Role of the Hematological Profile in Early Diagnosis of Neonatal Sepsis

Objective: To evaluate the utility of the hematological parameters for early detection of neonatal sepsis.

Study Design: A cross-sectional analytical study.

Place and duration of study: The Neonatal Intensive Care Unit of Pakistan Institute of Medical Sciences, Islamabad, over a period of ten months from January to October 2009.

Subjects and Methods: All those neonates were enrolled in the study that were suspected to have sepsis or had maternal history of infection. Babies who had a major congenital anomaly, inborn errors of metabolism or a hemolytic jaundice were excluded from the study. Each baby was examined and findings recorded. A septic work up was carried out in all these infants which included complete blood counts, C-reactive protein (CRP) and blood culture with antibiotic sensitivity. The analysis of the peripheral smear was done by the pathologist blinded to the diagnosis of the baby. The parameters studied were the abnormal number of total leucocytes and absolute neutrophil counts, thrombocytopenia and the C-reactive proteins.

Results: A total of 138 babies were evaluated for sepsis out which 48 were proved septic by the positive blood cultures. Acinetobacter [35%, n=17] and Klebsiella pneumoniae [25%, n=12] were the commonest organisms isolated. Analysis of the hematologic profiles revealed that the sensitivities of the parameters studied were below 60%. However the specificities were more than 70%. Though, the individual parameters had not the desired specificities but if put together can be a good tool in ruling out the possibility of the neonatal sepsis. The CRP was positive in 11/48 (23%) of proven septic babies and in 14/90 (16%) of babies with probable sepsis. Thrombocytopenia had a sensitivity of 61% and a specificity of 82%.

Conclusion: The hematologic profile that we studied can be a good test in detection of culture negative cases of neonatal sepsis. Though, not a very sensitive tool but it is a simple, quick, cost effective and readily available. It cannot provide a guideline to decisions regarding antibiotic therapy.

Keywords: C-reactive protein, Leukocyte count, Newborn, Sepsis, Absolute neutrophil count, thrombocytopenia

Introduction

Neonatal septicemia continues to be a major cause of morbidity and mortality in our country. It is one of the major causes of neonatal mortality in the developing countries contributing to 15% of all neonatal deaths. Though, it is a life-threatening condition, yet treatable if diagnosed early. Unfortunately, the early warning signs and symptoms are often nonspecific and can easily be confused with those from noninfective causes. These nonspecific signs and symptoms make it difficult to establish an early clinical diagnosis. The antibiotic therapy is usually initiated based on the clinical suspicion which may result in overtreatment ultimately leading to emergence of multiresistant organisms. In addition, the high cost of antibiotics overburdens the already underprivileged parents. Blood culture is still
considered to be the ‘gold standard’ for diagnosis of sepsis; however, its accuracy has been questioned because of spurious positive results due to contamination and negative blood cultures in fatal generalized bacterial infections. The yield of a positive blood culture ranges from 8-73% as shown in various studies. Moreover, the technique of blood culture is time consuming and demands a well equipped laboratory which is not available in most of the community hospitals. Therefore, the need is for a test that is cheap, easily performed with quick availability of reports. An ideal diagnostic test for neonatal sepsis should have maximum sensitivity and specificity. In recent years, various investigators have evaluated some highly sensitive and specific inflammatory markers (eg. ELIZA methods, haptoglobins, interleukins and highly sensitive and specific inflammatory markers (eg. ELIZA methods, haptoglobins, interleukins and counterimmunoelectrophoresis etc.) to diagnose neonatal sepsis. Although, these markers are sensitive and specific, but are sophisticated and expensive so impractical for developing countries.

Various cheap but reliable laboratory tests have been evaluated for the diagnosis of systemic infection in neonates. The complete blood count (CBC) with the various neutrophil parameters and C-reactive protein (CRP) are the most frequently used. The present study is aimed to evaluate the usefulness of various parameters of white blood cell count and C-reactive proteins, as an early indicator of neonatal septicemia because this is a simple bed-side test which can be done within a short time before putting the neonate on antibiotic therapy.

Materials and Methods

The study was conducted in the Department of Neonatology, Pakistan Institute of Medical Sciences, Islamabad, over a period of ten months from January 2009 to October 2009. All those neonates were enrolled in the study that were suspected to have sepsis or had maternal history of infection. The record of these neonates were kept and evaluated later. Some of these neonates were asymptomatic but were evaluated for sepsis because of maternal intrapartum sepsis risk factors like prolonged rupture of membranes, maternal urinary tract infection, maternal intrapartum fever >38°C, chorioamnionitis, and excessive vaginal discharge. Neonates were excluded if they had: a) major congenital anomaly; b) inborn errors of metabolism, c) hemolytic jaundice or respiratory distress syndrome (due to surfactant deficiency.) Each neonate was examined by a pediatric resident rotating in NICU or a neonatology fellow who recorded the signs and symptoms of the neonate, predisposing perinatal factors and the clinical assessment of the neonate. The sepsis work up included complete blood counts, C-reactive protein (CRP) measurements and blood culture with antibiotic sensitivity. A complete blood count, included platelet count, total leukocyte count (TLC), total neutrophil count (TNC). Absolute neutrophil counts (ANC) were calculated from the observed values. The reference values of the neonatal hematological parameters of Manroee, et al. were used as the standard values.

