Original Research Article

Prospective Analysis of B/N Ratio and Thrombocytopenia as Diagnostic Indicators of Neonatal Sepsis

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ABSTRACT

Introduction: Neonatal septicaemia is a major established cause of morbidity and mortality in new-born infants, has nonspecific features like positive blood culture, thrombocytopenia and elevated CRP, often making the presentation confusing, thus making diagnosis difficult and delayed especially in our country where patients present late. Early diagnosis prevents serious morbidity and facilitates early treatment. This study correlates thrombocytopenia, band forms and neutrophils ratio with bacterial or fungal organisms isolated from blood cultures of neonates with sepsis to ascertain ideal diagnostic indicators of neonatal sepsis.

Methods: A prospective diagnostic observational study from Dec'13-Nov'14. Following Peripheral blood collection, complete haemogram was performed using Sysmex ix800on 60 Neonates with suspected Septicaemia. Organisms were isolated by blood culture.

Results: In 60 neonates, 30%hadpositive culture, 38.3%infantshad probable sepsis and 31.6%non-infected. 75.6% neonates had early onset ($\leq$ 7 days) and 24.3% had late onset sepsis. Among 41neonates with sepsis; 58.53% neonate had low birth weight. Out of 41probable sepsis cases 28 had B/N $\geq$0.2 and 31 had thrombocytopenia. Sensitivity and specificity for Thrombocytopenia was 87.8%, 63.1%, for B/N ratio were 68.2%, 84.2%, and for combination of both tests was 75.6%, 89.4% respectively. Thrombocytopenia and B/N Ratio correlation >0.2.

Conclusion: Thrombocytopenia and Band forms are constant and significant association with B/N ratio and Thrombocytopenia being good early diagnostic indicators for neonatal sepsis. A combination of these tests has better specificity for neonatal sepsis than single tests and should be a part of every neonatal sepsis diagnostic protocol.

Keywords: Neonate, Sepsis, Thrombocytopenia, India, Band form, Diagnostic, Screening.

INTRODUCTION

Neonatal septicaemia is a major established cause of morbidity and mortality in new-born infants worldwide. Neonatal sepsis is a syndrome characterized by systemic signs of infection and bacteraemia in 1st month of life. [1] It has an overall incidence of 1-8 cases/1000 live births. [2] Systemic bacterial infection during the 1st month remain a major cause of infant

morbidity and mortality accounting to 50% neonatal deaths despite the development of broad spectrum antimicrobial agents and technological advancements in life supportive therapy.

Neonatal sepsis shares nonspecific serological features like positive blood culture, thrombocytopenia and elevated CRP, often making the presentation confusing, thus making diagnosis difficult and delayed especially in our country where patients present late. Early diagnosis prevents serious morbidity and facilitates treatment planning. Clinical presentation of neonatal septicaemia is mimicked by lot of other disorders affecting the new-born like transient Tachypnoea of new-born, hypoxia induced encephalopathy and hypoglycaemia.

Early diagnosis and treatment prevent morbidity and mortality caused by untreated or lately treated neonatal septicaemia; it is important that treatment is started early with rational antibiotic therapy, based on certain indirect markers such as Leukopenia, thrombocytopenia ,band form to neutrophil ratio and CRP, collectively known as sepsis screen. [3]

Even though positive blood culture is diagnostic (gold standard) of neonatal septicaemia, the technique of blood culture has its own limitations i.e. time consuming, requires a well-equipped laboratory and has a low success rate. In the presence of predisposing factors, early clinical suspicion coupled with "sepsis screen" detects neonatal septicaemia earlier, which enables the clinician to treat infection timely and adequately, thus reducing neonatal morbidity and mortality.

Our prospective diagnostic study correlates thrombocytopenia, band forms and mature neutrophils ratio with bacterial or fungal organisms isolated from blood cultures of neonates with sepsis to ascertain ideal early diagnostic indicators of neonatal sepsis.

MATERIALS AND METHOD

This is a prospective diagnostic observational study from Dec'13- Nov'14 performed in the pathology department of a medical college following clearance from institutional ethical committee clearance. Following Peripheral blood collection, complete haemogram was performed using Sysmex ix800 on 60 Neonates (<28 days) with suspected Septicaemia. Organisms were isolated by blood culture. Neonates admitted to the hospital from outpatient department and neonates born were included in study group.

Inclusion Criteria: Neonates admitted to the hospital (≤ 28 days) with clinical suspicion of septicaemia.

Exclusion Criteria: Congenital and acquired cases of thrombocytopenia other than sepsis, Parents refused consent.

Data Assessed: The data was collected under following headings:

Mother's Data: Name, Age, Weight, Parity, Type of Delivery, Maternal complications, Hb%, Blood Group and RH of the mother.

Baby's Data: Registration number, Birth Mark, Age, Gender, Blood Group, Rh, Weight, Gestational Age, Clinical diagnosis, Serology- Hb%, TLC, Platelets, Band forms, CRP and Blood culture parameters.

