ORIGINAL ARTICLE

Diagnostic Accuracy of CRP (C-Reactive Protein) Examination in Neonatal Infections

M. Sholeh Kosim, Bagus Ngurah Putu Arhana, Harry Mangunsong

(Department of Child Health, Medical School, Diponegoro University / Dr. Kariadi Hospital, Semarang)

Abstract. The incidence of neonatal infections has been still high in Indonesia. The main factors influencing success of treatment are early clinical diagnosis and rapid determination of the etiologic microorganisms. C-reactive protein (CRP) could be measured in the serum of baby suffering from bacterial infections. The aim of this study was to compare the capability of CRP examination in determining the diagnosis of neonatal infections, using blood culture examination as the gold standard. For comparison, white blood cell (WBC) and platelet counts, which have been used as indicators of neonatal infections, were also evaluated. This study was done prospectively on 30 cases with suspected neonatal infections admitted to the High Risk Baby Ward, Division of Perinatology, and 30 normal babies as control, during the period of April to December 1990. CRP examination was done by latex agglutination method. The results of this study revealed that CRP examination has good sensitivity, specificity, and predictive values. CRP was also proved to be superior to WBC and platelet counts. CRP examination was also easy to perform, cheaper, and faster than blood culture examination. It is concluded that CRP examination could be used as the confirmatory tool in the diagnosis of neonatal bacterial infections. **[Paediatr Indones 1933; 33:150-158]**

Introduction

Early diagnosis and prompt treatment are very important in overcoming health problems. This is particularly true in perinatal medicine. One of the most important problems in perinatology is neonatal infections, which are still common in Indonesia, with high morbidity and mortality rates.[1] The main factors influencing the success of treatment of neonatal infections are early clinical diagnosis and rapid determination of the etiologic bacteria with its antibiotic sensitivity. However, early diagnosis of neonatal infections is not easy because the signs and symptoms are usually not specific. WBC (white blood cell) and platelet counts, which have been used as the diagnostic tools for neonatal infections, frequently give doubtful result.[2,3] Blood culture examination, although provides almost 60% detection of neonatal infection cases, takes several days before the results are available.[4] For those reasons, alternative methods for diagnosing neonatal bacterial infections are needed. Serum C-reactive protein (CRP) examination has been used to support or confirm early diagnosis of neonatal bacterial infections. This diagnostic tool is sensitive, cheap, and easy to perform.[2,3,5] The purpose of this study is to compare the value of CRP examination (its sensitivity, specificity, and predictive values) as a diagnostic tool for determining neonatal infections, using blood culture test as the gold standard. In addition, similar evaluations were performed on WBC and platelet counts.

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Materials and Methods

This study was done prospectively during the period of April until December 1990 on 30 full- term babies with suspected neonatal infections admitted to the High Risk Baby Ward, Division of Perinatology, Karyadi Hospital, Semarang. Suspicion of neonatal infection was based on clinical findings.[1,6] Thirty full-term babies born spontaneously without any complication of pregnancy and delivery, notably no history of PROM (premature rupture of the membrane), served as controls. All cases of neonatal infection were subject to undergo 3 kinds of examination, i.e. WBC and platelet counts, blood culture, and CRP test. For normal babies only CRP examination was performed.

WBC and platelet counts were performed using standard procedures. Using normal values of our laboratory, we defined abnormal WBC counts ('positive WBC tests') when there was leukopenia (WBC<5.000/µL) or leukocytosis (WBC; 20.000/µL), while abnormal platelet count ('positive platelet count') was considered when the pletelets were <150.000/µL.

CRP measurement was done semi-quantitatively using latex agglutination method. The reagent used was "Rapi Tex CRP" (Behring Institute) with the dilution of 1/5, 1/10, 1/20, 1/40, 1/80, and 1/160. The procedure was as follows: Serum and reagent were placed at room temperature; serum was diluted with normal saline with dilution of 1/5, 1/10, etc. One drop of serum was placed on an object glass and one drop of reagent was added. They were then mixed slowly and carefully. After 2-3 minutes, evidence of agglutination reaction, which was categorized as positive reaction, was examined. The examination was repeated with subsequent dilution until a negative result was obtained. Equivalent values between positive result on dilution and CRP level by semi-quantitative examination were:

Positive agglutination (With dilution level)	CRP level (mg/L)
1/5	6
1/10	12
1/20	24
1/40	48
1/80	96
1/160+	192

We used 2 cutoff point for CRP results, i.e., 6 mg/L and 12 mg/L; in other words in the first analysis values of <6 mg/L were considered negative, while values of 6 mg/L or more were considered positive. In the second analysis, values of <12 mg/L were considered negative, while values of 12 mg/L or more were considered positive.

