Rapid Identification of Neonatal Sepsis

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Abstract

Objective: To achieve rapid identification of neonatal sepsis.
Setting: Neonatal Intensive care unit (NECU) of a teaching hospital.
Method: We evaluated fifty neonates who were admitted with clinical features suggcsting sepsis or who had principal risk lactors, e.g. Prematurity (<36 weeks), Low birth weight (<2. 5 kg), lIio maternal pyrexia or prolonged rupture of membranes, birth asphyxia, unbooked cases or instrumentation. Five tests, i.e., Total Leukocyte Count (T.L.C.), Absolute Neutrophil Count, Immature/Total Neutrophil ratio (I.T. ratio). Platelet count and C-Reactive protein were used for rapid diagnosis of neonatal sepsis.
Results: C-reactive protein (C.R.P.) and absolute Neutrophil count had a sensitivity of over 60% with a specificity of 50%.White blood cell count had a specificity of 93% but a sensitivity o 14%.
Conclusion: None of the tests used alone were reliable, but when in combination these five tests may help to diagnose sepsis within a few hours. Also, if the tests show a high negative predictive value, the neonate can be discharged early froni the hospital, stopping the antibiotics, thereby reducing the cost of treatment and anxiety ol the family (JPMA 50:94,2000).

Introduction

Sepsis iii neonates may be difficult to differentiate from other conditions because the clinical signs are non-specific[1,2]. Delay of even a few hours in initiating treatment can cosiderably increase the morbidity and mortality[3]. Traditional methods, such as blood cultures, do not provide a rapid diagnosis. However tests are available which can identify neonates who do have bacterial infection[4].
In acute phase the concentrations of many serum proteins rise in response to inflammation, infection, trauma, or tissue damage. Among these proteins, the important ones are C-reactive protein (CRP), haptoglobin and fibrinogen. They can be used as non-specific indicators of bacterial sepsis. As a result of increased hepatic protein synthesis (especially librinogen), the erythrocyte sedimentation rate rises, However, the ESR takes upto 24 hours to change in response to infection and therefore is not a sensitive test for the diagnosis of acute neonatal bacteremia[4].
The hematological response to inflammation in neonates includes changes in total white cell count, total neutrophil count, mature to total neutrophil ratio (liT ratio) and platelet numbers. The predictive value of a high white cell count (WBC) is poor and upto 30% of neonates with proven sepsis will have normal value[5]. While a decreased count (leucopenia) is a more specific indicator of bacterial infection then leucocytosis[6]. The normal value of neutrophil count varies with postnatal age and is a more sensitive and sepcific indicator of bacterial sepsis than the WBC count, particularly, if below the normal age hUt has the greatest specificity of all neutrophil indices[7-9]. The converse is true for the immature to tqtal neutrophil ratio. lt is elevated in more than 90% of infants with proven sepsis but is also increased in a high percentage of non-infectious respiratory illnesses[10]. It has limited predictive value as a prognostic indicator[11]. Platelet count represents an insensitive and nonspecific indicator of neonatal sepsis and is commonly noted in a variety of other neonatal disorderes. Upto 50% . of neonates with bacterial sepsis will develop low platelet levels as a late event[12],
A combination of hemátological and biochemical tests may provide a more rapid diagnosis of sepsis than conventional microbiological methods[6]. There are few comparative studies of these diagnostic

tests. The tests that have been used vary widely in their specificity and sensitivity[4-6,11]. Five tests, including C-reactive protein, have been performed to investigate sepsis. Selected tests are the reflection of hem atological and acute phase response of inflammations in neonates and can be performed rapidly and economically.

