Original Article

Immature-to-total neutrophil ratio as an early diagnostic tool of bacterial neonatal sepsis

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Abstract

Background Bacterial sepsis is the main cause of morbidity and mortality in neonates. Early diagnosis and appropriate treatment for the condition can reduce mortality rates. Blood cultures are the gold standard to diagnose bacterial sepsis, but they require 3-5 days for results, whilst the disease may progress rapidly in neonates. Examination of immature-to-total neutrophil ratio (I/T ratio) in peripheral blood smears is a quicker and less expensive method to diagnose bacterial sepsis in neonates. Some studies found the sensitivity of I/T ratio to be 88%-90% in predicting bacterial spesis.

Objective To assess the usefulness of the I/T ratio as an early diagnostic tool for neonatal bacterial sepsis.

Methods This cross-sectional study was conducted from February to March 2011. Subjects were collected by consecutive sampling. Fifty-three neonates suspected to have bacterial sepsis in the Perinatology Unit at H. Adam Malik Hospital were included. Subjects underwent routine blood examinations, C-reactive protein level measurements, blood cultures, and peripheral blood smears. All statistical analyses were conducted with SPSS (version 16.0 for Windows).

Results Of the 53 subjects, 26 had bacterial sepsis based on blood cultures. The I/T ratio had a sensitivity of 88.46%, specificity 81.84%, positive predictive value 82.14%, and negative predictive value 88%. The receiver operating characteristic curve showed a cut-off point of 83.3 (95%CI 71.3 to 95.3)%.

Conclusion The I/T ratio may be a good alternative to blood cultures as an early indicator of bacterial neonatal sepsis, as it is faster, less expensive and has good sensitivity and specificity. [Paediatr Indones. 2015;55:153-7.].

Keywords: bacterial sepsis, IT ratio, blood culture, neonate, diagnostic

Neonatal sepsis is an inflammatory response to bacteremia occuring during the first month of life. Infection remains a major problem in developing countries, including Indonesia. Infection rates in small infants and newborns are quite high in Indonesia and often develop into septicemia.[1] Septicemia in newborns is a systemic inflammatory reaction to local infection that may lead to the development of more grave conditions.[2] Blood cultures have been known as the gold standard for diagnosing neonatal sepsis and should be performed prior to antibiotic administration. Blood cultures require 48-72 hours for results, whilst the disease may progress rapidly, increasing mortality rates in these neonates.[3]

Neutrophils are the cellular components of the basic body defense mechanism. The number of neutrophils varies in the first week of life. Infection causes the release of neutrophils into the bloodstream, leading to increased true white blood cell count.[4]

Immature neutrophils are at a younger stage than neutrophil bands (metamyelocytes, myelocytes, promyelocytes and myeloblasts). These young granulocytes are usually found during infection and septicemia.[3,4] The ratio of immature neutrophils to total neutrophils (I/T ratio) increases when the white blood cell count differential shows a left shift. An I/T ratio > 0.2 has been considered to be a marker for septicemia in newborns.[3,5] We aimed to assess the usefulness of the I/T ratio as an early diagnostic tool for neonatal bacterial sepsis.

Methods

This cross-sectional study was conducted to determine the sensitivity and specificity of the I/T ratio method as an early diagnostic tool for neonatal bacterial sepsis in the Perinatology Unit of Haji Adam Malik Hospital from February to March 2011. The subjects were suspected of having neonatal bacterial sepsis based on clinical symptoms and risk factors. Subjects were collected by consecutive sampling. Blood specimens were taken from subjects ≤ 48 hours prior to antibiotic administration. We excluded neonates with multiple congenital anomalies, anemia, and icterus within the first 24 hours of life.

Blood specimens were obtained from 53 newborn babies (27 females and 26 males) with suspected septicemia. Blood smear specimens were taken from venous blood and blood cultures were performed to confirm diagnoses. Differential counts were perfomed manually using a binocular microscope.

All statistical analyses were conducted with SPSS (version 16.0 for *Windows*). Sensitivity, specificity,

Table 1. Demographic data of subjects

Characteristics	Blood cultures results	
	Positive (n = 26)	Negative (n = 27)
Gestational age, n		
26 – 28 weeks	3	0
28 – 30 weeks	6	3
30 – 32 weeks	0	5
32 – 34 weeks	1	0
34 – 36 weeks	11	14
36 – 38 weeks	2	3
38 – 40 weeks	1	2
40 – 42 weeks	2	0
Gender, n		
Male	14	14 (51.9)
Female	12	13 (48.1)
Mean birth weight, grams (SD)	2,329.2 (982.01)	2,509.2 (878.66)
Diagnosis, n		
TTN	1	0
Asphyxia	0	1
Anal atresia	2	0
ELBW	1	0
LBW	1	1
VLBW	2	1
Down syndrome	1	0
Gastroschisis	2	1
Umbilical hernia	0	1
Macrosomia	0	1
Hirschsprung's disease	3	1
Hypoxic encephalopathy	1	3
Meningocele	0	1
Omphalitis	1	0
Cephalohematoma	3	0
Omphalocele	0	1
Suspected sepsis	1	1
Respiratory distress	7	14

TTN=transient tachypnea of newborn; ELBW=extremely low birth weight; LBW=low birth weight; VLBW=very low birth weight

positive predictive value, and negative predictive value of the I/T ratio method were determined using a two-by-two table with 95% confidence intervals. The cut-off point for diagnosis was determined by receiver operating characteristic (ROC) curve.

