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Usefulness of C-Reactive Protein in the Diagnosis of Neonatal Sepsis

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ABSTRACT

Aim: Early diagnosis of sepsis in the neonate is often difficult because symptoms and signs are usually non-specific. A study was conducted to evaluate C-reactive protein (CRP) as a screening tool for neonatal sepsis among very low birth weight (VLBW) infants.

Method: The study population consisted of 70 VLBW infants suspected of sepsis. Sepsis was diagnosed from positive cultures of blood, cerebro-spinal fluid or bone/joint aspirate in the presence of signs. Positive cultures were the "gold standard" against which the performance of CRP ≥1.0 mg/dL, abnormal white cell counts (WCC), absolute neutrophil (ANC) and platelet counts were compared.

Results: Of 152 septic screens, 30 (20%) had positive cultures. From analysis of the receiver operating characteristic (ROC) curve, CRP ≥0.7 mg/dL rather than CRP ≥1.0 mg/dL appeared a better cut-off for screening. The sensitivity, specificity, positive and negative predictive values of CRP ≥0.7 mg/dL were 56%, 72%, 71% and 57% respectively. Only abnormal platelet counts had similar efficiency as CRP. Abnormal WCC had the lowest sensitivity and positive predictive value while abnormal ANC had the lowest specificity and negative predictive value among them.

Conclusion: CRP assay using laser nephelometry is a valuable adjunct in screening for neonatal sepsis, complementing clinical decision-making.

Keywords: C-reative protein, acute phase reactant, infection, neonatal sepsis, laser nephelometry

INTRODUCTION

Early recognition of sepsis in the neonate is one of the most difficult problems facing clinicians today. Such infants often present with non-specific symptoms and signs so that failure or delay in treatment may result in significant mortality and morbidity[1]. Although various haematological indices had been utilised to screen for sepsis, most were neither highly sensitive nor specific and were commonly affected by perinatal factors like maternal hypertension, asphyxia and haemolytic disease[2].

C-reactive protein (CRP) has been used as an acute phase reactant to diagnose and follow the course of infection in neonates[3-7]. Its advantages include its very low serum levels in normal infants, a rapid rise within 12 to 24 hours of sepsis and a large incremental increase thereafter. A study was conducted to evaluate the usefulness of serum CRP as a diagnostic tool of sepsis among very low birth weight infants in a neonatal intensive care unit (NICU).

MATERIAL AND METHODS

The study population consisted of very low birth weight (VLBW) babies (weighing less than 1,500 g) admitted to the neonatal intensive care unit (NICU) of a teaching institution between July 1990 and April 1993, and who developed symptoms or signs suggestive of sepsis. These symptoms included temperature instability, lethargy or irritability, apnoea, hypotension, feed intolerance, abdominal distension, bloody stools, hypo- or hyper-glycaemia and seizures. Infants suspected of sepsis underwent a septic screen consisting of a full blood count, chest X-ray and cultures of blood, cerebrospinal fluid and urine. Cultures of endotracheal aspirates and other normally sterile sites (like bone/joint cavity) were also taken where clinically indicated. All routine haematological investigations were carried out by the hospital laboratory. At the same time, CRP quantitation was performed on 20 μL of blood by medical staff using laser nephelometry (Laser CRP-1, Arrows Co Ltd, Osaka, Japan) at the bedside. All infants following the septic screen received antimicrobial therapy.

The diagnosis of sepsis was based on the isolation of bacteria or fungi from cultures of peripheral venous blood, cerebro-spinal fluid or joint aspirate in the presence of signs of infection. Positive culture results were considered the "gold standard" against which the performance of CRP, total white cell counts, absolute neutrophil counts and platelet counts were compared. A CRP value ≥1.0 mg/dL was deemed abnormal. Abnormal total white cell counts (WCC) were defined by either leucopenia ≤5,000 mm^3 or leucocytosis ≥20,000/mm^3. Abnormal absolute neutrophil counts (ANC) were defined by either neutropenia ≤1,750/mm^3 or neutrophilia ≥5,500 mm^3 according to Manroe et al[2]. Thrombocytopenia was defined by platelet counts of ≤150,000/mm^3.