Patients and Methods

Infants admitted to the Neonatal intensive Care Unit (NICU) of the Abbasi Shaheed Hospital, Karachi, between March 1st to October 31st, 1994 were evaluated for neonatal sepsis. The unit admits about 500 babies each year with both medical and surgical illnesses. Majority of admission in NICU is due to prematurity, low brith weight (LBW), respiratory distress syndrome (RDS), sepsis and Jaundice.
Infants were evaluated for neonatal sepsis based on certain risk factors and clinical manifestations, only inborn infants suffering from medical problems were included in the study Surgical cases were referred to National Institute of Child Health (N.i.C.H.). The principal risk factors were prematurity (less than 36 weeks), low birth weight (<2.5 kg), evidence of perinatal maternal infection prolonged rupture of membranes (>24 hours), birth asphyxia, home delivery, unbooked cases and instrumentation. The principal clinical factors were feeding problem, lethargy, temperature instability, respiratory distress, irritability (including convulsions), abdominal distention and unexplained apnea or cyanotic spells. Over 90% had these clinical features.
Fifty patients were evaluated for sepsis during this period. Each infant was examined by a registrar who recorded on a data acquisition sheet, predisposing perinatal factors, clinical features, and a subjective clinical impression as to the presence or absence of sepsis before investigations were instituted. Screening tests were performed along with blood culture. All 50 patients entered into the study had blood culture done. Liquid Thioglycolate liSP (OXOID) media was used for blood culture and 2 ml of blood was added to 20 ml of medium. lnoculated bottles were incubated at 36°C for 7 days and examined for growth every 24 hours. Negative reports were given after 7 days. Urine culture and C.S.F culture were done when indicated. The diagnosis of sepsis was made when there were positive findings on blood cultures. Test used for screening were (1) Total leukocyte count <5000 or >20,000/cmm, (2) Neutropenia /Neutrophilia (age adjusted count, described by Monroe et al 1979), (3) Immature to total neutrophil (l.T. ratio >0.2), (4) C-reactive protein positive (CRP), (5) platelet counts <50,000/mm.
The leukocyte count and platelet count were performed in the laboratory using a cou Iter counter. Differential counts were performed manually. Neutrophil is classified as Band forms when there is no nuclear segmentation. Band forms together with less mature cell forms were classified as immature neutrophils. C-reactive proteins were checked by latex method. The latex test is a slide agglutination test with addition of a drop of reagent (antisera) to a drop of serum with gentle agitation for four to five minutes, which can be easily performed at the bedside or in a laboratory. (Under Laboratory Supervision).

Results

Sepsis was confirmed in 42 % (21 of 50) cases on the basis of positive blood culture. Fourteen of 2 I (66.6%) were <48 hours old, while 33.3% (7 of 21) were >48 hrs. Mean age of onset was 4 days (range 12 hours to 20 days).
Sixty six percent neonates (33 of 50) were <2.5 kg. The mean weight was 2.32 kg (range 1.3-4.12 kg). Majority i.e., 36 out of 50 (72%) were born before term (<36 weeks). The mean gestational age was 35.5 weeks (range 3 1 .5-39.5 weeks).
The WBC count was abnormal 1113 out of2l culture proven cases (14%) but it was also abnormal in 7% (2 of 29) of culture negative cases. WBCs were normal in 85% (18 of 21) and 93% (27 of 29) in

culture +ve and culture-ye cases respectively. It showed less sensitivity but high specificity (truly do not have disease). While neutrophil count was less often normal when culture was positive (8 of 21). It was found abnormal in 62% (13 of 21) of proven sepsis, it was also abnormal in 14 of 29 culture negative cases (48%). An elevated lIT ratio identified 8 of 21 (sensitivity 38%) infants with sepsis but 13 of2I (62%) of proven sepsis showed normal lIT ratio (specificity 65%). 19 of 29 (65.5%) culture negative cases. Normal lIT ratio were had in but it was found abnormal in 10 of 29 (34.5%) culture negative (false positive) cases.

Upto 52 % of proven sepsis showed decreased platelet count (II of 21), platelet was also decreased in 38% (11 of 29) of non-infected cases. Eighteen of 29 and 10 of 21 in culture negative and proven cases respectively showed normal platelet count. The C-reactive protein was significantly elevated in 14 (66.66%) of 21 episodes associated with positive blood culture. In 15 of 29 (52%) culture negative cases it was also found positive (false positive) (Table 1).

Table 1. Results of culture positive and negative cases analysis of the study.

