The role of serum interleukin-6 and C-reactive protein levels for differentiating aetiology of neonatal sepsis

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ABSTRACT

***Introduction*:** In our clinical practice, we observed high interleukin-6 (IL-6) levels in gram-negative sepsis.

Objective: To investigate the relationship between IL-6 and C-reactive protein (CRP) levels and early determination of neonatal sepsis of gram-negative or gram-positive aetiology.

***Population and Methods*:** White blood cell count, IL-6 and CRP levels were compared among different groups.

***Results*:** Gram-negative, gram-positive and fungal infection groups consisted of 73, 82 and 15 patients, respectively. The optimal cut-off levels of IL-6 between gram-negative and gram-positive fungal infection groups were 202 and 57 pg/ml. The fungal infection group had higher CRP levels than gram-negative and positive infection groups.

***Conclusions*:** To our knowledge, this is the largest reported study aiming at determining of IL-6 cut-off levels to differentiate neonatal sepsis aetiology. Gram-negative microorganisms led to 10 fold higher IL-6 production. The evaluation of IL-6 and CRP is useful to diagnose and also differentiate neonatal sepsis aetiology.

Key words: *newborn, sepsis, interleukin-6, C-reactive protein, gram-negative infections, fungal disease.*

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INTRODUCTION

Neonatal sepsis continues to be a common and significant health care burden because of high mortality and morbidity rates despite advances in neonatology, especially in developing countries.[1] Several interleukins, tumor necrosis factor (TNF), procalcitonin (PCT), C-reactive protein (CRP), immunoglobins, and other markers have been used in the diagnosis of sepsis.[2]

Early recognition of signs of infection based on clinical or laboratory studies in the early stages of bacteremia could therefore, help to identify those patients who are likely infected with either gram-negative or gram-positive pathogens. Interestingly, some studies have shown significantly greater inflammatory response in gram-negative sepsis than in gram-positive ones.[3-5] Fungal infections also show different inflammatory responses.[6] Recently, we observed high IL-6 levels in some newborn infants with sepsis. During follow up, results showed that most of these patients had gram-negative infection. After this clinical observation, we decided to perform a subgroup analysis with the data obtained from a previously published study reporting cut-off levels of IL-6 and CRP in neonatal sepsis. [7]

OBJECTIVE

1. To investigate the value of IL-6 and CRP in the early establishment in neonatal sepsis aetiology as gram-negative or gram-positive,
2. To determine the cut-off value for each marker of neonatal sepsis,
3. To identify the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of each cut-off level.

METHODS

Patients

This retrospective study took place in Zekai Tahir Burak Maternity Teaching Hospital between January 2008 and December 2008; medical records were reviewed and the study was approved by the local Ethics Committee. Clinical findings for sepsis diagnosis required at least three of the following: bradycardia (<100/min), tachycardia (>200/min), hypotension, hypotonia, seizures, apnea, tachypnea, cyanosis, respiratory distress, unusual skin color and perfusion, feeding difficulty, irritability, lethargy, and laboratory results showing elevated levels of IL-6 (>70 pg/ml) or CRP (>10 mg/dl). [8]

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Inclusion criteria of groups

- Group Ia (Proven sepsis; 170 patients): Newborns with positive blood cultures, clinical findings of infection, and elevated IL-6 and/or CRP levels.
- Group Ib (Clinical sepsis; 62 patients): Newborns with clinical findings of infection, elevated IL-6 and/or CRP levels, 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 ablatio placentae, 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.

Statistical analyses

Statistical analyses were performed using the SPSS statistical package (v.15.0). Categorical variables were analyzed using the chi-squared test. The comparison of means was done using a t-test when the data fit a normal distribution, and a Mann–Whitney U test when the data was non-normal. In order to compare 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. A *p* value of <0.05 was deemed to indicate statistical significance.

RESULTS

There were 232 patients 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, male/female ratio, and vaginal delivery rate did not differ statistically. The work-up day of IL-6 and CRP was similar between proven and clinical sepsis groups, but was earlier for the control group. Blood culture results are listed in *Table 2*; 29 types of microorganism were isolated from blood cultures. The number of gram-negative microorganisms, gram-positive microorganisms and fungus was 73 (43%), 82 (48%) and 15 (9%), respectively. The most frequently isolated microorganisms were *Klebsiella pneumoniae* (44; 25.8%), *Staphylococcus epidermidis* (29; 17%), fungi (*Candida albicans* and *Candida tropicalis*, 15; 8.8%), and *Staphylococcus aureus* (10; 5.8%).

We previously found cut-off levels of IL-6 and CRP between proven sepsis and control group as 21.5 pg/ml and 5.82 mg/dl, respectively.[7] Levels of IL-6 and CRP in all groups are listed in Table 3. Between the sepsis groups and the control group, there were significant differences for both IL-6 and CRP levels ($p < 0.001$). Levels of IL-6 and CRP in groups of gram-negative, gram-positive and fungal infection groups are listed in Table 4. There were statistically significant differences between gram-negative and other groups according to levels of IL-6 ($p < 0.001$). The optimum cut-off values of IL-6 in the diagnosis of gram-negative infection were found to be 202 pg/ml versus gram-positive infection, and 57 pg/ml versus fungal infection. The optimum cut-off value of IL-6 in the diagnosis of gram-positive infection versus fungal infection was found to be 58 pg/ml. Sensitivity, specificity, NPV and PPV of IL-6 level of 202 pg/ml (gram-negative versus gram-positive infection) are 68%, 58%, 57% and 69%, respectively. Sensitivity, specificity, NPV and PPV of IL-6 level of 57 pg/ml (gram-negative versus fungal infection) are 76%, 42%, 24% and 71%, respectively. Sensitivity, specificity, NPV and PPV of IL-6 level of 58 pg/ml (gram positive versus

TABLE 1. *Clinical and demographic characteristics of proven, clinical sepsis and control 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, gram	1580 ± 685	1585 ± 718	1735 ± 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

mean±standard deviation (interquartile range)

fungal infection) are 76%, 29%, 27% and 55%, respectively. The fungal infection group had higher CRP levels than other groups (p <0.05). Mortality rates of gram-negative, gram-positive and fungal infection groups were 18%, 10% and 20%, respectively (p >0.05).

