Role of procalcitonin, C-reactive protein, interleukin-6, interleukin-8 and tumor necrosis factor-α in the diagnosis of neonatal sepsis

Emine Kocabaş[1], Aysun Sarıkçıoğlu[1], Necmi Aksaray[1], Gülşah Seydaoğlu[2]
Yalçın Seyhun[3], Akgün Yaman[4]

Departments of [1]Pediatrics, [2]Biostatistics, and [4]Microbiology, Çukurova University Faculty of Medicine, Adana, and [3]Department of Medical Biology, Istanbul University Istanbul Faculty of Medicine, Istanbul, Turkey

SUMMARY: Kocabaş E, Sarıkçıoğlu A, Aksaray N, Seydaoğlu G, Seyhun Y, Yaman A. Role of procalcitonin, C-reactive protein, interleukin-6, interleukin-8 and tumor necrosis factor-α in the diagnosis of neonatal sepsis. Turk J Pediatr 2007; 49: 7-20.

Diagnosis of neonatal sepsis may be difficult because clinical presentations are often nonspecific, bacterial cultures are time-consuming and other laboratory tests lack sensitivity and specificity. In this study, we aimed to investigate the role of procalcitonin (PCT), C-reactive protein (CRP), interleukin (IL)-6, IL-8 and tumor necrosis factor-alpha (TNF-α) in establishing the diagnosis and evaluating the prognosis of neonatal sepsis. Twenty-six neonates with blood-culture positivity and clinical sepsis, hospitalized for clinical suspicion of neonatal sepsis in neonatal intensive care units of Balcalı Hospital, Çukurova University and Adana State Hospital between May 2000 and January 2001 (Group I) and 29 healthy neonates followed at the neonatal units and outpatient clinics of these hospitals (Group II) in the same period were studied. Among the septic neonates, 13 had early-onset (Group Ia) and 13 had late-onset (Group Ib) neonatal sepsis, while 14 of the healthy neonates had perinatal risk factors (Group IIa) and 15 of them had no risk factors (Group IIb). The demographic and clinical characteristics of the septic and healthy neonates were recorded, blood samples for determining serum PCT, CRP, IL-6, IL-8 and TNF-α were collected from the healthy and the septic neonates before starting treatment, and these investigations were repeated on the 3rd and 7th days of treatment. In this study, it was found that: (a) pre-treatment mean serum PCT, CRP, IL-6, IL-8 and TNF-α levels were significantly higher in the septic neonates than in the healthy ones, (b) compared with the pre-treatment values, serum PCT, IL-6 and TNF-α had progressively decreased on the 3rd and 7th days of the treatment in the 17 recovered patients, though they progressively increased in nine patients who died during treatment, (c) the area under the receiver operating characteristic (ROC) curve (AUC) for PCT, TNF-α, IL-6, CRP, and IL-8 were 1.00, 1.00, 0.97, 0.90 and 0.68, respectively. For the cut-off value of PCT ≥0.34 ng/ml, the test was found to have a sensitivity of 100%, specificity of 96.5%, positive predictive value of 96.2%, negative predictive value of 100% and diagnostic efficacy of 98.3% for bacterial sepsis in neonates. For the cut-off value of TNF-α ≥7.5 pg/ml, sensitivity, specificity, positive predictive value, negative predictive value and diagnostic efficacy were found to be 100%, 96.6%, 96.2%, 96.5% and 98.3%, respectively. It was detected that sensitivity, specificity and diagnostic efficacy values were lower for IL-6, CRP and IL-8. We conclude that PCT and TNF-α are the best markers in the diagnosis of neonatal sepsis, and these markers are also valuable in following the effectiveness of treatment and determining the prognosis of the disease.

Key words: neonatal sepsis, procalcitonin, C-reactive protein, interleukins, cytokines.

Neonatal sepsis is a clinical syndrome characterized by systemic signs of infection, accompanied by bacteriemia in the first month of life. Two patterns of disease, early- (<7 days of birth) and late-onset (>7 days) have been associated with neonatal sepsis[1]. Despite major advances in neonatology in the past few decades, bacterial sepsis is still one of the most important causes of morbidity and mortality in this age group worldwide, especially in

developing countries[2]. Early detection of bacterial sepsis is difficult for various reasons: firstly, early warning signs and symptoms are often protean and non-specific. Then there is the difficulty of distinguishing the clinical picture of neonatal sepsis from non-infectious causes. Further, microbiological culture results are not usually available until at least 48-72 hours after the specimen reaches the laboratory, and high false-negative rates of culture results may occur[3]. The availability of a laboratory test to accurately and rapidly identify septic neonates would be of great value in improving the outcome of these patients. Equally difficult is the exclusion of infection in infants with suspected sepsis. The initiation of antibiotic treatment by physicians for all of the patients with sepsis suspicion, since the risk of mortality is very high in non-treated or inappropriately treated cases, leads to unnecessary intravenous antibiotics being administered in the case of 11-23 noninfected newborns for every one with proven sepsis[4,5]. Early detection of the absence of infection would decrease the number of children started on antibiotics, shorten the length of hospital stay, and lessen the treatment costs and potential for emergence of resistant organisms.

Several hematological tests [total leukocyte count, total neutrophil count, immature neutrophil count, immature/total neutrophil (I/T) ratio and morphological and degenerative changes in neutrophils] were used for the early and reliable diagnosis of neonatal sepsis in the early and mid 1980's. The non-specific nature of these tests has directed investigators towards finding more specific and earlier increasing infection markers. 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 neonatal sepsis. Today, procalcitonin (PCT), C-reactive protein (CRP), interleukin (IL)-6, IL-8, tumor necrosis factor-alpha (TNF-α), and some leukocyte surface antigens (CD11b, CD64) are the most hopeful markers amongst these[6]. In most of the studies, a positive correlation has been shown between these markers and neonatal sepsis. However, quite contradictory results have been reported because of the investigation of patients with different gestational ages and birth weights, use of different sepsis descriptions, study of only small sample groups and variability in the characteristics of the control groups.

In this study, we aimed to: (1) investigate the value of PCT, CRP, IL-6, IL-8 and TNF-α in establishing the early diagnosis of early-onset and late-onset neonatal sepsis; (2) determine the most appropriate cut-off value of each marker in detecting neonatal sepsis by using receiver operating characteristics (ROC) curves and identifying the diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), efficiency and area under ROC curve (AUC) of these markers according to those cut-off levels; and (3) follow the alterations in plasma concentrations by serial measurements of these markers during treatment and therefore investigate their roles in determining the efficacy of treatment and the prognosis of the disease.

