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Original Research Article

Diagnostic accuracy of haematological indices in the diagnosis of neonatal sepsis in a resource poor setting: a cross-sectional study

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ABSTRACT

Background: Where the newer methods for the diagnosis of neonatal sepsis are not available, reliability of simple and inexpensive haematological indices as predictors of neonatal infection becomes useful. This study aimed to determine the sensitivity and specificity of total white blood cell count (TWBC), immature-to-total neutrophil ratio (I/T ratio) and micro-erythrocyte sedimentation rate (micro-ESR) in the diagnosis of neonatal sepsis.
Methods: A hospital-based cross-sectional study of 120 neonates hospitalized at the Special Care Baby Unit (SCBU) of a tertiary institution whose parents/caregivers wrote informed consent were recruited while neonates with gross congenital abnormalities (obvious neural tube defects, gastroschisis) and those whose caregivers refused to give consent were not recruited. A proforma was used to document neonatal and laboratory information. Data were analysed, appropriate proportions were used to obtain the sensitivity and specificity of TWBC, I/T ratio and m-ESR. Test of association for categorical variables were done using Chi-square test and T-test for comparison of mean between babies with and without sepsis. P value was set at <0.05.
Results: Associations between TWBC, I/T ratio and neonatal sepsis were statistically significant (p=0.015, 0.020 respectively). There was a statistical difference between the mean I/T ratio in babies with and without sepsis. (p=0.032). The sensitivities of I/T ratio, m-ESR and TWBC were 83.2%, 53.3% and 46.7% respectively while their specificities were 40.0%, 61.1% and 76.7% respectively.
Conclusions: Hospitals in resource poor-settings, where blood culture cannot be obtained readily, I/T ratio can be used to detect neonatal sepsis.

Keywords: Cross-sectional study, Diagnostic accuracy, Haematological indices, Neonatal sepsis, Resource poor settings

INTRODUCTION

Blood culture is considered the standard for detection of neonatal sepsis.[1] Automated blood culture systems; such as the Bactenecin (BACTEC), BacT/Alert, BioArgos etc have been reported to be among the most sensitive methods and are used in most countries worldwide. They employs mechanisms that neutralise antimicrobial agents

from blood or render the circulating drugs inactive. They also, can detect bacterial presence even at concentrations of 1-2 colony-forming unit (CFU) per ml using the improved bacteriological techniques. Despite these advantages, these new culture methods are expensive, require high-tech laboratory equipment and well-trained laboratory staff which are not available in many developing countries in Nigeria.[2,3] Also, the long duration of incubation period and the long period before antibiogram sensitivity becomes available, all lead to delay in prompt intervention.[1]

Many attempts have been made to develop a set of screening tests which can rapidly diagnose presence of septicaemia in neonates and prevent a delay associated with waiting for blood culture results.[4,5] In settings where these newer methods (BACTEC, BacT/Alert etc) are not available due to their high cost and need for technical expertise, the reliability of simple and inexpensive haematological indices as predictors of neonatal infection becomes useful.

Various haematological indices such as total white blood cell count (TWBC), immature to total neutrophil ratio (I/T ratio) and micro-erythrocyte sedimentation rate (micro-ESR), when analyzed can serve as screening tests for early diagnosis of neonatal sepsis circumventing delays arising from waiting for blood culture results.[5-8] The presence of two or more abnormal haematologic parameters with strong clinical suspicion of sepsis is considered a positive sepsis screen.[6,9] These tests are simple, quick, and cost-effective. However, the sensitivity, specificity, positive and negative predictive values of these parameters have been shown to vary widely across studies.[6,7,10-13] They have also been found to be more useful in excluding neonates without infection rather than identifying those who are infected.[14] This study therefore aimed to determine the diagnostic accuracy of haematological indices (total white blood cell count, immature-to-total neutrophil ratio and micro-erythrocyte sedimentation rate) in the diagnosis of neonatal sepsis.

METHODS

This was a hospital-based cross-sectional study, carried out at the Special Care Baby Unit (SCBU) of the Nnamdi Azikiwe University Teaching Hospital Nnewi in Nigeria. Data collection was commenced on February 2019 and completed on July 2019. All neonates (both in-born and out-born) hospitalized in the SCBU were studied.

Inclusion criteria

All neonates hospitalized with not less than two clinical features indicative of sepsis and whose parents/caregivers wrote informed consent were recruited in the study.

Exclusion criteria

Neonates with gross congenital abnormalities such as obvious neural tube defects, gastroschisis and whose caregivers were not able to give accurate information and refused to give consent were not recruited.

