

Ursodeoxycholic acid in neonatal sepsis-associated cholestasis

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Abstract

Background Sepsis-associated cholestasis (SAC) is an intrahepatic cholestasis caused by inflammatory cytokines. Patients with this condition have poor prognoses. Antibiotics are the mainstay of therapy, however, other adjuvant therapies, such as ursodeoxycholic acid (UDCA), have not been well established.

Objective To assess the effect of UDCA for treatment of neonatal sepsis-associated cholestasis.

Methods We performed a randomized, double-blind, controlled trial in 37 neonates who were diagnosed with sepsis-associated cholestasis in the Neonatal Care Unit of Cipto Mangunkusumo Hospital. Subjects were divided into two groups, with 19 neonates randomly allocated to the intervention group (received UDCA at 30 mg/kg/day divided into 3 doses for 7 days) and 18 neonates to the control group (received placebo). After 7 days of treatment, we evaluated the subjects' liver function parameters and performed a survival analysis.

Results Liver function parameter improvements at day 7 were not significantly different between the UDCA group and the control group, including for mean decrease of total bilirubin (TB) levels [2.2 (SD 2.9) mg/dL vs 1.7 (SD 4.6) mg/dL; $P=0.080$], mean decrease of direct bilirubin (DB) levels [1.1 (SD 2.3) mg/dL vs 0.6 (SD 3.6) mg/dL; $P=0.080$], median indirect bilirubin (IB) levels [0.4 (range 0.1- 5.6) mg/dL vs 0.9 (range 0.1-4.1) mg/dL; $P=0.358$], mean decrease of alanine aminotransferase (ALT) levels [0.5 (-80.0 - 21.0) U/L vs -2.0 (ranged -167.0 - 85.0) U/L; $P= 0.730$], median aspartate aminotransferase (AST) levels [43.0 (range 14.0-297.0) U/L vs 150.0 (range 24.0-840.0) U/L; $P=0.081$], and median *gamma-glutamyl transpeptidase* (GGT) levels [125.0 (48.0-481.0) U/L vs 235.0 (56.0-456.0) U/L; $P=0.108$]. Five neonates in control group died compared to two in the UDCA group ($P=0.232$). In addition, UDCA did not significantly lengthen the survival time (hazard ratio/HR 3.62; 95%CI 0.69 to 18.77).

Conclusion Ursodeoxycholic acid tends to improve total bilirubin, direct bilirubin, and AST levels in sepsis associated cholestasis. [Paediatr Indones. 2014;54:206-12.].

Keywords: sepsis-associated cholestasis, liver function, mortality, ursodeoxycholic acid

Neonatal sepsis remains a major cause of morbidity and mortality among neonates.¹ Sepsis increases the cost and duration of hospital stay. The average length of hospitalization caused by sepsis is 3 weeks, resulting in an economic burden of nearly \$17 billion annually in the United States.² Sepsis is also an important cause of hospitalization in the Neonatal Care Unit of Cipto Mangunkusumo Hospital, Jakarta. A study reported that at our hospital, 13.5% of newborns needed to be hospitalized and 52% of those are due to sepsis.³

Cholestasis induced by inflammation, is a common complication in patients with extra-hepatic infection or inflammatory processes, and generally referred to as sepsis-associated cholestasis (SAC).¹ The incidence of cholestasis increases up to 20%-60% in neonates with sepsis.^{3,4} Neonates with sepsis and cholestasis had to be hospitalized longer and have a higher rate of death compared to neonates with sepsis without cholestasis. Neonates with SAC were reported to have a relative risk 2.25 times higher for mortality compared to neonates without cholestasis.³

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Sepsis-associated cholestasis is a form of hepatocellular cholestasis which develops because of bile acid transport dysfunction. Microbial products, including endotoxin, can lead to the activation of pro-inflammatory cytokines. Pro-inflammatory cytokines inhibit the transport of organic anions at both the sinusoidal and canalicular membrane of hepatocytes, affecting hepatocyte uptake and excretion of bile acids.⁴⁻⁷