Blood samples were collected before the initiation of antibiotic therapy. The analysis of the peripheral smear findings were done by the pathologist blinded to the infection status of the neonate.

Table I: Hematological scores used in the study

<table>
<thead>
<tr>
<th>Hematological test</th>
<th>Abnormality</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase or decrease WBC</td>
<td>≤ 5,000 mm³ or ≥ 25,000mm³, 30,000mm³, 21,000mm³ at birth, 12-24 hrs and day 2 onwards respectively</td>
<td>1</td>
</tr>
<tr>
<td>Increase or decrease total ANC</td>
<td>↓ or ↓</td>
<td>1</td>
</tr>
<tr>
<td>CRP</td>
<td>Positive</td>
<td>1</td>
</tr>
<tr>
<td>Platelet count</td>
<td>≤ 150,000 mm³</td>
<td>1</td>
</tr>
</tbody>
</table>

Normal values as defined by reference ranges of Manroee et al.
CRP = C reactive protein
ANC = Absolute neutrophil count

We assigned one number to each of the four hematological parameters. The hematological scores used are shown in Table I. Based on the clinical findings and laboratory data infants were classified into two categories: Proven sepsis and probable sepsis. The diagnosis of sepsis was made when there were positive findings on blood culture. Infant were classified as having probable sepsis when the blood culture was negative but there was a strong clinical history for infection. Comparison of the two variables was made by Chi-square. A level of 0.05 was considered statistically significant. The statistical analyses were performed using SPSS version 11 for windows.

Results

We admitted 691 neonates during our study period of ten months. A total of 138 neonates with suspected sepsis were analyzed. Out of 138 clinically suspected cases, 48 neonates were proved to be septic by blood culture and 90 neonates were of probable sepsis. A significant finding was the difference in the gender distribution of both groups. In the group of suspected sepsis the male were 48% [n=43] and the females were 52% [n=47] whereas the male babies in
the proven septic group were 59% [28/48] and the female babies were 41% [20/48]. The premature were 57% [51/90] in the group of probable sepsis where the group with confirmed sepsis consisted of 69% [33/48] preterm neonates. The youngest was of 25 weeks gestation and the oldest was of 41 weeks gestation. The birth weight ranged from 750 grams to 4200 grams. The demographic profile of the neonates in the proven septic group is shown in table II.

Table II: Basic characteristics of babies of proven sepsis, n= 48

<table>
<thead>
<tr>
<th>Sex</th>
<th>Male = 28 [59%]</th>
<th>Female = 20 [41%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premature</td>
<td>33 [69%]</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>750 grams - 3500 grams Mean = 2005 ± 706</td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td>25 weeks – 41 weeks Mean = 33 ± 3.1</td>
<td></td>
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</tbody>
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Analysis of the hematologic profile showed that the thrombocytopenia had the better sensitivity and specificity than others. Individually none of the tests had satisfactory levels of sensitivities.

Table III: Sensitivities and specificities of babies with suspected sepsis n= 138

<table>
<thead>
<tr>
<th>Hematological test</th>
<th>Sensitivity [%]</th>
<th>Specificity [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase or decrease WBC $\Delta$</td>
<td>35</td>
<td>77</td>
</tr>
<tr>
<td>Increase or decrease ANC $\Delta$</td>
<td>35</td>
<td>74</td>
</tr>
<tr>
<td>Platelets $\leq$ 150,000 mm$^3$</td>
<td>61</td>
<td>82</td>
</tr>
<tr>
<td>CRP</td>
<td>23</td>
<td>84</td>
</tr>
</tbody>
</table>

Normal values as defined by reference ranges of Manroe et al $^7$

$\Delta$ $\leq$ 5,000 mm$^3$ or $\geq$ 25,000mm$^3$, 30,000mm$^3$, 21,000mm$^3$ at birth , 12-24 hrs and day 2 onwards respectively

$\leq$ 1800 or $\geq$ 5400, 14000, 5400 at birth , 12- 48 hrs and 48 hrs onwards respectively $^{20}$

The detail of individual hematologic findings in 138 infants significantly associated with sepsis is shown in table III. As it is evident from the table that the sensitivities of the individual tests is below the satisfactory level but the specificity of the CRP and thrombocytopenia is in the satisfactory range. The CRP was positive in 11/48 (23%) of proven septic babies and in 14/90 (16%) of babies with probable sepsis which is a self explanatory. Likewise thrombocytopenia individually was not a good predictive screening test. It was present in 29/48 cases of proven sepsis with a sensitivity of 61% only. The study showed that the sensitivities of these tests were low but the specificities were better. The tests if combined can be a good screening tool for excluding sepsis.