Screening Test	Culture +ve (21)	Culture -ve (29)
WBC $\leq$5000/$\geq$20,000/mm^3	3 (14.28%)	2 (6.89%)
WBC Normal	18 (85.71%)	27 (93.10%)
*Abnormal Neutrophil count	13 (61.90%)	14 (48.27%)
Normal Neutrophil count	8 (38.09%)	15 (51.72%)
I:T Ratio $\geq$0.2	8 (38.09%)	10 (34.48%)
I:T Ratio <0.2	13 (62%)	19 (65.51%)
Platelet $\leq$150,000/mm^3	11 (52.38%)	11 (37.93%)
Platelet >150,000/mm^3	10 (47.61%)	18 (60.06%)
CRP +ve	14 (66.66%)	15 (51.72%)
CRP -ve	7 (33.33%)	14 (48.27%)

* Age adjusted as described by Monroe et al, 1979.

The sensitivity, specificity and poisitive and iiegative predictive values of each test are shown in (Table 2).

Table 2. Comparative analysis of tests used in the study.

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
WBC Count	14.28	93.1	60	60
Neutrophil Count	61.9	51.72	48.14	65.12
I:T Ratio	30.89	65.51	44.44	59.37
Platelet Count	52.38	62.06	50	64.28
CRP	66.66	48.27	48.27	66.66

PPV = Positive Predictive Value
NPV = Negative Predictive Value
I:T = Immature to Total Neutrophil Ratio
CRP = C-reactive protein.

All 21 (100%) cases showed one or more than one tests positive, so the sensitivity (true positive) was 100% and with all tests negative, tile likelihood of sepsis absent was 100% (NVP). Conversely only one patient, out of 21 culture proven sepsis, had all tests positive While not a single patient showed all tests positive in culture negative cases, so with all tests positive, the likelihood that sepsis was present was 100% (PPW). The likelihood of sepsis increased with the increasing number of positive tests (Table 3).

Table 3. Analysis of combinations of tests.

No. of tests +ve	Culture +ve (21)	Culture -ve (29)
≥1	21	27
≥2	16	14
≥3	7	4
≥4	2	1
5	1	0.0

The sensitivity, specificity and positive and negative predictive accuracy of combinations of tests is illustrated in Table 4.

Table 4. Comparative analysis of combination of test.

No. of tests +ve	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value
≥1	100	6.89	43.75	100
≥2	76.19	51.72	53.33	75.00
≥3	33.33	86.20	63.63	64.10
≥4	9.52	96.50	66.66	59.57
5	4.76	100	100	59.10

Discussion

The diagnosis of sepsis required microbiological and clinical correlation. Twenty-nine (29) infants classified as having probable infection had clinical evidence but lacked microbiological proof of infection. This was probably because of administration of intrapartuni antibiotic, which influenced culture results. Statistical analysis would be simplified if the latter infants were excluded tiom the study, but we cannot ignore these infants because Fatal infection has been reported in the presence of negative blood culture[13].

In this study five tests were evaluated (WBC count, neutrophil count, l/T ratio, platelet count and CRP) for use in the diagnosis of neonatal sepsis. None of the tests gave the sensitivity, specificity and positive predictive accuracy of the combined results of acridine orange leucocyte cytospin (Aolc), nitroblue tetrazolium (NBT) and C-reactive (CRP)[6], but the former two tests are not available in our setup. So the tests were selccted on the basis of ease, speed of performance, cost and availability. These tests have practical advantages: they are applicable to all infants including those who have received antibiotic therapy prior to evaluation": it saves time for the busy physician particularly the inexperienced house officer and it could allow a mere systeniic approach to decision regarding antibiotic therapy. We still stress importance of correlating both clinical and laboratory data. because Few infants in our study who had and were included in the study because of intrapartum risk factors. Similarly the infants who would have been missed by these test had clinical features of sepsis. Christensen et al[14] reported that on the first day of life there is a latent period of four hours between the onset of infection and hematological response and describes fatal early onset disease especially in preterm neonates. On the basis of this report, Rodwell et al[11], recommended clinical surveillance and repeat hematological profile 8 to 12 hours after the initial test in asymptomatic preterm infants <24 hours of age. Therefore, the individual hematological findings should be used with caution as the sole indicator of infection, because the hematological response may vary with both gestational and post natal ages[7,15], with the time interval between the onset of infection and blood sampling[16]. The variety of blood sampling sites may have influenced the results. Arterial and venous leucocyte counts are lower than capillary blood values[15]. To avoid erroneous interpretation of leucocyte values, each laboratory

should establish its own reference ranges and the precise postnatal age at the time of blood sampling should he ascertained before determining the interpretation of test.