Variables Assessed: Band form/Mature Neutrophil ratio (B/N Ratio), Thrombocytopenia, Birth weight distribution, Gestational Age distribution, Combination of B/N Ratio and Thrombocytopenia, Organism profile and associated severity of thrombocytopenia, Thrombocytopenia in relation to infection status.

Methodology:

After entering the preliminary details of the mother and child, neonates were investigated. Total platelet counts were counted by the Sysmex xs800i haematological analyser, using the electrical impedance principle. The particles with size

2-20 fl are counted as platelets by the analyser. To rule out cases of spurious thrombocytopenia, as a limitation of the haematological analyser, the Patients with total platelet counts < 1, 50,000 were screened out and manual Total Platelet count was done from peripheral smear with the microscope. Platelets were counted per 1000 RBCs, say 'n' where TPC = n x TRBC/1000. (TRBC = Total RBC/μL). [4]

Peripheral direct smear is prepared with a drop of blood from heel side prick and stained with Leishman's stain using standard principles of staining. [5] Neutrophils are 10-12 microns in diameter. The cytoplasm contains fine azurophilic granules and 3-4 nuclear lobes. Band forms are premature neutrophils with nucleus showing indentation >50 %. These are expressed in per 100 neutrophils. For Sepsis Screening Band forms to neutrophil ratio should be >0.2

For, Blood Culture samples were collected from peripheral vein under strict aseptic precautions before administration of antibiotic therapy. 1 ml of blood [6] was collected in Blood culture bottle for babies available at our hospital and sent immediately to in-house microbiology department for cultures. Three sub-cultures were performed and observed after 24hrs, 48hrs and 120 hrs. If no growth was observed, material kept for 7 days and checked. Cultures were performed for Gram +, Gram - and Aerobic/Anaerobic organisms. Prevalence of various organisms were confirmed by blood culture and correlated with thrombocytopenia, along with all the other data obtained.

Based on clinical findings and laboratory data neonates were classified in to 3 categories: Culture Positive, Probable sepsis and Non-infected. [7]

A. Culture Positive: The diagnosis of sepsis was made when there were positive findings on blood culture.

B. Probable infection: Neonates were classified as having infection when blood cultures were negative but there was a strong clinical history indicating infection. Certain high risk factors such as meconium aspiration, PROM (Pre-mature Rupture of Membrane) and maternal fever were noted and the neonates presented with clinical features such as respiratory distress, grunting, apnoea, lethargy, shock etc. along with high CRP.

C. Non-Infected: This consisted of neonates with negative blood culture, who initially presented with features of suspected sepsis or with associated risk factors, but later found to be suffering from other disorders like, transient tachypnoea of new-born, hypoxia induced encephalopathy and hypoglycaemia.

Statistical Analysis:

Sensitivity and specificity for thrombocytopenia, B/N Ratio alone and together in diagnosing neonatal sepsis were assessed. P-values and chi square test values were calculated.

OBSERVATIONS AND RESULTS

Out of the 60 neonates in the study group, 41 neonates (68.3%) had sepsis i.e. 18 (30%) neonates had culture positive, 23 (38%) had Probable Sepsis and 19 (32%) were non-infected.

Out of 41 sepsis proven and probable sepsis cases, 31(75.6%) neonates were <7 days old (early onset) and 10 (24.3%) neonates were >7 days old (late onset). Birth weight distribution was ≥ 2500 Kgs in 17 (41.4%) neonates, 1500-2400 Kgs in 19(46.3%) neonates and 1000- 1499 Kgs in 5 (12.1%) neonates. Gestational Age distribution is represented in Table.1.

Table.1: Gestational Age Distribution (n=41):

	Pre-Term	Term	Total
Culture Positive	15	3	18
Probable sepsis	13	10	23
Non Infected	8	11	19
Total	36	24	60

In thrombocytopenia, Organism *Klebsiella sp.* was associated in maximum (5) instances with 3 instances being moderate and 2 instances being severe thrombocytopenia. The organism profile (n=18) and associated severity of thrombocytopenia is represented in Table.2. Thrombocytopenia (n=60) in relation to infection status is represented in Table.3. Maximum instances of mild thrombocytopenia were seen in 33 cases, 17 instances being in the probable sepsis group. Thrombocytopenia had 87.8% sensitivity and 63.1% Specificity for neonatal sepsis. At significance value at 0.01, p value =0.000046. Chi square value = 16.605

Table.2: organism profile with degree of thrombocytopenia With organism isolated (n=18):

	Normal	Mild	Moderate	Severe	Total
Staphylococcus aureus		3			3
Pseudomonas aeruginosa	1	1	1		3
Klebsiella			3	2	5
Actinobacter		1			1
Proteus		1		1	2
Enterobacter		1			1
Enterococci Faecalis			1		1
E.coli		2			2
Total	1	9	5	3	18

Table.3: Thrombocytopenia In Relation To Infection Status (n=60).