The blood culture was positive in 48/138 (35%) of the babies suspected of sepsis. The commonest organism isolated was Acinetobacter [35%, n=17], followed by Klebsiella pneumoniae [25%, n=12] , Staphylococcus aureus [19% n=09] , E. coli [12%, n=06]. There were 2 isolates of Proteus vulgaris and one each of Pseudomonas aeruginosa and Salmonella.

Discussion

The present study evaluates the usefulness of the white blood cell count and the C-reactive proteins as an early indicator of neonatal septicemia. This study was undertaken because it is a simple bed-side and cost effective test which can be done even if the baby has had antibiotics. Though, the blood culture is a gold standard for diagnosing neonatal sepsis but this test is not readily available in our country whereas blood picture is available in all setups. In a systematic review of the literature of studies evaluating the usefulness of CRP and leukocyte indices the authors have found a wide range of sensitivity (17% to 90%) and specificity (31% to 100%).

The major problem in neonatal infections is the identification of the infected infant and the equally important task is of identifying the non-infected infant because of its nonspecific clinical symptoms. In the present study, 90 infants categorized as having probable sepsis had clinical evidence but lacked microbiologic proof of infection. These are the neonates who pose a diagnostic and therapeutic dilemma because fatal infections have been reported in the presence of negative blood cultures. Although various tests are used, as a diagnostic tool for neonatal sepsis, the complete blood count with differential is widely used, either singly or in conjunction with other test or clinical findings. The criteria of Manroe et al $^7$ is the most reliable of the published criteria evaluated which identifies almost all infants with sepsis or with probable sepsis. The advantage of Hematological Scoring System lies in the fact that it is easy and applicable to all infants, including those who have received antibiotic. Unfortunately due to some practical limitations we could not study the immature neutrophils and so therefore the ratio of immature to mature neutrophils could not be included in the study which is a major drawback.

Among the different parameters analyzed, we found that none of the parameters individually had the required sensitivity. This is in accordance with large number of studies who have observed that the total leukocyte count or the total neutrophil count show no significant association with sepsis. Our observations

are also in agreement with those of Monroe et al, who have cautioned that one should not be deceived by neutrophil count alone without noting alteration in the ratio of mature and immature neutrophils.

In our study the CRP was not found to have a good correlation with the neonatal sepsis. The sensitivity was only 23% with a specificity of 84%. There are conflicting results on the CRP levels in the literature. Zeeshan et al have also showed poor predictive value in their study.

In the neonatal hematological parameter, the association of low platelet count with neonatal systemic infection is significant. We observed thrombocytopenia in 61% of the septic group and in 18% of probable septic group rendering this a less ideal test as a single parameter for the screening of neonatal sepsis. The presence of any one factor had an unsatisfactory sensitivity which making the test unsuitable for diagnosing the neonatal sepsis. However the specificities were better and if combined together can have a good negative predictive value. It was observed that if the tests are combined together then the sepsis can be excluded with some confidence. The ratio of the immature neutrophils to the mature neutrophils has a good sensitivity and specificity but unfortunately this aspect is missing in our study which is set back for the study.

In the present study sepsis was confirmed in 48 infants based on positive blood culture. In contrast to the developed world where group B streptococcus (GBS) continues to be the most common bacterial pathogen, studies from developing countries have identified gram negative organism as the more frequent infecting agent. In our study also Klebsiella and Acinetobacter were the commonest organisms isolated together constituting 60% of all the organisms isolated. Arshad et al have reported positive blood cultures in 26% of their babies whereas we isolated the organisms in 35% of cases.

In our study the group with probable sepsis had 57% prematures whereas as the group with confirmed sepsis constituted 69% preterm. This was possibly due to impaired defense mechanisms and low immunoglobulin G levels in preterm neonates. The literature also supports this finding of the susceptibility of premature to the sepsis.

Conclusion

The Hematologic profile that we studied is a simple, quick and cost effective tool in the early diagnosis of neonatal sepsis but its sensitivity in detection of neonatal sepsis is unsatisfactory. Therefore it cannot provide a guideline to decisions regarding antibiotic therapy.

Recommendations: We need large databases to improve the accuracy of this screening test so it is recommended that more subjects should be included in future studies. The ratio of the immature to the mature neutrophils needs to be studied. There should also be a control group composed of healthy and asymptomatic neonates so that positive predictive and negative predictive values can be obtained.

References

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