

## CD64 and I/T Ratio as a Diagnostic Test on Neonatal Sepsis in Banjarmasin

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### ABSTRACT

Neonatal sepsis remains a major problem in the service and care of neonates. The clinical features of neonatal sepsis are non-specific, which makes it difficult to diagnose. The primary objective of this study was to assess the clinical use of cluster of differentiation 64 as a diagnostic marker of neonatal infection. This research used an analytical observational study. The research subjects consisted of 43 samples of neonates at Ulin Hospital, Banjarmasin who had met the inclusion criteria. The results of the study concluded that there was no significant difference between the I/T ratio and cluster of differentiation 64 values in patients with suspected neonatal sepsis with the gold standard procalcitonin and/or blood culture at Ulin Hospital with  $p=0.874$  for the I/T ratio and  $p=0.285$  for cluster of differentiation 64. The diagnostic test for the I/T ratio with a cut-off of 0.2 showed a sensitivity of 23.8%, specificity of 72.7%, positive predictive value of 45.5%, negative predictive value of 50%, mean of 0.16, and median of 0.11. The results of the cluster of differentiation 64 diagnostic test with a cut-off of 2025 showed a sensitivity of 42.9%, specificity of 72.7%, positive predictive value of 81.8%, negative predictive value of 50%, mean of 2487.93, and median of 1671. There was no significant difference between the I/T ratio and cluster of differentiation 64 values in patients with suspected neonatal sepsis with the gold standard procalcitonin and/or blood culture at Ulin Hospital, Banjarmasin.

**Keywords:** Cluster of differentiation 64, I/T ratio, neonatal sepsis

### INTRODUCTION

Neonatal sepsis remains a major problem of neonatal services and care.<sup>1</sup> The incidence of neonatal sepsis is 1-10 of 1000 live births and reaches 13-27 of 1000 live births in infants with birth weight <1500 grams and the mortality rate reaches 13-50%, especially in premature infants with an incidence of 5-10 times compared to term neonates and neonates with severe disease.<sup>2</sup> The incidence of neonatal sepsis in several referral hospitals in Indonesia ranges from 1.5-3.72% with a mortality rate ranging from 37.09-80%. According to WHO, there are 1 million deaths due to neonatal sepsis with 42% of deaths in the first week of life. Epidemiology in the United States shows that the incidence of bacterial neonatal sepsis is 1-4 per 1000 live births, which is smaller than in Southeast Asia (2.4-16 per 1000 live births). The mortality rate due to neonatal sepsis in Indonesia is 94,050 neonates per year.<sup>3</sup>

The clinical manifestation of neonatal sepsis is non-specific, which makes it difficult to diagnose. Early diagnosis and appropriate treatment can reduce mortality and morbidity; therefore, a laboratory test is needed to support the clinical

examination in patients.<sup>4</sup> Blood culture is a good gold standard for the diagnosis of neonatal sepsis.<sup>5</sup> One of the laboratory tests used to diagnose neonatal sepsis is procalcitonin (PCT). A procalcitonin value of 2-2.5 ng/mL indicates a systemic infection by bacteria or fungi, with a sensitivity of 85% and a specificity of 54%.<sup>6</sup>

CD64 is one of the markers for the diagnosis of neonatal sepsis. The results have suggested that CD64 Antibody-Binding Capacity (ABC) shows the highest sensitivity and specificity at the cut-off value of 2025 ABC with a sensitivity of 83.33% and specificity of 72.97%.<sup>7</sup>

The I/T ratio is the ratio of immature neutrophils to total neutrophils. The I/T ratio indicates the possibility of infection, making it able to be one of the markers for the occurrence of sepsis. The results showed a difference in the I/T ratio in the diagnosis of neonatal sepsis with the gold standard blood culture with a specificity of 83.82%, a sensitivity of 76.47%, Positive Predictive Value (PPV) of 54.16%, and Negative Predictive Value (NPV) of 93.44%.<sup>8</sup>

The primary objective of this study was to assess the clinical use of CD64 as a diagnostic marker of neonatal infection.

## METHODS

This study was an analytical observational study with a diagnostic test design approach to analyze differences in CD64 values and I/T ratio with the gold standard of blood culture and/or PCT for the diagnosis of neonatal sepsis. The population in this study were 43 neonates in the Neonatology Room of Ulin Hospital, Banjarmasin from November to December 2019 based on medical record data.

A total of 3 mL whole blood samples were taken from umbilical cord blood, which was put in an EDTA vacutainer and into a BacT Alert bottle of 3 mL. Blood collected was used for complete blood count, blood chemistry, immunoserology test, and blood culture at the same time to determine the value of CD64, I/T ratio, PCT, and blood culture.