Material and Methods

In this study, all consecutive neonates admitted with a suspected clinical sepsis and hospitalized in the neonatal intensive care units at Balcalı Hospital, Çukurova University and Adana State Hospital, all consecutive healthy neonates who had no signs of infection but had been hospitalized for their perinatal risk factors in the neonatal units of these hospitals, and all consecutive healthy neonates without infectious risk factors admitted to the well-baby outpatient clinics of these hospitals were prospectively enrolled between May 2000 and January 2001. Newborns that had been started on antibiotic treatment earlier and had a history of maternal antibiotic administration were excluded from the study. Variables of each case recorded at baseline and during follow-up included newborns' demographics, antenatal, prenatal and postnatal history, physical examination findings, principal diagnosis, vital signs, routine blood tests, microbiologic culture results and applied therapies. Samples for hematological tests (complete blood count, WBC differentiation-leukocyte formula, thrombocyte count) from healthy newborns, and before starting the antimicrobial therapy, whole blood count, urine examination, sedimentation rate, CRP, biochemical tests including blood glucose, hepatic and renal function tests, arterial blood

gases, chest and abdominal X-ray images and cultures (blood, throat, urine, cerebrospinal fluid-CSF and tracheal aspiration etc.) from neonates with suspected sepsis were obtained. In addition, blood samples for CRP, PCT, IL-6, IL-8 and TNF-α were obtained from healthy neonates and from neonates with suspected sepsis before starting the antimicrobial therapy (Day 0). While blood samples for sepsis markers were reobtained only from neonates with positive blood cultures (patient group) on the 3rd (Day 3) and 7th days (Day 7) of treatment, for ethical reasons, they were taken only once from the healthy neonates (control group) in the neonatal unit or the well-baby outpatient clinics. Written consent was obtained from the families of all the investigated neonates, and the study protocol was approved by the ethical committee of the Faculty of Medicine, Çukurova University.

Patient group (Group I): Of a total of 157 premature and term newborns hospitalized in the neonatal intensive care units at Balcalı Hospital, Çukurova University and Adana State Hospital between May 2000 and January 2001, 53 had been diagnosed as suspected clinical sepsis based on their Hematological Score and Töllner Score results (hematological score ≥3 and Töllner Score ≥10)[7,8]. Eight of them were excluded from the study since either their mothers had used antibiotics or the newborn had been given antibiotics before, and four were excluded because permission was not obtained from one or both of the parents. Thus, 41 neonates with suspected sepsis were included in the study. Before starting the antimicrobial therapy, laboratory tests were performed and blood samples were taken for sepsis markers from these 41 neonates with suspected sepsis. Blood cultures were found to be positive in the first 48 hours of treatment in 26 of those neonates that were started on antibiotic treatment. These 26 patients who had positive blood cultures and clinical findings of sepsis (change in skin color, peripheral circulation impairment, hypotonia, bradycardia, respiratory distress, hepatomegaly, leukocytosis/leukopenia, left shift, thrombocytopenia, metabolic acidosis) were finally investigated as a patient group (Group I) in the study. Second and third blood samples for sepsis markers were obtained from these patients on the 3rd (Day 3) and 7th days (Day 7) of treatment. Nine of the 26 investigated patients died during the treatment period. Five of the patients with early-onset neonatal sepsis died in the first 48 hours of treatment, so blood samples from those patients could be obtained only once before the treatment and once before death. However, four of the patients with late-onset neonatal sepsis also died, but since these deaths occurred after the 7th day of treatment, blood samples could be obtained before treatment and on the 3rd and 7th days of the treatment. The patient group was evaluated in two subgroups according to their birth ages: (a). Early-onset neonatal sepsis (Group Ia): 13 septic neonates whose birth ages were between 0-5 days, (b). Late-onset neonatal sepsis (Group Ib): 13 septic neonates whose birth ages were between 5-30 days.

Control group (Group II): Healthy neonates who were brought to the well-baby outpatient clinics for check-ups, and healthy neonates who were born and followed for 0–5 days in neonatal units because of their perinatal risk factors, who were not supposed to be clinically septic and who had normal physical examination findings, hematological tests and CRP results (CRP ≤6 mg/L) were investigated as the control group (Group II). The control group was evaluated in two subgroups according to whether or not perinatal risk factors were present: (a) Healthy neonates with perinatal risk factors (Group IIa): 14 neonates with risk factors such as prematurity, diabetic mother, mother with systemic lupus erythematosus, anoxic delivery, and fetal distress, and (b) Healthy neonates with no perinatal risk factors (Group IIb): 15 healthy neonates without risk factors.

Laboratory analyses: Complete blood counts were carried out with an automatic counter (Medonic CA 530 Thor Model Coulter-Counter) in the patient and control groups. By examining peripheral blood smears prepared with Giemsa stain, band forms, myelocytes and metamyelocytes in leukocyte formula evaluated as immature neutrophils and immature/total neutrophils (I/T) ratio was calculated. I/T neutrophil ratios greater then 0.2 were evaluated as pathologic[9].

Before starting the antimicrobial treatment, blood cultures, and urine, CSF and tracheal aspiration cultures (TAC), when needed,

were obtained from all of the neonates with suspected sepsis. Cultures were examined using the BACTEC 9240 (Becton Dickinson) automation system. Microbiological sorting of growing microorganisms was made by SPECTOR (Becton Dickinson) equipment. For urine culture, urine samples were planted in bloody and eosin-methylene blue (EMB) agars and incubated for 24–48 hours under aerobic conditions. Identification and antibiograms of growing microorganisms were evaluated with Vitek-2 Systems (BioMérieux) equipment.

Blood samples that were obtained from the control group and from neonates with suspected sepsis before treatment were centrifuged at 2500 cycles for 15 mins and the serum part was preserved by freezing it at –20°C for PCT and cytokine (IL-6, IL-8, TNF-α) studies, while CRP investigation was made immediately. This process was repeated on the 3rd and 7th days of treatment of the patient group. CRP level measurement was studied by the latex immunonephelometric method (BNA analyser, Behring-Werke AG, Marburg, Germany). CRP was measured twice, once before the treatment period and once on the 7th day of the treatment. CRP levels ≤6 mg/L were accepted as normal. PCT levels were measured by the immunoluminometric method with Auto-Clini Lumat LB 952 equipment using Lumitest-®PCT kits (Brahms-Diagnostica, Berlin, Germany). The lowest detection limit of this test is at the level of 0.08 ng/ml[10]. IL-6 and IL-8 levels were measured by the ELISA (enzyme linked immunosorbent assay) method using cytoscreen immunoassay kits (Pierce Endogen, USA). TNF-α levels were measured by the solid phase sandwich ELISA method using immunoassay kit (Biosource International, USA)[11]. All of the laboratory analyses in this study were carried out in the central laboratory of Balcali Hospital, Çukurova University.

