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IMMATURE-TO-TOTAL NEUTROPHIL RATIO (I/TR) IN RELATION TO NEONATAL SEPSIS AND IT'S OUTCOME

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ABSTRACT **Introduction:** The gold standard of positive blood culture for diagnosis and prognostication of neonatal sepsis is time consuming and resource intense. Immature-to-Total Neutrophil ratio (I/TR) is suggested as a surrogate quick and cost-effective marker. This study focuses on its relation to neonatal sepsis, duration of hospital stay and outcome.
Aims and Objectives:
1. To assess I/TR among neonates admitted to NICU with probable sepsis
2. To compare the outcome and duration of hospital stay among those with I/TR>0.2 and <0.2
Methodology: 86 neonates admitted to NICU of a tertiary-care teaching hospital from September 2018-September 2019 with potential risk factors for sepsis were enrolled. Maternal factors predisposing to sepsis, gestational age, birth weight, indication for admission, investigations including sepsis screen: rapid diagnostic tests (RDT); CBC, micro-ESR, CRP and I/TR from peripheral smear and blood culture, management and outcome were recorded. The data was analysed using SPSS 23.0 version and Chi-square test & Fischer's exact t-test. **Results:** Study characteristics were as follows: Preterm -45.4%, LBW -43%, SGA -11.6%, M:F ratio -1.8:1, Early Onset Sepsis (EOS) -89.5%, I/TR≥0.2% - 39.5%, positive CRP -25.6%, Micro-ESR>15mm/hr -80.2%, presumed sepsis -59.3%, probable sepsis -22.1%, positive blood culture - 18.6%, duration of antibiotics for 3, 7, 14, and 21 days-55.8%, 24.4%, 14% & 5.8% respectively, NICU stay for 3, 7, 21 days -44.2%, 20.9% & 14% respectively. I/TR≥0.2 had a significant association with probable and confirmed sepsis (p<0.001). 29.4% with positive blood culture was associated with I/TR ≥0.2% (p-0.037). Klebsiella was isolated in 6/16 cases, followed by E. Coli, pseudomonas, S. aureus and candida albicans. Those with I/TR≥0.2 had longer duration of hospital stay and antibiotic course (p-0.031). Sensitivity and specificity of I/TR≥0.2 was 62.5% & 65.7% and positive and negative predictive values were 29.4% & 88.4% respectively. **Conclusion:** The findings that I/TR≥0.2 was significantly associated with probable and confirmed sepsis, longer duration of hospital stay and antibiotic course and the high negative predictive value of 88.4%, confirms the usefulness of this test. Hence it is recommended as a quick and cost-effective surrogate marker of sepsis and it's outcome among neonates.

KEYWORDS : Sepsis screen, Immature-to-Total Neutrophil ratio, neonatal outcome

BACKGROUND:
Neonatal sepsis is the third leading cause of neonatal mortality, and is due to prematurity and intrapartum-related complications or birth asphyxia. Newborns in NICU are more prone to develop sepsis due to more exposure to infections, low birth weight, increased handling etc. Thus, early identification & proper treatment can reduce the morbidity and mortality. Developing countries lack a surveillance system, and a high proportion of new-borns in these countries die at home before they are registered (1). Blood culture is the gold standard to diagnose bacterial sepsis, but requires 3-7 days for results, while the disease may progress rapidly in neonates (2). I/TR from a peripheral blood smear is a surrogate marker of sepsis, which is a quick and cost-effective method. Other biomarkers of sepsis are TLC, ANC, platelets, ESR, Interleukins (IL6, IL8), CD64 and TNF-a, pro-calcitonin and CRP. But these are costly and not readily available in resource limited Low and Middle-income countries (LMICs).

AIMS AND OBJECTIVES:
1. To assess I/TR among neonates admitted to NICU with probable sepsis
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Operational Definition:
Presumed sepsis: Blood culture was negative but with a strong clinical suspicion for infection.

Probable sepsis: Signs and symptoms of infection and at least two abnormal laboratory results when blood culture is negative.