	Normal	Mild	Moderate	Severe	Total
Culture +ve	1	9	5	3	18
Probable sepsis	4	17	1	1	23
Non infected	12	7	0	0	19
Total	17	33	6	4	60

Table.4: Combination of B/N Ratio and Thrombocytopenia (n=60)

	Positive	Negative	Total
Culture Positive & Probable sepsis	31	10	41
Non Infected	2	17	19
			60

Among the 60 cases, Band form/Mature Neutrophil ratio (B/N Ratio) was≥ 0.2 in 28 instances and < 0.2 in 13 instances in the Culture positive and Probable sepsis group. B/N Ratio was ≥ 0.2 in 3 instances and < 0.2 in 16 instances in the Non-infected group. Thus, Sensitivity being 68.2% and Specificity being 84.2%. At significance value at 0.01, p value =0.000153. Chi square value = 14.3318

For the Combination of B/N Ratio and Thrombocytopenia see Table.4, cases with both Thrombocytopenia and B/N ≥ 0.2 is taken as positive, cases with any of the two negative were taken as negative for the comparison. Thus, Sensitivity being 75.6% and Specificity being improved to 89.4%. At significance value at 0.01, p value =0.000002. Chi square value = 22.2204 Figure.1 represents the Specificity and sensitivity of various tests alone and in combination.
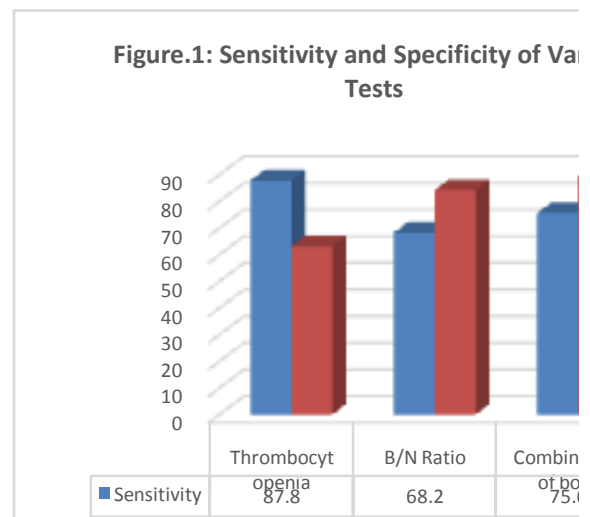

Figure.1: Chart comparing the sensitivity and Specificity of Various Tests used alone or in combination.

DISCUSSION

This study was undertaken to evaluate thrombocytopenia and band form variation in neonatal sepsis and to look into various haematological parameters both

individually and in combination as part of sepsis screening. Blood cultures, being gold standard for diagnosis of neonatal sepsis requires a minimum period of 48-72 hours and yields positive results in 25-70% of cases. [8]

We found Band – Total Neutrophil ratio (B/N), an important tool in the investigative workup, out of 41 culture positive and probable sepsis cases, 28 cases were with B/N (Band form: Neutrophil ratio) ≥ 0.2. Whereas other published literatures report a sensitivity of 62%, [9] 82% [10] and 88%, [11] we observed a sensitivity of 68.2% when used alone. Not many studies have reported the specificity; we observed specificity of 84.2% when B/N ratio was used alone. Predictive value of elevated band count and simplicity of the test justifies its routine use in early diagnosis of neonatal sepsis. [12]

Out of 41 culture positive and probable infection cases 36 had thrombocytopenia and 7 non-Infected cases had thrombocytopenia. Specificity and sensitivity of thrombocytopenia when used alone was 87.8%, 63.1% respectively. Whereas other published literatures report a sensitivity of 64.3%. [13] Thrombocytopenia is also known to be of prognostic value. Thrombocytopenia was found to be consistently associated with poor prognosis, confirming the finding of other studies. [14,15]

The above two tests showed more promising results with improved sensitivity and specificity for early detection of neonatal sepsis when used in combination (p value =0.000002). Sensitivity being 75.6% and Specificity being improved to 89.4%.

The advantage of studying the haematological profile of neonates suspicious of having sepsis is that these tests can be done rapidly even in small hospitals, allowing prompt treatment to neonates with sepsis and minimizing therapy to those without infection.

There was a marked degree of thrombocytopenia in neonatal sepsis cases. Low birth weight cases were more common in with neonatal sepsis. Pre-term cases were more commonly associated with neonatal sepsis. Pre-term cases were associated with early onset sepsis. Early onset sepsis cases were more common than late onset sepsis. Early onset sepsis was more commonly associated with low birth weight cases.

Thrombocytopenia was more severe in Gram negative cases. B/N ratio, Platelet count, TWBC count all are very good tests for sepsis screening. Sepsis screen is simple, cheap, less time consuming and easy to perform even at bedside. B/N ratio has excellent sensitivity and good specificity.

CONCLUSION

Thrombocytopenia and Band forms are constant and significant association with B/N ratio and Thrombocytopenia being good early diagnostic indicators for neonatal sepsis. A combination of these tests has better specificity for neonatal sepsis than single tests and should be a part of every neonatal sepsis diagnostic protocol.

The combination of the two tests (Thrombocytopenia and B/N ratio) hassuperior sensitivity and specificity when used together. The combination of parameters yielded better results than single tests and proved to be an invaluable aid for early diagnosis of neonatal sepsis.

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