International Journal of Contemporary Pediatrics
Mehrotra G. Int J Contemp Pediatr. 2017 May;4(3):890-895
http://www.ijpediatrics.com
pISSN 2349-3283 | eISSN 2349-3291

DOI: http://dx.doi.org/10.18203/2349-3291.ijcp20171693

Original Research Article

Study of C - reactive protein in neonatal sepsis

Gunjan Mehrotra*

Department of Paediatrics, SAMC and PGI, Indore, Madhya Pradesh, India

Received: 17 January 2017
Accepted: 17 February 2017

***Correspondence:**
Dr. Gunjan Mehrotra,
E-mail: drgunjankela@yahoo.com

ABSTRACT

Background: Sepsis is one of the most common causes of morbidity and mortality in the newborn. Early diagnosis and treatment is vital to improve outcome. Neonatal sepsis in newborn is characterized by paucity of signs and symptoms and is due to invasion and spread through the body of non-pathogenic/ pathogenic & Gram positive / negative organism. It is subtle disease, the general characteristic of bacterial infection in neonatal period are influenced more by response of the infant than the causative organism. The present study was therefore carried out to determine the usefulness of C-reactive protein (CRP) for evaluation of neonatal sepsis in tertiary care hospital.
Methods: Neonates with clinical suspicion of sepsis were prospectively studied out from June 2006 to January 2008. Blood was obtained from each subject recruited for the qualitative estimation of CRP. Blood culture was used as gold standard for diagnosis of NNS.
Results: Of 50 neonates studied, 34 (68%) had positive CRP while 31 (62%) had positive blood culture. The sensitivity, specificity, positive and negative predictive values of CRP were 90.32%, 42.10%, 71.79% and 72.72% respectively.
Conclusions: The qualitative method of estimating CRP which is cheap and rapid has moderate sensitivity, specificity and negative predictive value.

Keywords: C-reactive protein, Neonatal sepsis

INTRODUCTION

During the last decades advances in neonatal intensive care have led to an impressive decrease of neonatal mortality and morbidity. However, infectious episodes in the early postnatal period still remain serious and potentially life-threatening events with a mortality rate of up to 50% in very premature infants.[1] The signs and symptoms of neonatal sepsis can be clinically indistinguishable from various non-infectious conditions such as respiratory distress syndrome or maladaptation. Therefore rapid diagnosis is crucial for preventing the child from an adverse outcome. The current practice of starting empirical antibiotic therapy in all neonates showing infection-like symptoms results in their exposure to adverse drug effects, nosocomial complications, and in the emergence of resistant strains.[2]

Sepsis results from the complex interaction between the invading microorganism and the host immune, inflammatory, and coagulation response.[3,4] Inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-8, IL-15, IL-18, MIF) and growth factors (IL-3, CSFs), and their secondary mediators, including nitric oxide, thromboxanes, leukotrienes, platelet-activating factor, prostaglandins, and complement, cause activation of the coagulation cascade, the complement cascade, and the production of prostaglandins, leukotrienes, proteases and oxidants.[5] Laboratory sepsis markers represent a helpful tool in the evaluation of a child with clinical signs and complement the evaluation of a neonate with a potential infection.

During the last decades efforts were done to improve laboratory sepsis diagnosis and a variety of the above mentioned markers and more were studied with different success. Despite the promising results for some of them current evidence suggests that none of them can consistently diagnose 100% of infected cases. C-reactive protein (CRP) is the most extensively acute phase reactant studied so far and despite the ongoing rise (and fall) of new infection markers it still remains the preferred index in many neonatal intensive care units.

There is great interest in rapid diagnostic tests that are able to safely distinguish infected from uninfected newborns, especially in the early phase of the disease.[6] In fact, a delayed start of the antibiotic treatment may be no more able to stop the fulminant clinical course with development of septic shock and death within hours after the first clinical symptoms.[7]

In the era of multi-resistant microorganisms, it is as well important to avoid the unnecessary use of antibiotics in sepsis-negative infants.

