Serum Interleukin-6 Level as a Diagnostic Test in Children with Sepsis

**Background.** This study was performed to determine the serum concentrations of interleukin-6 (IL-6) during the early course of bacterial infection in children, and to evaluate the usefulness of IL-6 as a diagnostic test alone and in combination with C-reactive protein (CRP).

**Methods.** We measured serum IL-6 values in three groups of children on their first day of admission, from January 2001 to December 2001: group 1, patients with clinical and microbiological evidence of sepsis (n = 13); group 2, patients with clinical and chest radiographical evidence of pneumonia (n = 18); and group 3, patients with no signs of infection (control group) (n = 16). Kruskal-Wallis tests were used to compare the difference of IL-6 values between groups and control subjects.

**Results.** IL-6 values were significantly higher in groups 1 and 2 compared with those in the control group (p < 0.001). No significant differences were found between the groups 1 and 2. As a diagnostic test, IL-6 (≥ 20 pg/mL) alone yielded a sensitivity of 68%, a specificity of 88%, a positive predictive value of 71%, and a negative predictive value of 58%. A combined parameter of IL-6 (≥ 20 pg/mL) and CRP (≥ 1 mg/dL) yielded a sensitivity of 94%, a specificity of 63%, a positive predictive value of 79%, and a negative predictive value of 87%.

**Conclusion.** IL-6 levels increase in children with sepsis. In combination with CRP, IL-6 seems to be a valuable parameter in the early diagnosis of pediatric infections.

**Keywords**
children; C-reactive protein; cytokine; interleukin-6; sepsis

Sepsis is a major cause of morbidity and mortality in pediatric patients. The varying and non-specific symptoms make it difficult to distinguish from other conditions in patients with clinical signs of acute inflammation. Several studies have reported on searches for parameters that could be helpful in the early diagnosis of infection. Increased pro-inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), occur in sepsis. Serum C-reactive protein (CRP), an acute phase reactant that increases in the presence of inflammation and is caused by in fec tion or tissue injury, is syn the sized in the liver. IL-6 is the major regulator of this synthesis. Calandra et al. showed that IL-6 serum concentrations contributed more significantly to the prediction of outcome. Some studies have shown that IL-6 is an early and sensitive marker of sepsis in adults, children, and neonates. The combination of CRP and IL-6 has recently proven to be helpful in new borns evaluated for sepsis.

This study was designed and performed: (1) to determine the serum concentrations of IL-6 during sepsis in children other than neonates in the early course of disease, and (2) to evaluate the usefulness of IL-6 as a diagnostic test alone or in combination with CRP.

**Patients and Methods**

**Patients**

From January 2001 to December 2001, children who were admitted to Taipei Veterans General Hospital with the diagnosis of septicemia, bacterial meningitis or pneumonia were included in this study. Sepsis was defined as a clinical suspicion and a positive blood culture as well as clinical evidence of sepsis in children.
ated with 2 or more of the following: a body temperature of > 38°C or < 36°C, tachycardia with a heart rate > 95th percentile for age, respiratory rate of > 95th percentile for age and/or a white blood cell count of > 12,000 cells/mm³ or < 4,000 cells/mm³. Bacterial meningitis was defined as clinical signs consistent with sepsis in addition to a positive bacterial culture of the cerebrospinal fluid (CSF). The positive blood cultures of seven children were: *Staphylococcus aureus* (n = 2), *Coagulase-negative staphylococcus* (n = 2), *Escherichia coli* (n = 1), *Salmonella* species (n = 1), and *Klebsiella pneumoniae* (n = 1). The positive CSF cultures of 6 children were: *Klebsiella pneumoniae* (n = 2), *Streptococcus pneumoniae* (n = 1), *Staphylococcus aureus* (n = 1), *Escherichia coli* (n = 1) and *Haemophilus influenzae* (n = 1). Pneumonia was defined as the presence of respiratory symptoms and patchy consolidation or pleural effusion on chest X-ray films. Children who were admitted with out signs of infection were defined as the control group (Table 1).

### Samples

Peripheral blood samples were obtained on the first day of admission. Blood samples were centrifuged after collection, and sera were stored at -70°C until assayed.

### Analysis of CRP and IL-6

CRP in the sera was measured with commercially available immunonephelometry (Behring, San Jose, CA, USA). IL-6 concentrations in the sera were measured with enzyme-linked immunosorbent as say (R & D systems, Minneapolis, MN, USA).

### Statistical analysis

All results were expressed as mean±SD. To analyze whether the means of the various groups were significantly different, Kruskal-Wallis tests were used to compare the difference between groups and control subjects. An analysis of the receiver operating characteristic (ROC) curve was performed to determine the best cut-off value for IL-6 and CRP. A *p* value less than 0.05 was considered to be significant.