Statistical analysis was performed using the student's t-test for continuous variables and two-tailed Fisher's exact test for categorical variables. Only p values <0.05 were considered significant. The sensitivity, specificity, positive and negative predictive

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values of CRP ≥1.0 mg/dL and that of abnormal haematological indices were calculated according to the method of Feinstein[8] and compared. Sensitivity was calculated from the proportion of septic patients testing positive, while specificity was calculated from the proportion of non-septic patients with negative tests. The positive predictive value was the proportion of patients with positive tests who actually had sepsis, while the negative predictive value was that with negative tests who were nonseptic. In addition, the receiver operating characteristic (ROC) curve for CRP was graphed by plotting the sensitivity (true positive rate) against "one minus specificity" (false positive rate) for different threshold levels of CRP[9]. The point on the curve nearest the top left-hand corner would be the sensitivity and specificity of the optimum CRP value providing a trade-off between the true and false positive rates[9]. The area under the ROC curve was calculated after drawing a step-wise plot through all points.

RESULTS

One hundred and fifty two septic screens were performed in 70 VLBW infants during the study period with a median (range) of 2 (1-8) screens per baby. The septic screens were performed when the babies were between 4 and 178 days old. The mean (S.D.) birth weight of the infants was 1,094 (222) g while the mean (S.D.) gestational age was 29 (2) weeks. Their mean (S.D.) one and five minute Apgar scores were 5.0 (2.4) and 7.4 (1.9) respectively. Fifty-three infants (76%) were born to mothers with risk factors for sepsis which included maternal pyrexia in labour, prolonged rupture of membranes for >24 hours, maternal chorio-amnionitis and unexplained preterm labour.

Sepsis was diagnosed in 26 (37%) of 70 infants. All septic infants had positive blood cultures, including two with positive joint aspirate cultures due to septic arthritis and one with brain abscess at autopsy. There was no infant with meningitis. Comparison of the septic with non-septic infants is shown in Table I. Septic infants were of significantly lower birth weight and gestational age than the non-septic ones.

Table I - Comparison of demographic characteristics of septic and non-septic infants (n=70)

	Septic	Non-septic	p-value
Number	26	44	
Birth weight (g)	990 (241)	1156 (188)	0.002
Gestation (wks)	28.1 (2.2)	29.3 (2.3)	0.03
Males	18 (69)	23 (52)	ns
Caesarean section	13 (50)	16 (36)	ns
Apgar ≤5 at 5 min	4 (16)	4 (10)	ns
Deaths	3 (12)	6 (14)	ns

values expressed as mean (S.D.) for birth weight and gestation, and number (percent) for other variables; ns - not significant

Table II - Causative organisms of sepsis (n=30)

	No.	%
Staphylococcus epidermidis *	11	37
Staphylococcus aureus #	6	20
Candida rugosa, parapsilosis	5	16
Pseudomonas aeruginosa	2	7
Klebsiella	2	7
Bacillus	2	7
Group D *Streptococcus*	1	3
Escherichia coli	1	3

* including 9 methicillin-resistant and 2 methicillin-sensitive S. epidermidis

\# including 3 methicillin-resistant and 3 methicillin-sensitive S. aureus

The sepsis prevalence rate was 20% (30/152 septic screens). Analysis of the 30 episodes of sepsis revealed that the median age at diagnosis was 20.5 (range 4-47) days. Table II lists the causal organisms in decreasing order of frequency. The commonest organism was *Staphylococcus epidermidis* (11), followed by *Staphylococcus aureus* (6) and *Candida* species (5). In two infants with signs of sepsis, *Bacillus* was cultured from two sources in each of them, including peripheral blood and percutaneous central silastic cannula tip. Nine infants died, including three who succumbed from overwhelming infection with *Escherichia coli, Pseudomonas aeruginosa* and *Candida.*