Statistical analyses: Statistical analyses were performed using the statistical package SPSS v 10.0. For continuous variables, normality was checked. Since the data were not distributed normally, an appropriate non-parametric test was chosen. Comparisons of continuous variables between groups were applied using the Mann-Whitney U test. Time-dependent within group data were analyzed by the Wilcoxon rank sum test. Categorical variables between groups were analyzed using the chi-square test. A ROC curve was constructed to determine the optimal laboratory diagnostic parameters (PCT, CRP, IL-6, IL-8, TNF-α) in predicting a newborn with sepsis. A ROC curve displayed the false-positive rate on the x axis (specificity), and the true-positive rate on the y axis (sensitivity) for varying test cut-off values, thus plotting the performance of a diagnostic test[12]. The ideal cut-off criteria for the laboratory results were chosen by determining the point lying geometrically closest to an ideal test with 100% specificity and sensitivity[13]. Diagnostic efficiency was defined as (sensitivity+specificity)/2. Results were presented as number (n), percent (%), mean, standard deviation (SD), median, and minimum-maximum (min-max). A p value <0.05 was considered as significant.

Results

In this study, 26 neonates with positive blood cultures and clinical sepsis (Group I) and 29 healthy neonates (Group II) were investigated. The patient group was evaluated in two subgroups as early-onset (Group Ia) (n=13) and late-onset neonatal sepsis (Group Ib) (n=13). The control group consisting of healthy neonates was also evaluated in two subgroups: healthy neonates with perinatal risk factors (Group IIa) (n=14) and healthy neonates with no perinatal risk factors (Group IIb) (n=15).

Blood culture was positive for all patients. The identified bacteria included Klebsiella pneumoniae (n=11), Escherichia coli (n=5), Staphylococcus epidermidis (n=5), Pseudomonas aeruginosa (n=2), S. aureus (n=1), S. warneri (n=1) and Acinetobacter baumannii (n=1). In addition to the blood cultures, positive urine cultures in six of the patients (all E. coli), positive CSF cultures in two of them (1 K. pneumoniae, 1 S. pneumoniae) and positive tracheal aspiration cultures in nine of them (7 K. pneumoniae, 2 P. aeruginosa) were found. One or more perinatal risk factors were detected in healthy neonates with risk factors (Group IIa): prematurity (n=6), respiratory distress syndrome (n=4), preeclamptic mother (n=4), eclamptic mother (n=2), transient tachypnea of newborn (n=2), mother with placenta previa (n=1), diabetic mother (n=1), mother with systemic lupus erythematosus (n=1) and low Apgar score (n=1).

The demographic data of the patient and control groups are shown in Table I. There was no significant difference in mean gestational age, age, weight, gender and premature/term born newborns ratio between the investigated patient (n=26) and control (n= 29) groups (p>0.05). The baseline (Day 0) laboratory values of the patient and control groups are shown in Table II. We found that mean serum PCT, IL-6, IL-8, TNF-α and CRP levels were significantly higher in the patient group compared with the control group (p<0.001, p<0.001, p<0.05, p<0.001 and p<0.001, respectively). When newborns with early-onset neonatal sepsis (age range: 1-4 days) (Group Ia) were compared with newborns with late-onset neonatal sepsis (age range: 8-30 days) (Group Ib), mean serum IL-6 values were found to be lower (X±SD: 16.5±15.5 pg/ml) in newborns with early-onset neonatal sepsis (p<0.05), while no significant difference was detected in the mean gestational age, weight, gender, premature/term born newborns ratio and serum PCT, IL-8, TNF-α and CRP values (Tables I, II). When healthy neonates carrying perinatal risk factors (age range: 1-4 days) (Group IIa) were compared with healthy neonates who had no risk factors (age range: 5-29 days) (Group IIb), it was found that in neonates with risk factors, mean gestational age and weight were lower than those of the neonates without risk factors (p<0.01 and p<0.001, respectively), while male/female ratio was higher (p<0.05). No significant difference was found in mean serum PCT, CRP, IL-8 and TNF-α values between the two groups, but serum mean IL-6 levels (3.4±2.9 pg/ml) were found higher in newborns with risk factors (p<0.05).

Serum values of CRP, PCT, IL-6, IL-8 and TNF-α before beginning treatment (Day 0) and on the 3rd (Day 3) and 7th days (Day 7) of treatment are shown in Table III. The values of nine septic newborns who died during the treatment period and of 17 septic newborns who recovered are given separately in this Table. Serum CRP measurements could only be studied before treatment and on the 7th day of treatment. In septic neonates who recovered with treatment, it was detected that serum median values of CRP, PCT, IL-6, IL-8 and TNF-α progressively decreased on the 3rd and 7th days of treatment when compared with pretreatment values and this decrease was statistically significant, while low CRP values on the 7th day of treatment were not at a significant level (p=0.8). In septic neonates who died during the treatment period, it was observed that, when compared with pretreatment values, serum median PCT, IL-6, TNF-α and CRP values had been progressively rising, and this rise was of a significant dimension, while the increase in mean serum IL-8 levels was of limited significance (p=0.066). When pretreatment serum median PCT, IL-6, IL-8, TNF-α and CRP levels of septic neonates who recovered during the treatment period were compared with those of the septic neonates who died during the treatment period, it was detected that serum median PCT, IL-6 and TNF-α levels were significantly higher in the recovered patients, while no significant difference was found in serum median IL-8 and CRP levels between the two groups.

The optimal cut-off values were identified by drawing ROC curves of tests (CRP, PCT, IL-6, IL-8, TNF-α) that were investigated in distinguishing septic neonates from the healthy ones (Fig. 1). The sensitivity, specificity, PPV, NPV, diagnostic efficiency and the AUC of the tests for the optimal cut-off values selected were identified using ROC analysis for Day 0 (Table IV). The optimum cut-off value was found to be 10 mg/L for CRP, 0.34 ng/ml for PCT, 3.6 pg/ml for IL-6, 0.65 pg/ml for IL-8 and 7.5 pg/ml for TNF-α in the diagnosis of neonatal sepsis. For these cut-off values, AUC values were found to be 0.90 for CRP (95%CI; 0.81-1), 1.00 for PCT (1-1), 0.98 for IL-6 (0.94-0.99), 0.69 for IL-8 (0.54-0.82), and 1.00 for TNF-α (0.99-1) (Table IV). At a cut-off of 0.34 ng/ml, PCT was found to have a sensitivity of 100%, specificity of 96.6%, PPV of 96.2%, NPV of 100% and diagnostic efficacy of 98.3% for bacterial sepsis in neonates. For the cut-off value of TNF-α of 7.5 pg/ml, sensitivity, specificity, PPV, NPV and diagnostic efficacy of the test were found to be 100%, 96.6%, 96.2%, 96.5% and 98.3%, respectively. It was detected that diagnostic efficacy values were lower for IL-6, CRP and IL-8 (Table IV).

