

Correlation of blood culture and band cell ratio in neonatal septicemia.

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Abstract: Background: Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteraemia in the first month of life. Incidence differs among hospitals depending on variety of factors. Blood culture is considered gold standard for the diagnosis, but does not give a rapid result. Hence, there is a need to look for a surrogate marker for diagnosing neonatal septicemia.

Material & Methods: 335 neonates were studied for clinically suspected septicemia over a period of one year. Blood was cultured and organism identified biochemically. Parameters of subjects like EOS, LOS and Band cell counts were recorded. Results analysed statistically. Results: Male preponderance was observed. Majority of the cases had a normal vaginal delivery. 47.46% cases had early onset septicemia. Meconium stained liquor was the predominant risk factor. Culture positivity was found to be 32.24% and 87.96% of them also had band cells percentage ranging from 0 to >25.

Conclusion: Band cell count can be used as a surrogate marker for neonatal septicemia. An upsurge of Candida species as a causative agent in Neonatal septicemia has been observed.

I. Introduction:

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteraemia in the first month of life. It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infections. Superficial infections like conjunctivitis and oral thrush are not usually included under neonatal sepsis. (1)

According to the data from National Neonatal Perinatal Database (NNPD, 2002-03) the incidence of neonatal sepsis is 30 per 1000 live births. The database comprising 18 tertiary care neonatal units across India found sepsis to be one of the commonest causes of neonatal mortality contributing to 19% of all neonatal deaths.(2)

Infection is the primary cause of mortality in 18.6% of intramural neonates and 38% of extramural babies. Klebsiella pneumoniae being the most frequent bacterial isolate 32.5% and 27.5% in both intramural and extramural admissions respectively. Followed by Staphylococcus aureus in intramural (13.6%) and extramural (14.9%) admissions. (2) A rising trend of the role of Candida species in the pathogenicity of neonatal sepsis has also been observed by several workers.(3-7)

Incidence differs among hospitals depending on various factors such as obstetric and nursery practices, perinatal care, health and nutrition of mother and incidence of prematurity. Though a positive blood culture is the gold standard for the diagnosis of neonatal septicemia, it does not provide a rapid diagnosis. (3,8) Hence, there is a need to look for a reliable surrogate marker for neonatal septicemia to start an empirical treatment till culture report is awaited. (8)

The band cell ratio (ratio of mature to immature neutrophils) can be included in rapid diagnosis of neonatal septicemia. The ratio is considered increased when it is more than 0.2 (Normal Band cell ratio is 0.03-0.05). Band neutrophil counts are helpful for the detection of neonatal sepsis. Modern automated hematology instruments provide an accurate and precise total neutrophil count but do not report band numbers which need to be calculated manually. (1, 9,10)

Our goal was to detect elevated band neutrophils whenever present and correlate it with blood cultures from NICU in the suspected cases of neonatal sepsis admitted in our hospital (Shri Mahant Indiresh Hospital, Dehradun).

II. Study design:

In this prospective study blood samples from 335 neonates admitted for clinically suspected neonatal sepsis in the NICU in SMIH, Dehradun were collected, from January 2012 to December 2012.
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Selection criteria for Subjects

Inclusion criteria:
1. Neonates of both sexes were included in this study.
2. Neonates presenting with signs and symptoms such as refusal to feed, lethargy, fever, hypothermia, vomiting, diarrhoea, abdominal distension, jaundice, respiratory distress, seizures, or any external evidence of sepsis like umbilical cord infection, skin infection etc. were taken up for study.
3. A sample showing the growth of organisms of low pathogenicity, on repeat culture was included in this study.

Exclusion criteria:
1. Neonates with absence of signs of sepsis were excluded from this study.
2. Low pathogenic organisms like CoNS, Candida spp. unless grown on repeat culture were excluded.

III. Material and methods:

BacT/ALERT automated culture system was used for blood culture.

Positive culture bottles were promptly removed and a smear was made for Gram staining. Then a subculture was done on Columbia blood agar and MacConkey Agar and incubated aerobically at 37°C for 16-18 hrs. After 18 hours plates were observed for growth and colonies were processed according to standard Microbiological procedures. (11, 12)

Antibiotic sensitivity was done by Kirby Bauer’s disc diffusion method as per CLSI guidelines. (13)

Total & differential neutrophil counts were performed in Beckman Coulter automated system and Band cell ratio was calculated manually.