Results

During the study period, fifty-three neonates suspected of having neonatal sepsis in the Perinatology Unit underwent routine blood examinations, blood cultures, and peripheral blood smears to examine I/T ratios.

Table 1 shows the subjects' characteristics including gestational age, gender, birth weight, and diagnosis. We found 26 infants with sepsis diagnosed by positive blood cultures. The incidence of sepsis in this study was 45.6%. Subjects' mean gestational age was 34-36 weeks. More males had sepsis than females, and respiratory distress was the most common diagnosis accompanying neonatal sepsis in our subjects.

Table 2 shows the microorganisms found in blood cultures of the septic newborns. Most of the bacteria were gram-positive. *Staphylococcus epidermidis* was the most commonly found species.

Table 2. Microorganisms identified in blood cultures of septic newborns (N=26)

Microorganisms	n
Enterobacter aerogenes	1
Enterobacter agglomerans	1
Klebsiella oxytoca	1
Pseudomonas sp	6
Staphylococcus aureus	1
Staphylococcus epidermidis	9
Staphylococcus saprophyticus	4
Streptococcus faecalis	1
Streptococcus viridans	2

Table 3. Sensitivity and specificity of the I/T ratio method compared to blood cultures

	Blood culture		Total
	Positive	Negative	
I/T Ratio			
>0.2	23	5	28
≤0.2	3	22	25
Total	26	27	53

The sensitivity of I/T ratio was 88.46%; specificity was 81.48%; positive predictive value was 82.14%; and negative predictive value was 88%. The positive likelihood ratio was 4.776 and negative likelihood ratio was 0.142. The area under the curve in this study was 83.3% (95%CI 71.3 to 95.3; P< 0.05) (**Figure 1**). This result was indicative of good accuracy of the diagnostic test.
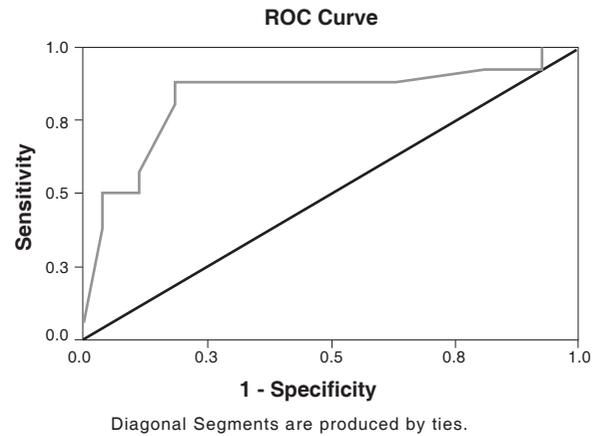
Figure 1. ROC curve of sensitivity and specificity of the I/T ratio

Discussion

Of the 53 subjects, we found 49% had bacterial sepsis based on positive blood cultures. Subjects were of various gestational ages, but those of 34-36 week gestational age had the most bacterial sepsis (42.3%). This finding is consistent with theory that a risk factor for bacterial sepsis is prematurity due to underdeveloped organs.[6] Premature infants may be predisposed to respiratory distress, limited calorie intake, increased risk of bleeding and infection. They are, therefore, at higher risk for neonatal sepsis than full term infants.[7]

Neonatal sepsis remains a major problem in developing countries including Indonesia and is the major cause of mortality in neonates.[3] As the signs and symptoms are non-specific, it is important to diagnose sepsis early, so that appropriate treatment may be administered.[8] In addition to clinical manifestations, various hematological changes are associated with sepsis including total white blood cell count, total neutrophil count, platelet count and increased C-reactive protein level.[9]

Other risk factors for sepsis in neonates are low birth weight, respiratory distress syndrome, and a history of aggressive resuscitation. Severe asphyxia causes infection due to cellular hypoxia and inflammation.[6] We found that neonates of low birth weight were more likely to have sepsis than those of normal birth weight. More males (53.8%) than females had neonatal sepsis, this difference was not significant.[10] The most common working diagnosis in this study was respiratory distress (26.95%), similar to an American study that found the most common causes of sepsis in neonates to be respiratory tract infection (38%), gastrointestinal infection (18%), post-operative infection (5%), and unidentified causes (24%).[11] *Staphylococcus epidermidis* was the most commonly cultured species (17%) found in our subjects, similar to other studies which reported that many hospitalized infants have *Staphylococcus epidermidis* growth on the skin.[12,13]