Confirmed sepsis: Neonates with positive blood culture

Criteria for Screen Positive Test:
• Total WBC count<5000/mm3
• Absolute Neutrophil Count (ANC)<1800/mm3
• I/TR≥0.2
• Micro-ESR>15mm 1st hour
• CRP Positive

MATERIALS AND METHODS:
86 neonates admitted to NICU of a tertiary-care teaching hospital from September 2018-September 2019 with potential risk factors for sepsis were enrolled in this descriptive analytical study. Babies with chromosomal abnormalities, features of intrauterine infections, metabolic disorders, congenital heart diseases, haemolytic jaundice due to blood group incompatibility and mothers on drugs (steroids, immunosuppressives) were excluded. Parental informed consent and IEC approval were obtained prior to study. Maternal risk factors predisposing to sepsis, type of delivery, gestational age, gender, birth weight, clinical signs suggestive of sepsis, postnatal day of NICU admission, indication for admission, investigations including sepsis screen: rapid diagnostic tests (RDT); CBC, micro-ESR, CRP and I/TR from peripheral smear and blood culture, management, length of hospital stay and the outcome were recorded. I/TR was calculated from the sum of all forms of immature neutrophils - band cells, myelocytes, metamyelocytes and divided by total mature neutrophils. The I/TR was interpreted as normal if <0.2, suggestive of infection if ≥0.2 and high risk of death if >0.8. The data was analysed using SPSS 23.0 version and Chi-square test & Fischer's exact t-test.

RESULTS:

Among the study participants, 54.6% were term, 45.4% preterm; 65.1% were born out of LSCS, 34.9% delivered vaginally; 52.3% had normal BW, 43% were LBW, 4.7% macrosomic; 70.9% AGA, 17.4% LGA and 11.6% SGA, M:F ratio was 1.8:1, Most (79.1%) were admitted on PND 1, followed by 8.1% each on PND 2 and PND 4. 89.5% had EOS and 10.5% had LOS. 45.3% had no risk factors for sepsis, while gestational diabetes mellitus (14%) and PPROM (12.8%) were most common, followed by anemia (9.3 %). 60.5% had I/TR<0.2% and 39.5% ≥0.2%. No neonatal death was recorded. 74.4% had a negative CRP and 25.6% had positive CRP. 80.2% had <15mmHr and 19.7% had >13mmHr. 81.3% had no growth in blood culture, 18.6% had a positive growth. 59.3% had presumed sepsis, 22.1% probable sepsis. 55.8% received antibiotics for 3 days, 24.4% for 7 days, 14% for 14 days and 5.8% for 21 days course. 44.2% had an NICU stay of 3 days, 20.9% for 1 week and 14% for 3 weeks. The study variables are summarised in Table1.

Table2 depicts the comparative data between those with IT/R≥0.2 and <0.2. There was a significant association between I/TR and confirmed, presumed and probable sepsis (p value <0.00*). Around 30% of the growth in culture and sensitivity was seen in the group with the I/TR≥0.2% (p value -0.037*). The percentage of participants who had 14 and 21 days of antibiotics course was higher among the group which had I/TR≥0.2% (p value -0.031*). Longer duration of hospital stays also observed among I/TR≥0.2% (p value -0.031*). Sensitivity and specificity of I/TR were 62.5% and 65.7% respectively. The positive and negative predictive values were 29.4% and 88.4% respectively.

DISCUSSION:

This is a unique study comparing the outcome of neonates with I/TR≥0.2 and <0.2 admitted to NICU with presumed sepsis.

Majority of the study participants were enrolled on postnatal day 1 (79.1 %), followed by postnatal day 2 (8.1 %) and day 4 (8.1%). In a similar study done by Makkar et al (3) from Haryana, found the maximum cases admitted on the 1st day of life, with majority in less than 12hrs, i.e., 51.81% neonates were less than 24hrs.

Most of the sepsis was EOS (89.5 %) while 10.5 % of sepsis was LOS, comparable to the studies done by Gebremedhin et al (4), Gebrehiwot A et al (5) and Woldu et al (6), where EOS was 76.9%, 81.8% and 81.4 % respectively. In contrast to this, Krediet et al (7) found that the incidence of EOS in the group of 185 patients was only 7.5%. The difference may be due to the fact that we have taken less than 3 days as EOS while in other studies EOS is calculated as less than 7 days (5,6).

