ORIGINAL RESEARCH

Usefulness of immature to total neutrophil ratio (I/T ratio) in the diagnosis of early neonatal sepsis

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Abstract
Background: To evaluate the usefulness of immature to total neutrophil ratio (I/T Ratio) in early neonatal sepsis diagnosis.
Materials & methods: A total of 115 neonates included 4-5 days old who were admitted to neonatal intensive care unit (NICU) and suspected to have sepsis based on peri-natal risk factors which included low birth weight, prematurity, abnormal amniotic fluid, premature rupture of membranes, foetal distress and maternal pyrexia together with clinical features were taken as the study group. The unpaired student t test will be used to determine the statistical significance between the study groups. A P value of <0.05 will be considered to be statistically significant.
Results: The difference of mean positive blood culture was significantly higher in I/T ratio, and WBCs as compared negative blood culture. p value were 0.001 &<0.0001 respectively. Although, the mean weight with positive blood culture 2.451 ± 0.456 kg was slightly more as compared to negative BC 2.391 ± 0.714 kg but it was statistically non-significant. The mean I/T in positive CRP 0.204 ± 0.04 was non-significantly higher as compared to negative CRP 0.162 ± 0.038 (p =0.074).
Conclusion: The I/T ratio is a useful test for detecting early sepsis (EOS) with high specificity.
Keywords: neonatal sepsis, CRP, WBC.

Introduction
Neonatal sepsis is described as a suspected or demonstrated infection in newborn, a systemic inflammatory response syndrome with variable sign and symptoms caused by pathogens with or without accompanying bacteremia. [1] The incidence of Neonatal sepsis is approximately 8 per 1000 live births and as high as 13 to 27 per 1000 for newborns weighing < 1500 gms. [2] Neonatal septicemia is responsible for 1.5 to 2.0 million deaths/year or between 4000 to 5000 deaths/day in the underneath advanced countries of the world. [3] Two thirds of the world's neonatal deaths occur in just 10 countries, frequently in Asia. Pakistan accounts for 7% of global neonatal deaths. The predominant causes are infections (36%), preterm births (28%) and birth asphyxia (23%) accounting for about 87% of neonatal deaths worldwide. [4]Early-onset sepsis(EOS) is generally caused by the transmission of pathogens from the female genitourinary system to the newborn or the fetus. These pathogens can ascend the vagina, the cervix, and the uterus, and can also infect the amniotic fluid. Neonates can also become

infected in utero or during delivery as they pass through the vaginal canal. Typical bacterial pathogens for EOS include Group B streptococcus (GBS), Escherichia coli, coagulase-negative Staphylococcus, Haemophilus influenza, and Listeria monocytogenes. Maternal factors that increase the risk of neonatal sepsis include chorioamnionitis, GBS colonization, delivery before 37 weeks, and prolonged rupture of membranes greater than 18 hours. [5]

A complete blood count (CBC) with white blood cell (WBC) differential is often done as part of an evaluation for sepsis in newborns. Beginning with Manroe et al [6], many investigators have shown that a low WBC count, a low absolute neutrophil count (ANC) and a high proportion of immature neutrophils (I/T, where I is the immature and T is the total neutrophil count) predict infection.[7-9] Recently showed that the CBC is more informative if its performance is deferred until at least 4 hours of age and the results are categorized into intervals, rather than simply dichotomized into "normal" and "abnormal" ranges.[8] Hence, this study was conducted to evaluate the usefulness of immature to total neutrophil ratio (I/T Ratio) in early neonatal sepsis diagnosis.

Materials & methods

A total of 115 neonates included 4-5 days old who were admitted to neonatal intensive care unit (NICU) and suspected to have sepsis based on peri-natal risk factors which included low birth weight, prematurity, abnormal amniotic fluid, premature rupture of membranes, foetal distress and maternal pyrexia together with clinical features were taken as the study group. A combination of clinical and laboratory parameters was used to identify the neonates with presumed sepsis. The samples for Complete Blood Count (CBC) were taken at the same time into microtainer tripotassium EDTA tubes and analyzed by fully auto analyzer in the manual mode within 4 hours of collection to obtain total WBC count. The samples for CRP were taken at 48 hrs into plain bottles and sent for analysis. As there was no single laboratory parameter available as the diagnostic tool in early neonatal sepsis; high clinical suspicion, positive blood culture and increased CRP value (>6 mg/L) were considered. All the results will be tabulated as mean and standard deviation. By using the SPSS 23.0 version for statistical analysis. The unpaired student t test will be used to determine the statistical significance between the study groups. A P value of <0.05 will be considered to be statistically significant.