Figure 1 shows the ROC curve constructed for various cut-off levels of CRP ranging from 0.1 to 8.0 mg/dL. At the cut-off level of 1.0 mg/dL, CRP had a sensitivity of 48% and specificity of 79%. However, from the ROC curve the better cut-off CRP value appeared to be 0.7 mg/dL, where sensitivity was 56% and specificity 72%. The area under the ROC curve was found to be 0.643.

The diagnostic indices of CRP ≥1.0 mg/dL, CRP ≥0.7 mg/dL and abnormal haematology are tabulated in Table III. The positive and negative predictive value of CRP ≥1.0 mg/dL was 74% and 55%, while that of CRP ≥0.7 mg/dL was 71% and 57% respectively. Thrombocytopaenia was found to have similar sensitivity, specificity, positive and negative predictive values as CRP. Abnormal WCC had the lowest sensitivity (13%) and positive predictive value (39%) while abnormal ANC had the lowest specificity (55%) and negative predictive value (39%) among the indices.

DISCUSSION

In our evaluation of CRP assay, the diagnosis of sepsis was based on positive cultures of blood, cerebrospinal fluid or joint aspirate in the presence of signs of infection. The definition excluded pneumonia, gastro-intestinal, urinary tract and skin infections which may not result in CRP changes[10,11]. Comparison of the performance of CRP and abnormal haematology was thus made against a well-defined "gold standard".

Table III - Comparison of CRP and abnormal haematological indices in 152 septic screens

	Sensitivity	Specificity	PPV	NPV
CRP ≥1.0 mg/dL	48	79	74	55
CRP ≥0.7 mg/dL	56	72	71	57
abnormal WCC	13	75	39	42
abnormal ANC	37	55	52	39
thrombocytopaenia	54	81	77	60

PPV positive predictive value; NPV negative predictive value; abnormal WCC abnormal white cell count defined as $\leq 5,000/mm^3$ or $\geq 20,000/mm^3$; abnormal ANC abnormal absolute neutrophil count defined as $\leq 1,750/mm^3$ or $\geq 5,500/mm^3$; thrombocytopaenia defined as platelet count $\leq 150,000/mm^3$.