### RESULTS

#### Characteristics of the study population

In all, 47 children met our study criteria and were enrolled in the study. Seven children had positive blood cultures and 6 had positive CSF cultures. Thirteen children were included in group 1. The average duration of illness prior to hospitalization was 1 day. None of the patients were in shock on admission and none died during their hospital stay. The most common signs of sepsis in order of frequency included fever (100%), vomiting (85%), headache (77%) and lethargy (69%). Upper respiratory symptoms, irritability, crying, neck stiffness, abdominal pain, loose stool and drowsiness were seen with decreasing frequency. All of the patients received antibiotic administration and recovered with no sequelae.

#### Samples

Peripheral blood samples were obtained on the first day of admission. Blood samples were centrifuged after collection, and sera were stored at -70°C until assayed.

### Analysis of CRP and IL-6

CRP in the sera was measured with commercially available immunonephelometry (Behring, San Jose, CA, USA). IL-6 concentrations in the sera were measured with enzyme-linked immunosorbent assay (R & D systems, Minneapolis, MN, USA).

### Statistical analysis

All results were expressed as mean±SD. To analyze whether the means of the various groups were significantly different, Kruskal-Wallis tests were used to compare the difference between groups and control subjects. An analysis of the receiver operating characteristic (ROC) curve was performed to determine the best cut-off value for IL-6 and CRP. A *p* value less than 0.05 was considered to be significant.

### RESULTS

#### Characteristics of the study population

In all, 47 children met our study criteria and were enrolled in the study. Seven children had positive blood cultures and 6 had positive CSF cultures. Thirteen children were included in group 1. The average duration of illness prior to hospitalization was 1 day. None of the patients were in shock on admission and none died during their hospital stay. The most common signs of sepsis in order of frequency included fever (100%), vomiting (85%), headache (77%) and lethargy (69%). Upper respiratory symptoms, irritability, crying, neck stiffness, abdominal pain, loose stool and drowsiness were seen with decreasing frequency. All of the patients received antibiotic administration and recovered without sequelae. Eighteen patients were diagnosed with pneumonia in group 2. The frequency of signs and symptoms were characterized by fever, cough and rhinorrhea. Other less common symptoms included head ache, vomiting and abdominal pain. All of the patients recovered with no death or complication. Sixteen patients with no infection comprised group 3 (control group) (Table 2). There were no significant differences in mean age and gender distribution among all the groups (Table 2).

### Samples

Peripheral blood samples were obtained on the first day of admission. Blood samples were centrifuged after collection, and sera were stored at -70°C until assayed.

### Analysis of CRP and IL-6

CRP in the sera was measured with commercially available immunonephelometry (Behring, San Jose, CA, USA). IL-6 concentrations in the sera were measured with enzyme-linked immunosorbent assay (R & D systems, Minneapolis, MN, USA).

### Statistical analysis

All results were expressed as mean±SD. To analyze whether the means of the various groups were significantly different, Kruskal-Wallis tests were used to compare the difference between groups and control subjects. An analysis of the receiver operating characteristic (ROC) curve was performed to determine the best cut-off value for IL-6 and CRP. A *p* value less than 0.05 was considered to be significant.

### RESULTS

#### Characteristics of the study population

In all, 47 children met our study criteria and were enrolled in the study. Seven children had positive blood cultures and 6 had positive CSF cultures. Thirteen children were included in group 1. The average duration of illness prior to hospitalization was 1 day. None of the patients were in shock on admission and none died during their hospital stay. The most common signs of sepsis in order of frequency included fever (100%), vomiting (85%), headache (77%) and lethargy (69%). Upper respiratory symptoms, irritability, crying, neck stiffness, abdominal pain, loose stool and drowsiness were seen with decreasing frequency. All of the patients received antibiotic administration and recovered without sequelae. Eighteen patients were diagnosed with pneumonia in group 2. The frequency of signs and symptoms were characterized by fever, cough and rhinorrhea. Other less common symptoms included headache, vomiting and abdominal pain. All of the patients recovered with no death or complication. Sixteen patients with no infection comprised group 3 (control group) (Table 2). There were no significant differences in mean age and gender distribution among all the groups (Table 2).

### Samples

Peripheral blood samples were obtained on the first day of admission. Blood samples were centrifuged after collection, and sera were stored at -70°C until assayed.

### Analysis of CRP and IL-6

CRP in the sera was measured with commercially available immunonephelometry (Behring, San Jose, CA, USA). IL-6 concentrations in the sera were measured with enzyme-linked immunosorbent assay (R & D systems, Minneapolis, MN, USA).

### Statistical analysis

All results were expressed as mean±SD. To analyze whether the means of the various groups were significantly different, Kruskal-Wallis tests were used to compare the difference between groups and control subjects. An analysis of the receiver operating characteristic (ROC) curve was performed to determine the best cut-off value for IL-6 and CRP. A *p* value less than 0.05 was considered to be significant.