The mainstay treatment for SAC is antibiotics. Nevertheless, other substances have been reported to have potential benefits for SAC. Ursodeoxycholic acid (UDCA) has been reported to benefit cholestatic jaundice.^{4,8} Ursodeoxycholic acid improves the flow of bile acid, prevents apoptosis of hepatocytes, changes bile acid compounds, and has an immunomodulatory effect.⁹ An animal study showed that UDCA improved liver function parameters in SAC by increasing the transporter protein.¹⁰ Few human studies have investigated a potential benefit of UDCA for SAC. We hypothesized that UDCA may be beneficial for neonates with SAC, thus, we conducted this study to assess for an effect of UDCA in neonates with SAC.

Methods

We conducted this randomized, double-blind, placebo controlled trial study at the Neonatal Care Unit of Cipto Mangunkusumo Hospital from January to October 2012.

Subjects were neonates who were diagnosed with SAC and selected by consecutive sampling. Inclusion criteria were full term infants aged 0-28 days or premature infants with correctional age 42 weeks, diagnosed with sepsis proven by positive blood cultures, and the presence of cholestasis. Cholestasis was defined as increased direct bilirubin >20% with total bilirubin >5 mg/dL, or direct bilirubin >1 mg/dL with total bilirubin <5 mg/dL. We excluded patients who refused to participate in the trial or if their 3-portion stool test was all white or clay-colored.

The minimum sample size required was calculated to be 25 for each group. Patients were randomized by a 6-block technique. Patients in the intervention group received UDCA (*Urdafalk*[®]) at 30 mg/kg/day divided into 3 doses for 7 days, while they in placebo group received an identical-looking placebo.

Primary outcomes were improvement in liver

function parameters. We measured total bilirubin (TB), direct bilirubin (DB), indirect bilirubin (IB), aminotransferase (AST and ALT), and gamma-glutamyltransferase (GGT). Those parameters were examined on the 1st day and 7th day of drug administration. After therapy, subjects were followed until the time of hospital discharge or death. Duration of hospital stay and mortality status were secondary outcomes. Duration of hospitalization was defined as the number of days from the time that sepsis was established until the day of discharge or death.

Infection parameter tests were conducted in the Neonatology Unit Laboratory. Leukocytes and platelets counts, as well as immature/total neutrophil (IT) ratio were counted manually, while C-reactive-protein (CRP) was measured by *NycoCard*[®] CRP single test. Normal values were considered to be 5,000-34,000/ μ L for leukocytes counts, 150,000-300,000/ μ L for platelets counts, <10 mg/dL for CRP, and <0.2 for IT ratio.¹¹ Liver function tests were conducted in the Clinical Pathology Laboratory with colorimetric methods by *Cobas c311*[®]. Normal values were considered to be <12 mg/dL for TB, <0.3 mg/dL for DB, <0.1-0.7 mg/dL for IB, <84 U/L for AST, <60 U/L for ALT, and <204 U/L for GGT.¹²

Demographic and clinical data is presented in numbers and percentages. We performed independent T-test and Mann-Whitney test to compare parameters between the intervention and control groups. Intention-to-treat was applied to analyze mortality outcome and duration of hospitalization. In addition, survival analysis by Kaplan-Meier method was conducted to assess the survival rate, and Cox proportional-hazards model was used to calculate relative risks. Data analyses were performed with a type I error of 5% and power of 80%. This study was approved by Ethical Clearance Committee of University of Indonesia Medical School.

Results

During the study periods there were 109 neonates with sepsis confirmed by positive blood cultures. We excluded 13 neonates who died, 2 neonates due to being discharged against medical advice, and 6 neonates with gastrointestinal tract abnormalities. Of 88 subjects who met the inclusion criteria, 48 neonates (54.4%) had

SAC, but only 37 (78.0%) could receive oral treatment. Hence, 19 neonates received the UDCA treatment and 18 received a placebo. In the follow up, 4 subjects in the intervention group had to discontinue treatment because they were unable to take oral medication and 1 subject was discharged against medical advice before the 7-day treatment period ended. In the placebo group, 3 subjects had to discontinue treatment (unable to take oral medication) and 1 was discharged before the 7-day treatment period ended (Figure 1).