Discussion

In this study, in which 26 septic neonates with positive blood culture and 29 healthy neonates were investigated to evaluate the

Table I. Demographic Data of the Patient and Control Groups

	Septic neonates			Healthy neonates		
Demographic data	Early-onset sepsis (n:13) Mean±SS Median (Min-Max)	Late-onset sepsis (n:13) Mean±SS Median (Min-Max)	All patients (n:26) Mean±SS Median (Min-Max)	With risk factor (n:14) Mean±SS Median (Min-Max)	Without risk factor (n:15) Mean±SS Median (Min-Max)	All controls (n:29) Mean±SS Median (Min-Max)
Gestational age (week)	34.64±4.32 34.0 (28-40)	37.07±3.63 38.0 (28-39)	35.8±4.10 38.0 (28-40)	35.8±2.7 36.5 (31-40)	38.7± 0.9♥♥ 39.0 (37-40)	37.3±2.4 38.0 (31-40)
Age (day)	1.9±1.1♣♣♣ 2.0 (1-4)	20.61±8.42ϕ 22.0 (8-30)	11.2±11.2 6.0 (1-30)	1.9±1.14 1.5 (1-4)	12.7±6.0♥♥♥ 11.0 (5-29)	7.5±7.0 5.0 (1-29)
Weight (g)	2719.2±1172.3 2450.0 (1300-4700)	3156.9±1103.1 3020.0 (1660-5400)	2938.1±1137.3 2860 (1300-5400)	2627.7±630.3 2650 (1698-3600)	3573.3±612.3♥♥♥ 3400 (2800-5100)	3116.8±776.6 3200.0 (1698-5100)
Sex (M/F)#	9/4	7/6	16/10	11/3	5/10♥	16/13
Premature/term#	7/6	3/10	10/16	6/8	2/13	8/21

data was expressed as number.
α : Comparison between all septic and all healthy neonates.
♣ : Comparison between neonates with early-versus late-onset sepsis.
♥ : Comparison between healthy neonates with versus without risk factor.
* : Comparison between neonates with early-onset sepsis and healthy neonates with risk factor.
ϕ : Comparison between neonates with late-onset sepsis and healthy neonates without risk factor.
(α, ♣, ♥, *, ϕ): p< 0.05, (α α, ♣♣, ♥♥, **, ϕ ϕ): p< 0.01, (α α α, ♣♣♣, ♥♥♥, ***, ϕ ϕ ϕ): p<0.001.

Table II. Laboratory Values of the Patient and Control Groups

	Septic neonates			Healthy neonates		
Laboratory values	Early-onset sepsis (n:13) Mean±SS Median (Min-Max)	Late-onset sepsis (n:13) Mean±SS Median (Min-Max)	All patients (n:26) Mean±SS Median (Min-Max)	With risk factor (n:14) Mean±SS Median (Min-Max)	Without risk factor (n:15) Mean±SS Median (Min-Max)	All controls (n:29) Mean±SS Median (Min-Max)
PCT (ng/ml)	18.7±29.1 4.11 (0.39-88.96)	19.4±21.8ϕϕϕ 10.38 (1.78-58.38)	19.0±25.2ααα 4.7 (0.39-88.9)	0.18±0.1 0.16 (0.08-0.39)	0.14±0.04 0.16 (0.08-0.2)	0.16±0.66 0.16 (0.08-0.39)
IL-6 (pg/ml)	16.5±15.5♣ 12.80 (3.08-54.50)	29.4±22.5ϕϕϕ 14.0 (9.20-77.80)	22.9±20.0ααα 13.3 (3.08-77.8)	3.4±2.9 2.9 (0.50-9.8)	1.4±0.95♥ 0.9 (0.50-3.5)	2.37±2.32 1.80 (0.50-9.8)
IL-8 (pg/ml)	0.42±0.36 0.28 (0.01-1.41)	1.69±1.63ϕϕ 0.82 (0.18-5.0)	1.05±1.32α 0.38 (0.01-5)	0.35±0.22 0.29 (0.13-0.87)	0.30±0.23 0.25 (0.05-0.87)	0.33±0.22 0.26 (0.05-0.87)
TNF-α (pg/ml)	451.6±903.7 180.0 (10-3364.0)	747.6±1020.2ϕϕϕ 208.0 (39.60-2964.0)	599.6±956.2ααα 180.5 (10-3364)	1.5±2.5 0.9 (0.30-10.10)	1.58±1.63 0.9 (0.50-5.20)	1.53±2.05 0.90 (0.30-10.10)
CRP (mg/L)	28.8±2.2 16.0 (0.0-96.0)	49.4±38.0ϕϕϕ 45.0 (0.0-128.0)	39.1±36.0ααα 24.0 (0-128)	1.7±2.8 0.0 (0.0-6.0)	1.2±2.48 0.0 (0.0-6.0)	1.45±2.61 0.0 (0.0-6.0)

data was expressed as number.
α : Comparison between all septic and all healthy neonates.
♣ : Comparison between neonates with early- versus late-onset sepsis.
♥ : Comparison between healthy neonates with versus without risk factor.
* : Comparison between neonates with early-onset sepsis and healthy neonates with risk factor.
ϕ : Comparison between neonates with late-onset sepsis and healthy neonates without risk factor.
(α, ♣, ♥, *, ϕ): $p<0.05$, (α α, ♣♣, ♥♥, **, ϕ ϕ): $p< 0.01$, (α α α, ♣♣♣, ♥♥♥, ***, ϕ ϕ ϕ): $p<0.001$.

Table III. Serum PCT, IL-6, IL-8, TNF-α, and CRP Values of Recovered and Exitus Patients Determined Before (Day 0) and During (Days 3 and 7) Treatment

		Day 0	Day 3		Day 7	
		Median (Min-Max)	Median (Min-Max)	$^{\&}p_1$ value	Median (Min-Max)	$^{\&}p_2$ value
PCT (ng/ml)	Alive	9.8 (2.3-88.96)	1.3 (0.25-92.10)	0.019	0.7 (0.25-69.0)	0.002
	Dead	2.87 (0.39-68.20)#	9.3 (0.45-94.10)*	0.008	92.0 (4.83-96)***	0.008
IL-6 (pg/ml)	Alive	20.0 (4.8-77.8)	9.4 (2.10-42.5)	0.000	3.7 (1-23.5)	0.000
	Dead	9.8 (3.1-18.0)**	21.9 (9.8-39.0)**	0.012	39.8 (11-54.8)***	0.008
IL-8 (pg/ml)	Alive	0.57 (0.18-5)	0.32 (0.10-1.24)	0.001	0.19 (0-5)	0.004
	Dead	0.33 (0.01-4)	0.32 (0.19-2.0)	0.906	0.57 (0.1-5.5)#	0.066
TNF-α (pg/ml)	Alive	228.0 (101-3364)	116 (7.9-1690)	0.000	62.0 (3.2-181.0)	0.000
	Dead	52.0 (10-243)***	111 (39.3-1358)	0.008	3024.0 (154-7305)***	0.008
CRP (mg/L)	Alive	24.0 (0-128)			10.0 (0-138)	0.800
	Dead	24.0 (0-46)			56.0 (6-384)#	0.008

$^{\&}p_1$ value: Comparison between Day 0 and Day 3, p_2 value: Comparison between Day 0 and Day 7 (Wilcoxon test).
(*): Comparison between recovered and exitus patients (Mann-Whitney U test), (*): $p<0.05$, (**): $p<0.01$, (***): $p<0.001$.
(#): $p=0.07$.