Sample size determination

The estimated average admission in the SCBU per annum is 420. The minimum sample size for the study was calculated using the formula;[15]

$$n = \frac{z^2 pq}{d^2}$$

Where n = minimum sample size, Z = standard normal variate (standard value of 1.96 for 95% confidence interval), p = prevalence of NNS from a previous study in the study center = 10.8%[16], q = 1-p, d = degree of precision which was set at 5% (standard value of 0.05).

Therefore,

$$n = \frac{1.96^2 \times 0.108 \times (1 - 0.108)}{0.05^2}$$

$$n = \frac{3.8416 \times 0.108 \times 0.892}{0.0025}$$

$$n = \frac{0.3701}{0.0025}$$

$$n = 148.04$$

Thus, n = 149

But $nf = \frac{n}{1 + (n/N)}$

Where; nf = the desired sample size when the population is less than 10,000, n = 149, N = 420 (the estimate of the population size, which is the total number of admissions in SCBU of the tertiary institution over one year).

Therefore, $nf = \frac{149}{1 + \frac{149}{420}}$

$$nf = \frac{149}{1.35}$$

nf = 110.37, which is approximately 111. A minimum sample size of 111 was used for the study.

Sampling method

Total 120 eligible neonates were recruited consecutively until the needed sample size was obtained. Identification numbers were given to each participant recruited for easy identification and to avoid double enrolment.

Data collection

A proforma was used to collect basic demographic details from the participants clinical records and to record the results of the Micro-ESR, TWBC and the I/T ratio. The blood sample collection was done under aseptic conditions and standard precautions were maintained. The site for venepuncture was first prepared to minimize contamination by skin flora with sterile cotton wool soaked with 70% alcohol. The skin was scrubbed in a widening spiral pattern for 30 seconds with the alcohol swap followed by 10% povidone-iodine. The site was then allowed to dry for about 30-60 seconds. Two Millilitres of blood sample was put into a coded plastic blood collection tube containing ethylenediamine-etetraacetic acid (EDTA) as the anticoagulant for the total white blood cell count and blood film for I/T ratio. The tube was gently rocked to ensure adequate mixing of the blood sample with the anticoagulant. Afterwards, the pre-heparinized capillary tube was used to collect the blood sample for micro-ESR by heel-prick technique after cleaning the site with 70% alcohol. Micro-ESR was said to be elevated if the height of plasma column measured was greater than the sum of the age in days and a constant (3) for neonates aged 0 to 14 days; and greater than 15 mm/hr for neonates aged 15 to 28 days. The complete blood count estimation was performed using the Mindray BC-5300 haematology autoanalyzer. Blood film was also reported manually to determine corrected white blood cell count as well as differential white cell count. TWBC <5000/mm^3 or >20,000/mm^3 was taken as suggestive of sepsis. Immature to total neutrophil ratio (I/TNR) was estimated on peripheral blood smear (PBS) stained with Leishmann's stain and a ratio of ≥0.2 was regarded as abnormal.[11,17-19] One milliliter of the blood sample was introduced into a properly coded BACTEC-Ped plus aerobic blood culture bottle after disinfecting the cover cap with alcohol. Blood samples were collected before the commencement of antibiotics and where the delay was unavoidable, the antibiotics were commenced before sample collection. The BACTEC Ped Plus blood culture bottles were incubated in the BACTEC 9050 automated system at a temperature of 37^{0}C for five days with strict adherence to the manufacturer's instruction.[20] Bacterial growths was indicated by a signal alarm system in the BACTEC automated blood culture machine indicating a presumptive presence of a viable microorganism in the vial. Samples with bacterial growth were sub-cultured on blood agar and MacConkey agar and incubated at 35°C-37^{0}C for 18-24 hours. A positive culture on solid media was determined as up to one colony-forming unit on the surface of the culture media along the line of streaking. The vials with no signal alarm were sub cultured terminally by the end of the 5th day and were recorded as negative if no growth was observed on any of the culture plates.

All isolates were identified based on colonial morphology, Gram-staining and results of biochemical testing such as catalase, coagulase, indole, citrate utilization, sugar utilization (with Kligler iron agar-KIA) tests using standard techniques for identification of organisms.[21,22] This test process was carried out by residents in clinical microbiology with the researcher participating in all the steps of the process, under the supervision of a Consultant Clinical Microbiologist.

Data analysis

All completed proforma were cleaned and coded before entry for analysis. Data were analysed using the Statistical Package for the Social Sciences (SPSS) statistics for window, version 23.0 (IBM Corp., Armonk, N.Y., USA). Frequency tables were generated for categorical variables. Appropriate proportions were used to derive the sensitivity, specificity, positive and negative predictive values of TWBC, I/T ratio and micro-ESR. Test of association between categorical variables was done using Chi-square test (or Fisher's exact test as appropriate). A p value of <0.05 was taken as statistically significant.

RESULTS

One hundred and twenty (120) participants who met the inclusion criteria were recruited consecutively for the study.