Subjects' characteristics were similar in the two groups. Subjects were predominantly male (23/37), preterm (27/37), and with low birth weight <2,500 grams (27/37). The most common clinical presentations were hyperthermia (19/37), lethargy (12/37), and respiratory distress (10/37). The most prevalent infection markers were thrombocytopenia (30/37) and elevated CRP > 10 mg/dL (32/37). Gram-negative bacteria as the etiology of sepsis were found in 21 subjects. *Acinetobacter sp.* were the most common bacteria found (8 subjects). Systemic fungal infection occurred in 2 neonates.

We examined urine bilirubin prior to blood examination of liver function parameters. There were no differences between the onset of cholestasis in the two groups, which occurred predominantly at day 6 (5.9 vs 7.4; P = 0.180).

Nine neonates failed to complete 7 days of treatment. Therefore, we performed liver function parameter assessments in only 28 neonates. Data were interpreted with median values due to non-homogenous distribution of data. On the first day of therapy, liver function parameters were higher in the placebo than in the UDCA group (Table 1).

We used differences between TB, DB, and ALT values before and after intervention to analyze the outcome of liver function after seven days of treatment. Because the initial AST, IB and GGT between the two groups did not differ significantly, we used the after intervention value as the outcomes. Decreases of TB and DB levels were greater in UDCA group than in the placebo group (Table 2), but these differences were not statistically significant (P >0.05). Levels of AST and GGT at the end of treatment were lower in