CRP can be assayed quantitatively or qualitatively. The quantitative method is more widely used in developed countries.[8] It provides rapid, highly sensitive and specific results but requires more time (about 15 to 30 minutes) and is more complex and expensive to perform.[8,9] The test kits may also not be readily available in some health centres in developing countries. The qualitative method provides rapid results within 15 minutes. However, it is less specific but has the advantage of being simple and easier to perform and interpret and as such can be performed at side laboratory.[9,10] It is also less expensive and requires less skill. The qualitative method may therefore, be more feasible in resource poor centres.

METHODS

The present study was conducted in Department of Pediatrics, Bhandari Hospital and Research Centre, Indore, Madhya Pradesh, India**.**

From June 2006 to January 2008, neonates between the ages 0-28 days admitted to Bhandari Hospital and Research Center, Indore were included in the study. After taking a detailed history, a thorough clinical examination was done. The findings were recorded on proforma, specially designed for the study. Sepsis was suspected in the presence of clinical features like fever, respiratory distress, poor feeding, jaundice, hypothermia, convulsion, vomiting, irritability, lethargy and abdominal distension. Risk factors for sepsis included outborn delivery, perinatal asphyxia, and preterm delivery, prolonged rupture of membranes, maternal peripartum pyrexia and foul smelling amniotic fluid.

Infants of mothers who had intrapartum antibiotics within 1 week of delivery as well as babies with prior antibiotic therapy for present illness before admission were excluded from the study.

C-reactive protein was estimated qualitatively using the CRP latex kit. The specific performance characteristics of the CRP latex reagent was standardized to detect serum CRP levels at or above 6 mg/l, which is considered the lowest concentration of clinical significance. Half a milliliter of venous blood was collected in plain bottles and centrifuged. C-reactive protein was estimated using a drop of undiluted serum placed onto the circle of the agglutination slide with the use of disposable pipettes provided in the kit.

One drop of CRP latex reagent was added to the drop of serum and the broad end of the pipette was used to spread the latex reagent over the entire area of the test circle. The agglutination slide was gently tilted backwards and forwards approximately once every two seconds for two minutes. The results were read using the positive and negative controls as reference for agglutination. Visible agglutination of latex particles constituted a positive result which indicated a level of CRP > 6 mg/l while negative result was the reverse. After the results were read, the glass slide was rinsed with distilled water and air dried properly for re-use.

Blood culture was done for all neonates using two milliliter of venous blood collected from a peripheral vein after adequate skin preparation and before the commencement of antibiotics. The blood was aseptically introduced into aerobic and anaerobic culture media. The specimens were processed according to standard methods in the microbiology laboratory.[11] Inoculated blood culture media were considered negative if there was no growth after continuous incubation for up to 7 days, subcultures being made each day. Antibiotic sensitivity was done using Kirby-Bauer disc diffusion method.[11]

The results of laboratory investigations and other relevant data such as age, sex, birth weight and gestational age as well as symptoms present and risk factors for sepsis of recruited babies were recorded in a proforma. The results were analysed. The sensitivity, specificity, positive and negative predictive values of CRP were calculated.

RESULTS

Table 1: Sex wise distribution of the case studied.

Sex	Cases	Percentage
Male	31	62
Female	19	38
Total	50	100

The above table shows that 31, i.e. 62% of the total cases studied were male and 19, i.e. 38% were female.

Table 2: Relation of birth weight of neonatal sepsis.

Weight in gm	Cases	Percentage
1000-1500	14	28
1501-2000	14	28
> 2000	22	44
Total	50	100

The above shows that the incidence of neonatal sepsis is highest amongst neonates below 2000 gm that is 56% as compared to the incidence amongst these above 2000 gm weight which is 44%.

Table 3: Relation between periods of gestation to neonatal sepsis.

Period of gestation	Cases	Percentage
Less than 37 weeks	21	42
More than 37 weeks	29	58
Total	50	100

This table shows that the incidence of neonatal sepsis is highest in neonate more than 37 weeks of gestation than in the neonate less than 37 weeks, i.e. 58% and 42% respectively.

Table 4: Clinical features of neonatal sepsis.