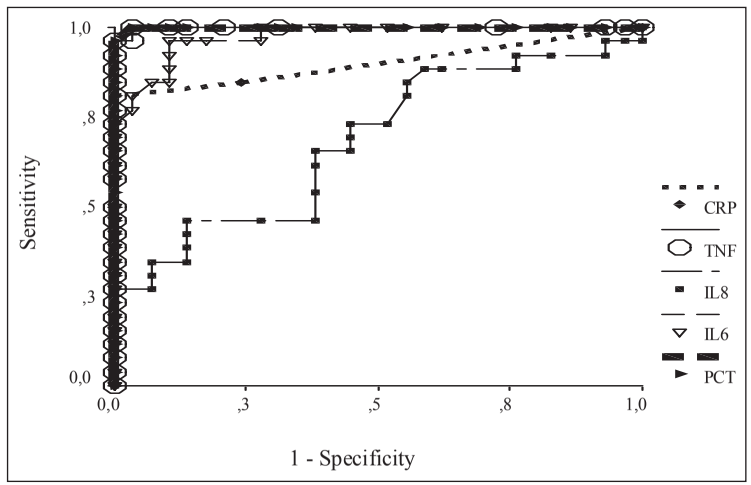

Fig. 1. Receiver operating characteristic (ROC) curves comparing procalcitonin (PCT), C-reactive protein (CRP), interleukin (IL)-6, IL-8 and tumor necrosis factor-alpha (TNF-α) for prediction of neonatal sepsis in the groups of neonates. The area under the curve (AUC) was 1 for PCT, 1 for TNF-α, 0.97 for IL-6, 0.90 for CRP and 0.68 for IL-8.

Table IV. Sensitivity, Specificity, PPV, NPV, Diagnostic Efficiency and AUC of PCT, CRP, IL-6, IL-8 and TNF-a for Neonatal Sepsis Using Optimum Cut-off Levels for Day 0, Derived from the ROC Curves

Laboratory tests and optimum cut-off levels	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Efficiency (%)	AUC (95% CI)
CRP≥10 (mg/L)	80.8	100	100	85.2	90.4	0.90 (0.81-1)
PCT≥0.34 (ng/ml)	100	96.5	96.2	100	98.3	1 (1-1)
IL-6≥3.6 (pg/ml)	96.2	89.7	86.2	96.1	93	0.97 (0.94-1)
IL-8≥0.65 (pg/ml)	34.6	86.2	69.2	59.5	60.4	0.68 (0.54-0.82)
TNF-α≥7.5 (pg/ml)	100	96.6	96.2	96.5	98.3	1 (0.99-1)
CRP≥10 and IL-6≥3.6	80.7	100	100	85.3	90.4	
CRP≥10 and IL-8≥0.65	34.6	100	100	63.0	67.3	

PPV: Positive predictive value. NPV: Negative predictive value. AUC: Area under the curve.
ROC: Receiver operating characteristics.

value of serum CRP, PCT, IL-6, IL-8 and TNF-α in determining early diagnosis and prognosis of neonatal sepsis, it was established that: (a) mean serum CRP, PCT, IL-6, IL-8 and TNF-α values before treatment were significantly higher in septic neonates compared with healthy ones, (b) PCT and TNF-α detection had high sensitivity and specificity in the early diagnosis of neonatal sepsis, followed by IL-6, CRP and IL-8 detection, respectively, (c) serum PCT, IL-6 and TNF-α detection were particularly valuable in determining the efficacy of treatment and the prognosis of the disease.

While evaluating the findings obtained from this study, methodological limitations such as the small number of investigated newborns and the absence of investigations of newborns with clinical sepsis suspicion who were either bacteriologically negative or had viral infections must be taken into account. However, the findings that were obtained in this study were strengthened by the following factors: the presence of positive blood cultures in all of the septic neonates; the investigation of both the early- and late-onset neonatal septic neonates, taking both healthy newborns and newborns at risk of neonatal sepsis development as the control group; the exclusion of neonates who were either delivered by a mother that was using antibiotics or had used antibiotics before; identification of the serum levels of investigated markers before treatment and on the 3rd and 7th days of treatment; identification of the sensitivity and specificity of each marker using optimum cut-off levels at Day 0, derived from the ROC curves; and the inclusion of investigated patients consecutively among the ones hospitalized at neonatal intensive care units and its reflection of clinical practice in these units.

Since the symptoms and findings are non-specific, neonatal sepsis diagnosis is quite difficult. There is a great need for new diagnostic laboratory methods for the early diagnosis of the disease and the evaluation of prognosis and treatment efficacy. Hopeful findings concerning the efficacy of several acute phase reactants (CRP, PCT) and cytokines (IL-6, IL-8 and TNF-α) in the early diagnosis of neonatal sepsis have been reported in recent years.

When the 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 unnecessary use of antibiotics in false-positive cases, a competent diagnostic marker also needs to have a 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%[14,15].