Results

A total 115 neonates were registered after screening, out of which 59 (51.3%) were males and 56 (48.7%) were females. The mean age and weight of the neonates were 1.82±0.0.89days and 2.69±0.85kg (maximum 4 kg and minimum 1.1 kg) respectively.

Table: 1. Categorical variable of study population (n=115)

Variables	Mean/SD, No(%)
Mean age (days)	1.82±0.0.89
Mean weight (kg)	2.69±0.85
Male	59(51.3%)
Female	56(48.7%)
Blood culture positive	41(35.6%)
Blood culture negative	74(64.4%)

Furthermore, the difference of mean positive blood culture was significantly higher in I/T ratio, and WBCs as compared negative blood culture.p value were 0.001 &<0.0001 respectively. Although, the mean weight with positive blood culture 2.451 ± 0.456 kg was slightly more as compared to negative BC 2.391 ± 0.714 kg but it was statistically non-

significant. The mean I/T in positive CRP 0.204 ± 0.04 was non-significantly higher as compared to negative CRP 0.162 ± 0.038 (p =0.074).

Table: 2 Blood culture with I/T ratio, age, weight and total WBC count & CRP.

Parameters	Blood culture		p-value
	Negative (n=74)	positive (n=41)	
I/T ratio	0.1634± 0.0392	0.210± 0.034	0.001*
Age	1.612±0.652	2.101±0.742	0423
Weight	2.451±0.456	2.391±0.714	0.587
WBC	15874.25±6854.23	19861.2±8350.35	<0.001*
CRP	0.162±0.038	0.204±0.04	0.074

Table: 3 Association of Blood Culture with I/T ratio and CRP

Parameters	Blood culture		p- value
	Negative (n=74)	Positive (n= 41)	
I/T Ratio			
>0.2	12 (16.2%)	32 (78%)	<0.001
<0.2	62 (83.8%)	9 (22%)	
CRP			
Negative	60 (81.1%)	8 (19.5%)	<0.001
Positive	14 (18.9%)	33 (80.5%)	

A difference in proportion of I/T ratio ≥ 0.2 was statistically significant higher in positive blood culture p value was< 0.001).

Table: 4. Diagnostic Accuracy of I/T ratio in cases with neonatal sepsis.

	Proven sepsis (CS positive) (n=41)	Presumed sepsis (CS negative) (n=74)
I/T Ratio >0.2	True positive (TP) = 32	False positive (FP) = 12
I/T Ratio <0.2	False negative (FN) =9	True negative (TN) = 62

Statistic	Value	95% CI
Sensitivity	78.05%	62.39% to 89.44%
Specificity	83.78%	73.39% to 91.33%
Positive likelihood ratio	4.81	2.80 to 8.28
Negative likelihood ratio	0.26	0.15 to 0.47
Disease prevalence *	35.65%	26.94% to 45.12%
Positive predictive value*	72.73%	60.78% to 82.11%
Negative predictive value *	87.32%	79.32% to 92.52%
Accuracy *	81.74%	73.45% to 88.33%

Specificity = 83.78%, Sensitivity = 78.05%, PPV = 72.73%, NPV = 87.32%, Accuracy= 81.74%.

Discussion

Risk factors for neonatal sepsis are low birth weight infants, birth asphyxia, respiratory compromise at birth, maternal risk factors and congenital anomalies. [5] Diagnostic tools for identification of EOS includes prenatal screening of high risk mothers to clinical and laboratory identification of newborns with presumed sepsis. Various diagnostic tools have been extensively studied over many years for EOS like WBC, BANDS, ANC, immature to total neutrophil ratio, CRP, interleukin six, procalcitonin. Blood culture remains a gold standard for diagnosing neonatal sepsis but results are typically obtained after three to five