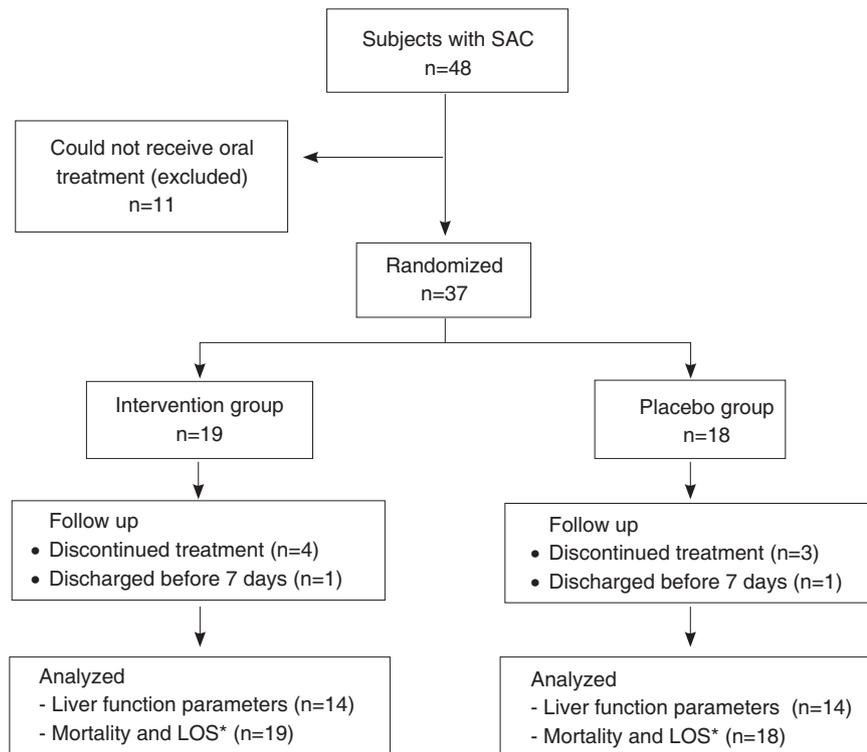


Figure 1. Flow diagram of the study

*LOS=length of stay

Table 1. Initial laboratory values for liver function

Variables	Placebo n=14	UDCA n=14
Median total bilirubin level (range), mg/dL	8.8 (1.9-21.8)	5.2 (2.1-10.5)
Median direct bilirubin level (range), mg/dL	7.1 (1.4-20.1)	2.1 (1.2-7.5)
Median indirect bilirubin level (range), mg/dL	0.9 (0.6-6.8)	1.7 (0.4-6.4)
Median ALT* level (range), U/L	65.0 (6.0-281.0)	15.5 (8.0-92.0)
Median AST* level (range), U/L	95.0 (15.0-703.0)	49.5 (17.0-155.0)
Median gamma-GT** level (range), U/L	302.5 (108.0-869.0)	265.0 (88.0-928.0)

*ALT: alanine aminotransferase; AST: aspartate aminotransferase; Gamma-GT: gamma-glutamyl transferase

Table 2. Comparative analyses of liver function improvement as the outcomes (n= 28)

Variables	Placebo n=14	UDCA n=14	P value
Mean TB decrease (SD), mg/dL*	1.7 (4.6)	2.2 (2.9)	0.080 ¹
Mean DB decrease (SD), mg/dL*	0.6 (3.6)	1.1 (2.3)	0.080 ¹
Median IB (range), mg/dL**	0.9 (0.1-4.1)	0.4 (0.1-5.6)	0.358 ²
Median ALT (range), U/L**	-2.0 [(-167.0) – (85.0)]	0.5 [(-80.0) - (1.0)]	0.730 ²
Median AST (range), U/L**	150.0 (24.0-840.0)	43.0 (14.0-297.0)	0.081 ²
Median GGT (range), U/L**	235.0 (56.0-456.0)	125.0(48.0-481.0)	0.108 ²

¹Unpaired t-test

² Mann-Whitney test

*(initial minus final laboratory result), **the final laboratory result

TB=Total bilirubin, DB=Direct bilirubin, IB=Indirect bilirubin, ALT=Alanine aminotransferase, AST=Aspartate aminotransferase, GGT=gamma-glutamyl transferase

Table 3. Clinical trial analysis with mortality as the outcome

Group	Mortality		Total	RR	95%CI	P value ¹
	Yes	No				
UDCA, n	2	17	19	0.810	0.583 to 1.118	0.232
Placebo, n	5	13			18	
Total, n	7	30			37	

¹Chi-square test; relative risk reduction (RRR) = 62.1%; number needed to treat (NNT) = 6

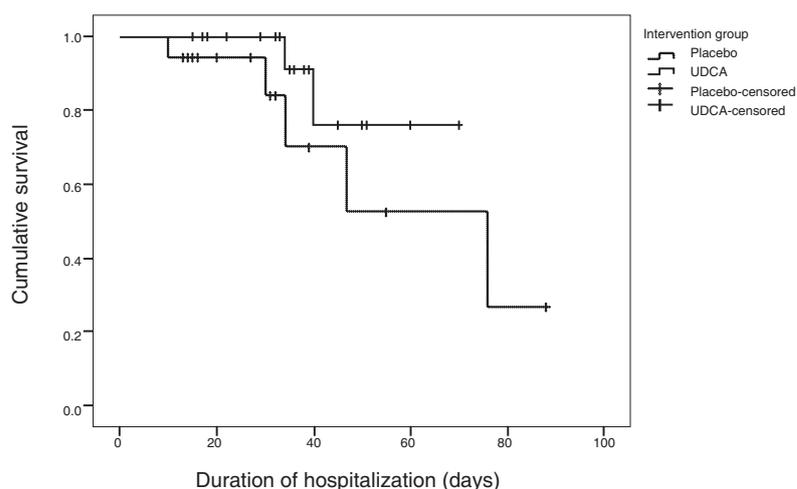


Figure 2. Kaplan-Meier curve for survival of subjects in the placebo and UDCA groups

the UDCA than in the placebo groups, but again the results were not statistically significant ($P > 0.05$).

