Original research article

Diagnostic Accuracy of Immature-to-Total Neutrophil Ratio in Neonatal Sepsis - A Descriptive Study.

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Abstract

Background: Neonatal sepsis accounts for early-half of all neonatal death in India. Although the gold standard for the diagnosis of neonatal sepsis is by blood culture, there is a need for a test that is cheap, accurate, easily performed with quick availability of reports so that prompt and appropriate treatment is ensured.

Aim: In this study we try to analyse the diagnostic value of I/T2ratio in comparison to I/T ratio and absolute neutrophil count (ANC) independently to correctly predict neonatal sepsis.

Materials and Methods: A prospective comparative study was done on neonates with suspected neonatal sepsis. Neonates were then analysed in three groups depending on their blood culture results as no sepsis, probable sepsis and proven sepsis. I/T2was calculated for all the babies and an optimum cut off was found for the same. The diagnostic accuracy of I/T2 ratio with septic screen positivity and blood cultures were assessed.

Results: 245 neonates included in the study were divided into three groups depending on whether they were blood culture positive sepsis, probable or clinical sepsis. The means of septic screen parameters including I/T2 were highly significant when compared between the three groups (p value <0.001). ROC curve created showed the AUC for I/T, I/T2 and ANC was 0.822, 0.809, 0.610 (p value <0.001) and a higher specificity.

Conclusion: I/T2 shows promise as a parameter to screen for sepsis in neonates. Calculating the I/T2 ratio in conjunction with established septic screen parameters could enhance prediction of sepsis in newborns.

Keywords: I/T2 ratio, neonatal sepsis, I/T ratio, ANC.

Introduction

Neonatal sepsis is frequent and important cause of morbidity and mortality particularly in the developing countries, like India and accounts for early-half of all neonatal death in our country. The incidence of neonatal sepsis in India is 30 per 1000 live births.[1] Some population-based studies have reported clinical sepsis rates ranging from 49 to 170/1000 live births in rural

India.[2] Incidence is not changed much over the past decade, and the fatality due to sepsis is between 30% and 65%.[3]

Gold standard for the diagnosis of neonatal sepsis is by demonstration of the organisms in blood culture, but it takes a long time of 2-8 days for positive culture, the cost is relatively expensive and has low positive rate and hence various other investigations are necessary for early diagnosis or to rule out sepsis.[4] Therefore, there is a need for a test that is cheap, accurate, easily performed with quick availability of reports so that prompt and appropriate treatment is ensured. Many studies have investigated the utility of ratio of immature neutrophils compared to total neutrophils (I/T ratio), the results of which might have to be read with caution in neonates in whom the total white blood cell (WBC) counts may be lowered. But not many studies have been done on the utility of immature to total neutrophil square ratio (IT2) which might be a better indicator of neonatal sepsis. In this study we try to analyse the diagnostic value of IT2 ratio in comparison to IT ratio and absolute neutrophil count (ANC) independently to correctly predict neonatal sepsis

Materials and Method
This prospective randomized study was conducted at Upgraded Department of Paediatric and Neonatology, at Patna Medical College and Hospital, Patna. The study was approved by the institutional research and ethical committee. The study was conducted between September 2020 and August 2021. An informed and written consent was taken from the participating subjects prior to the commencement of the study.

A time bound, prospective comparative study was done on all neonates admitted to neonatal intensive care unit with suspected neonatal sepsis. All neonates >32 weeks of gestation admitted to neonatal intensive care unit (NICU) with at least one of the clinical features suggestive of sepsis[5] were included in the study.

The exclusion criteria were neonates who have already been started on antibiotics before admission to NICU. Babies with jaundice were included had all other regular causes ruled out.

Data collection
Demographic data, clinical symptoms and examination findings were recorded for all the neonates who were fitting into the inclusion criteria. This included sex, gestational age, age of onset of symptoms, clinical features suggestive of sepsis, vital signs and systemic findings.

