in 12 samples. *Klebsiella pneumonia, E. baumanii,* and *Serratia liquifeciens* were the most etiologic factor for neonatal sepsis in this study, and followed by negative coagulase *Staphylococcus sp.* Simonsen and colleagues had described that GBS and *E.coli* were the most important pathogen in early onset neonatal sepsis both in preterm and aterm newborn (20). Gold standard for diagnosis neonatal sepsis was the presence of bacterial in blood culture. Blood culture had

several weaknesses such as duration of method (more than 3 days), false positive result, inadequate volume, intermitten bacteremia condition or low density bacteremia, suppression of bacterial growth caused by mother antibiotic administration (26,27). Negative result of blood culture in this study might be caused by blood sampling method (standardized method, blood sampling was 2 times from different extremities). In this study, one time blood sampling and using of antibiotics were limitations of study. Early onset neonatal sepsis research should differentiate positive and negative culture group, and should be analyzed in each group in hematological score, AST, ALT and AST:ALT ratio.

TNF-α, IL-1β and IL-6 were endogenous mediator for immune response toward bacterial infection, and thereby used as non-specific screening (20,28). Bender and colleagues had reported that IL-6 combined with procalcitonin could be used for detection of early onset neonatal sepsis, but not with TNF-α (29). Meta-analysis study showed that TNF-α had moderate accuracy for diagnosing early onset neonatal sepsis (sensitivity 66%, specificity 76%) and delayed onset neonatal sepsis (sensitivity 84%, specificity 83%) (30). Ying Fan and colleagues had reported that pro-inflammatory cytokines and other biomarkers such as CRP could not be used alone to diagnose or exclude neonatal sepsis, such as pro-calcitonin, IL-6 and IL-8 which were more superior compared to CRP, TNF-α, and IL-1β (28).

Hepatocyte injury was marked by elevation of several biomarkers such as aminotransferase, alkaline phosphatase, glutamil transpeptidase, 5-nukleotidase, leucine aminopeptidase (17). ALT or known as alanin aminotransferase was specific enzyme for hepatocyte necrosis, while AST or aspartat aminotransferase was not specific enzyme and could be found in skeletal, myocardial cells, renal, and brain tissue. Elevated level of those enzymes indicated cell lysis. Acute liver injury was defined as impairment of hepatic function marked by elevated amino transferase serum more than 2 times above normal level. Normal level of ALT in neonates was 1,2-23,1U/L. Normal level of AST in neonates was 10-40 U/L. AST:ALT ratio <1 indicated acute liver injury (16,17). In this study, acute liver injury was marked by mean of AST level 31,95 U/L, mean of ALT level 43,59 U/L, and AST:ALT ratio 0,7. Acute liver injury correlated with mortality in septic patients. However, in clinical practice, impairment of hepatic function could not be easily detected. In the other hand, impairment of hepatic function induced by sepsis contributed in severity of disease (4), thereby biomarker for hepatic injury was needed. Several cytokines such as TNF-α and IL-6 could induce hepatocellular function disability in response toward endotoxin (4). This study showed significant positive correlation between TNF-α and IL-6 level and AST, ALT, and AST:ALT ratio. Furthermore, TNF-α level influences AST level, ALT level, and AST:ALT ratio more than IL-6 in neonates with clinical early onset neonatal sepsis. The limitation of this study is that we did not examine AST and ALT levels in serial before and after neonate diagnose early onset neonatal sepsis to examine the effectof early onset neonatal sepsis toward *acute liver injury.*

The result of this study shows moderate correlation between TNF-α, IL-6 and AST, ALT, AST:ALT ratio. The remaining indicates other factors contribute. The possibility is that TNF-α has double role, including induction of hepatocyte proliferation and liver regeneration (35). Study done by Xiaoling and colleagues showed that IL-6 also affect in liver regeneration in early phase through IL-6 transignalling process. Correlation TNF-α and IL-6 to AST:ALT ratio should have negative correlation, the more level of TNF-α and IL-6, the more AST:ALT ratio until <1. This indicates increasing AST more than increasing ALT. AST increase not only in liver injury but also injury of skeletal, heart, brain, and kidney (16,17).

Kramer and colleagues had reported that early stage of acute liver injury occurred in 11% patient with critical condition, thus hepatic dysfunction represented worse prognosis (7). Infection and metabolic disorders were leading cause of hepatic failure in neonates (31,32). In early phase of septic shock, hepatic dysfunction was induced by gut-derived norepinephrin, thus it activated α-2 adrenoceptor. This activation was followed by stimulation of Kupffer cells to release TNF-α and suppress hepatocellular function (33). Ding and colleagues showed that IL-6 was increaseing in animal model of sepsis and elevation of IL-6 significantly induced elevation of ALT and AST plasma (34). Xiaoling and colleagues had described the effect of IL-6 in acute liver injury. IL-6 plays an important role in regeneration of hepatocellular (1-2 days) and then impaires regeneration of hepatocellular in next phase (35).

Sepsis has correlation with hepatic ischemia and reperfusion injury. In septic and hepatic failure condition, there was elevated hepatosplanchnic blood flow, oxygen consumption, and delivery. In septic shock condition, splanchnic tissue oxygenation was caused by elevated metabolic demand, reflected by elevated consumption of tissue oxygen and tissue oxygenation impairment. Elevated proinflammatory cytokines production and reactive oxygen species (ROS) were contributing increased oxygen demand. Septic condition together with respiratory impairment characterized by elevated intrathoracal tension caused by mechanical ventilator could contribute to decreasing hepatic blood flow (6). Inflammatory process also involves another immune cell, including netrophyl. Netrophyl consists granules with many enzymes, like gelatinase, specific granule and azurophyl granule, and antimicrobial peptide. Netrophyl products have important role in inflammation due to infection process, which in this study was not performed (35).

The impact of TNF-α and IL-6 in clinically early onset neonatal sepsis in this study has not been distinguished with other conditions such as low birth weight, prematurity, asphyxia, and etiology early onset neonatal sepsis. TNF-α and IL-6 levels should also be checked to know the influence of neonatal sepsis to that condition. This study demonstrates that TNF-α and IL-6 levels in clinical early onset neonatal sepsis correlated with acute liver injury, whereas early onset neonatal sepsis was correlated with acute liver injury. In clinical practice, this study demonstrates the possibility to develop new drugs, for example anti TNF-α, as therapy for clinical early onset neonatal sepsis.

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