days and its accuracy varies between eight and 73% in various studies. [8] However, there are some screening tests (WBC, Platelets, Micro Erythrocyte sedimentation rate(ESR), Absolute Neutrophilic Count (ANC), C-Reactive Protein (CRP), (I/T) ratio, nitroblueteterazolium (NBT), serial Interleukin-6 (IL-6) and pro-calcitonin) that could predict sepsis within 6 to 8 hours. [10] The ANC (<1000/ul) and the I/T ratio(≥ 0.2)give the clue about the early-onset sepsis in newborns. [11] Rapid diagnostic tests like CRP, WBC indices may be used as a screening approach for early diagnosis and treatment of sepsis. [12] Hence, this study was conducted to evaluate the usefulness of immature to total neutrophil ratio (I/T Ratio) in early neonatal sepsis diagnosis.

In the present study, a total 115 neonates were registered after screening, out of which 59 (51.3%) were males and 56 (48.7%) were females. The mean age and weight of the neonates were $1.82\pm0.0.89$days and 2.69 ± 0.85kg (maximum 4 kg and minimum 1.1 kg) respectively. A study by Saboohi E et al, showed a descriptive cross-sectional study conducted in Neonatal Intensive Care Unit of Liaquat College of Medicine & Dentistry (LCMD) Hospital from January 2016 to January 2017. Out of 85 neonates, 13 had positive blood cultures (15.29%). The mean white blood count was 18761.18 ± 8570.75 and mean I/T ratio was 0.1622 ± 0.0419. About 50% of proven sepsis cases had WBC higher than 26000 as compared to 50% of cases for negative diagnoses that had WBC <15500. The mean I/T in positive CRP 0.204 ± 0.04 was non-significantly higher as compared to negative CRP 0.151 ± 0.034 (p =0.084). The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of I/T ratio were 76.47%, 83.82%, 54.16% and 93.44% respectively. Similarly majority of neonates having high I/T ratio also depicts positive C-reactive protein (CRP) (NPV 91.23%). Therefore, both I/T and CRP showed a high negative predictive value (I/T = 93.44% and CRP = 91.23%). [13]

In the present study, the difference of mean positive blood culture was significantly higher in I/T ratio, and WBCs as compared negative blood culture. P-value were 0.001 &<0.0001 respectively. Although, the mean weight with positive blood culture 2.451 ± 0.456 kg was slightly more as compared to negative BC 2.391 ± 0.714 kg but it was statistically non-significant. The mean I/T in positive CRP 0.204 ± 0.04 was non-significantly higher as compared to negative CRP 0.162 ± 0.038 (p =0.074). A difference in proportion of I/T ratio ≥ 0.2 was statistically significant higher in positive blood culture p value was< 0.001). Specificity = 83.78%, Sensitivity = 78.05%, PPV = 72.73%, NPV = 87.32%, Accuracy= 81.74%. Another study by Newman TB et al, obtained electronic records of blood cultures and of complete blood counts with manual differentials drawn <1 hour apart on 66,846 infants $\geq$ 34 weeks gestation and <72 hours of age born at Kaiser Permanente Northern California and Brigham and Women's Hospitals. They evaluated the ability of the I/T to discriminate newborns with pathogenic bacteremia from other newborns tested using the area under the receiver operating characteristic curve (c). Discrimination of the I/T (c = 0.79; 95% confidence interval: 0.76-0.82) was similar to that of logistic models with indicator variables for each of 24 combinations of the absolute neutrophil count and the proportion of immature neutrophils (c = 0.80, 95% confidence interval: 0.77-0.83). Discrimination of the I/T improved with age, from 0.70 at <1 hour to 0.87 at $\geq$ 4 hours. However, 60% of I/T had likelihood ratios of 0.44-1.3, thus only minimally altering the pretest odds of disease. [10]

I/T ratio reference ranges are obtained from Schmutz chart.[14] A ratio of $\geq$0.2 is highly sensitive marker of neonatal septicemia. [15] Some previous studies have shown different results in this parameter which may be due to variation in blood sampling, severity of sepsis, age of patient and investigative criteria followed, sensitivity: 90% to 100%, specificity: 30% to 78%, PPV: 11% to 51% and NPV: 99% to 100%. [16]

Conclusion

The I/T ratio is a useful test for detecting early sepsis (EOS) with high specificity.

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