What are the Cut-Off Levels for IL-6 and CRP in Neonatal Sepsis?

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Neonatal sepsis, characterized by systemic signs of infection in the first month of life, remains an important clinical syndrome. Despite advances in neonatology, it has high rates of mortality and morbidity. The combine or alone usage of interleukin-6 (IL-6) and C-reactive protein (CRP) has recently been proven to be useful in the early diagnosis of sepsis in newborns. The study included 282 patients; there were 232 in Group I (170 proven and 62 clinical sepsis) and 50 in Group II (control group). The optimum cut-off value in the diagnosis of neonatal sepsis was found to be 24.65 pg/ml for IL-6 and 4.82 mg/l for CRP. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV)

of this IL-6 cut-off for neonatal sepsis were 72, 84, 95, and 42%, respectively. Sensitivity, specificity, PPV, and NPV of the CRP cut-off for neonatal sepsis were 67, 97, 99, and 39%, respectively. The combination of IL-6 (> 24.65 pg/ml) and CRP (> 4.82 mg/l) in the diagnosis of neonatal sepsis gave sensitivity, specificity, PPV, and NPV of 53, 100, 100, and 33%, respectively. To our knowledge, this is the largest reported study seeking to determine cut-off levels for IL-6 and CRP in the diagnosis of neonatal sepsis. In conclusion, we think that it is useful to evaluate IL-6 and CRP, in combination, for the early diagnosis of neonatal sepsis. *J. Clin. Lab. Anal.* 24:407–412, 2010. © 2010 Wiley-Liss, Inc.

Key words: neonatal sepsis; IL-6; CRP; cut-off value

INTRODUCTION

Neonatal sepsis, characterized by systemic signs of infection in the first month of life, remains an important clinical syndrome. Despite advances in neonatology, it has high rates of mortality and morbidity, especially in developing countries. Early diagnosis and adequate antibiotic treatment are required (1,2). Early diagnosis of sepsis is difficult for various reasons: firstly, early warning signs and symptoms are often protean and non-specific. Then there is difficulty of distinguishing the clinical picture of neonatal sepsis from non-infectious causes. Diagnosis is typically made by clinical and laboratory findings. The availability of a laboratory test to accurately and rapidly identify septic neonates would be great value in improving the outcome of these patients. Several interleukins, tumor necrosis factor (TNF), procalcitonin (PCT), C-reactive protein (CRP), immunoglobulins, and other markers have been used in the diagnosis of sepsis (3). Blood culture is the gold standard laboratory technique for the diagnosis of

infection, but culture results may take 48–72 hr. If there is a suspicion of sepsis, treatment should be started before culture results, because of its high mortality rate. False-negative culture results may also occur (4). On the other hand, if a sepsis diagnosis is made based on clinical signs and symptoms, without laboratory findings such as interleukin-6 (IL-6), CRP, or cultures, unnecessary treatment may be given. Infections and non-infectious diseases, such as malignancies and inflammatory disease, can cause CRP production (5). IL-6 is one of the proinflammatory cytokines, and is detected in serum in the early stages of infections (6). IL-6 has a role in the production of CRP and fibrinogen

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from the liver. Particularly in bacterial infections, IL-6 levels may be higher than CRP in early disease stages, and this may be helpful for early diagnosis (7). Early in infection, the CRP level may be low, but serial measurements can provide useful results and can be helpful in deciding when to discontinue antibiotic treatment (8). The combination of IL-6 and CRP has recently been proven to be useful in the early diagnosis of sepsis in newborns (9).

We sought to (1) investigate the value of IL-6 and CRP in establishing the early diagnosis of neonatal sepsis, and (2) determine the most appropriate cut-off value for each marker of neonatal sepsis, using receiver operating characteristic (ROC) curves, and by identifying the diagnostic sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each cut-off.

MATERIALS AND METHODS

Patients

In total, 282 newborns admitted to the neonatal intensive care unit at Zekai Tahir Burak Women's Health Education and Research Hospital were included. This retrospective study took place between January 2008 and December 2008, and was approved by the local ethics committee. Patients were grouped into an infection group, including clinical sepsis and proven sepsis patients (Group I), and a non-infection group (Group II).

Classification of Newborns

Clinical findings for a diagnosis of sepsis required at least three of the following: bradycardia ($<100/\text{min}$), tachycardia ($>200/\text{min}$), hypotension, hypotonia, seizures, apnea, tachypnea, cyanosis, respiratory distress, poor skin color and perfusion, feeding difficulty, irritability, lethargy, and laboratory results showing elevated levels of IL-6 or CRP (10).

Group Ia (Proven sepsis; 170 patients)

Newborns with positive blood cultures, clinical findings in agreement with the diagnosis, and elevated IL-6 and/or CRP levels during the clinical course.