### RESULTS

#### Characteristics of the study population

In all, 47 children met our study criteria and were enrolled in the study. Seven children had positive blood cultures and 6 had positive CSF cultures. Thirteen children were included in group 1. The average duration of illness prior to hospitalization was 1 day. None of the patients were in shock on admission and none died during their hospital stay. The most common signs of sepsis in order of frequency included fever (100%), vomiting (85%), headache (77%) and lethargy (69%). Upper respiratory symptoms, irritability, crying, neck stiffness, abdominal pain, loose stool and drowsiness were seen with decreasing frequency. All of the patients received antibiotic administration and recovered without sequelae. Eighteen patients were diagnosed with pneumonia in group 2. The frequency of signs and symptoms were characterized by fever, cough and rhinorrhea. Other less common symptoms included headache, vomiting and abdominal pain. All of the patients recovered with no death or complication. Sixteen patients with no infection comprised group 3 (control group) (Table 2). There were no significant differences in mean age and gender distribution among all the groups (Table 2).
IL-6 and CRP levels

Serum IL-6 levels were higher in groups 1 and 2 than in group 3 (the control group) \((p < 0.001, \text{multiple comparisons})\); and there was no difference between groups 1 and 2 (Table 2).

Serum CRP levels were higher in groups 1 and 2 than in group 3 \((p < 0.005, \text{multiple comparisons})\) (Table 2). However, there were no significant differences between groups 1 and 2.

IL-6 and CRP as diagnostic tests

To evaluate the IL-6 and CRP as diagnostic tests, we used data obtained by measurements of IL-6 and CRP in children who were initially suspected of having an infection to compare the difference in those with no infection (group 1 to group 3). The most appropriate cut-off value for IL-6 used alone as a diagnostic test was 20 pg/mL. For CRP alone, a value above or equal to 2 mg/dL was used as the cut-off value. By using IL-6 and CRP in combination, the appropriate cut-off values were 20 pg/mL for IL-6 and 1 mg/dL for CRP. The combined use of IL-6 and CRP as a diagnostic test yielded a sensitivity of 94%, a specificity of 63%, a positive predictive value of 79%, and a negative predictive value of 87% (Table 3).

DISCUSSION

The host response to bacteremia, if not identified and treated early, may progress to a clinical situation of sepsis as so-called with tachypnea, tachycardia, pyrexia or hypothermia and neutropenia or neutrophilia. This can subsequently advance to altered or granulocytic response with fever, refeeding associated with septic shock and multiorgan failure.

Increased IL-6 concentrations have also been associated with a number of disease states, including glomerulonephritis, malignancy, autoimmune disease, burn and renal transplant rejection. IL-6 is also increased in infants with bacterial sepsis and necrotizing enterocolitis, children with sepsis, and adults with sepsis, septic shock and multiorgan failure.

CRP has a high specificity for bacterial infections in children, but a CRP increase is often not seen until 12 to 24 hours after the start of the infection, so it seems to be less useful as a diagnostic test at the early stage of infection. In our study, the combined use of IL-6 and CRP showed a high sensitivity and high negative predictive value. As a diagnostic test for early-onset bacterial infection in children, the combined use was superior to the single use of either IL-6 or CRP alone. Similar conclusions have been reached by Buck et al. and Doellner et al.

However, because of its low specificity and low positive predictive value, the usefulness of the combined parameter is limited in its ability to distinguish infection from non-infected children.
In this study, IL-6 val ues at acute phases of dis ease could not dis tin guish sep sis, pneu mo nia and pro ba ble in fec tion. This in di cated that IL-6 me di ated a non-spe cific in flam mar tory re sponse in tis sue in jury and in fec tions.\textsuperscript{4}

The po si tion ing of the cut-off value, whether IL-6 is posi tive or not, is de bat able. Some authors have pro posed a cut-off value as low as 20 pg/mL, and oth ers have pro posed up to 500 pg/mL.\textsuperscript{9,27} Vari a tions in cut-off val ues re sult main ly from the dif fer ent meth ods of mea sur ing IL-6. In this study, we used a cut-off value of 20 pg/mL. At this point, IL-6 had a sen si tiv ity of 68%, a specific ity of 88%, a positive pre di c tive value of 91%, and a neg a tive pre di c tive value of 58%.

In sum mar y, the IL-6 value is use ful in the pre di c tion of sep sis and se vere in fec tion in the acute phase of dis ease in chil dren, es pecially when com bined with CRP. But as says of IL-6 take sev eral hours to com plete, and the lack of an op ti mal thresh old limit its prac ti cal ap pli ca tion.

ACKNOWLEDGEMENTS

This study was sup ported by grants from Tai pei Vet er ans Gen er al Hos pi tal.

REFERENCES