Deaths from sepsis-related cholestasis occurred in 7 of 37 subjects, 2 from the UDCA group and 5 from the placebo group. Clinical trial analysis revealed that UDCA treatment could decrease mortality 62.1% compared to placebo with number needed to treat was 6 (Table 3), however the Kaplan-Meier curve showed no statistically significant differences in survival and mortality rates between the two groups ($P=0.232$). Cox-regression analysis also showed that UDCA lengthened the survival time [hazard ration/HR = 3.62 (95%CI 0.69 to 18.77)], but it was not statistically significant.

There was no significant difference in median duration of hospitalization between the two groups [28.5 (range 10-88) days vs. 35 (range 15-70) days; ($P=0.150$)].

Discussion

The World Health Organization (WHO) reported infectious disease to be the highest cause of death in neonates, with 5 million deaths per year. Sepsis is still a major problem in neonates. The prevalence of sepsis in Cipto Mangunkusumo Hospital, Jakarta is 8%, higher than the 1-5% previously reported in developed countries.³ A previous study has described how sepsis may trigger the development of intrahepatic cholestasis, which in turn increases the mortality rate in neonates.⁴

We found the prevalence of SAC in neonates to be 54.4%. Bachtiar *et al.* showed a slightly higher prevalence, 65.9%, in 2007.³ Tufano *et al.* found that its prevalence in Naples, Italy in 2009 was 20%.¹³ The varying results may be due to differences in study design and the multifactorial nature of SAC pathogenesis.¹⁴

Almost three out of four neonates with SAC had body weight under 2,500 grams, which may be associated with premature birth, similar to a previous report.¹⁵ Sepsis-associated cholestasis occurs mostly in preterm infants and is related to the immaturity of their immune systems. Although SAC is found more frequently among preterm infants, Bachtiar *et al.* reported that gestational age <38 weeks was not a significant risk factor for developing SAC.³

Sepsis is a clinically-based diagnosis, but many studies have attempted to justify the use of laboratory parameters that are strongly associated with sepsis in an effort to improve management. One of the parameters for sepsis is CRP, which has high sensitivity and specificity, but it appears 6-8 hours after the onset of sepsis and its rise starts after 20-48 hours.¹⁶ In this study, we found two altered laboratory parameters, CRP and thrombocytopenia.

Hospitals have different patterns of microorganisms. In this study, more than half of the microorganisms found in blood cultures were Gram-negative bacteria. Tiker *et al.* reported that *Escherichia coli* was the main cause of sepsis (46.6%) among Gram-negative bacteria.¹⁵ However, Bachtiar *et al.* found no association between Gram-negative bacteria and the incidence of cholestasis.³

Urine bilirubin has been used as a non-invasive screening tool for SAC. In our study, positive bilirubin strip tests that were confirmed with positive bilirubin serum tests were used to establish the time of onset of cholestasis. This test showed that most cholestasis occurred on the 6th day of sepsis. Similarly, another study reported that cholestasis occurred on the 7th day of sepsis.³ However, Tiker *et al.* reported a longer onset, on the 10th day.¹⁵ These differences may be explained by the diversity of sepsis severity in these studies.

Bilirubin levels in sepsis are positively correlated with the outcome. This hypothesis was supported by the findings of Tufano *et al.* who reported that elevated bilirubin (12.23 ± 3.76 mg/dL) was an independent predictor of mortality.¹³ The other liver function parameters such as ALT and AST indicate hepatocyte damage. Both ALT and AST levels rise during sepsis, but AST and ALT levels in sepsis accompanied by cholestasis are generally higher than in sepsis without cholestasis.¹⁴ In the early stages, ALT and AST levels may be as high as 3-6 times the normal values.¹³ Aspartate amino transferase (AST) is also produced by other cells, therefore, in sepsis with multiorgan failure, AST level will be elevated higher than ALT level.^{4,7}

In our study, the initial liver function levels were found to be higher in the placebo group, which may have affected the outcomes. The limitations of this study were that we did not have the required sample size (37 instead of 50) due to time limitation and not all subjects underwent liver function examinations

after 7 days of treatment. The median total bilirubin level in the placebo group was higher than in the UDCA group, but both were below the bilirubin level correlated with poor prognosis. Levels of AST and GGT were also higher in the placebo group, but the difference was not statistically significant. Oswari *et al.* evaluated the prognostic value of bilirubin, AST, ALT, and GGT and reported that only AST and GGT had prognostic value to predict the outcomes of SAC patients.¹⁷ The prognosis of the two groups in our study did not differ significantly, although the initial liver function parameters were different.