Laboratory Diagnosis
All newborns included underwent a septic screening[6,7] as per the NICU protocol. Blood was collected from peripheral vein under all aseptic precautions prior to starting antibiotic treatment. 1 ml of blood was collected for blood cultures and transferred to BACTEC bottles. Blood sample of 2 ml each was then collected in plain vacutainer as well as in EDTA vacutainer. Plain vacutainer for CRP estimation and EDTA vacutainer for total leucocyte count, platelet count, preparation of peripheral smears and for measurement of micro ESR.
The neonates were then divided into three groups depending on blood culture and septic screen as follows: culture positive sepsis, probable sepsis and clinical sepsis. Baseline characteristics like sex, gestational age, birth weight, number of days the newborn received antibiotics and number of days of NICU stay were compared in each group as depicted in Table 1. All were subjected to septic screening, along with which I/T2 ratio was calculated, the means of which were highly significant when compared between the three groups as shown in [Table 1].

Findings which were consistent with sepsis were considered if at least 2 out of 5 criteria were fulfilled in the haematological scoring system. Cultures were considered positive if it has a significant yield of an organism known to cause bacteremia. Organisms usually known to contaminate skin were also included if the yield was significant as the neonates included were symptomatic. Neonates were then analysed in three groups depending on their results –

Limb 1: Proven sepsis,

Limb 2: Probable sepsis and

Limb 3: Clinical sepsis.

Calculation of IT2

Calculation of I/T ratio was done by dividing the total immature neutrophil count by total neutrophil count. IT2 was taken by dividing the total neutrophil count again with this value. Normal IT ratio was considered to be <0.2.

IT2 = Total immature neutrophil count Total neutrophil count

Statistical analysis

Statistical analysis was done using Statistical Package of Social Sciences (SPSS) version 17.0. Demographic data were analysed by mean and standard deviation. Pearsons chi square tests were done to analyse categorical data. Continuous variables were analysed by ANOVA. P value

<0.05 will be considered as significant. The diagnostic accuracy of IT2 ratio with septic screen positivity and blood cultures were assessed by creating a receiver operator curve (ROC) and the area under the curve (AUC) was calculated.

Table 1: Clinical features suggestive of neonatal sepsis	
Sl. No.	Clinical Features
1	Neurological: convulsions, drowsy or unconscious, decreased activity, bulging fontanelle
2	Respiratory: Respiratory rate >60/min, grunting, severe chest indrawing, central cyanosis
3	Cardiac: poor perfusion, rapid and weak pulse
4	Gastrointestinal: Jaundice, poor feeding, abdominal distension
5	Dermatological: skin pustules, periumbilical erythema or purulence
6	Musculoskeletal: edema or erythema overlying bones or joints
7	Temperature >37.7 C or <35.5 C

Results

Of the 304 newborns eligible for the study, 254 were included. 8 newborns were discharged against medical advice, 29 newborns were already started on antibiotics before including in the study, 7 newborns had laboratory error in reporting and 6 newborns had congenital anomalies.

Table 2:		
Sl. No.	Laboratory Parameter	Positive For Sepsis
1.	Total leucocyte count	<5000/mm3, >30,000/mm3
2.	Immature/total neutrophils	>0.2
3.	Micro-ESR	>15 mm in first hour
4.	C reactive protein (CRP)	>6 mg/dl
5.	Absolute neutrophil count	As per Manroe and Mouzinho's chart[8]
6.	Platelet count	$< 1.5 * 10^5$ cells/mm3

Table 3: Baseline Characteristics – mean and standard deviation				
Variable	Proven sepsis	Probable sepsis	No sepsis	p value
Sex (n,%) Male Female	31 (47) 35 (53)	26 (36.1) 46 (63.9)	50 (43.5) 65 (56.5)	0.410
Gestational age (weeks)	34.6 ± 2.6	34.4 ± 3.2	36.1 ± 4	< 0.001
Birth weight (kg)	2 ± 0.83	1.9 ± 0.68	2.3 ± 0.65	0.002
Number of days of antibiotics	16.1 ± 8.3	11 ± 5.8	4.8 ± 2	< 0.001
Days in NICU	20 ± 12.4	14.6 ± 9.9	5 ± 3.4	<0.001