The data were analyzed by analytical observation by conducting an independent T-test analysis if the data was normally distributed or Mann-Whitney if the data was not normally distributed on each variable through statistical software. Furthermore, the data were tabulated significant differences were determined.

This research had been approved by the research ethics committee of the Dr. Ulin Hospital in Banjarmasin with ethical feasibility article number No.223/VIII-Reg Riset/RSUDU/19.

## RESULTS AND DISCUSSIONS

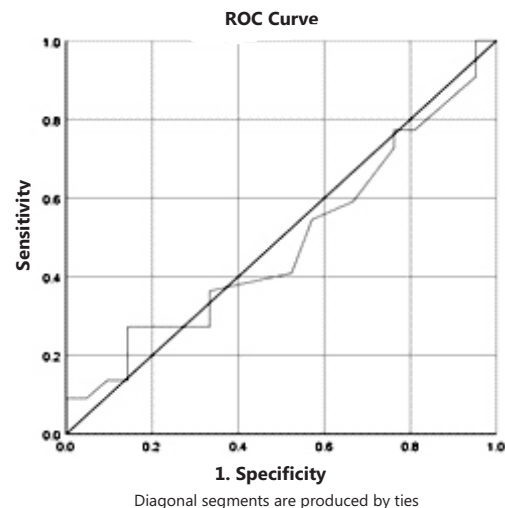
The study was carried out on patients with suspected neonatal sepsis. The characteristics of the research subjects can be seen in Table 1 and Table 2, and the diagnostic test of the I/T ratio can be seen in Table 3.

Table 3 illustrates that the I/T ratio diagnostic test with a cut-off of 0.2 shows a sensitivity value of 23.8%, specificity of 72.7%, PPV of 45.5%, and NPV of 50% with an ROC curve, which can be seen in Figure 1. The results showed that the data were not normally distributed; therefore, the Mann-Whitney statistical test was used and showed showed p-value

**Table 3.** Diagnostic test of I/T ratio with gold standard PCT and/or blood culture

I/T	Sepsis (PCT $\geq$ 0.5 and/or Blood Culture (+))		p-value
	(+)	(-)	
$\geq$ 0.2	5	6	0.874
$<$ 0.2	16	16	
Total	21	22	

Sensitivity 23.8%; Specificity 72.7%; PPV 45.5%; NPV 50%



**Figure 1.** ROC curve of the I/T ratio test

=0.874. It was concluded that there was no significant difference between the I/T ratio values in patients with suspected neonatal sepsis with the gold standard PCT and/or blood culture at Ulin Hospital.

I/T ratio is the ratio between immature neutrophils to the total number of neutrophils (total). Immature neutrophils include band neutrophils, metamyelocytes, myelocytes, and promyelocytes. Mature neutrophils are part (segments) of neutrophils.<sup>9</sup>

The I/T ratio is still believed to be one of the most useful single tests (tests) available. Several previous studies have shown that the I/T value is a sensitive indicator of sepsis; however, this study found

**Table 1.** The characteristics of subjects by risk factors

Way of Birth					APGAR Score		Risk Factor of Sepsis	
Normal	SC	Vacuum	BW	BL	$\leq$ 5	$>$ 5	(+)	(-)
23(57.5%)	15(37.5%)	2(5%)	2747	47	7(17.5%)	33(82.5%)	35(81.4%)	8(18.6%)

**Table 2.** The characteristics of subjects by laboratorium results

PCT		I/T Ratio		CD64		Blood Culture	
$\geq$ 0.5	$<$ 0.5	$>$ 0.2	$\leq$ 0.2	$\geq$ 2025	$<$ 2025	(+)	(-)
6(14%)	37(86%)	11(26%)	32(74%)	15(35%)	28(65%)	18(42%)	25(58%)

contradictory results. This study obtained a p-value of 0.874 with low sensitivity. This indicated that the diagnostic value of the I/T ratio was not high enough. A study by Diakite *et al.* showed that the I/T ratio is quick, simple, and cost-effective routine laboratory tests, which help in the early diagnosis of neonatal sepsis. Although there are many serological markers available, the I/T ratio serves as a reliable predictor of neonatal sepsis. With a good sensitivity, high specificity, and a good NPV these parameters can therefore help in the timely and early identification of neonatal sepsis.<sup>10</sup>