Group Ib (Clinical sepsis; 62 patients)

Newborns with clinical findings of infection, plus a significant increase in IL-6 and/or CRP levels during the clinical course, but with negative blood cultures.

Group II (Control group; 50 patients)

IL-6 and CRP levels of newborns admitted to the hospital for perinatal risk factors such as ablation

placenta, Rh isoimmunization, transverse position in utero, or non-infectious diseases, such as hypoglycemia, intrauterine growth restriction, transient tachypnea, indirect hyperbilirubinemia, without clinical findings of infection, were used to define control levels. Infants in this group had normal physical examination findings, and were matched as far as possible in demographic characteristics to those in the proven and clinical sepsis groups.

Before antibiotic therapy, blood samples for culture were taken from patients with a diagnosis of sepsis. Urine and cerebrospinal fluid were cultured only when clinically indicated.

CRP Determinations

Serum concentrations of CRP were measured by a Tinaquant CRP (Latex) high-sensitive immunoturbidimetric assay on the Roche Modular P analyzer, according to the manufacturer's instructions (CRP latex HS, Roche kit; Roche Diagnostics, GmbH, D-68298 Mannheim, Germany).

IL-6 Determinations

Plasma levels of IL-6 were analyzed by IL-6 solid-phase, enzyme-labeled, chemiluminescent sequential immunometric assay on IMMULITE 1,000 analyzer, according to the manufacturer's instructions (Siemens Diagnostic Product Corporation, Los Angeles, CA).

Statistical Analyses

Statistical analyses were performed using the SPSS statistical package (v. 15.0). Categorical variables between groups were analyzed using the chi-squared test. Comparison of mean between two groups was examined using a *t*-test where the data fit a normal distribution, and the Mann-Whitney *U* test where the data was non-normal. For comparisons of more than two groups, ANOVA was used for normal distributions, and the Kruskal-Wallis test for non-normal distributions. ROC analysis was used to determine the power of variables to differentiate groups, and the area under the curve was calculated; significant cut-off levels were calculated using a Youden index. Logistic regression analysis was used to compare independent variables. A *P* value of <0.05 was deemed to indicate statistical significance.

RESULTS

The study included 282 patients; there were 232 in Group I and 50 in Group II. The characteristics of patients and their distribution within the groups are listed in Table 1. Gestational age, birth weight,

TABLE 1. Characteristics of Patients According to the Groups

	Group Ia (n = 170)	Group Ib (n = 62)	Group II (n = 50)
Male/female	87/82	38/24	30/20
Sepsis work-up day	14.3 ± 10.0	14.8 ± 10.2	3.9 ± 3.4
Birth weight (g)	1,580 ± 685	1,585 ± 718	1,735 ± 760
Gestational age (weeks)	30.6 ± 3.4 (23–41)	30.8 ± 3.6 (24–38)	31.7 ± 3.9 (25–42)
Vaginal delivery (%)	27.6	24.2	30.6
Age of mother (years)	26.8 ± 5.2	27.7 ± 6.7	28.4 ± 6.1

TABLE 2. Blood Culture Results

Microorganism	Number of patients
<i>Klebsiella pneumoniae</i>	44
<i>Staphylococcus epidermidis</i>	29
Fungus (<i>Candida albicans</i> , <i>Candida tropicalis</i>)	15
<i>Staphylococcus aureus</i>	10
<i>Staphylococcus hominis</i>	9
<i>Klebsiella oxytoca</i>	8
<i>Enterococcus faecium</i>	7
<i>Escherichia coli</i>	6
<i>Enterobacter cloacae</i> , <i>Staphylococcus haemolyticus</i>	5
<i>Staphylococcus warnerii</i> , <i>Enterococcus faecalis</i>	4
<i>C group streptococcus</i> , <i>Acinetobacter baumannii</i> , <i>Streptococcus sanguis</i> , <i>Staphylococcus capitis</i> , <i>Enterobacter species</i> , <i>Serratia marcescens</i> , <i>Pseudomonas aeruginosa</i>	2
<i>Streptococcus acidominimus</i> , coagulase negative staphylococcus	
<i>Staphylococcus saprophyticus</i> , <i>Streptococcus mitis</i> , <i>Streptococcus intermedius</i> , <i>Pantoea agglomerans</i> , <i>Stenotrophomonas maltophilia</i> , <i>Staphylococcus</i> <i>chromogenes</i>	1
Total	170

male/female ratio, and vaginal delivery rate were similar between groups and did not differ statistically. Work-up day was similar between proven and clinical sepsis groups, but was earlier for the control group, where the work-up was performed on the application day. In group I, 170 had proven sepsis and 62 had clinical sepsis. Blood culture results are listed in Table 2; 29 types of microorganism were isolated from blood cultures. The most frequently isolated microorganisms were *Klebsiella pneumoniae* (44 cases, 25.8%), *Staphylococcus epidermidis* (29, 17%), fungi (*Candida albicans* and *C. tropicalis*, 15, 8.8%), and *Staphylococcus aureus* (10, 5.8%).