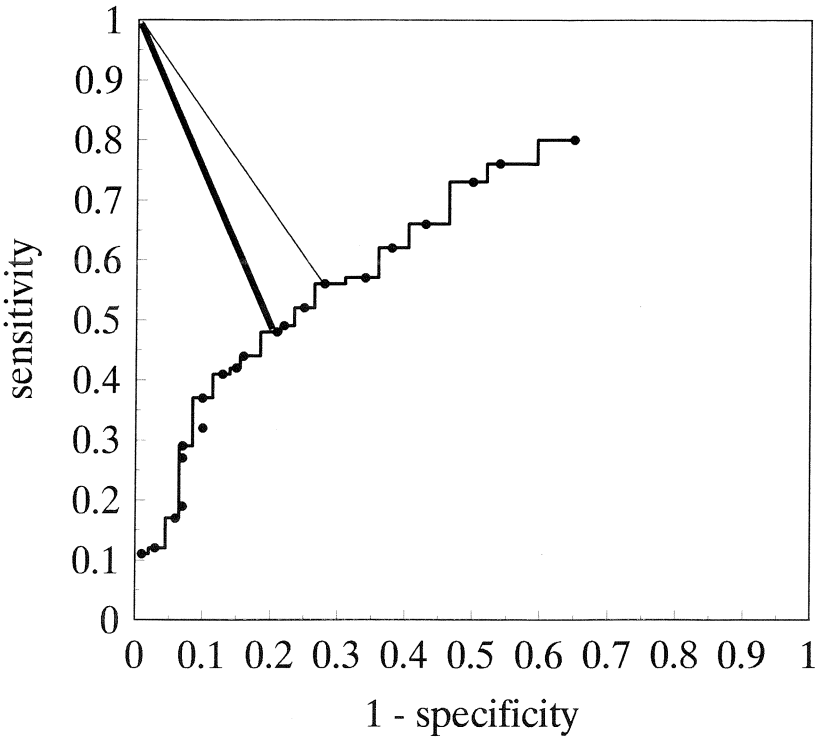

Fig 1 - Receiver operating characteristic (ROC) curve by plotting sensitivity (true positive rate) against "one minus specificity" (false positive rate) at different cut-off levels of CRP. A step-wise plot has been drawn through all points. The lines join the top left-hand corner to points representing CRP ≥1.0mg/dL (thick line) and CRP ≥0.7 mg/dL (thin line).

Like the report of Wagle et al[12], septic infants had significantly lower birth weight and gestational age than non-septic ones. Slightly over half of all infecting organisms were the *Staphylococcus* (17/30 or 57%), which form a leading cause of nosocomial infections in the susceptible neonate[1].

The ROC curve (Fig 1) provided a comprehensive and elegant graphical display of the whole spectrum of sensitivities and specificities for CRP. From the curve, CRP ≥0.7 mg/dL appeared to have better efficiency than CRP ≥1.0 mg/dL for screening of neonatal sepsis. Using CRP ≥0.7 mg/dL, CRP values were found to be positive in 56% of septic episodes but only 28% of non-septic ones, similar to the findings in previous reports[3-7, 10-12]. The false negative rate of 44% may be due to the earlier detection of sepsis before the infant could respond to infection by elevation of CRP. Other studies had reported that CRP demonstrated increased sensitivity when assayed serially during the course of infection[12-14].

The calculation of both sensitivity and specificity depend on knowing which infants were already septic when CRP assay was performed. Greater practical value is derived from knowing its predictive accuracy[8], although these are dependent on prevalence rate. In other words, when CRP showed a positive result, one wants to know what proportion of infants were in fact septic. In our study, CRP ≥0.7 mg/dL had slightly better positive than negative predictive values for diagnosis of neonatal sepsis, so that 71% of positive tests were correctly classified as compared to only 57% of negative tests.

Comparison of the indirect indices of infection and CRP showed that only thrombocytopaenia had sensitivity, specificity, positive and negative predictive values comparable with those of CRP. In contrast, an abnormal WCC had the lowest sensitivity (13%) and positive predictive value (39%) while an abnormal ANC had the lowest specificity (55%) and negative predictive value (39%) among the indices. The low sensitivity and specificity of the leucocyte indices render them less valuable than CRP for screening purposes, similar to the findings of others[10, 15-18]. Furthermore, Manroe[2] had reported that abnormal haematology may be affected by non-septic processes like steroid treatment as part of therapy for chronic lung disease. CRP on the other hand was unaffected by arterial catheterisation, intraventricular bleeding or steroid therapy[18,19].

Our study showed that the measurement of C-reactive protein by laser nephelometry is a useful adjunctive tool in screening for neonatal sepsis. Quantitative assay of CRP is simple to perform at the bedside by medical staff. It is readily completed within 10 minutes, utilising only 20 μL of the infant's blood. The optimum CRP value for screening of neonatal sepsis appeared to be 0.7 mg/dL. Abnormal haematology especially leucocyte indices did not have as comparable a sensitivity or specificity as CRP. Sound clinical judgement combined with quantitative CRP assay should provide a rational basis for treatment decisions in the management of neonatal sepsis[1]. Such a strategy may significantly reduce unnecessary antimicrobial therapy which could otherwise permit the emergence of resistant strains of organisms as well as place these immature infants at risk for allergic and adverse side-effects with increased hospitalisation costs.

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