The Role of Serial Serum C-Reactive Protein in the Diagnosis and Duration of Antibiotic Therapy in Neonatal Sepsis

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الخلاصة

الخلفية : تشخيص و علاج الأنتان أليوالدي و خصوصاً في الدول النامية صعب و لا يوجد فحص موثوق به للاستدلال على الإصابة في الوقت الحاضر. بروتين c التفاعلي لطالما استخدم كعلامة للإنتان يوصى بقياس البروتين c التفاعلي المتسلسل كدليل للتشخيص وتحديد فترة العلاج بالمضادات الحيوية.

الهدف : تقييم قياس البروتين c التفاعلي كدليل لتشخيص و مراقبة و تحديد فترة العلاج بالمضادات الحيوية للانتان أليوالدي.
الطرق :٢٠٠ طفل حديث الولادة مشخص سريريا كانتان وليدي (مجموعة المرضى) و ٢٠٠ طفل حديث الولادة ادخل المستشفى أو زار العيادة الخارجية لأسباب أخرى (المجموعة الضابطة) أشركوا في هذه الدراسة المستقبلية. زرع الدم و فحص البروتين c التفاعلي اجري لكافة الأطفال في مجموعة المرضى بينما اجري فحص البروتين c التفاعلي مرة واحدة لأطفال المجموعة الضابطة.
النتائج : من ٢٠٠ طفل حديث الولادة (مجموعة المرضى). ٩٠ (٤٥%) كان زرع الدم موجباً. فحص البروتين c التفاعلي كان سالباً في اليوم الأول و الثالث عند ٥٤ (٢٧%) طفل حديث الولادة في مجموعة المرضى بينما كان الفحص موجباً لدى ٨ أطفال (٤%) من ألمجموع الضابطة. فحص البروتين c التفاعلي يملك درجة حساسية ٧٣% و درجة نوعية ٩٦% بينما قيمة التنبئية الموجبة ٩٤.٨% و قيمة التنبئية السالبة ٧٨%.

الاستنتاج : فحص واحد للبروتين c التفاعلي لا ينفي الإصابة بالانتان أليوالدي بينما فحصان سالبان ليومين متتاليين يستبعدان الإصابة بالإنتان أليوالدي و يمكن الطبيب المعالج من إيقاف المضادات الحيوية

ABSTRACT

Background : the management of neonatal sepsis(**N.S.**) especially in developing countries is problematic. There is no single reliable marker of infection available at the present. C-reactive protein (**CRP**) has long been used as a marker of infection. Serial measurements of **CRP** are recommended as a guide for duration of antibiotic therapy.

Objective : to evaluate the serial **CRP** measurements as a guide line for diagnosis and monitoring therapy and determining the duration of antibiotic treatment in suspected neonatal sepsis.

Methods : Two hundreds neonates with clinical diagnosis of neonatal sepsis (patients group) and 200 neonates admitted or visited the outpatient clinic of the hospital for causes other than neonatal sepsis (control group) were enrolled in this prospective study. Blood culture & serial **CRP** were done for all patients group while single **CRP** was done for control group.

Results : Of 200 infants (patients group), 90 (45%) had positive blood culture. **CRP** was negative on first and third day in 54 neonates (27%) of patients group while it was positive in 8 neonates (4%) of control group. **CRP** had sensitivity of (73%) and specificity of (96%). It's positive predictive value was (94.8%) whereas it's negative predictive value was (78%).

Conclusion : single negative **CRP** value does not exclude **N.S.** and two negative **CRP** values, 24 hours apart can exclude the probability of **N.S.** and allow pediatricians to discontinue antibiotics.
Key words : Neonatal sepsis; C-reactive protein, Diagnosis, Antibiotic therapy.

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INTRODUCTION

Neonatal sepsis (**N.S.**) may have subtle, diverse and non-specific symptoms and signs; moreover, a delay in the diagnosis and commencement of treatment results in a high morbidity and mortality rates [1]. It is estimated that about 5 million neonates die every year in low-income countries. Infection contributes to approximately 30 to 40% of neonatal deaths in these countries [2], however, early diagnosis of **N.S.** has remained a frustrating experience even in high-income countries [3].

Isolation of microorganisms from one or more blood cultures is the gold standard to establish a definitive diagnosis of **N.S.** [4-6]. The sole use of blood culture to diagnose **N.S.** has a number of limitations. It may take 24 to 72 hours to obtain culture results [7,8]. The sensitivity of blood cultures may be impaired by exposure to intrapartum antibiotics, which are administered to 15% to 40% of mothers in labor [9-11]. With the development of multiple drug resistant bacteria and the cost of therapy with multiple antibiotics ,the ability to diagnose or rule out sepsis is an essential tool to limit inappropriate antibiotic exposure [12-14].

Over the last decade , a variety of laboratory tests have been developed to enhance the early and accurate identification and treatment of infant with suspected sepsis like total white blood cell count and differential , immature to total neutrophil ratio , platelets count , erythrocyte sedimentation rate and C- reactive protein (**CRP)**.