Hemoglobin, white blood cell count, and platelet levels were evaluated, and there was no statistically significant difference between the groups ($P > 0.05$).

Levels of IL-6 and CRP in all groups are listed in Table 3. There was no difference in IL-6 or CRP levels ($P > 0.05$) between the proven sepsis and clinical sepsis groups. Between the sepsis groups and the control

group, there were significant differences for both IL-6 and CRP levels ($P < 0.05$).

The optimal cut-off values for IL-6 and CRP were identified by drawing ROC curves. ROC curves of proven and clinical sepsis versus control, and proven sepsis alone versus control are shown in Figure 1. Sensitivity, specificity, NPV, PPV, and cut-off levels are listed in Table 4. The optimum cut-off value in the diagnosis of neonatal sepsis was found to be 24.65 pg/ml for IL-6 and 4.82 mg/l for CRP. Sensitivity, specificity, PPV, and NPV of this IL-6 cut-off for neonatal sepsis were 72, 84, 95, and 42%, respectively. Sensitivity, specificity, PPV, and NPV of the CRP cut-off for neonatal sepsis were 67, 97, 99, and 39%, respectively. The combination of IL-6 (> 24.65 pg/ml) and CRP (> 4.82 mg/l) in the diagnosis of neonatal sepsis gave sensitivity, specificity, PPV, and NPV of 53, 100, 100, and 33%, respectively. For proven sepsis, the combination of IL-6 (> 21.5 pg/ml) and CRP (> 5.82 mg/l) had sensitivity, specificity, PPV, and NPV of 56, 100, 100, and 42%, respectively. According to their ROC curves, the combination of IL-6 and CRP was more helpful in the diagnosis of neonatal sepsis than was IL-6 or CRP alone.

DISCUSSION

This study of 170 septic neonates with positive blood cultures, 62 neonates with clinical sepsis, and 50 healthy neonates evaluated serum IL-6 and CRP in the early diagnosis of sepsis, and sought to establish the cut-off levels, sensitivity, specificity, PPV, and NPV. To our knowledge, this is the largest reported study seeking to determine cut-off levels for IL-6 and CRP in the diagnosis of neonatal sepsis. Study populations in the literature have previously ranged from 9 to 66 (10). The large size of the population is a strength of this study.

Neonatal sepsis is a major issue in neonatal intensive care units all over the world. Mortality, although declining, is still 10–20% (11). The increased survival rate of very low birth weight infants has also led to an increased incidence of sepsis.

Early diagnosis and treatment of neonatal sepsis are very important to prevent mortality and morbidity.

TABLE 3. IL-6 and CRP Results

	Group I (n = 232) Mean $\pm$ SD		Group II (n = 50) Mean $\pm$ SD	P
	Group Ia	Group Ib		
IL-6 (pg/ml)	349 $\pm$ 422	257 $\pm$ 358	35 $\pm$ 104	<0.05
CRP (mg/ml)	18.2 $\pm$ 14.5	13.6 $\pm$ 13.5	1.6 $\pm$ 2.6	<0.05

Fig. 1. (A) Shows ROC of proven and clinical sepsis versus control group; (B) shows proven sepsis versus control group on IL-6 levels.

TABLE 4. Cut-Off Levels, Sensitivity, Specificity, Negative Predictive Value, and Positive Predictive Values of IL-6 and CRP

		Cut-off level	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
IL-6	Group Ia versus Group II	21.5	75	82	92	52
	Group I versus Group II	24.65	72	84	95	42
CRP	Group Ia versus Group II	5.82	71	97	99	49
	Group I versus Group II	4.82	67	97	99	39

Clinical signs and symptoms are usually non-specific, and may be difficult to distinguish. Laboratory studies, such as white blood cell count, interleukins, and CRP, may be used to diagnose sepsis. Although blood culture remains the gold standard, it can have false-negative results; also, culture results are not available before 48 hr, and culture positivity rates range from 8 to 73% (12). When high mortality and serious morbidity of neonatal sepsis are taken into consideration, it is desirable for the ideal diagnostic marker to have about 100% sensitivity (infected infants have a positive test) and NPV (a negative test confidently rules out infection). To minimize the unnecessary use of antibiotics in false-positive cases, a competent diagnostic marker also needs to have reasonably high specificity (the test is negative if infection is absent) and a good PPV (infection is present when the test is positive), preferably better than 85% (13).