The predictive value of high WBC is poor[17] and upto 30% of neonates with proven sepsis have normal value[5] but the result obtained in our study is different. About 85% of neonates had normal WBC count in culture proven cases. The reason for this high false negative result might be due to time interval between the onset of bacterernia and sampling[16]. The WBC count was found to he the most specific of all tests but least sensitive. Neutrophil count was found more sensitive than WBC counts. The neutrophil count, particularly if below the normal age adjusted range, is a more sensitive and specific indicator of bacterial sepsis than the WBC count[18]. In our study the specificity of neutrophil count was not high compared with the specificity of WBC count. In about 48% of cases, neutrophil count was abnormal ui culture negative cases, possibly because the neutropenia is more common in non-infective respiratory illness. The absolute neutrophil count appears to be most useful as a screen for sepsis among infants who develop respiratory distress during the first few hours of life[19]. Nearly 90% of these infants exhibit either neutropenia or neutrophilia[7]. We found this figure (90%) reduced to 62% but still had highest sensitivity, when CRP is excluded. Siegel[26] recommends an elevated liT ratio to identi& infected infunts and indicates that further tests are of no value; our study For an elevated lIT ratio conflicts this recommendation. It showed comparatively low sensitivity (38,09%). But the result obtained, was almost similar to the sensitivity mentioned by Kite et al[4]. The poor sensitivity of the immature to total neutrophil ratio in this study may have been the result of sampling at wrong time and also because a significant percentage of Neonates were brought late to this unit i.e. after first week of life when the test becomes less sensitive[15].

tipto 50% of neonates with bacterial sepsis develop low platelet levels (thrombocytopenia) as a late event[12]. This was almost confirmed in our study (52%). Thrombocytopenia was also found in less than half of the culture negative cases.

Raised serum level of CRP are found in 50-90% of neonates from six hours of onset of bacteremia, but raised level are not specific for bacterial infection[21]. Recently many investigators have considered CRP estimation to be of value in early diagnosis and monitoring of neonatal sepsis[2,22]. However, in a large study from New York, CRP values were found to be moderately raised in sepsis but the serum levels were also found high with asphyxia. shock and other problems not related to infection[23]. The frequent occurrence of raised CRP in sera of uninfected newborn infants eliminates it as a useful indicator of infection but may suggest an active tissue daniaging process1 In our study CRP was found positive in 52 percent of culture negative cases while it was negative in 33 percent of culture proven sepsis. Thus the question, with a positive test that how likely is the disease to be present and with a negative test how likely is the disease not to be present remains to be answered.

We used latex agglutination slide test for CRP, which is simple to perform and easy to interpret but CRP can also be detected by quantitative rod ioi mm unodi ffLlsion technique. Squire and Co-worker[23] found that CRP was elevated in 88% of patients with severe infection. It emphasizes that qualitative tests are non-specific and must be correlated with other laboratory data and clinical findings, while on the other hand quantitative technique is comparatively less non-specific but tlie Process requires more time which may be too long a period to deal with an acutely ill neonate. Serial levels are useful in diagnostic evaluation of neonate with suspected sepsis[22].

Each test used in this study had different specificity, sensitivity and positive predictive accuracy. It has demonstrated that combination of tests increases the sensitivity, specificity and positive predictive accuracy compared with a single test for the diagnosis of neonatal sepsis[4-6]. This was found true in our study that specificity and positive predictive value were improved with the increased number of positive tests used for screening and with all five tests positive it reached to 100 percent. Conversely

with all negative tests, the likelihood of sepsis being absent was 100 percent (NPV).
It is known that these tests can heconie positive in a variety of non-infective disorders, conversely septicemia can occur with normal findings. Therefore we do not advocate relying on the result of a single test. Even with the combination of tests; we still stress on the importance of correlating the clinical and laboratory data. This is an initial workup study and we recommend that scoring system should be designed for our setup, using those tests that are easy to perform, economical, available and should ideally identify all infected infants (high sensitivity), so that disease can be confidently excluded with negative test results (high negative predictive value) and stop the antibiotic early, thereby reducing the cost, duration of hospital stay and anxiety of the parents.

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