Table 1: General characteristics of the study population (n=120).

Variable	Frequency	%
Gender		
Male	68	56.7
Female	52	43.3
Maturity		
Preterm < 37 weeks GA	43	35.8
Term ≥ 37-42 weeks GA	77	64.2
Mode of delivery		
Spontaneous vaginal delivery	90	75.0
Assisted vaginal delivery	2	1.7
Caesarean section	28	23.3
Resuscitation at delivery		
Yes	59	49.2
No	61	50.8
Birth weight (g)		
Low <2500	47	39.1
Normal 2500-3999	65	54.2
High ≥ 4000	8	6.7

General characteristics of the study population

There were 68 (56.7%) males and 52 (43.3%) females giving a male to female ratio of 1.3:1. The median age at admission was 48(14.75-96.0) hours while the mean birth weight was 2,610.83±913.30g. Seventy-seven (64.2%) were term delivery and 47 (39.2%) of the study population were low birth weight. The mode of delivery was spontaneous vaginal in 90 (75%) of the subjects (Table 1).

Association between haematological indices and a positive blood culture

Fourteen (40%) participants with abnormal total WBC had positive blood culture while 16 (18.8%) with normal total WBC were culture positive. The associations between total WBC, immature to total neutrophil ratio and positive blood culture (NNS) were statistically significant. (p-values = 0.015, 0.020 respectively). There was a statistical difference between the mean I/T ratio and positive blood culture (NNS). (p-value = 0.032, T-test) (Table 2).

Table 2: Association between haematological indices and a positive blood culture.

Haematological indices	Neonatal sepsis			Test statistic	P value
	Culture positive n=30	Culture negative n=90	Total n=120		
TWBC					
Abnormal, N (%)	14 (40.0)	21 (60.0)	35 (100.0)	5.929[a]	0.015*
Normal, N (%)	16 (18.8)	69 (81.2)	85 (100.0)		
Mean	16,860.3	14,483.1	15,077.4	1.060[b]	0.291
SD	13.591.8	9,474.4	10,640.6		
I/T ratio					
Abnormal, N (%)	25 (31.6)	54 (68.4)	79 (100.0)	5.446[a]	0.020*
Normal, N (%)	5 (12.2)	36 (87.8)	41 (100.0)		
Mean	0.42	0.32	0.35	2.171[b]	0.032*
SD	0.23	0.22	0.22		
m-ESR					
Elevated, N (%)	16 (31.4)	35 (68.6)	51 (100.0)	1.921[a]	0.166
Not elevated, N (%)	14 (20.3)	55 (79.7)	69 (100.0)		
Mean	8.87	6.93	7.42	1.778[b]	0.078
SD	4.72	5.29	5.20		

[a]Chi square; [b]T-test; *Statistically significant; SD, standard deviation

Table 3: Diagnostic value of total white blood cell count (TWBC) in diagnosis of NNS.

Variable		Culture result		
		Positive	**Negative**	**Total**
TWBC	Abnormal	a (TP) 14	b (FP) 21	a + b 35
	Normal	c (FN) 16	d (TN) 69	c + d 85
	Total	a + c 30	b + d 90	 120

TP: true positives, FN: false negative, FP: false positive, TN: true negatives

Table 4: Diagnostic value of immature to total neutrophil ratio (I/T RATIO) in diagnosis of NNS.

Variable		Culture		
		Pos	**Neg**	**Total**
I/T ratio	Abnormal	a (TP) 25	b (FP) 54	a + b 79
	Normal	c (FN) 5	d (TN) 36	c + d 41
	Total	a + c 30	b + d 90	120

TP: true positives, FN: false negative, FP: false positive, TN: true negatives

Diagnostic value of haematological indices TWBC in the diagnosis of NNS

Out of the 120 study subjects, 35 (29.2%) had abnormal TWBC while 85 (70.8%) had normal TWBC. The sensitivity of the TWBC was 46.7% while its specificity was 76.7%. Of the 35 abnormal TWBC, there were 14 (40.0%) true positives and 21 (60.0%) false positives. Also, there were 16 (18.8%) false negatives and 69 (81.2%) true negatives (Table 3).

Diagnostic value of immature to total neutrophil ratio (I/T ratio) in diagnosis of NNS

Out of the 120 subjects, 79 (65.8%) had an abnormal I/T ratio while 41 (34.2%) had a normal I/T ratio. The sensitivity of the I/T ratio was 83.3% while its specificity was 40.0%. Of the 79 abnormal I/T ratio, there were 25 (31.6%) true positives and 54 (68.4) false positives. Also, there were 36 (87.8%) true negatives and 5 (12.2%) false negatives (Table 4).

