

# Diagnostic Value of Immature-to-Total Neutrophil Ratio in Neonatal Sepsis

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## ABSTRACT

**Background:** Neonatal sepsis is the third most common reason of neonatal mortality in Pakistan. Blood culture, the gold standard test for diagnosis of neonatal sepsis (NS), is time consuming. Therefore, rapid diagnostic tests with good specificity and sensitivity is needed for accurate and early diagnosis of this condition. The objective of this study was to determine the diagnostic value of abnormal ( $\geq 0.2$ ) immature-to-total-neutrophil ratio in neonatal sepsis.

**Material and Methods:** This cross-sectional study was carried out on 288 neonates, aged 0-28 days, admitted with suspected sepsis. Detailed history of the neonates was recorded including gender, age, birth weight, maternal age, gestational age and clinical features. Blood culture and Peripheral blood films were done in each case. Differential leucocyte counts, total Polymorphoneutrophil count (PMN), immature neutrophil count, mature neutrophil count and calculation of I/T ratio was carried out in the Pathology Department of PIMS, Islamabad. The study outcome was divided into three groups on the basis of positive or negative blood culture and I/T ratio as normal, probable sepsis and proven sepsis group. Data was analyzed by SPSS version 21.0. Diagnostic value of I/T ratio was determined in NS by calculating values of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) by considering the blood culture as the gold standard test of NS.

**Results:** The mean age and weight of the neonates at the time of admission was 1.1 ( $\pm 0.6$ ) days and 2.51 ( $\pm 0.40$ ) kg, respectively. About 60% of the neonates were males and 118(41%) neonates had I/T ratio of  $\geq 0.2$ . On the basis of positive or negative blood culture and I/T ratio, 82 (28.5%) neonates were diagnosed as proven sepsis, 43 (14.9%) neonates had probable sepsis and remaining 163 (56.6%) neonates were declared as normal. Out of 82 neonates with positive blood cultures, 75 (91.5%) had I/T ratio  $\geq 0.2$ , while 7 (8.5%) had I/T ratio  $\leq 0.2$ . The sensitivity, specificity, positive and negative predictive value and diagnostic accuracy of abnormal I/T ratio to diagnose neonatal sepsis was 91%, 79%, 64%, 96% and 83%, respectively.

**Conclusions:** Due to substantially high diagnostic accuracy of I/T ratio  $\geq 0.2$ , we recommend it as a useful, rapid and cost-effective tool in accurate diagnosis of neonatal sepsis.

**Key words:** Diagnostic value, Immature-to-total neutrophil ratio, Neonatal sepsis

### Authors' Contribution:

<sup>1,2</sup> Conception, synthesis, planning of research and manuscript writing  
<sup>1</sup> Interpretation, discussion, <sup>3,4</sup> Active participations in data collection

<sup>5,6</sup> Data analysis.

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## Introduction

Neonatal sepsis (NS) is defined as "a clinical syndrome of systemic illness accompanied by bacteremia occurring in the first month of life".<sup>1</sup> Up to 25% of children in intensive

care units have sepsis.<sup>2</sup> According to UNICEF statistics of 2015, neonatal mortality rate in Pakistan is 46 deaths per 1,000 live births.<sup>3</sup> In Pakistan, NS is the third most

common cause of neonatal mortality after prematurity and birth asphyxia, resulting in 17.2% of neonatal deaths.<sup>3</sup> However developed countries have very low incidence of NS i.e. 1/1000 in term neonates and 4/1000 in preterm neonates.<sup>4</sup>

Early diagnosis of NS is primarily based on clinical presentation, while laboratory diagnosis mainly depends on blood cultures. The definitive diagnosis of NS on the basis of blood cultures takes at least 36 hours, which is quiet time consuming.<sup>5</sup> On the other hand, the standard hematological investigations in terms of white blood cell (WBC), neutrophils and platelet counts are all simple tests which are easily available, time saving and cost effective compared to blood cultures.<sup>6</sup> It should also be noted that negative blood culture cannot always rule out NS. On the other hand, blood culture can become positive due to contamination from skin or asymptomatic bacteremia.<sup>7</sup>

Therefore, hematological parameter should be considered for quick diagnosis of bacterial infections in neonates and ruling out neonatal sepsis to prevent over-usage of antibiotics and concomitant emergence of antibiotic resistant strains. Timely and effective management of neonatal sepsis can significantly reduce neonatal mortality. Band cells are defined as "neutrophils that have not reached full maturation. They are characterized by absence of complete separation of the lobes, which gives the nucleus a characteristic horse-shoe shape with a uniform thickness". On stimulation by pro-inflammatory cytokines, the bone marrow releases these cells into the circulation.<sup>8,9</sup>

Raised immature neutrophil count in blood is usually considered as an indicator of sepsis. In 2005, the International Consensus Conference on Pediatric Sepsis rephrased the definition of NS by including "the presence of immature neutrophils >10%".<sup>10</sup> However the diagnostic accuracy of immature neutrophil count in sepsis has become debatable, as it is affected by many causes other than infection including perinatal asphyxia, maternal hypertension, hemorrhage, hemolysis, corticosteroids, parenteral nutrition and surgery, etc.<sup>11,12</sup>

This study was planned to assess the diagnostic accuracy of abnormal ( $\geq 0.2$ ) immature-to-total neutrophil (I/T) ratio in neonatal sepsis at our clinical setting.

## Material and Methods

After seeking permission from Institutional Ethics Review Board of Pakistan Institute of Medical Sciences (PIMS), Islamabad, this cross-sectional study was carried out at Neonatology Department of PIMS from 1<sup>st</sup> November, 2014 till 30<sup>th</sup> April, 2015. This study included 288 neonates between 0-28 days admitted in Neonatal ICU with suspicion of sepsis. Neonates of diabetic mothers and those with severe neonatal jaundice resulting from blood group incompatibilities and congenital anomalies were excluded from the study. The sample size was calculated with WHO sample size calculator with following values of calculations; 95% confidence interval, 5 % margin of error and 75% true prediction of neonatal sepsis by I/T ratio  $\geq 0.2$ .<sup>13</sup>

Neonates between 0-28 days admitted in the NICU with suspected sepsis were screened for enrollment. Those who fulfilled the above-mentioned inclusion criteria were registered in the study after informed consent of parents/ caretaker of the neonates. Once enrolled, a careful history was taken from the parents/caretakers of each enrolled neonate regarding gender, age, birth weight, maternal age, gestational age and clinical features and recorded in a preset structured proforma.

Once the information was obtained, necessary investigations were done within 24 hours of admission. The skin was cleaned with a spirit swab and blood sample was taken after skin became dry. About 1 mL of blood sample was collected in tubes containing EDTA and sent to laboratory for blood cultures. Beckman Coulter HMX automated Hematology Analyzer (USA) was used to obtain values of total WBCs and platelets. Peripheral blood films were stained by Leishman staining method. Differential leucocyte counts, total Polymorphoneutrophil count (PMN), immature neutrophil count, mature neutrophil count and calculation of I/T ratio was carried out in the Pathology Department of PIMS, Islamabad.

The study outcome was divided into three groups: 1. Normal group – both blood culture and I/T ratio were negative, 2. Probable sepsis group – blood culture was negative but the I/T ratio was  $\geq 0.2$  along with clinical features of NS, 3. Proven sepsis group – blood culture was positive along with clinical features of NS with or

without I/T ratio  $\geq 0.2$ . Data was recorded on a structured proforma and analyzed by SPSS version 21.0. Mean and standard deviation were calculated for quantitative variables such as age of neonate, maternal age, gestational age, WBCs, PMN and platelet count. Frequencies and percentages were calculated for qualitative variables like gender, clinical signs, prematurity and neonatal sepsis. Moreover, diagnostic value of I/T ratio was determined in NS by calculating values of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) by considering the blood culture as the gold standard test of NS.

## Results

In this study, 288 neonates with clinical features of NS were enrolled. The mean age was 1.1 ( $\pm 0.6$ ) days (Table I). Out of 288, 173 (60%) neonates were males while 115 (40%) were females with a male-to-female ratio of 1.5:1. The mean birth weight was 2.51 ( $\pm 0.40$ ) kg with 21 (7.3%) neonates having a birth weight less than 2 kg and 111 (38.5%) having birth weight between 2.0 and 2.5 kg. The mean maternal age of the neonates was 25.8 ( $\pm 5.3$ ) years and the mean gestational age of neonates was 36.9 ( $\pm 1.8$ ) weeks. About 91 (31.6%) neonates were premature while 197 (68.4%) were full term neonates. Regarding clinical symptoms, 108 (37.5%) had tachypnea, 89 (30.9%) lethargy, 88 (30.6%) reduced sucking, and 119 (41.3%) had vomiting during their stay in the hospital. The mean WBC count was 6905 ( $\pm 221.7$ )  $\text{mm}^3$  with 39 (13.5%) neonates having a WBC count more than 10,000  $\text{mm}^3$ . The mean PMN count was 4833 ( $\pm 155.2$ )  $\text{mm}^3$  and the mean platelet count was 216972 ( $\pm 2566.1$ )  $\text{mm}^3$ .

The diagnostic value of I/T ratio in NS was determined by calculating its sensitivity, specificity, PPV and NPV by considering blood culture as gold standard. Out of 82 neonates with positive blood cultures, 75 (91.5%) had I/T ratio  $\geq 0.2$ , while only 7 (8.5%) neonates had I/T ratio  $\leq 0.2$ . Therefore, the sensitivity of I/T ratio to diagnose neonatal sepsis was 91%, specificity was 79%, PPV was 64%, NPV was 96% and diagnostic accuracy was 83%. Out of 82 neonates with proven sepsis, 49 (60%) neonates were males. While, out of 43 neonates with probable sepsis, 26 (60%) were males.

**Table I: Descriptive Statistics of Neonates**

Characteristics	Mean $\pm$ SD
Age (days)	1.1 $\pm$ 0.6
Maternal Age (years)	25.8 $\pm$ 5.3
Birth Weight (kg)	2.51 $\pm$ 0.40
Gestational Age (weeks)	36.9 $\pm$ 1.8
White Blood Cells ( $\text{mm}^3$ )	6905 $\pm$ 221.7
Neutrophils ( $\text{mm}^3$ )	4833 $\pm$ 155.2
Platelets ( $\text{mm}^3$ )	216972 $\pm$ 2566.1
Characteristics	n (%)
<b>Gender</b>	
Male	173 (60%)
Female	115 (40%)
<b>Gestational Age</b>	
Premature	91 (31.6%)
Full Time	197 (68.4%)
<b>I/T Ratio</b>	
$\leq 20\%$	170 (59%)
$\geq 20\%$	118 (41%)
<b>Blood Culture</b>	
Positive	82 (28.5%)
I/T ratio $\leq 0.2$	7 (8.5%)
I/T ratio $\geq 0.2$	75 (91.5%)
Negative	206 (71.5%)
I/T ratio $\leq 0.2$	163 (79.1%)
I/T ratio $\geq 0.2$	43 (20.9%)

## Discussion

Neonatal sepsis is still one of the most common reasons of neonatal mortality and morbidity despite the great advancements in neonatal care. Clinical features of neonatal sepsis are often confused with those of noninfectious and metabolic diseases. In such cases, often empirical antibiotics are started early to avoid neonatal mortality which can result in adverse effects and emergence of resistant strains. In order to prevent these complications and timely management of neonatal sepsis, rapid, cost effective and accurate diagnostic test is needed.<sup>14</sup>

In our study, out of 288 neonates, 82 (28.5%) were diagnosed as proven sepsis, 43 (14.9%) neonates had probable sepsis and remaining 163 (56.6%) neonates were normal. Out of 82 neonates with positive blood cultures, 75 (91.5%) neonates also had I/T ratio  $\geq 0.2$ , while 7 (8.5%) had I/T ratio  $\leq 0.2$ . Therefore, the sensitivity of I/T ratio to diagnose neonatal sepsis was 91%, specificity was 79%, PPV was 64%, NPV was 96%

and diagnostic accuracy was 83%. Our findings are comparable with those of other studies done in different parts of the world.

An Egyptian study on 285 neonates showed that 65.6% of patients had abnormal I/T ratio with sensitivity, specificity, PPV and NPV of 82.4%, 81.3%, 92.5% and 62.2%, respectively.<sup>15</sup> This study also showed that I/T ratio of more than 0.3 was associated with increased odds of mortality, suggesting a prognostic role of I/T ratio in predicting the patients outcome.<sup>15</sup> Sensitivity and specificity of this study can be compared to our study but PPV and NPV are quite different.

Similarly, a retrospective study on 125 newborns by Bernhard and colleagues in Austria found that an abnormal I/T ratio  $\geq 0.2$  was a more common finding than an abnormal WBC count in early onset sepsis.<sup>16</sup> A study conducted in India by Annam and colleagues analyzed cord blood for various hematological parameters and found that an abnormal I/T ratio is the most sensitive parameter for early onset neonatal sepsis.<sup>17</sup> Makkar and coworkers from India also evaluated the importance of hematological scoring system in the early diagnosis of NS. This study revealed that I/T ratio of  $\geq 0.2$  had a high sensitivity (94%), high specificity (94%) and NPV (94%) but low PPV (8%) for the diagnosis of neonatal sepsis. These values were the highest among all other hematological parameters.<sup>18</sup> A retrospective study in United States by Hornik and colleagues on a large cohort of neonates (n=166,092) from 1996 to 2009 observed that I/T ratio  $\geq 0.2$  was significantly associated with NS with a sensitivity of 54.6% and specificity of 73.7%.<sup>13</sup> Specificity of this study is comparable to that of our study but sensitivity is much lower than that of our study.

Murphy and Wiener also conducted a retrospective study on neonates who were assessed for NS within the first 24 hours of life. Of the 3,154 included neonates, 1,539 (49%) had two normal ( $\leq 0.2$ ) I/T ratios and a negative blood culture at 24 hours. None of these neonates with normal I/T ratios were subsequently diagnosed with NS (NPV 100%).<sup>19</sup> The results are similar to those of our study.

Yet in another study conducted in USA on 163 infants, Bhandari and colleagues found very low sensitivity (33%), specificity (1%), PPV (1%) and NPV (66%) of I/T ratio ( $\geq$

0.2) for the diagnosis of NS. The findings of this study are quite different as compared to our study mainly due to smaller sample size used in this study.<sup>20</sup> Another prospective study from Bangladesh conducted on 80 neonates also showed low sensitivity and specificity of I/T ratio  $\geq 0.2$  lower than those of our study mainly due to low sample size.<sup>21</sup>

## Conclusion

We recommend I/T ratio  $\geq 0.2$  as very useful, rapid and cost-effective investigation in early detection of NS. It can save life of many neonates by early administration of antibiotics in cases of NS and unnecessary use of antibiotics in those with no sepsis thus preventing antibiotics resistance and adverse effects.

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