Data analysis

The difference of proportion of CRP results between suspected neonatal infection cases and normal control was analyzed using Fischer exact test; $p<0.05$ was used for statistical significance. Sensitivity, specificity, and predictive values of CRP and WBC and platelet counts were determined using the usual 2 x 2 tables. Results of blood culture were served as the gold standard; 95% confidence intervals, calculated with the help of True Epistat Program, were supplied where appropriate.

		Gold standard	
		Positive	Negative
Result	Positive	True Positive	False Positive
	Negative	False Negative	True Negative

Sensitivity = True positive/(True Positive + False Negative) x 100%, or the percentage of positive test in patients with positive blood culture.

Specificity = True Negative/(True Negative + False Positive) x 100%, or the percentage of negative test in patients with negative blood culture.

Positive predictive value = True Positive/ (True Positive + False Negative) x 100%, or the percentage of positive blood culture in patients with positive CRP.

Negative predictive value = True Negative/(True negative + False negative) x 100%, or the percentage of negative blood culture in patients with negative CRP.

Fig 1. Table for calculating sensitivity, spesificity, and predictive values

Results

During the period of April until December 1990, 30 cases of neonatal infections and 30 normal babies were studied. CRP test was positive in 20 cases (66,7%) of neona-

Table 1. Result of CRP measurement in neonatal infection cases and control group

	Result of CRP				
Group	Positive	%	Negative	%	Total
Neonatal infection	20	66.67	10	33.33	30
Control	2	6.67	28	93.33	30

$p < 0.05$

Table 2. Result of WBCand platelet count, CRP, and blood culture in 30 cases with suspected neonatal infection

Examination	Positive	Negative	Total
WBC (/µl)			
<5.000 or >20.000	3	4	7
5.000-20.000	15	8	23
Platelet (/µl)			
<150.000	2	4	6
>150.000	16	8	24
CRP, cutoff 6 mg/L			
> 6 mg/L	15	5	20
<6 mg/L	3	7	10
CRP, cutoff 12 mg/L			
CRP >12 mg/L	15	3	18
CRP <12 mg/L	3	9	12

Table 3. Sensitivity and specificity, and predictive values of WBC (<5,000 or >20,000 /μl) in the diagnosis of neonatal infections

		Blood culture		
		Positive	Negative	Total
WBC	Positive	3	4	7
	Negative	15	8	23
		18	12	30

Sensitivity = 3/18 (16.7%), CI 95% from 3.6% to 41.4%
Specificity = 8/12 (66.7%), CI 95% from 34.9% to 90.1%
Positive Predictive Value = 3/7 (42,9%), CI 95% from 9.9% to 81.6%
Negative Predictive Value = 8/23 (34.8%), CI 95% from 16.4% to 57.3%

Table 4. Sensitivity and specificity, and predictive values of platelet count (<150,000 /μl) in the diagnosis of neonatal infections

		Blood culture		
		Positive	Negative	Total
Platelet	Positive	2	4	6
	Negative	16	8	24
		18	12	30

Sensitivity = 2/18 (11.1%), CI 95% from 1.4% to 34.7%
Specificity = 8/12 (66.7%), CI 95% from 34,9% to 90.1%
Positive Predictive Value = 2/6 (33.4%), CI 95% from 4.3% to 77.7%
Negative Predictive Value = 8/24 (33.4%), CI 95% from 15.6% to 55.3%

Table 5. Sensitivity and specificity, and predictive values of CRP (>6 mg/L) in the diagnosis of neonatal infections

		Blood culture		
		Positive	Negative	Total
CRP	Positive	15	5	20
	Negative	3	7	10
		18	12	30

Sensitivity = 15/18 (83.3%), CI 95% from 58.6% to 96.4%
Specificity = 7/12 (58.3%), CI 95% from 27.7% to 84.8%
Positive Predictive Value = 15/20 (75%), CI 95% from 50.9% to 91.3%
Negative Predictive Value = 7/10 (70%), CI 95% from 34.8% to 93.3%,

Table 6. Sensitivity and specificity, and predictive values of CRP (>12 mg/L) in the diagnosis of neonatal infections

		Blood culture		
		Positive	Negative	Total
CRP	Positive	15	5	18
	Negative	3	9	12
		18	12	30

Sensitivity = 15/18 (83.3%), CI 95% from 58.6% to 96.4%
Specificity = 9/12 (75%), CI 95% from 42.8% to 94.5%
Positive Predictive Value = 15/18 (83.3%), CI 95% from 58.6% to 96.4%
Negative Predictive Value = 9/12 (75%), CI 95% from 42.8% to 94.5%

tal infections and in 2 (6.7%) normal babies (Table 1); this was statistically significant. Blood culture tests showed positive results in 18 (60%) of cases with suspected neonatal infections. Types of bacterial encountered were *Enterobacter* (27.8%), *Pseudomonas* (22.2%), *Escherichia coli* and *Streptococcus* (16.7% each), *Salmonella* (11.1%) and *Staphylococcus* (5.6%).