CRP, an acute phase reactant, is synthesized in the liver in response to inflammatory cytokines and may rise more than 1000 times during an acute phase response. It falls quickly after efficient elimination of microbial stimulus, due to it's short half-life of 19 hours [15-19].

CRP is a sensitive and rapid reacting index in bacteremic infections, however, because other factors than septicemia also increase **CRP** (intraventricular hemorrhage, meconium aspiration, necrotizing enterocolitis, pneumothorax , maternal fever during labor , prolonged rupture membrane and stressful delivery) [20], we deem a negative **CRP** value most informative, if two negative determinations, taken several hours a part, the patient is very unlikely to have invasive bacterial infection [21].

The objective of the present study is to evaluate the role of **CRP** as a parameter in the diagnosis and treatment of proved & suspected N.S..

PATIENTS AND METHODS

The study was conducted in Karbala teaching hospital for children, Karbala, Iraq , over eighteen months period from 1st April 2005 to 30th September 2006. The study subjects included 200 neonates { patients group) admitted in the neonatal intensive care unit and general pediatric wards with a clinical diagnosis of **N.S.** having either non specific signs and symptoms or focal signs of infection and 200 neonates (control group) admitted or visited the outpatient clinic for causes other than **N.S.**

N.S. was diagnosed according to a sepsis score which included the following signs and symptoms such as refusal to feed, lethargy, poor cry, vomiting, diarrhea, excessive cry, jaundice, hypothermia, fever, apnea, tachypnea . poor capillary refill, abdominal distention, seizure, omphalitis and cyanosis [22]. If a baby had three or more of the above signs or symptoms, septicemia was suspected.

The neonates who had congenital malformations, birth asphyxia, inborn error of metabolism, hemolytic jaundice, meningitis and those who had undergone surgery were excluded. Neonates with gestational age less than 37 weeks & weight less than 1500 gm were also excluded.
All patients group underwent a detailed history, complete physical examination and relevant hematological, microbiological and radiological investigations to explore all possible sources

of infection. Under strict aseptic measures blood samples for culture and sensitivity were collected, One ml of blood was added to a bottle containing 10 ml of brain-heart infusion broth. Serum **CRP** were done for all patients and control groups. **CRP** value was estimated by latex agglutination method with **CRP** kit manufactured by Biocon Diagnostik, Hecke 8, 34516 Vohl / Marienhagen, Germany. The **CRP** value of more than 6 mg/l was taken as abnormal. Serum **CRP** was estimated every 2-4 days for patients group & once for control group.
Intravenous antibiotic amino glycoside (gentamycin) and a third generation cephalosporin (cefotaxime or ceftriaxone) were administered to all patients group and stopped when two **CRP** results, 24 hours apart, were negative. After stopping the treatment, babies were observed for 48 hours in the hospital and followed up to 4 weeks for any relapse.
The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of **CRP** test were calculated.

RESULTS

In the present study, 200 (108 male, 92 female) neonate, admitted in NICU and general wards with clinical features of **N.S.** (patients group) and 200 (120 male, 80 female) admitted or visited the outpatient clinic for other causes (control group) were investigated. Out of 200 sick neonate (patients group), 92 neonates (46%) were less than 7 days old, 54 neonates (27%) weighed 1500-2000 gm, 25 neonates (12.5%) weighed 2001-2499, 121 neonates (60.5%) weighed more than 2500 mg.

Table (1) shows the presenting symptoms and signs.

Table (1) Neonatal sepsis : symptoms and signs. (N=200)

Symptoms and signs	No.	%
Refusal to feed	170	85
Lethargy	163	81.5
Poor cry	132	66
Vomiting	47	23.5
Excessive cry	19	9.5
Diarrhea	9	4.5
Hypothermia	87	43.5
Cyanosis	47	23.5
Poor capillary refill	45	22.5
Apnea	42	21
Tachypnea	27	13.5
Fever	17	8.5
Abdominal distention	17	8.5

Seizures	15	7.5
Conjunctivitis	14	7
Omphalitis	12	6

Blood culture was positive in 90 cases (45%). **Table (2)** shows the organisms isolated.

Table (2) The Organisms isolated from 90 blood culture positive neonates

Organisms	No.	%
Staphylococcus epidermidis	55	61.11
Escherichia coli	17	18.89
Staphylococcus aureus	10	11.11
Proteus	5	5.56
Pseudomonas	3	3.33

Forty eight neonates (24%) died of N.S., out of which 26 (13%) were blood culture positive and **CRP** positive while 22 (11%) were blood culture negative (17 of 22 were **CRP** +ve).
Out of 200 cases of patients group., **CRP** was negative on first and third day of treatment in 54 patients (27%), in 49 patients (24.5%), **CRP** was positive on first day & negative on third day. in 45 patients (22.5%) **CRP** was positive on third day, negative on fifth day, in 20 patients (10%) **CRP** was positive on fifth day, negative on seventh day, in16 patients (8%) **CRP** was positive on seventh day, negative on tenth day, in13 patients (6.5%) **CRP** was positive on tenth day, negative on fourteenth day while only in 3 patients **CRP** was positive up to fourteenth day & became negative on sixteenth, eighteenth and twentieth day respectively.