Since the end of the 1980s, an acute phase reactant, CRP, has been used and investigated as a common practice for the diagnosis of neonatal sepsis. CRP is synthesized by the liver in response to, and as part of, the inflammatory response. IL-6 is the major stimulus to production of CRP, along with IL-1 and TNF-α. CRP is synthesized within six to eight hours of exposure to an infective process or tissue damage, peaks at 24 to 48 hours, and then diminishes over time as the inflammation

resolves[16,17]. In variable studies using CRP ≥1 mg/dl as the cut-off value, the range of reported statistical outcomes is as follows: sensitivity 70% to 93%; specificity 41% to 98%; positive predictive accuracy 6% to 83%; and negative predictive accuracy 97% to 99%[18,21]. In our study, CRP ≥10 mg/L was found to be the most appropriate cut-off value by using ROC curves, and at this cut-off value, test sensitivity was 80.8%, specificity was 100%, PPV was 100%, NPV was 85.2% and AUC value was 0.90 (95%CI 0.81-1) (Table IV). The increase in the serum concentrations of CRP is rather slow during the first 12-24 hours of infection, and this may negatively affect the sensitivity of the test. In addition, increase in CRP concentrations in non-infected clinical conditions (meconium aspiration, recent vaccination, surgery, prolonged rupture of membranes, fetal distress, perinatal asphyxia, intraventricular hemorrhage) are thought to affect the specificity of the test[14,18]. However, we found no significant difference in mean serum CRP values between neonates with perinatal risk factors (Group IIa) and neonates with no perinatal risk factors (Group IIb) and between neonates with early-onset neonatal sepsis (Group Ia) and neonates with risk factors (Group IIa) in our study. This suggests that the presence of perinatal risk factors other than infection does not affect the diagnostic value of CRP. However, low test sensitivity and low NPV values for CRP in both our study and other studies have led us to think that this test alone will not be sufficient in the early diagnosis of neonatal sepsis. In our study, we observed that a combination of CRP with IL-6 or IL-8 did not change this result.

Another acute phase marker, PCT, has been intensively investigated for its diagnostic role in neonatal sepsis since the mid 1990s. It has been reported that serum concentrations of PCT begin to rise four hours after exposure to the bacterial endotoxin, peak at six to eight hours, and remain elevated for at least 24 hours[22]. A preliminary molecule of the calcitonin hormone, PCT is a 116 amino-acid polypeptide with a molecular weight of 14.5 kDa. While it is at undetectable levels in the blood of healthy people, the concentrations of PCT in the blood increase in relation to the intensity of inflammatory reaction due to infection in septic patients without causing any rise in calcitonin. PCT is synthesized in the liver and induced by similar means with acute phase reactants such as CRP. In fact, it has been shown that a great amount of PCT is produced in human liver cells after TNF-α and IL-6 stimulation[23]. The PCT level remains continuously high despite a decrease in TNF-α and IL-6 levels in parallel with the severity of the ongoing infection. In many studies, PCT sensitivity in the early diagnosis of neonatal sepsis was found to be 83-100% while the specificity was 70-100%[25-28]. In these studies, it has been suggested that PCT is a more powerful diagnostic marker then CRP in early diagnosis of sepsis, and it is a convenient diagnostic test in both early- and late-onset neonatal sepsis since it responds to a suitable stimulus at an earlier stage than CRP. However, in some of the studies it was reported that PCT is not a better marker than CRP in the early diagnosis of neonatal sepsis[29-31]. In these studies, it was reported that serum PCT levels had also increased in non-infected neonates with either perinatal asphyxia, intracranial hemorrhage, pneumothorax, or after resuscitation, and these conditions had negatively affected the specificity of PCT. Physiological alterations seen in serum PCT levels during the first 48 hours after birth and the effect of prenatal, intranatal and postnatal antibiotic application on serum PCT levels have been functioning as a confounder of the relation between PCT and infection. In addition, lack of correction for reference ranges for neonatal PCT values may also have influenced the outcome of PCT as a marker for bacterial infection. In their study, Chiesa et al.[32] reported that a serum PCT rise caused by perinatal events other than infection was smaller than the PCT response against infection. In our study, it was detected that mean serum PCT levels in septic neonates were significantly higher compared with healthy neonates, and when PCT ≥0.34 ng/ml was taken as the cut-off value, the sensitivity of PCT in the diagnosis of neonatal sepsis was 100%, specificity was 96.5%, PPV was 96.2% and NPV was 100%. With these findings, it was seen that that diagnostic value of PCT is excellent and better than CRP. Significantly higher mean serum PCT values of infants with early-onset neonatal sepsis than of the healthy infants with risk factors, and, on the other hand, the absence of significant differences

between the mean serum PCT values of healthy neonates with and without risk factors, lead us to believe that the effect of perinatal events other than infection on the concentrations of PCT is not at an advanced level.

The concentrations of some proinflammatory cytokines, especially TNF-α, IL-6 and IL-8, in systemic circulation were reported to increase in severe infections and septic shock[33]. TNF-α and IL-6 production occurs before PCT synthesis in bacterial infections. The presence of bacteria or bacterial endotoxin causes cytokine secretion by variable cell types, particularly by the mononuclear cells. Alterations in concentrations of these proteins and cytokines, peculiar to the acute inflammation phase, have been used as diagnostic indicators of bacterial infection[34]. However, methodological difficulties in detecting cytokines, the absence of their routine usage in all centers and the increase in cytokines in all non-specific infections and in shock have limited their use in daily practice.

Interleukin-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. In the studies, IL-6 has been reported to have the highest sensitivity (89%) and NPV (91%) at the onset of infection, compared with other biochemical markers[14,35]. However, it has a very short half-life, and the concentrations fall precipitously with treatment and become undetectable in most infected patients within 24 hours. The sensitivity is therefore reduced to a much lower concentration at 24 and 48 hours (67% and 58%, respectively)[14]. IL-6 can be considered as an early and sensitive marker of neonatal infection. In addition, it has been reported that IL-6 levels show natural fluctuations immediately after the postnatal period just as PCT does, and its serum levels are affected by gestational age and by perinatal complications other than infection. This situation affects the sensitivity and the specificity of the test[36]. The combination of IL-6 (early and sensitive marker) with CRP (late and specific marker) has been reported to have a better sensitivity than either marker alone[14,35,37]. In our study, it was observed that mean serum IL-6 levels of septic neonates were significantly higher than of the healthy ones. However, patients with early-onset neonatal sepsis when compared with patients with late-onset neonatal sepsis, and healthy neonates with risk factors when compared with healthy neonates without risk factors, had lower IL-6 values. We thought that these findings might be due to natural fluctuations immediately after the postnatal period of IL-6 and serious effects of perinatal complications other than infection on the IL-6 levels. When IL-6 ≥3.6 pg/ml is considered as the cut-off value, sensitivity is 96.2%, specificity is 89.7%, PPV is 86.2% and NPV is 96.1%. In addition, it was detected that the combination of the test with CRP did not make a significant contribution to the accuracy of the test. These findings suggest that IL-6 is an important marker in the diagnosis of neonatal sepsis.