ROC curve was created to assess the value of I/T2 ratio in diagnosing true blood culture positive sepsis in comparison with ANC and I/T ratio individually. ROC curve is depicted in [Figure 1]. AUC was then calculated for all the three and found to be 0.822, 0.809 and 0.610 for I/T2 ratio, I/T ratio and ANC respectively; the p value of which is 0.001. While plotting the ROC curve, we tried to find an ideal cut off point for the I/T2 ratio which was 0.01. Subsequently, we also tried to find out according to our data, the sensitivity and specificity of the known cut off of I/T ratio of 0.2.

Surprisingly, we found the highest specificity and sensitivity for a cut off of 0.12, suggesting that the newborns with proven, culture positive sepsis had a value of I/T ratio much lower than the established cut off. The sensitivity, specificity, positive predictive value and negative predictive value of IT2 was 62.1%, 91.4%, 71.9% and 87.2% respectively. Similarly, the sensitivity, specificity, positive predictive value and negative predictive value of IT was 65.2%, 87.8%, 65.2% and 87.8% respectively. Also, the sensitivity, specificity, positive predictive value and negative predictive value of Absolute neutrophil count(ANC) was 45.4%, 76.6%, 40.5% and 80% respectively. This shows that I/T2 has a considerably higher specificity and negative predictive value when compared to I/T ratio, while the sensitivity and specificity of ANC in detecting a proven sepsis is much lower than I/T or I/T2 ratios. A detailed subgroup analysis of I/T2 ratio (< 0.01) in different ranges of ANC (< 500, 500 – 999, 1000 – 1499 and >1500), a p value of 0.06 was attained which did not show any significant difference.

Table 4: Mean and standard deviation of septic screen and other haematological parameters				
Blood investigation	Proven sepsis	Probable Sepsis	No sepsis	p value
Hemoglobin (g/dl)	14.7 ± 3.2	16.7 ± 3.4	17.1 ± 2.3	< 0.001
WBC (cells/mm3)	9.8 ± 5.1	12.8 ± 6.3	16.6 ± 7	< 0.001
Platelets (* 105 cells/mm3)	1.7 ± 0.95	2.3 ± 1	2.5 ± 0.83	< 0.001
ANC (cells/mm3)	578.6 ± 400	759.9 ± 457	945.2 ± 590	<0.001
I/T ratio	0.24 ± 0.14	0.13 ± 0.13	0.04 ± 0.03	< 0.001

I/T2 ratio	0.033 ± 0.03	0.012 ± 0.012	0.003 ± 0.004	< 0.001
CRP (mg/dl)	27.9 ± 25.2	17.2 ± 22.7	9.2 ± 13.5	< 0.001
Micro ESR (mm of H2O)	10.3 ± 4.5	9.3 ± 4.4	7.6 ± 3.9	< 0.001

Table 5: Comparison of sensitivity, specificity, positive predictive value and negative predictive value of I/T ratio, IT2 ratio and ANC.

Parameter	Sensitivity	Specificity	PPV	NPV
I/T	65.2%	87.8%	65.18%	87.8%
I/T2	62.1%	91.4%	71.9%	87.2%
ANC (< 500/mm3)	45.4%	76.6%	40.5%	80%
ANC (< 1500/mm3)	95.3%	10.7%	27.1%	86.9%

Discussion

Sepsis is a systemic inflammatory response to infection and so isolating the causative organism is a must. Although blood culture is the gold standard, it might be important to remember that a larger amount of blood might yield a better culture which may not be possible in neonates. Also, the time taken for a blood culture to grow an organism might vary from 24 – 48 hours.[8] This makes it imperative to device an effective screening tool for neonatal sepsis which is close to blood culture in terms of accuracy.