We found the I/T ratio to have sensitivity 88.46%, specificity 81.48%, positive predictive value 82.14%, and negative predictive value 88%. A New York study found that an I/T ratio of > 0.2 had sensitivity of 90% and specificity of 78%.[8] Another study in Australia found that the I/T ratio had sensitivity 96% and specificity 71%.[10] Furthermore, a study in northern California using an I/T ratio >0.15 found sensitivity to be 89% and specificity 94%.[14] A Netherlands study using an I/T ratio >0.2 found it had sensitivity of 89% and specificity 82%.[15] Lastly, an Indian study using an I/T ratio > 0.2 had sensitivity 93.75% and specificity 85.48%.[4]

The I/T ratio test was sufficiently accurate to diagnose sepsis in potentially infected neonates. As such, the I/T ratio can be used as an early diagnostic tool for neonatal bacterial sepsis because of its high sensitivity and specificity. It is also quicker and less expensive than blood cultures, so this test may help reduce the mortality and morbidity due to neonatal sepsis.

To generalize these findings, studies with a large number of neonates are needed. Clinical sepsis and I/T ratio are sufficiently accurate as diagnostic tests for sepsis in neonates. Parallel test increase sensitivity, the negative result revealed that it is not sepsis. Serial test will increase specificity. There is high probability of having sepsis, if the result was positive.

In conclusion, I/T ratio can be used as an early diagnostic tool for neonatal sepsis because of its high

sensitivity and specificity. It may be a good alternative to blood cultures, as it is less expensive and takes less time to perform.

Conflict of interest

None declared.

References

1. Amirullah A. Sepsis pada bayi baru lahir. In: Kasim SM, Yunanto A, Dewi R, Sarosa IG, Usman A, editors. Buku ajar neonatologi. Jakarta: Ikatan Dokter Anak Indonesia; 2008. p. 170-87.

2. Gomella TL. Infectious disease. In: Gomella TL, Cuningham MD, Eyal FG, Zenk KE, editors. Neonatology: management, procedures, on-call problems, disease and drugs. New York: Lange, McGraw-Hill; 2004. p. 434-40.

3. Aulia D, Sanjaya AI, Timan IS. The use of immatane to total neutrophil (IT) ratio to detect bacteriemia in neonatal sepsis. J Lab Med Quality Assurance. 2003;25:237-42.

4. Jackson GL, Engle WD, Sendelbach DM, Vedro DA, Josey S, Vinson J, *et al*. Are complete blood cell count useful in the evaluation of asymptomatic neonates exposed to suspected chorioamnitis? Pediatrics. 2004;113:1173-80.

5. Mishra UK, Jacobs SE, Doyle LW, Garland SM. Newer approaches to the diagnosis of early onset neonatal sepsis. Arch Dis Child Fetal Neonatal Ed. 2006;91:F208-12.

6. Klinger G, Levy I, Sirota L, Boyko V, Lerner-Geva L, Reichman B, *et al*. Outcome of early-onset sepsis in national cohort of very low birth weight infants. Pediatrics. 2010;125:e736-40.

7. Osrin D, Vergnano S, Costello A. Serious bacterial infections in newborn infants in developing countries. Curr Opin Infect Dis. 2004;17:217-24.

8. Phillip AG, Hewitt JR. Early diagnosis of neonatal sepsis. Pediatrics. 1980;65:1036-41.

9. Rodwell RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr. 1988;112:761-7.

10. Stoll BJ, Hansen N, Fanaroff A, Wright LL, Carlo WA, Ehrenkranz RA, *et al*. Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. N Engl J Med. 2002;347:240-7.

11. Saez-Llorens X, Vargas S, Guerra F, Coronado L. Application of new sepsis definitions for evaluate outcome of pediatric

patients with severe systemic infections. Pediatr Infect Dis J. 1995:14;557-60.

12. Baltimore RS, Huie SM, Meek JI, Schuchat A, O'Brien KL. Early-onset neonatal sepsis in the era of group B streptococcal prevention. Pediatrics. 2001;108:1094-8.

13. Stoll BJ. Infections of the neonatal. In: Behrman RE, Kiegman RM, Jenson HB, Stanton BF, editors. Nelson textbook of pediatrics. 18th ed. Philadelphia: Saunders Company; 2007. p. 794-811.

14. Manroe BL, Rosenfeld CD, Weinberg AG, Browne R. The differential leukocyte count in the assessment and outcome of early-onset neonatal group B streptococcal disease. J Pediatr. 1977;91:632-7.

15. Franz AR, Kron M, Pohlandt F, Steinbach G. Comparison of procalcitonin with interleukin 8, C-reactive protein and differential white blood cell count for the early diagnosis of bacterial infections in newborn infants. Pediatr Infect Dis J. 1999;18:666-71.