Interleukin-8 is a cytokine that has a role in the release, activation and chemotaxis of neutrophils. Serum IL-8 level has been reported to increase both in early- and late-onset neonatal sepsis and to have a sensitivity of about 80–91% and a specificity of about 76–100%[38-40]. The diagnostic accuracy is further enhanced by simultaneous measurement of CRP. TNF-α is an important mediator that has a role in the pathogenesis of sepsis and septic shock. In the studies, the sensitivity of the test was reported to range between 73% and 88% while the specificity ranged between 43% and 94%[14,41,42]. In our study, it was detected that baseline mean serum IL-8 and TNF-α levels were significantly higher in septic neonates than in the healthy ones, and that there was no significant difference between the mean serum IL-8 and TNF-α levels of neonates with early-onset versus late-onset sepsis. Furthermore, the presence or absence of perinatal risk factors did not affect mean serum levels of these cytokines in healthy neonates. When a cut-off value of IL-8 ≥0.65 pg/ml is used, the diagnostic sensitivity, specificity, PPV and NPV of IL-8 are 34.6%, 86.2%, 69.2% and 59.5%, respectively. On the other hand, when a cut-off value of TNF-α ≥7.5 pg/ml is used, the diagnostic sensitivity, specificity, PPV and NPV of TNF-α are 100%, 96.6%, 96.2% and 96.5%, respectively. These findings have shown that IL-8 does not have the characteristics of a good diagnostic test but TNF-α has characteristics of a diagnostic test as powerful as those of PCT. When TNF-α is thought of as the most potent inducer of PCT, the two are expected

to have similar specificity and sensitivity. Our findings suggest that TNF-α is as nearly a perfect marker as PCT and more sensitive and specific then CRP, IL-6 and IL-8 in the diagnosis and differentiation of neonatal sepsis. Similar results have been obtained in many of the published studies[41-44].

Another characteristic of the markers that are used in the diagnosis of neonatal sepsis is that it gives information about the prognosis of the disease and helps in coming to a decision as to whether to stop or continue antibiotic treatment. In our study, when blood samples obtained before treatment (Day 0) and on the 3rd (Day 3) and 7th days (Day 7) of treatment both from patients recovered and from those who died were compared, it was observed that mean serum PCT, IL-6, IL-8 and TNF-α values significantly decreased in recovered patients during the treatment. On the other hand, mean serum CRP, PCT, IL-6 and TNF-α values were detected to significantly increase in the exitus patients. With these findings, it is observed that PCT, IL-6 and TNF-α are quite useful markers in determining the prognosis of the disease and treatment efficacy, while CRP and IL-8 do not have similar efficacy. Similar results have been obtained in many studies[35,45-47]. In contrast to the results of some studies[46-49], we found that pretreatment mean serum values of IL-6 and TNF-α were significantly lower in patients who died than in patients who recovered. We thought that presence of excessive antiinflammatory response might explain this result[6,50]. However, since we did not measure serum values of anti-inflammatory mediators (IL-4, IL-10) in this study, we could not reach a precise conclusion.

Reports in the literature on the use of CRP, PCT, IL-6, IL-8 and TNF-α as early markers of neonatal sepsis are contradictory. Variations in study design, definition of neonatal sepsis, number and characteristics of enrolled subjects (wide-ranging differences in postnatal age, gestational age and risk factors), cut-off points of the markers, methods of laboratory measurement, data analysis and reporting of results lead to difficulties in comparing studies. In particular, the predominance of studies at single medical centers with small sample sizes makes it difficult to apply the tests in clinical decision making[51,52]. Thus, it is often difficult to formulate a definitive opinion on the clinical usefulness of infection markers from the published reports.

In conclusion, in this prospective study, PCT and TNF-α proved to be the best indicators of early- and late-onset neonatal sepsis. It was also determined that these markers are valuable in following the efficacy of the treatment and determining the prognosis of the disease. However, it is thought that these findings need to be confirmed by means of future studies examining different categories of infections and larger number of neonates using rigorous methods to give clinicians firmer recommendations.

REFERENCES

1. Remington JS, Klein JO. Current concepts of infections of the fetus and newborn infant. In: Remington JS, Klein JO (eds). Infectious Diseases of the Fetus and Newborn Infant. Philadelphia: Saunders; 1995: 1-19.
2. Osrin D, Vergnano S, Costello A. Serious bacterial infections in newborn infants in developing countries. Curr Opin Infect Dis 2004; 17: 217-224.
3. Gerdes JS. Diagnosis and management of bacterial infections in the neonate. Pediatr Clin North Am 2004; 51: 939-959.
4. Hammerschlag MR, Klein JO, Herschel M, et al. Patterns of use of antibiotics in two newborn nurseries. N Engl J Med 1988; 235: 1268-1269.
5. Philip AG, Hewitt JR. Early diagnosis of neonatal sepsis. Pediatrics 1980; 65: 1036-1041.
6. Ng PC. Diagnostic markers of infection in neonates. Arch Dis Child Fetal Neonatal Ed 2004; 89: F229-F235.
7. Rodwel RL, Leslie AL, Tudehope DI. Early diagnosis of neonatal sepsis using a hematologic scoring system. J Pediatr 1988; 112: 761-767.
8. Töllner U. Early dagnosis of septicemia in newborn. Clinical studies and sepsis score. Eur J Pediatr 1982; 138: 331-337.
9. King JC Jr, Berman ED, Wright PF. Evaluation of fever in infants less than 8 weeks old. South Med J 1987; 80: 948-952.
10. Meisner M. PCT, Procalcitonin - A New, Innovative Infection Parameter. Berlin: Brahms-Diagnostica; 1996.
11. Prince WS, Harder KJ, Saks S, Reed BR, Chen A. ELISA for quantitation of tumor necrosis factor-alpha in serum. J Pharm Biomed Anal 1987; 5: 793-796.
12. McNeil BJ, Keller E, Adelstein SJ. Primer on certain elements of medical decision making. N Engl J Med 1975; 293: 211-215.
13. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. Radiology 1983; 148: 839-848.
14. Ng PC, Cheng SH, Chui KM, et al. Diagnosis of late onset neonatal sepsis with cytokines, adhesion molecules, and C-reactive protein in preterm very low birthweight infants. Arch Dis Child Fetal Neonatal Ed 1997; 77: F221-F227.
15. Ng PC, Li K, Wong RP, et al. Neutrophil CD64 expression: a sensitive diagnostic marker for late-onset nosocomial infection in very low birthweight infants. Pediatr Res 2002; 51: 296-303.

16. Gabay C, Kushner I. Mechanisms of disease: acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999; 340: 448-454.

17. Gallou S, Kushner I. C-reactive protein and acute phase response. Adv Intern Med 1992; 37: 313-336.

18. Çaksen H, Kurtoglu S, Hallaç IK, Üstünbaş HB, Uzun K, Kılıç H. The relationship between scoring systems and cytokine levels in neonatal sepsis. Ann Acad Med Singapore 2003; 32: 418-420.

19. Russell G, Smyth A, Cooke R. Receiver operating characteristic curves for comparision of serial neutrophil band forms and C-reactive protein in neonates at risk for infection. Arch Dis Child 1993; 67: 808-812.