I/T2 ratio has shown to have better specificity and almost similar sensitivity to ANC and I/T ratio individually and hence is ideal to be used as an independent marker for screening neonatal sepsis.[8] The importance of I/T2 ratio lies in the fact that in the case of low total neutrophil count, a high I/T ratio may be seen which might screen falsely negative for sepsis. This can be avoided by calculation of I/T2, especially where the sepsis was detected in newborns due to low total count.[8]

There is a lot of discrepancy in the cut-off values taken for I/T ratio in different studies evaluating different parameters of neonatal sepsis. While many standard references mention an I/T ratio of more than 0.3 to be indicative of sepsis,[9] most of the studies done on neonatal sepsis considers a cut-off of more than 0.2 for its diagnosis. Schmutz et al showed that a single value of I/T ratio of > 0.3 had a high NPV(99%)but a very poor PPV(25%).[7,10-13] Recently, increasing I/T ratios were shown to have increasing logistic regression values, highest for a cut-off >0.60 at ≥4 hours (10.7) in 245 neonates with culture-proven sepsis.[14] However in our study, we initially considered any value >0.2 in our inclusion criteria, but we found out highest specificity and sensitivity for a cut off of 0.12, suggesting that the newborns with proven, culture positive sepsis had a value of I/T ratio much lower than the established cut offs of either 0.2 or 0.3.This is consistent with a study done by Diana et al,[15] where the reference value of I/T ratio in healthy newborns were 0.01-0.13 (mean 0.06), in sepsis neonates 0.13-0.60 (median 0.26) and the cut off value for sepsis detection was 0.13 and they concluded that I/T ratio could be used as a marker for early detection of newborn septicaemia.

In a prospective study of 150 neonates 32–38 weeks gestational age with early onset sepsis, the sensitivity of an ANC <8000/mm3 was 76% and the negative predictive value was 94%.[16] In the study done by Newman et al, an ANC <1000/mm3 had a +LR of 7.5, 33.5 and 115 when drawn at <1, 1–3 and ≥4 hours, respectively, in patients ≥34 weeks with positive culture

results.[14] In the study done by Shalini et al, positive septic screen was considered when there was presence of two of the four parameters namely, TLC (< 5000/mm3),band to total polymorphonuclear cells ratio of>0.2, absolute neutrophil count < 1800/mm3,C- reactive protein (CRP) >1mg/dl and micro ESR > 10 mm- first hour.1In our study, we tried to compare the sensitivity and specificity in predicting sepsis with ANC cut offs of 500 cells/mm3 with 1500 cell/mm^3 and found a higher specificity and PPV when the value of 500 cells/mm^3 was considered as the cut-off while sensitivity and negative predictive value increased to 95.4% was 86.9% with 1500 cells/mm3 at the cut-off, but it lacked good specificity and positive predictive value.

In our study we found that the culture positivity rate was 25.9% and Klebsiella was the most common organism isolated followed by Staphylococcus epidermidis/ Methicillin Resistant Staphylococcus aureus (MRSA) as opposed to other studies. In a review of blood culture results of neonatal sepsis in the first 90 days of life from 63 centres, the South Asian region appeared to have 9.8% S. aureus, 0.8% Group B Streptococcus, 23% Klebsiella, and 12% E. coli during the first 28 days of life.[17,18] In a study done by Hamer et al in 2015, S.aureus was the predominant organism in neonatal sepsis, followed by Acinetobacter species, Enterobacter species, E.coli, and Klebsiella.[19]

Conclusion

I/T2 shows promise as a novel, effective, simple method to screen sepsis when used in conjunction with established parameters like ANC and I/T ratio. Its advantage lies in the fact that it does not require special laboratory facilities which makes it useful in the early detection of neonatal sepsis. Additional studies may be required to validate this index in larger populations.

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