The main therapy for SAC is administration of antibiotics.^{4,7} Other adjuvant therapies are drugs that improve bile acid flow, such as UDCA.⁴ Maldonado *et al.* compared the efficacy of UDCA (10 mg/kg/day) and phenobarbital (3 mg/kg/day) in neonates with cholestasis caused by prolonged parenteral nutrition. They concluded that UDCA was superior to phenobarbital, with a significant improvement in bilirubin of about 2.29 mg/dL ($P < 0.01$).¹⁸

Ursodeoxycholic acid may enhance protein transporter production in the basolateral membrane and canaliculi membrane, thereby improving bile acid secretion and decreasing bilirubin serum levels. Furthermore, UDCA may reduce hepatonecrosis by decreasing mitochondrial permeability and improving the delivery of cytochrome c, as clinically confirmed by improved AST and ALT levels.

In our study, TB and DB improvement in the UDCA group was higher than in the placebo group. Although this difference was not statistically significant, this finding was clinically important, since lower bilirubin values are indicative of better prognoses. The median level of AST, another liver function parameter, at the end of therapy was reduced in the UDCA group. In contrast, the AST value in the placebo group was 3 times higher than the UDCA group. The difference was not statistically significant, but like bilirubin values, AST has also been related to prognosis. Similarly, Al-Hathol *et al.* showed an improvement in AST level after administration of UDCA (15-20 mg/kg/day), while the ALT and GGT levels did not significantly improve.¹⁹ However, Tufano *et al.* reported no improvement in ALT, AST, and GGT levels with administration of UDCA (30 mg/kg/day).¹³ Varying study results and the lack of statistically significance in our study may be explained by the smaller

than required sample size and the length of UDCA administration. Tufano *et al.* reported that there were no changes in AST, ALT, and GGT values after 10.5 days of UDCA treatment.¹³ However, Hathol *et al.* reported significantly decreased TB and DB levels after 2 weeks, and decreased AST level after 3 weeks of treatment.¹⁹ Oswari *et al.* reported that improvement and resolution of cholestasis occurred on the 19th day after the onset of sepsis.¹⁷ Administration of UDCA for only 7 days might not have allowed time for cholestasis resolution.

In this intention-to-treat analysis, mortality rate was 19% (7 cases), with 2 cases in the UDCA group and 5 cases in the placebo group. Number needed to treat from our study was 6 patients. Among the 6 patients treated with UDCA, the risk of mortality can be reduced by 62.10% (relative risk reduction). However, analysis of an association between mortality and administration of UDCA, revealed that the result was not statistically significant ($P = 0.232$).

Another issue in patients with SAC is duration of hospitalization. In this study, we found that the median duration of hospitalization was 28.5 (range 10-88) days in the placebo group and 35 (range 15-70) days in UDCA group ($P = 0.15$). As such, the administration of UDCA did not shorten the duration of hospitalization.

In conclusion, administration of UDCA in neonates with SAC tends to improve total bilirubin, direct bilirubin, and AST levels, although these improvement of liver function parameters were not statistically significant. Further study with a larger sample size is needed.