Acute phase reactants have been used to diagnose infections since the 1980s. IL-6 and CRP have been most commonly used. In the last 25 years, acute phase proteins, complement system components, chemokines, cytokines, adhesion molecules, cell surface markers, and combinations of these were investigated for the early and reliable diagnosis of the neonatal sepsis. Today, PCT, CRP, IL-6, IL-8, TNF- α , and some leukocyte surface antigens (CD11b and CD64) are the most hopeful markers among these. PCT, TNF- α , and IL-8 may also be useful in the detection of infection, but they are not yet routinely used worldwide. None is a perfect early indicator of infection. IL-6 is an important cytokine of the early host response to infection. Its concentration increases sharply after exposure to bacterial products, and precedes the increase in CRP. It has a very short half-life, and the concentration falls

precipitously with treatment, becoming undetectable in most infected patients within 24 hr. CRP is synthesized by the liver within 6–8 hr in an inflammatory response, peaks at 24–48 hr, and then diminishes over time as the inflammation resolves. We investigated IL-6 and CRP because our study is a retrospective study. But new studies with other markers to compare with IL-6 and CRP are necessary.

Some other studies have calculated cut-off levels for interleukins and CRP. Cut-off levels for IL-6 have been shown to be between 10 and 500 pg/ml, with most falling between 10 and 30 pg/ml (14). Onal et al. found a cut-off level for IL-6 at 20 pg/ml in 14 patients with proven sepsis and 20 patients with clinical sepsis, with 91% sensitivity, 74% specificity, 78% PPV, and 89% NPV (15). Ng et al. reported an IL-6 cut-off level at 31 pg/ml in 45 patients with proven sepsis, with 89% sensitivity, 96% specificity, 95% PPV, and 91% NPV (16). In the study by Ng et al., the patients were very low birth weight infants. Because of incomplete immune responses of these infants to infection, levels of IL-6 and CRP may have been lower at the beginning of their disease course, and higher levels may be seen through the infection. Our own patient population included both preterm and term infants. Cut-off levels for CRP were determined at 6–10 mg/l. Mathai et al. found a cut-off level for CRP at 6 mg/l, with sensitivity, specificity, PPV, and NPV of 60, 77, and 98.6%, respectively (17). Cord blood CRP levels were measured by Mathai et al. at 24 hr after birth. In our study, the sampling time was approximately 14 days. Benitz et al. gave a 10 mg/l CRP cut-off, with sensitivity, specificity, PPV, and NPV of 78, 78, 67, and 97%, respectively (18). In one study, cut-off levels for IL-6 and CRP were 18.2 pg/ml and 4 mg/l, respectively, in a population of 11 sepsis, 6 clinical sepsis, and 31 control patients. For IL-6, they found 87% sensitivity and 50% specificity. The CRP cut-off gave 95.7% sensitivity, 88.9% specificity, 78.6% PPV, and 98% NPV (3). In our study, cut-off levels for IL-6 were 21.5 pg/ml for proven sepsis and 24.65 pg/ml for both proven and clinical sepsis. Cut-off levels for CRP were 5.82 mg/l for proven sepsis and 4.82 mg/l for proven and clinical sepsis. In previous studies, cut-off levels of both IL-6 and CRP were given in large intervals. Because of our study population is the biggest one in literature, we calculated the cut-off levels in low-ranking interval with adequate sensitivity, specificity, PPV, and NPV. Our results for IL-6 were 75% sensitivity, 82% specificity, 92% PPV, and 52% NPV for proven sepsis. CRP results for proven sepsis were 71% sensitivity, 97% specificity, 99% PPV, and 49% NPV. The combination of IL-6 and CRP may lead to rapid and correct diagnosis of neonatal sepsis. IL-6 (>70 pg/ml) and CRP (>10 mg/l), found in combination, has been suggested to give

sensitivity, specificity, PPV, and NPV of 91, 74, 43, and 98%, respectively (10). We suggest the combination of IL-6 (>24.65 pg/ml) and CRP (>5.82 mg/l) for the rapid and accurate diagnosis of neonatal sepsis because of high sensitivity, specificity, and PPV. This combination is superior to the use of IL-6 or CRP alone.

Sampling time is another important issue in diagnosis. IL-6 levels increase before those of CRP; when IL-6 is high, CRP level may be low, or high CRP levels may be found with low IL-6 levels, after the second or third day of response. These differing peak times mean that serial measurements of more than one acute phase reactant may be indicated for diagnosis.

Neonatal sepsis remains one of the most important clinical syndromes, leading to high morbidity and mortality, despite advances in neonatology. Although early diagnosis and treatment is needed, its non-specific clinical symptoms and signs, together with difficulties and limitations of culturing procedures, can lead to wasted time before treatment of septic patients, as well as to unnecessary treatment of others. Laboratory studies with appropriate cut-off levels, sensitivity, specificity, PPV, and NPV for each test should help us to diagnose neonatal sepsis early and to guide the appropriate timing of treatment. In conclusion, we think that it is useful to evaluate IL-6 and CRP, in combination, for the early diagnosis of neonatal sepsis.

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