Table (3) shows **CRP** guided duration of treatment in days

Table (3) Duration of treatment in days according to results of **CRP** (- or +)

Days						Number	%
1	3	5	7	10	14		
-	-					54	27
+	-					49	24.5
-	+	-				45	22.5
-	+	+	-			20	10
+	+	+	+	-		16	8
+	+	+	+	+	-	13	6.5
+	+	+	+	+	+	3	1.5

Antibiotics were stopped in all cases when **CRP** became negative on two successive days ,but patients kept in hospital for two days for observation then discharged & followed for four weeks. There was no relapse in any group within four weeks of discontinuation of antibiotics (negative predictive value of 100%)

C-reactive protein was negative on first & third day in 54 neonates (27%) of patients group while it was positive in 8 neonates (4%) of control group.
C-reactive protein had **sensitivity of 73%** & **specificity of 96%**. It's **positive predictive value** was **94.8%** whereas it's **negative predictive value** was **78%**.

DISCUSSION

Bacterial infection stimulates the hepatocytes to produce **CRP**, a non specific immune response, which is a useful clinical marker for the individual host-pathogen interaction. Since **CRP** does not cross the placenta [23], its half life is short (19 hours), a rapid fall is seen with successful therapy, as demonstrated in the present study.
The most common symptoms, by which 85% of these patients presented were refusal to feed, followed by lethargy & poor cry which is similar to the observation of Guha et al [24].

Fever was not a prominent feature in the present study as demonstrated by other workers [24,25]. Instead hypothermia was more common (43.5% vs. 8.5%).
The incidence of blood culture positivity was (45%) in the present study, which is similar to other studies [25-27]. and higher than other studies Zeeshan A. et al [28] and Arshad et al [29] with a positivity of 28% and 25% respectively, the low sensitivity of blood culture in the last two series may be due to intrapartum administration of antibiotics to mothers which can affect the blood culture results.
Although essential for diagnosis and appropriate management, blood culture results are not immediately available and their yield is low. The yield depend on skin disinfection, sample volume and sampling site.
The pattern of bacterial isolates was different in different studies due to geographical variations and use of different antibiotics at different centers .
Staphylococcus epidermidis was the commonest bacterial pathogen isolated in blood culture, 55 cases (61.11%) which could be a contaminant [30] or true sepsis, 50 cases of them revealed +ve **CRP**, so we can use **CRP** to differentiate true staphylococcus epidermidis sepsis from contamination [31] .
In the present study 48 neonates (24%) died of **N.S.**, 26 neonate (13%) were blood culture +ve & **CRP** +ve, 17 neonate (8.5%) were blood culture –ve & **CRP** +ve ,while 5 neonates (2.5%) were blood culture –ve & **CRP** –ve ,which means that –ve blood culture and –ve **CRP** doesn't exclude the diagnosis of **N.S.** in sick neonates.
CRP was negative in 119 neonates (59.5%) on first day & became +ve in 65 neonates (32.5%) on third day of admission which means that single –ve reading on first day of admission doesn't exclude the diagnosis of **N.S.**, which is similar to the suggestion of Chiesa C et al [32] and the study of Garland SM et al [33].
Antibiotics were stopped in 103 neonates (51.5%) by the third day, 45 neonate (22.5%) by the fifth day and 20 neonate (10%) by the seventh day while 32 neonates (16%) required longer duration of antibiotic therapy , 28 of them (87.5%) had positive blood culture, suggesting that, those with positive blood culture & +ve **CRP** needed longer duration of antibiotics therapy which is in line with the study of R.S. Jaswal [34].
In the present study, **CRP** had sensitivity, specificity, PPV and NPV of 73%, 96%,94.8% and 78% respectively. These figures were 79%, 85%, 36% and 97% in Hajiehe Boma study [35], which shows clear difference in PPV (94.8% vs. 36%) while Nuntnarumit P et al [36] reported a sensitivity of 100%, specificity 94%, PPV and NPV of 91.6% and 100% respectively of **CRP** for detecting proven sepsis and localized infection.

Santana et al [37] reported 80% sensitivity and 92% specificity for **CRP** which is in line with the present study while an Australian study has documented 67% sensitivity and 86% NPV of **CRP** in diagnosis of **N.S.** [33]

CONCLUSION

- Single negative **CRP** value does not exclude **N.S.** since false negative values can occur in preterm neonates, overwhelming sepsis and the first 24 hours of **N.S.**
- Two negative **CRP** values, 24 hours a part can exclude the probability of **N.S.** and allow clinicians to discontinue antibiotics, limiting extended unnecessary antibiotic exposure.

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