Diagnostic value of micro-erythrocyte sedimentation rate in the diagnosis of NNS

Out of the 120 neonates studied, 51(42.5%) had elevated m-ESR while 69 (57.5%) did not have elevated m-ESR. The sensitivity and specificity of the m-ESR was 53.3% and 61.1% respectively. Of the 51 participants with elevated m-ESR, 16 (31.4%) had true positives, while 35 (68.6%) had false positives (Table 5).

Table 5: Diagnostic value of micro-erythrocyte sedimentation rate in the diagnosis of NNS.

Variable		Culture		
		Pos	**Neg**	
m-ESR	Elevated	a (TP) 16	b (FP) 35	a + b 51
	Not elevated	c (FN) 14	d (TN) 55	c + d 69
	Total	a + c 30	b + d 90	120

DISCUSSION

The evaluation of inexpensive screening investigations for neonatal sepsis is salient because sepsis may pose a serious menace to the new-born. Confirmation of diagnosis using blood culture may take time. This study evaluated the diagnostic accuracy of haematological indices in the detection of neonatal sepsis. In as much as blood culture is the benchmark for the diagnosis of neonatal sepsis, it has some limitations; it can be falsely negative due to intermittent/low density bacteraemia, suppression of bacterial growth by intrapartum administration of antibiotics, improper inoculation and incubation of blood culture.[4] The automated system despite the advantages has some limitations; it may be time consuming for detecting slow-growing organisms, its performance is dependent on the quality of the blood samples, contaminated or improperly collected samples can lead to false-positives and false-negatives. [23]

In this study, the associations between total WBC, immature to total neutrophil ratio and NNS were statistically significant. (p values = 0.015, 0.020 respectively). There was a statistical difference between the mean I/T ratio and NNS (p value = 0.032, T-test). Pius et al, Monroe et al and Ottolini et al in their various studies reported similar findings.[7,13,24]

With regards to alternative inexpensive confirmatory investigation for the detection of neonatal sepsis, the sensitivity of TWBC was found to be low (46.7%) though the specificity was high (76.7%) indicating that the test is poor in ruling in NNS because 53.3% of neonates with presumed sepsis had a normal result. Bhalodia et al and Ahmed et al in their studies also reported a low sensitivity and high specificity of TWBC.[12,17] Contrary to this, a study carried out in Pakistan reported high sensitivity (75%) and low specificity (57%) of TWBC.[4] Also a study done in Maiduguri Nigeria reported a low sensitivity and specificity of TWBC which was different from the present study.[7] These variations may be due to the age of the neonates as the test becomes less sensitive after the first week of life, also the time interval between the onset of bacteraemia and sampling may be contributory.[17]

On the other hand, the sensitivity of the I/T ratio was high (83.3%) while the specificity was low (40.0%). This finding is similar to the study by Pius et al in Maiduguri Nigeria and Ali et al in Mumbai India who reported high sensitivity and low specificity of I/T ratio.[7,25] This finding is not surprising because it has been reported that the I/T ratio is the single most important parameter for early diagnosis of NNS.[12,26-27] However, Bhalodia et al and Nayana et al in their studies reported high sensitivity and specificity for the I/T ratio.[12,27] The differences could be attributed to the inter-observer variation in the interpretation of the peripheral smear, the variations in the blood sample time and severity of infection.[9,28]

The micro-ESR levels were elevated in 16 out of 30 neonates who had positive culture result giving a sensitivity of 53.3%. This finding is comparable to the works done by Vartak et al, Buch et al and Kutty et al who in their study found the sensitivities of micro-ESR to be 56%, 63.1% and 68.8% respectively.[29,5,30] In contrast, works by West et al, and Sriram had higher sensitivities levels of 75.7% and 73.1% respectively.[11,28] Micro ESR was unable to identify 14 of the 30 neonates who had culture-positive result giving a specificity of 61.1%. This is comparable with the 52.6% reported by Sriram but at variance with the findings of Kutty et al, Buch et al and Vartak et al with specificities of 76.8%, 72.7% and 95% respectively.[28,30,5,29]

This study has few limitations; being a cross-sectional study, it is difficult to derive causal relationships. Also, there was lack of randomness due to the consecutive sampling method applied in this study.

CONCLUSION

The findings of this study showed that the sensitivities of TWBC, I/T ratio and micro-ESR were 46.7%, 83.2% and

53.3% respectively while their specificities were 76.7%, 40.0% and 61.1% respectively.

Recommendations

Hospitals in resource poor-settings, where blood culture cannot be obtained readily, the use of I/T ratio which is less time consuming and cost effective is recommended for the diagnosis neonatal sepsis.

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Conflict of interest: None declared
Ethical approval: The study was approved by the arch Ethics Committee of the Nnamdi Azikiwe University Teaching Hospital Nnewi with protocol number NAUTH/CS/66/VOL.11/087/2018/049 dated 9th October, 2018

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