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References

1. Wang P, Chaudry IH. Mechanism of hepatocellular dysfunction during hyperdynamic sepsis. *Am J Physiol.* 1996;270:927-38.
2. Angus DC, Linde-Zwirble WT, Lidicker J, Clermont G, Carcillo J, Pinsky MR. Epidemiology of severe sepsis in

- United States: analysis of incidence, outcome, and associated costs of care. *Crit Care Med.* 2001;29:1303-10.
- Bachtiar KS, Oswari H, Batubara JRL, Amir I, Latief A, Firman K. Cholestasis sepsis at neonatology ward and neonatal intensive care unit Cipto Mangunkusumo Hospital 2007: incidence, mortality rate and associated risk factors. *Med J Indones.* 2008;17:107-13.
 - Chand N, Sanyal AJ. Sepsis-induced cholestasis. *Hepatology.* 2007;45:230-41.
 - Bolder U, Ton-Nu HT, Schteingart CD, Frick E, Hofmann AF. Hepatocyte transport of bile acids and organic anions in endotoxemic rats: impaired uptake and secretion. *Gastroenterology.* 1997;112:214-25.
 - Whiting JF, Green RM, Rosenbluth AB, Gollan JL. Tumor necrosis factor-alpha decreases hepatocyte bile salt uptake and mediates endotoxin-induced cholestasis. *Hepatology.* 1995;22:1273-8.
 - Gilroy RK, Mailliard ME, Gollan JL. Gastrointestinal disorders of the critically ill. Cholestasis of sepsis. *Best Pract Res Clin Gastroenterol.* 2003;17:357-67.
 - Ng VL, Balistreri WF. Treatment options for chronic cholestasis in infancy and childhood. *Curr Treat Options Gastroenterol.* 2005;8:419-30.
 - Beuers U. Drug insight: mechanisms and sited of action of ursodeoxycholic acid in cholestasis. *Nat Clin Pract Gastroenterol Hepatol.* 2006;3:318-28.
 - Oswari H. Mekanisme peran asam ursodeoksikolat terhadap kolestasis terkait sepsis. [dissertation]. Jakarta: Universitas Indonesia; 2008.
 - Benitz WE. Adjunct laboratory tests in the diagnosis of early-onset neonatal sepsis. *Clin Perinatol.* 2010;37:421-38.
 - Heil W, Koberstein R, Zatz B. Reference ranges for adults and children. Mannheim: Roche Diagnostic GmbH; 2004.
 - Tufano M, Nicastro E, Giliberti P, Vegnente A, Raimundi F, Iorio R. Cholestasis in neonatal intensive care unit: incidence, aetiology and management. *Acta Paediatr.* 2009;98:1756-61.
 - Shamir R, Maayan-Metzger A, Bujanover Y, Ashkenazi S, Dinari G, Sirota L. Liver enzyme abnormalities in gram-negative bacteremia of premature infants. *Pediatr Infect Dis J.* 2000;19:495-8.
 - Tiker F, Tarcan A, Kilicdag H, Gurakan B. Early onset conjugated hyperbilirubinemia in newborn infants. *Indian J Pediatr.* 2006;73:409-12.
 - Rohsiswatmo R. Kontroversi diagnosis sepsis neonatorum. In: Hegar B, Trihono PP, Ifran EB, editors. Update in neonatal infection. Jakarta: Departemen Ilmu Kesehatan Anak FKUI RSCM; 2005. p. 32-43.
 - Oswari H, Widjaja RK, Rohsiswatmo R, Cleghorn G. Prognostic value of biochemical liver parameters in neonatal sepsis-associated cholestasis. *J Paediatr Child Health.* 2013;49:E6-11.
 - Maldonado SR, Tellez NCG, Yescas-Buendia G, Fernan-Carrocer LA, Echaniz-Aviles OL, Rios ERR. Effectiveness of ursodeoxycholic acid vs phenobarbital for the treatment of neonatal cholestasis: a cross-randomized clinical trial. *Bol Med Hosp Infant Mex.* 2010;67:418-23.
 - Al-Hathlol K, Al-Madani A, Al-Salf A, Abulaimon B, Al-Tawil K, El-Demerdash A. Ursodeoxycolic acid therapy for intractable total parenteral nutrition-associated cholestasis in surgical very low birth weight infants. *Singapore Med J.* 2006;47:147-51.