20. Benitz W, Han M, Madan A, et al. Serial serum C-reactive protein levels in the diagnosis of neonatal infection. Pediatrics 1998; 102: E41.

21. Chiesa C, Pellegrini G, Penero A, et al. C-reactive protein, interleukin-6 and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection. Clin Chem 2003; 49: 51-59.

22. Dandona P, Nix D, Wilson MF, et al. Procalcitonin increase after endotoxin injection in normal subjects. J Clin Endocrinol Metab 1994; 79: 605-608.

23. van Rossum AM, Wulkan RW, Oudesluys-Murphy AM. Procalcitonin as an early marker of infection in neonates and children. Lancet Infect Dis 2004; 4: 620-630.

24. Nijsten MW, Olinga P, The TH, et al. Procalcitonin behaves as a fast responding acute phase protein in vivo and in vitro. Crit Care Med 2000; 28: 458-461.

25. Gendrel D, Raymond J, Coste J, et al. Comparion of procalcitonin with C-reactive protein, interleukin-6 and interferon-alpha for differentiation of bacterial vs. viral infections. Pediatr Infect Dis 1999; 18: 875-881.

26. Hatherill M, Tibby SM, Sykes K, Turner C, Murdoch IA. Diagnostic marker of infection: comparison of procalcitonin with C-reactive protein and leucocyte count. Arch Dis Child 1999; 81: 417-421.

27. Prat C, Dominguez J, Rodrigo C, et al. Use of quantitative and semiquantitative procalcitonin measurement to identify children with sepsis and meningitis. Eur J Clin Microbiol Infect Dis 2004; 23: 136-138.

28. Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. CID 2004; 39: 206-217.

29. Monneret G, Labaune JM, Isaac C, Bienvenu F, Putet G, Bienvenu J. Procalcitonin and C-reactive protein levels in neonatal infections. Acta Paediatr 1997; 86: 209-212.

30. Lapillonne A, Basson E, Monneret G, Bienvenu J, Salle BL. Lack of specificity of procalcitonin for sepsis diagnosis in premature infants. Lancet 1998; 351: 1211-1212.

31. Janota J, Stranak Z, Belohlavkova S, Mudra K, Simak J. Postnatal increase of procalcitonin in premature newborns is enhanced by chorioamnionitis and neonatal sepsis. Eur J Clin Invest 2001; 31: 978-983.

32. Chiesa C, Panero A, Rossi N, et al. Reliability of procalcitonin concentrations for the diagnosis of sepsis in critically ill neonates. Clin Infect Dis 1998; 26: 664-672.

33. Saez-Llorens X, Lagrutta F. The acute phase host reaction during bacterial infection and its clinical impact in children. Pediatr Infect Dis J 1993; 12: 83-87.

34. Urbach J, Shapira I, Branski D, Berliner S. Acute phase response in the diagnosis of bacterial infections in children. Pediatr Infect Dis J 2004; 23: 159-160.

35. Messer J, Eyer D, Donato L, et al. Evaluation of interleukin-6 and soluble receptors of tumor necrosis factor for early diagnosis of neonatal infection. J Pediatr 1996; 129: 574-580.

36. Chiesa C, Signore F, Assumma M, et al. Serial measurement of C-reactive protein and interleukin-6 in the immediate postnatal period: reference intervals and analysis of maternal and perinatal confounders. Clin Chem 2001; 47: 1016-1022.

37. Doellner H, Arntzen KJ, Haereid PE, et al. Interleukin-6 concentrations in neonates evaluated for sepsis. J Pediatr 1998; 132: 295-299.

38. Franz AR, Steinbach G, Kron M, et al. Reduction of unnecessary antibiotic therapy in newborn infants using interleukin-8 and C-reactive protein as markers of bacterial infections. Pediatrics 1999; 104: 447-453.

39. Franz AR, Steinbach G, Kron M, et al. Interleukin-8: a valuable tool to restrict antibiotic therapy in newborn infants. Acta Paediatr 2001; 90: 1025-1032.

40. Nupponen I, Andersson S, Jorvenpaa AL, et al. Neutrophil CD 11b expression and circulating interleukin-8 as diagnostic markers for early-onset neonatal sepsis. Pediatrics 2001; 108: E12-17.

41. Silveira RC, Procianoy RS. Evaluation of interleukin-6, tumor necrosis factor alpha and interleukin-1 beta for early diagnosis of neonatal sepsis. Acta Paediatr 1999; 88: 647-650.

42. deBonte ES, Martens A, vanRaan J, et al. Diagnostic value of tumor necrosis factor alpha (TNF alpha) and interleukin-6 (IL-6) in newborn sepsis. Acta Paediatr 1994; 83: 696-699.

43. Atıcı A, Satar M, Çetiner S, Yaman A. Serum tumor necrosis factor alpha in neonatal sepsis. Am J Perinatol 1994; 14: 401-404.

44. Girardin EP, Berner ME, Gran GE, Paunier I. Serum tumor necrosis factor in newborns at risk for infections. Eur J Pediatr 1990; 149: 645-647.

45. Oberhoffer M, Vogelsang H, Russwurm S, et al. Outcome prediction by traditional and new markers of inflammation in patients with sepsis. Clin Chem Lab Med 1999; 37: 363-368.

46. Casado-Flores J, Blanco-Quiros A, Asensio J, et al. Serum procalcitonin in children with suspected sepsis: a comparison with C-reactive protein and neutrophil count. Pediatr Crit Care Med 2003; 4: 190-195.

47. Athan F, Akagündüz B, Genel F, Bak M, Can D. Procalcitonin: a marker of neonatal sepsis. J Trop Pediatr 2002; 48: 10-14.

48. Van der Kaay DC, De Kleijn ED, De Rijke YB, Hop WC, De Groot R, Hazelzet JA. Procalcitonin as a prognostic marker in meningococcal disease. Intensive Care Med 2002; 28: 1606-1612.

49. Harbarth S, Holeckova K, Froidevaux C, et al. Diagnostic value of procalcitonin, interleukin-6, and interleukin-8 in critically ill patients admitted with suspected sepsis. Am J Respir Crit Care Med 2001; 164: 396-402.

50. Van Dissel JT, van Langevelde P, Westendorp RG, Kwappenberg K, Frölich M. Anti-inflammatory cytokine profile and mortality in febrile patients. Lancet 1998; 351: 950-953.

51. Malik A, Hui CP, Pennie RA, Kirpalani H. Beyond the complete blood cell count and C-reactive protein. A systematic review of modern diagnostic tests for neonatal sepsis. Arch Pediatr Adolesc Med 2003; 157: 511-516.

52. Chiesa C, Panero A, Osborn JF, Simonetti AF, Pacifico L. Diagnosis of neonatal sepsis: a clinical and laboratory challenge. Clin Chem 2004; 50: 279-287.