Statistical analysis:

Results were analysed using Chi square method for statistical significance.

IV. Results:

A total of 335 neonates were studied for clinically suspected septicaemia. Male preponderance was observed with 213/335 (63.55%) males and 122/335 (36.41%) females. 192/335 (57.31%) neonates had a normal vaginal delivery, 134/335 (40%) of the neonates were delivered by LSCS. Other methods including instrumentation and vacuum assisted delivery were 9/335 (2.68%). So, majority of our study population had a normal vaginal delivery.

Early onset septicaemia was observed clinically in 159/335 (47.46%) neonates, of which 36/159 (22.64%) were culture positive. While 176/335 (52.53%) had a late onset of septicaemia and 72/176 (40.90%) were culture positive. Statistically this finding was highly significant (P=0.0004). (Table 1)

When various risk factors were analysed we found that meconium stained liquor (MSL) dominated followed by premature rupture of membranes (PROM), mechanical ventilator support (MVS) and twin deliveries. P value was highly significant when compared with clinically suspected cases of neonatal sepsis. (Table 2)

Of the 108/335 culture positive cases, band cells were observed in 95/108 cases (87.96%). As the band cell ratio increased so did the culture positivity. Band cell ratio above 11% was found to correlate 100% with culture positivity. On analysis of the above data P value was found to be statistically highly significant. (Table 3)

Blood cultures when analysed showed that 227/335(68.05%) blood cultures were sterile while 108/335 (32.24%) were positive. (Table 4) Bacterial culture positivity was 67.76% and fungi 35.51%. (Table 5)

Table 1: Distribution of Early and Late onset sepsis (N=335)

<table>
<thead>
<tr>
<th>ONSET OF SEPSIS</th>
<th>CULTURE NEGATIVE</th>
<th>CULTURE POSITIVE</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early onset sepsis</td>
<td>123</td>
<td>36</td>
<td>159</td>
</tr>
<tr>
<td>Late onset sepsis</td>
<td>104</td>
<td>72</td>
<td>176</td>
</tr>
</tbody>
</table>

Chi square = 3.338,  P=0.0004 (highly significant)

Table 2: Distribution of Risk factors amongst subjects (N=335)

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Present</th>
<th>Absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSL</td>
<td>130 (38.8%)</td>
<td>205 (61.19%)</td>
</tr>
<tr>
<td>PROM</td>
<td>111 (33.13%)</td>
<td>224 (66.87%)</td>
</tr>
<tr>
<td>Mechanical ventilation</td>
<td>27 (8.05%)</td>
<td>308 (91.95%)</td>
</tr>
<tr>
<td>Twin delivery</td>
<td>10 (2.98%)</td>
<td>325 (97.02%)</td>
</tr>
</tbody>
</table>

Chi square = 194.78, P = 0.0001 (highly significant)
Correlation of blood culture and band cell ratio in neonatal septicaemia.

Table 3: Correlation of band cells with culture results

<table>
<thead>
<tr>
<th>BAND CELLS (%)</th>
<th>CULTURE NEGATIVE</th>
<th>CULTURE POSITIVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 5</td>
<td>47</td>
<td>24</td>
</tr>
<tr>
<td>6 – 10</td>
<td>4</td>
<td>25</td>
</tr>
<tr>
<td>11 – 15</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>16 – 20</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>21 – 25</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>&gt; 25</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Chi square = 60.00, P=0.0001 (highly significant)

Table 4: Blood culture results

<table>
<thead>
<tr>
<th>NUMBER OF CASES</th>
<th>CULTURE REPORT</th>
</tr>
</thead>
<tbody>
<tr>
<td>227 (67.76)</td>
<td>Negative</td>
</tr>
<tr>
<td>108 (32.24)</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Figures in parentheses are percentages.

Table 5: Isolate wise distribution (N=108)

<table>
<thead>
<tr>
<th>ISOLATES</th>
<th>NUMBERS</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial</td>
<td>69</td>
<td>63.89</td>
</tr>
<tr>
<td>Fungal</td>
<td>39</td>
<td>36.11</td>
</tr>
</tbody>
</table>

In our study fungal isolates contributed to about 36.11%, of which *Candida albicans* was isolated in 23.08% and *Non albicans Candida* species were 76.92%.