Among the study participants 59.3 % had presumed, 22.1% had probable and 18.6% had confirmed neonatal sepsis. Among full term pregnancies, the participants are equally divided between the I/TR<0.2% and >0.2% while in late preterm 73.3% of the participants had I/TR<0.2%. This difference was not found to be statistically significant (p value -0.376). Those with I/TR ≥0.2% was higher among early term (36-37 weeks) followed by late preterm (34-37 weeks). Similar findings were reported by Darnifayanti et al (2), and had reported that babies in the 34-36 weeks gestational age had most bacterial sepsis. Thus, early term babies seem to be at high risk for neonatal sepsis than full term infants.

Majority (88.5%) with I/TR<0.2% had shorter duration of antibiotic therapy of 3-7 days whereas ITR≥0.2% had longer days of antibiotic course of 8-14 days in 20.6% and 15-21 days in 11.8%. I/TR is recommended as a marker so that we can prevent unnecessary exposure of antibiotics and also can predict the duration of antibiotics. The association between antibiotics course and I/TR was found to be statistically significant (p value -0.031). All neonates with sepsis admitted at NICU, were discharged almost with good outcomes. There was no mortality. Most (44.1%) of the babies with I/TR≥0.2% had longer duration of stay of >7days. However, prolonged stay of 6-8weeks was noted among preterm babies with I/TR<0.2, due to other major morbidities which required more hospital stay apart from sepsis like respiratory distress, feeding problems etc. There are no similar studies comparing the two groups with ITR≥0.2 and <0.2.

Sensitivity and specificity of I/TR≥0.2 was 62.5% & 65.7% and positive and negative predictive values were 29.4% & 88.4% respectively. This was similar to the findings of Darnifayanti et al (2), Krediet T et al (7) and Erum Saboohi et al (8) with a high negative predictive value of I/TR 88%, 90-98% and 93.44% respectively. Thus,

a high negative predictive value and a reasonable positive predictive value, sensitivity and specificity suggest that I/TR may be a good alternative to blood cultures to exclude neonatal sepsis.

CONCLUSION:

There was a significant association of I/TR≥0.2 with probable and confirmed sepsis. Those with I/TR≥0.2 had longer duration of antibiotic course and hospital stay. The high negative predictive value of I/TR highlights its usefulness in ruling out neonatal sepsis. Hence it is recommended as a quick and cost-effective surrogate marker of sepsis and outcome among neonates.

Limitations:

1. Only a small proportion of the study population were blood culture positive
2. Single-centre study

Table 1: Baseline Parameters in the Study (n=86)

Parameter	no (%)	
Maternal Risk factors	Present	47 (54.7)
	Absent	39 (45.3)
Type of delivery	Vaginal delivery	30 (34.9)
	Caesarean section	56 (65.1)
Gestational Age	Term	47 (54.7)
	Preterm	39 (45.3)
Gender	Male	55 (64)
	Female	31 (36)
Birth weight	Normal	45 (52.3)
	Low Birth Weight	26 (30.2)
Onset of sepsis	Early Onset Sepsis	77 (89.5)
	Late Onset Sepsis	9 (10.5)
I/TR	≥0.2	34 (39.5)
	<0.2	52 (60.5)
Sepsis screen	Positive	34 (39.5)
	Negative	52 (60.5)
Blood culture	Positive	16 (18.6)
	Negative	70 (81.3)

Table 2: Comparative Data Between Those With IT/R≥0.2 And <0.2

Parameters		I/TR		Total	Significance P value
		≥0.2 no (%) (n=34)	<0.2 no (%) (n=52)		
Confirmed sepsis		10 (29.4)	6 (11.5)	16	<0.001*
Probable sepsis		14 (41.2)	5 (9.6)	51	
Presumed sepsis		10 (29.4)	41 (78.8)	19	
Blood culture positive		10 (29.4)	6 (11.5)	16	0.037*
Blood culture negative		24 (70.5)	46 (88.5)	70	
Duration of antibiotics	3-7days	23 (67.6)	46 (88.5)	69	0.031*
	8-14days	7 (20.6)	5 (9.6)	12	
	15-21days	4 (11.8)	1 (1.9)	5	
Hospital stay	3-7days	19 (55.9)	37 (71.1)	56	0.031*
	8-14days	6 (17.6)	5 (9.6)	11	
	15-21days	6 (17.6)	6 (11.5)	12	
	>21days	3 (8.8)	4 (7.6)	7	

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