TABLE 2. *Microorganisms isolated (blood culture)*

	Number of patients (%)
Gram negative microorganisms	**73 (43%)**
Klebsiella pneumoniae	44
Klebsiella oxytoca	8
Escherichia coli	6
Enterobacter cloacae	5
Acinetobacter baumannii, Enterobacter species, Serratia marcescens, Pseudomonas aeruginosa	2
Pantoea agglomerans, Stenotrophomonas maltophilia	1
Gram positive microorganisms	**82 (48%)**
Staphylococcus epidermidis	29
Staphylococcus aureus	10
Staphylococcus hominis	9
Enterococcus faecium	7
Staphylococcus haemolyticus	5
Staphylococcus warneri, Enterococcus faecalis	4
C group *streptococcus, Streptococcus sanguis, Staphylococcus capitis*	2
Streptococcus acidominimus, coagulase negative staphylococcus	
Staphylococcus saprophyticus , Staphylococcus chromogenes,	1
Streptococcus intermedia, Streptococcus mitis	1
Fungus	**15 (9%)**
Candida albicans	11
Candida tropicalis	4
Total	**170**

There was no statistically significant difference between the groups as regards hemoglobin and white blood cell count (p>0.05). Platelet counts of control, gram-negative, gram-positive and fungal infection groups were $199x10^3 \pm 131x10^3$, $153x10^3 \pm 126x10^3$, $207x10^3 \pm 138x10^3$, $101 x10^3 \pm 79 x10^3/\mu L$. According to platelet counts, the gram-negative and fungal infection groups had lower platelet levels than control and gram-positive infection group (p <0.05). The fungal infection group had lower platelet levels than the gram-negative group (p <0.05).

DISCUSSION

IL-6 is an important cytokine of the host's early response to infection. After exposure to bacterial products, concentration of IL-6 increases sharply, and leads the increase of CRP. It has a very short half-life, and the concentration falls with the treatment, becoming undetectable in most infected patients within 24 hr. The CRP is synthesized within 6-8 hr in an inflammatory response by the liver, peaks at 24-48 hr, and diminishes over time as the inflammation resolves.

Differences in mechanisms of bacterial virulence result in differences in the host response, the extent of activation of various signaling cascades and the stimulation/inhibition of host cell apoptosis, which influence the prognosis.[9,10] Pathogen-associated molecular patterns (PAMPs) have been already recognized.[11] PAMPs from gram-negative and gram-positive bacteria are known to act as ligands for mutually different pattern recognition receptors including Toll-like receptors.[12]

TABLE 3. *IL-6 and CRP levels of proven, clinical sepsis and control groups (mean±standard deviation)*

	Group Ia (proven sepsis) (n= 170)	Group Ib (clinical sepsis) (n= 62)	Group II (control) (n= 50)	*p*
IL-6 (pg/ml)	349 ± 422	257 ± 358	35 ± 104	<0.001
CRP (mg/dl)	18.2 ± 14.5	13.6 ± 13.5	1.6 ± 2.6	<0.001

TABLE 4. *IL-6 and CRP levels of gram negative, gram positive, fungal infection and control groups (mean ± standard deviation)*

Infection group	Gram negative (n= 73)	Gram positive (n= 82)	Fungal (n= 15)	Control (n= 50)
IL-6 (pg/ml)	500 ± 439	320 ± 418	45 ± 64	35 ± 104
CRP (mg/dl)	19 ± 14.2	17.9 ± 14.9	22.6 ± 13	1.6 ± 2.6

Our study showed that gram-negative microorganisms lead to a 10 fold increase of IL-6 cut-off level in comparison to the general proven sepsis group. Fungal infection seems to cause lower cytokine production. Studies on predictability of microorganism types according to cytokines are mostly at adult age. Fisher et al. previously reported that plasma IL-6 levels were significantly higher in patients with gram-negative bacteremia and predicts fatal outcome.[13] Abe et al. showed that IL-6 and CRP levels were higher in gram-negative bacteremia in the Intensive Care Unit.[3] They found that the incidence of gram-negative bacteremia and mortality were significantly higher in the septic shock than in the sepsis and severe sepsis groups.

In children with sepsis, IL-6 was found to predict mortality better than clinical or other laboratory tests.[14] In our study, mortality rate of gram-negative group was slightly higher than the gram-positive group with no statistical difference. Kumar et al. showed that TNF-α levels were higher in pediatric gram-negative than gram-positive bacteremia, but found no difference of CRP levels in the study population.[15]

Patients with fungal sepsis have lower IL-6 levels than gram-negative and gram-positive groups, but interestingly, have higher CRP levels. Oguz et al. previously reported high CRP levels in fungal sepsis patients, and showed that fungal sepsis should be suspected in patients with high persistent CRP levels.[6]

Neonatal sepsis treatment is initiated empirically and covers both gram-negative and gram-positive microorganisms. If aetiology is determined, adequate treatment may be started. These findings suggest that differences in host responses and virulence mechanisms of different pathogenic microorganisms should be considered in the treatment of bacteremic patients. Our findings may help clinicians to start adequate therapy and predict outcome.

In conclusion, we think that IL-6 and CRP are useful to determine the aetiology and implement empirical treatment of neonatal sepsis. ■

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