WBC and platelet counts showed that 7 (23.3%) of 30 cases with suspected infection had leukopenia or leukocytosis, and 6 (20%) of 30 cases had thrombocytopenia. With a cutoff point of 6 mg/l, CRP examination gave positive result in 20 (66.7%) cases, but using cutoff point of 12 mg/L, positive result was found in 18 (53.3%) of 30 cases. These results were summarized in Table 2.

The sensitivity, specificity, and predictive values of WBC and platelet counts and their corresponding 95% confidence intervals were depicted in Tables 3 and 4, respectively, while those values of CRP using cutoff points of 6 mg/L and 12 mg/L were presented in Tables 5 and 6, respectively. These tables revealed that both WBC and platelet counts have a low sensitivity, i.e., 16.7 and 11.1%, respectively. The specificity of both WBC and platelet counts was 66.7 %. On the other hand CRP (with 12 mg/L cutoff point) gave higher sensitivity and specificity, i.e. 83.3% and 58.3%, respectively. With a cutoff point of 6 mg/L, the sensitivity and specificity of CRP were 83.33% and 75%, respectively.

Tables 3 to 6 clearly showed that both positive and negative predictive values of CRP examination were superior than those of WBC and platelet counts. With WBC and platelet counts, both the positive and negative predictive values were consistently below 50%, while with CRP, either using the cutoff point of ;6 or ;12 mg/L , the positive and negative predictive values were more than 70%.

It should be noted, however, that most of the confidence intervals were very broad, certainly because the number of patients was too small.

Discussion

The diagnosis of neonatal infections is one of the most difficult problems in perinatal medicine, since early signs and symptoms of neonatal infections are not specific. On the other hand, failure or delay in treatment of neonatal infections is likely to result in a significantly high mortality and morbidity.[7] In sick newborns, antibiotics are commonly given routinely after birth or at any time when clinical deterioration occurs prior to infection. Concerned with this problem several previous studies were attempted and performed to make early diagnosis of neonatal sepsis or infections, including as neutrophil and band counts, buffy coat smears, erythrocyte sedimentation rate, immunoglobulin M, and CRP.[4,5,7-9] CRP is known to be produced by the fetus and has been found in high concentrations in the sera of newborn infants with variety of infections.[10,11] It is logical, therefore,

to assume that increased CRP level may be used as a diagnostic procedure of neonatal infections.

This study is proposed to compare the value of CRP test in neonatal infections with blood culture examination as the gold standard. Our results revealed that *Enterobacter* and *Pseudomonas* were the most common bacteria encountered. Kosnadi *et al.*[4] at the same place in 1982 found that *E.coli* was the most pathogenic bacteria. Faden[9] found the similar etiologic bacteria in Utah, and Kite *et al.*[8] found that the most common was coagulative streptococcus. Our study also showed that the sensitivity and specificity of WBC count were 16.7% and 66.7%, respectively, while Kite *et al.*[8] found 70.6% and 57.8%.Platelet count in our study had 11.1% sensitivity and 66.7% specificity. Squire *et al.*, cited by Aminullah,[1] mentioned that thrombocytopenia was found in their study in 40% of neonatal cases. With a cutoff point of 6 mg/L this study showed that CRP examination had a sensitivity of 83.3% and specificity of 58.3%, but with a cutoff point of 12 mg/L, the values were 83.3% and 75%, respectively. Philip *et al.*[5] found 47% sensitivity and 86% specificity, while Siebert *et al.*[7] found 67% sensitivity and 82% specificity in early infections, and 57% and 90% in late infections. Analysis of WBC and platelet counts and CRP in determining infection as represented in positive and negative predictive values showed that CRP has higher positive and negative predictive values; it means that CRP had lower false positive and false negative values.

The time needed for receiving the result of each examination varied. WBC and platelet counts, and CRP examination gave direct result, while blood culture result took 5-7 days. The cost per test in our laboratory was as follow: WBC and platelet counts = Rp. 2.500,-; CRP = Rp. 2.000,; Blood culture = Rp. 18.000,- These indicated that CRP examination is both quick and cheap.

Eventhough the increase of CRP level as one of indicators for diagnostic confirmation of neonatal infection is better than WBC and platelet counts, the combination of several tests is better and more suitable.[4,5,11,12]

Conclusions

Blood culture is still a standard procedure for the diagnosis of neonatal infection; however it usually takes several days before the results are available. Delay in diagnosis of neonatal infections may worsen the baby's condition, not only because it leads to the delay in giving prompt and proper treatment, but also it sometimes gives also other disadvantages. WBC and platelet counts, as seen in this study often give doubtful results. A semiquantitative agglutination method of CRP examination has a good sensitivity, specificity, and predictive values. In addition, it is cheap, easy to perform, and gives immediate result. For those reasons, CRP examination can be used as a confirmatory test in the diagnosis of clini-

cal neonatal sepsis; it has been proven in many studies as better than other diagnostic tests not including blood culture.

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