Clinical feature	Cases	Percentage
Lethargy	36	72
Irritability	7	14
Poor acceptance of milk	48	96
Jaundice	9	18
Cyanosis	15	30
Vomiting	21	42
Loose motions	11	22
Abdominal distension	22	44
Convulsion	25	50
Apneic spells	20	40
Child doesn't look well	40	80
Hyperthermia	20	40
Hypothermia	7	14
Sclerema	0	0

This table shows that the commonest mode of presentation in the cases studied is poor acceptance of milk i.e. 96%, child does not look well is 80%, lethargy is 72%, convulsion 50% and less common feature in study was hypothermia, irritability 14%.

Table 5: Incidence of positive blood culture in the cases studied.

Total cases	Cases with positive culture	Percentage
50	31	62

The above table shows that 50 of the total cases studied, 31 cases, i.e. 62% shows positive blood culture.

Table 6: Microorganism grown in the positive blood culture.

Organisms	Cases	Percentage
Klebsiella	8	26.66
E. coli	6	20.00
Pseudomonas	4	13.33
S. aureus	5	16.66
Enterobacteria	5	16.66
S. viridans	3	10.00
Total	31	100.00

Table 6 depicts that klebsiella was that most common offending organism in this study constituting next in order is E. coli followed by staph aureus, pseudomonas and s. viridans.

Table 7: CRP values.

Gestation	No. of Cases	CRP positive	CRP negative	CRP range in µg/ml
< 37 weeks	21	14	7	12 to 24
> 37 weeks	29	20	9	6 to 12
Total	50	34	16	

Table 8: Association of risk factor with CRP levels and blood culture.

Risk factor	No. of Cases wit CRP positive	CRP (neonatal blood on admission		Blood C/S Positive
		< 6 µg/ml	> 12 µg/ml	
PROM				
< 12 hours	5	0	5	3
> 12 hours	15	6	9	8
> 24 hours	30	10	20	20
Labour > 12 hours	40	6	34	19
Maternal fever	5	-	5	5
Examination after PROM				
Foul smelling liquor	17	8	9	13
Meconium in liquor	5	-	5	5
Postpartum infection	7	3	4	5
Gestation < 37 weeks	21	7	14	22
Male neonate	31	11	20	23
Female neonat	19	5	14	8

Total 50 cases of suspected Neonatal sepsis CRP is Done out of which, less than 37 weeks gestation were 21were studied and out which 14 cases were found to positive in the range of 12 µg to 24 µg/ml and remaining 7 cases were found negative. In more than 37 weeks of gestation 29 cases of suspected Neonatal Sepsis study for CRP, out of which 20 were found to be positive in the range of 6 to 12 µgram / ml. and remaining 9 cases were found to be negative (Table 7).

Table 8 shows risk factors, which were associated with elevation of CRP. Positive blood C/S like PROM more than 12 hours, more than 24 hours maternal fever, number of per vaginal examinations more than 3 times, foul smelling discharge, meconium in liquor, postpartum metritis, gestational age less than 37 weeks and male infants.

Table 9 shows that the mortality is highest in neonates in the weight group 1000-2000 gm. Constituting 28% and 21% in weight group >2000 gm. The overall mortality is 14%.

Table 9: Relation of mortality rate with weight.

Weight in gm	Total number of cases	Death	Percentage
1000-1500	14	4	29
1500-2000	14	3	21
> 2000	22	0	0
Total	50	7	14

Table 10: The relation of mortality rate with gestational age.

Gestational age	Total number of cases	Death	Percentage
< 37 weeks	21	5	24
> 37 weeks	29	2	7
Total	50	7	14

This table shows that the mortality rate is higher in neonates with gestational age less than 37 weeks, i.e. 24% as compared to mortality rate in those with gestational period greater than 37 weeks, i.e. 7%.

Table 11: Screening test.

CRP	Blood culture	
	Positive	Negative
Positive	28 (a)	11 (b)
Negative	3 (c)	8 (d)

Sensitivity = a/(a+c) x 100 = 28/31 x 100 = 90.32%

Specificity = b/(b+d) x 100 = 8/19 x 100 = 42.10%

PPV = a/(a+b) x 100 = 28/39 x 100 = 71.79%

NPV = d/(c+d) x 100 = 8/11 x 100 = 72.72%

DISCUSSION

The incidence of neonatal sepsis is more in male than female. In the part to biological difference it is proposed to be due to genetic origin for the origin of the sex different in vulnerability to infection. The special source of vulnerability open to females by virtue of her possession of to 'X'-chromosomes in contrast to the single 'X' of the male. Hence it is genetic locus on the 'X' Chromosomes involve with synthesis of immunoglobulins responsible for the sex difference as per Thomas C. Washburn et al.[12]

As per study done by Hengst JM et al, RNC, MSN, ARNP, in 2003 Low-birth-weight (LBW) infants are at the highest risk for both early- and late-onset neonatal sepsis.[13] This is caused, in Dart by an immature inexperienced immune system; a fragile cutaneous barrier; and a prolonged hospital stay with increased exposure to the neonatal intensive care unit (NICU) environment, including various invasive devices and procedures. In the current study the incidence of Neonatal sepsis is More in Neonates weighing less than 2000 gms i.e. 56% as compare whose weight is > than 2000gms i.e. 44%. It is because of lung immaturity and lack specific and non-specific antibodies in the preterm neonates. Ratio of low birth weight <2000gms with >2000gms is 1.27: 1.

Among the clinical features of neonatal sepsis in the present study, CRP performance was highest in neonates with apnoea, vomiting and lethargy and lowest in those with hypothermia and convulsion. The lower levels in neonates with hypothermia and convulsion may be due to the fact that there are other commoner causes of neonatal hypothermia and convulsions other than sepsis.

Among the risk factors for neonatal sepsis, CRP performance was highest in neonates born to mothers with foul smelling amniotic fluid, followed by peri-partum pyrexia. This is similar to findings by Mathai et al. in Tamil, Nadu who reported a significant association between maternal peri-partum pyrexia and neonatal positive CRP levels.[14] Unlike the present study however, they found no significant association between foul smelling amniotic fluid and positive CRP levels.

The prevalence of blood culture proven sepsis in the present study was 62%. This is similar to the 41.7% reported by Chako and Sohi, 42% by Mustafa et al. and 47.5% by Roy et al.[15-17]

The commonest organism isolated was klebsiella pneumonia followed by staphylococcus areus. From 1974 to 1978, Omene et al in-Benin City found Escherichia coli to be the predominant bacterial isolate in neonatal sepsis. AntiaObong and Utsalo in Calabar reported Staphylococcus aureus as the predominant bacterial isolate from 1985 to 1987.[18,19] Ugochukwu et al also found Staphylococcus aureus as the predominant

bacterial isolate in neonatal sepsis in Nnewi Nigeria, from 1998 to 2001.[14] This shows a changing pattern of bacterial isolates over the years in the Southern region of Nigeria. Similar to the finding in the present study, Roy et al in India reported Klebsiellaspp as the predominant bacterial isolate in neonatal sepsis.[17]

Role of CRP in deciding antibiotics therapy

As per study done by Jaswal et al 20 in 44% of cases therapy was stopped on 3rd day, as CRP was normal. In 8% antibiotics could be stopped within 5-7 days as CRP values returned to normal and in 48% therapy was extended beyond 7th day, as CRP values were high or rising persistently. Negative predictive value of serial CRP was 100% in deciding duration of antibiotic therapy 'in suspected neonatal septicemia up to 7 days. The correlation between positive CRP, raised micro ESR and positive blood culture was significant.

Current study

Bacterial infection stimulates the hepatocytes to produce CRP: a nonspecific immune response, which is a useful clinical marker for the individual host-pathogen interaction. Since the half-life of CRP is less than 3 days, a rapid fall is seen with successful therapy.

There was no relapse in any group within 4 weeks of discontinuation of antibiotics (negative predictive value of 100%). The antibiotics were required for more than 10 days in all neonates with raised CRP and positive blood culture. Current study also showed similar observations.

CONCLUSION

CRP is one of the most widely available, most studied, and most used laboratory tests for neonatal bacterial infection and despite the continuing emergence of new infection markers it still plays a central role in the diagnosis of early onset sepsis of the neonate. CRP has the advantage of being well characterized in numerous studies and the extensive knowledge on its properties and limitations makes it safer compared to other, newer markers. Still, further research is needed on the topics of the influence of gestational age on CRP kinetics in infection, non-infectious confounders, and the evaluation of dynamic and gestational age dependent reference values.

The qualitative method of CRP estimation, which is a rapid, inexpensive and simple test to perform, was found to have moderate sensitivity, specificity and NPV of 92.0%, 43% and 72.0%, respectively. This implies that CRP would correctly identify close to three quarters of neonates with sepsis and would have 72.0% probability in excluding sepsis. The C-reactive protein may therefore, help in the early detection of neonatal sepsis while awaiting blood culture results. CRP may also be invaluable in the management of neonatal sepsis in resource poor centres where facilities for blood culture may not be readily available.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Fanaroff A, Stoll BJ, Wright LL, Carlo WA, Ehrenkranz RA, Stark AR. Trends in neonatal morbidity and mortality for very low birthweight infants. Am J Obstet Gynecol. 2007;196(2):147-8.
2. Murray BE. Can antibiotic resistance be controlled? New England J Med. 1994;330(17):1229-30.
3. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. New England J Med. 2003;348(2):138-50.
4. Russell JA. Management of sepsis. New England J Med. 2006;355(16):1699-713.
5. Chirico G, Loda C. Laboratory aid to the diagnosis and therapy of infection in the neonate. Pediatr Reports. 2011;3(1):1-5.
6. Mishra UK, Jacobs SE, Doyle LW, Garland SM. Newer approaches to the diagnosis of early onset neonatal sepsis. Arch Dis Childhood Fetal Neonatal. 2006;91(3):208-12.
7. Ng PC, Li K, Leung TF, Wong RPO, Li G, Chui KM, et al. Early prediction of sepsis induced disseminated intravascular coagulation with interleukin-10, interleukin-6, and RANTES in preterm infants. Clini Chem. 2006;52(6):1181-9.
8. Clyne B, Olshaker JS. The C - reactive protein. J Emerg Med. 1999;17:1019-25.
9. Deodhare SG. C-reactive protein: clinical applications, pathology. Update: Microbiol Clini Pathol Series. 2001.
10. Mustafa S, Farooqui S, Waheed S, Mahmook K. Evaluation of c-reactive protein as early indicator of blood culture positivity in neonates. Pak J Med Sci. 2005;21:69-73.
11. Standard operating procedure manual. Department of medical microbiology and parasitology. University of Port Harcourt teaching hospital. African educational services. Owerri. 2007:146-62.
12. Washburn TC, Medearis DN. Childs, sex difference in susceptibility to infection ped. Pediatr. 1965;35(1):57-64.
13. Hengst, RNC, MSN, ARNP. The Role of C-reactive, protein in the evaluation and management of infants with suspected sepsis. Adv Neonatal Care. 2003;3(1):3-13.
14. Mathai E, Christopher U, Mathai M, Jana AK, Rose D, Bergstrom S. Is C - reactive protein level useful in differentiating infected from uninfected neonates among those at risk of infection? Indian Pediatr. 2004;41:895-900.
15. Chako B, Sohi I. Early onset neonatal septicaemia. Indian J Pediatr. 2005;72:23-6.

16. Mustafa S, Farooqui S, Waheed S, Mahmook K. Evaluation of c-reactive protein as early indicator of blood culture positivity in neonates. Pak J Med Sci. 2005;21:69-73.
17. Roy I, Jain A, Kumar M, Agarwal SK. Bacteriology of neonatal septicemia in a tertiary hospital of northern India. Indian J Med Microbiol. 2002;20:156-9.
18. Omene JA. Neonatal septicaemia in benin city, nigeria: a review of 74 cases. Trop Geogr Med. 1979;31:35-9.
19. Obong OE, Utsalo SJ. Bacterial agents in neonatal septicaemia in Calabar, Nigeria. West Afr J Med. 1993;12:114-7.
20. Jaswal RS, Kaushal RK, Goel A, Pathania K. Role of C - reactive protein in deciding duration of antibiotic therapy in neonatal septicemia. Indian Pediatr. 2003;40:880-3.

Cite this article as: Mehrotra G. Study of C - reactive protein in neonatal sepsis. Int J Contemp Pediatr 2017;4:890-5.