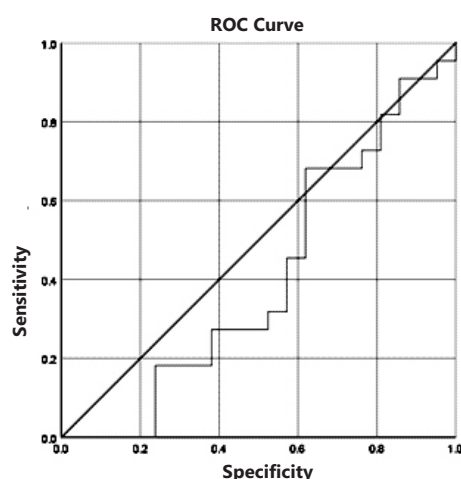
Other studies reported contradictory results too with this study. Jethani *et al.* concluded that the I/T ratio is still an indicator of the susceptibility (sensitive indicator) of sepsis.<sup>11</sup> Tiwari *et al.* reported that the I/T ratio can be used as a marker for early detection of sepsis with high specificity (more than 80%).<sup>12</sup>

Palupi *et al.* stated that the I/T ratio is a diagnostic marker that is more sensitive for bacterial meningitis in children compared to the total leukocyte count.<sup>13</sup> The differences in the results of these studies indicated variation that might occur in the method, patient selection, or observer error. Based on this, the I/T ratio cannot be used as a single parameter and it is necessary to use other parameters together to diagnose neonatal sepsis.

**Table 4.** Diagnostic test of CD64 with gold standard PCT and/or blood culture

CD64	Sepsis (PCT $\geq$ 0.5 and/or Blood Culture (+))		p-value
	(+)	(-)	
$\geq$ 2025	9	6	0.285
<2025	12	16	
Total	21	22	

Sensitivity 42.9%; Specificity 72.7%; PPV 81.8%; NPV 50%



**Figure 2.** ROC curve of CD64 value

Table 4 illustrates that the CD64 diagnostic test with a cut-off of 2025 showed a sensitivity of 42.9%, specificity of 72.7%, PPV of 81.8%, and NPV of 50% with ROC curve can be seen in Figure 2. The results of the p-value = 0.285. Therefore, it was concluded that there was no significant difference between CD64 in patients with suspected neonatal sepsis with PCT gold standard and/or blood culture at Ulin Hospital.

CD64 is one of the markers to diagnose neonatal sepsis. The expression of neutrophil CD64 (CD64) is considered to be the earliest phase of the immune response to bacterial infection. CD64 increases approximately one hour after invasion, making it able to be used as a means to exclude infection in blood cultures of cases with proven neonatal sepsis. The results have suggested that CD64 ABC shows the highest sensitivity and specificity at the cut-off value of 2025 ABC with a sensitivity of 83.33% and specificity of 72.97%.<sup>7</sup>

The CD64 data taken in this study were ABC type with a cut-off of 2025 ABC referring to previous studies. The results of this study showed that the diagnostic value of CD64 was not high enough. Similarly, a study by Tang *et al.* in 2018 found no association between an increase in CD64 and bacterial infection. It was reported in that study that CD64 had a higher diagnostic value than WBC, CRP, and PCT in diagnosing early-onset sepsis. However, PCT has a better advantage in the diagnosing of late-onset sepsis. Despite having a higher diagnostic value, unfortunately, there was no significant difference between the CD64 values in the sepsis and non-sepsis patients.<sup>14</sup>

Other authors reported different things. Pratiwi *et al.* concluded that CD64 ABC showed the highest sensitivity and specificity at the cut-off value of 2025 ABC with a sensitivity of 83.33% and a specificity of 72.97%.<sup>7</sup> In addition, according to Cui *et al.* in a study of neonates in China, it was found that CD64 is a valuable diagnostic test for diagnosing early-onset neonatal sepsis and there was a significant difference in the different tests of CD64 values in neonates with sepsis.<sup>15</sup> Neutrophil CD64 is a good test to assess diagnostic, prognostic, and as a marker for monitoring neonatal sepsis. The diagnostic value of CD64 will increase when combined with CRP. However, the CD64 cut-off value has not yet been determined and further studies are needed before it can be determined with certainty and applied in daily practice.<sup>16</sup>

The differences in the results of these studies indicated that there was variation that might occur in the laboratory technique used, patient selection, or

observer error. Based on this, the CD64 value also cannot be used as a single parameter and it is necessary to use other parameters together to diagnose neonatal sepsis.

## CONCLUSIONS AND SUGGESTIONS

There was no significant difference between the I/T ratio and CD64 values in patients with suspected neonatal sepsis with the gold standard PCT and/or blood culture at Ulin Hospital, Banjarmasin. The examination of the I/T ratio and CD64 as a diagnostic test showed low sensitivity but was not significant. Based on this, both the I/T ratio and CD64 value cannot be used as a single parameter for diagnosing neonatal sepsis.

It was recommended that further research be conducted on the combined test of several indicators in establishing the diagnosis of neonatal sepsis. Using a combination of several indicators is expected to provide meaningful results for the diagnosis of neonatal sepsis.

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