V. Discussion:

Neonatal sepsis is the most common cause of neonatal mortality despite treatment. Neonatal sepsis can be classified into two subtypes depending upon onset of symptoms. Signs of sepsis in neonates are often non-specific and high degree of suspicion is needed for early diagnosis. Some laboratory parameters can be helpful for screening of newborns with neonatal sepsis, but none of it is specific and sensitive enough to be used singly. Delay of even a few hours in initiating treatment can considerably increase the morbidity and mortality.

Male preponderance observed in this study was similar to most of the other studies. (14-17) The high incidence of culture positivity in extramural cases could be due to unhygienic conditions, untrained (dai) nurse deliveries and premature deliveries being referred to our hospital from far off places. The culture positivity in intramural deliveries were owing to associated multiple risk factors and possible nosocomial infections.

Culture positivity of 22.64% in EOS and 40.90% in LOS falls into the similar range as observed by various other workers, reporting EOS ranging from 24-77.6% and LOS ranging from 22.4-76%. (18, 19, 20) MSL, MVS and PROM as risk factors for development of sepsis were of great significance in the present study. It is thus important to keep a strict vigil in neonates falling into any of these categories.

Though blood culture is a gold standard for diagnosis of neonatal sepsis, but other factors like Absolute neutrophil count, Band cells, ESR, C-reactive protein, Procalcitonin and other serological markers are important in diagnosis of clinically suspected culture positive cases of neonatal sepsis. We found high percentage of band cells in culture positive cases of neonatal sepsis. This correlation was highly significant with band cell ratio above 11%. We suggest that band cell count be used as a surrogate marker till the blood culture report is awaited. The limitation of this study was that blood culture for anaerobic pathogens was not performed so it is not fit to comment upon raised band cell ratio and culture negativity. It is thus recommended to put a parallel aerobic as well as anaerobic culture where possible.

The spectrum of organisms causing neonatal sepsis is quite different in developed and developing countries, like India. Both bacterial and fungal organisms are implicated in neonatal sepsis.

The association between prematurity and blood borne candidial infections has been recognised for many years in the past. Over the same period of time the incidence of candidemia has escalated from 25 to 123 cases per 10,000 NICU admissions. Isolation of *Candida* species from a blood culture should never be regarded as a contaminant and should prompt an immediate search for evidence of dissemination, which occurs in approximately 10% of premature newborns with candidemia. (3) Despite the fact that fungal isolates which are often missed or considered contaminants also play an important role. A rising trend in pathogenicity of *non albicans Candida* is observed in our study.

Rates of bloodstream infection due to *Candida* species have increased steadily.(3, 4, 5)National nosocomial infection surveillance data of United States show that *Candida* species were the fifth leading cause of bloodstream infection hospital wide and the fourth in the intensive care units. Current data from the SCOPE (Surveillance and control of pathogens of epidemiologic importance) surveillance system confirm that *Candida*

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species were the fourth leading cause of blood stream infection in United States (21, 22). An upsurge of previously ignored pathogens like *non albicans Candida* was also observed in our study.

### VI. Conclusion:

Neonatal septicemia is one of the leading causes of mortality and morbidity in developing countries like India. In neonates, sepsis is difficult to diagnose clinically. They may be relatively asymptomatic until hemodynamic and respiratory collapse becomes evident. So, if there is even a remote suspicion of sepsis, neonates are frequently treated with antibiotics empirically until cultures are proven to be negative. We recommend band cell ratio as a surrogate marker for neonatal sepsis to bridge the gap between suspicion and confirmation by culture results.

Continued surveillance of neonatal sepsis should be undertaken by hospitals at their levels due to changing scenario of the spectrum of organisms and changing pattern of antibiotic susceptibility. This in turn would act as a guideline in formulating rational empirical treatment strategy.

In resource-limited countries, hand washing or hand hygiene program still remains the most effective evidence-based strategy that will reduce the rates of nosocomial infection in NICUs. Fumigation of ICUs at regular intervals, avoiding overcrowding in ICUs and controlled patient nurse ratio would go a long way in reducing the morbidity and mortality due to neonatal septicemia.

### References:


