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Circulating Interleukin (IL)-1 Beta, IL-6 and Tumor Necrosis Factor-Alpha in Children with Febrile Infection - A Comparison with C-Reactive Protein

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SUMMARY **Circulating interleukin (IL)-1 beta, IL-6, and tumor necrosis factor (TNF)-alpha were examined in 42 febrile children with fever lasting more than 4 days. Their diagnosis were probable viral syndrome in 22, urinary tract infection(UTI) in 10, and probable bacterial pneumonia in 10. None of our study patients had detectable serum IL-1 beta. TNF-alpha levels were significantly higher in children with pneumonia than in those with viral syndrome ($p < 0.01$). Children with UTI and pneumonia had significantly higher IL-6 and CRP, compared to those with probable viral syndrome($p < 0.01$ for both IL-6 and CRP). When appropriate cutoff values are chosen, IL-6 had greatly improved specificity (86.4%, > 20 pg/ml) to demonstrate UTI and pneumonia, as compared to that using CRP (48%, > 40 mg/l). After three days' antibiotic treatment, IL-6 fell to control levels in children with UTI and pneumonia, while CRP remained elevated. There was no difference in TNF-alpha values before and after treatment. Thus, IL-6, rather than IL-1 beta and TNF-alpha, may be a helpful diagnostic tool for evaluation of pediatric febrile infection. Sequential studies involving more patients are needed to determine whether IL-6 is better than CRP in this clinical setting.**

It's a common diagnostic dilemma for the pediatrician to differentiate between viral and bacterial infection when caring for children with prolonged febrile illness. C-reactive protein (CRP) is an acute phase protein synthesized by hepatocytes in response to tissue injury and inflammation. Elevation of serum CRP levels may suggest the possibility of bacterial infection in situations where microbiological diagnosis is difficult or too slow in the clinical context.[1,2] In general, CRP elevations in acute bacterial infections tend to be in the range of 100 to 350 mg/l, while CRP values in most acute viral infections tend to be much lower, < 20-40 mg/l.[3,4] However, this distinction is by no means absolute, and CRP values > 100 mg/l can occur in common pediatric infections caused by adenovirus, cytomegalovirus, influenza virus, etc.[5]

Proinflammatory cytokines like interleukin(IL)-1 beta, tumor necrosis factor (TNF)-alpha, and IL-6 are important in the induction of acute phase protein production by hepatocytes.[6] They are mainly produced by macrophages and monocytes in patients with severe infection and inflammation, particularly when associated with sepsis and endotoxic shock.[7,8] The value of serum IL-6 measurement in neonatal sepsis[9] and children with neutropenic fever[10] has been extensively studied, and generally regarded as a sensitive marker of acute bacterial infection. However, the role of cytokine measurement in pediatric community-acquired infection has been scarcely discussed.[11]

In this preliminary study, we investigated the value of serum IL-1 beta, TNF-alpha, and IL-6 measurement in differentiating the cause of

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infection in children with prolonged febrile illness, in comparison with CRP. We also evaluated the cytokine profiles during the acute and convalescent phase of the illness in children with probable bacterial infections.

PATIENTS AND METHODS

Serum IL-1 beta, IL-6, TNF-alpha, and CRP levels were examined in 42 previously healthy children who visited our clinic or Emergency room with acute febrile infections fulfilling the following criteria: 1) fever over 38°C lasting for at least 4 days and peak body temperature over 39°C; 2) febrile when the serum sample was obtained; 3) no evidence of acute otitis media nor documented use of antibiotics within 1 week. The mean age of the subjects was 4.7 ± 2.5 years (mean ± standard error of mean), and varied between 5 months and 15 years. The male to female ratio was 24:18. Serum samples were obtained on day 5.3 ± 0.4 of the illness. Sera were immediately separated and stored at -20°C until assayed. Follow-up studies were performed in 10 patients suspected of bacterial infection, and comparisons were made among various parameters during the febrile and convalescent phase.

Serum CRP levels were measured by immunoturbidimetry (Olympus Au 560; Olympus, Tokyo, Japan). Serum IL-1 beta, IL-6, TNF-alpha were determined by using a solid phase, two site chemiluminescent assay (IMMULITE® system, DPC Biermann, Bad Nauheim, Germany). The lower limit of detection in the IL-1 beta, IL-6, and TNF-alpha assay was 5, 2, 5 pg/ml respectively. Data from ten age-matched healthy control subjects showed IL-1 beta < 5 pg/ml, IL-6 < 15 pg/ml, and TNF-alpha < 15 pg/ml in all samples tested.

Statistical analysis was performed using Mann-Whitney rank-sum test for comparison of serum cytokine and CRP levels between groups. Wilcoxon signed rank test was applied for analysis of the difference between cytokine levels before and after treatment. Correlation coefficients(r) were generated by using Pearson product-moment. A P value of < 0.05 was considered statistically significant.

RESULTS

Disease category

Ten of 42 children had urinary tract infections (UTI), as evidenced by pyuria and positive urine bacterial cultures with a colony count greater than 10^5/ml. The etiological agent of all urinary tract infections was *Escherichia coli*. Ten children had clinical diagnosis of bacterial pneumonia due to the presence of leukocytosis, findings on chest films (alveolar infiltration in four, and lobar consolidation in six), and good response to appropriate antibiotic treatment within 12-24 hours. Four of them had serological evidence of *Mycoplasma pneumoniae* infection, and one with *Staphylococcus aureus* bacteremia. The remaining 22 patients were considered to have probable viral syndrome presenting clinically with symptoms of upper respiratory tract infections or gastroenteritis. No radiological or microbiological evidence suggesting bacterial infection was present in these patients. All of the 42 patients recovered without complication.

Serum cytokines and CRP levels in various infections

Serum IL-1-beta, IL-6, and TNF-alpha, and CRP levels were measured in all 42 patients. None of our patients had detectable serum IL-1 beta (< 5 pg/ml). Significant correlation was found between serum CRP and IL-6 ($r = 0.55$, $P < 0.01$). However, there was no significant correlation between serum levels of CRP and TNF-alpha ($r = 0.19$, $P = 0.26$).

Table 1 delineates the mean serum IL-6, TNF-alpha, and CRP levels during the acute stage of various infections. Serum IL-6 levels in children with UTI and pneumonia were significantly higher than those in children with viral syndrome ($P < 0.01$ for both UTI and pneumonia). Patients with UTI and pneumonia also had significantly higher serum CRP levels than those with viral syndrome ($P < 0.01$ for both). Higher levels of serum TNF-alpha were also observed in patients with pneumonia ($P = 0.006$), but not in children with UTI ($P = 0.24$), as compared to children with viral syndrome.

To determine the ability of CRP, TNF-alpha, and IL-6 to detect bacterial infection (UTI and pneumonia as a whole), we arbitrarily used cut-off values of CRP > 40 mg/l, TNF-alpha > 20 pg/ml, and IL-6 > 20 pg/ml to evaluate our patients. As shown in Table 2, use of the CRP levels had highest sensitivity, while the specificity of CRP (48%) was much inferior to that of IL-6 (86.4%). Use of the IL-6 > 20 pg/ml had the highest specificity and the best positive predictive value, while TNF-alpha had the lowest sensitivity and specificity of the three.

Table 1 Circulating Interleukin (IL)-6, tumor necrosis factor (TNF)-alpha, and C-reactive protein (CRP) levels in febrile children with various infections

	Probable viral syndrome (n = 22)	Urinary tract infections (n = 10)	Probable bacterial pneumonia (n = 10)
IL-6 (pg/ml)	10.3 ± 1.7	65.8 ± 23.6*	66.9 ± 34.5*
TNF-alpha (pg/ml)	18.1 ± 1.7	23.2 ± 3.4	26.4 ± 7.5*
CRP (mg/l)	45.4 ± 9.8	134.1 ± 13.3*	118.7 ± 18.2*

Data are expressed as mean ± SEM; * P < 0.01, compared to probable viral syndrome.

Table 2 Evaluation of various laboratory parameters

Parameters	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
CRP	100	48	66.6	100
TNF-alpha	65	45.9	59.1	65
IL-6	85	86.4	85	86.4

Cut-off: C-reactive protein (CRP), 40 mg/l; tumor necrosis factor (TNF)-alpha, 20 pg/ml; interleukin (IL)-6: 20 pg/ml.

Serum cytokine and CRP levels in patients with UTI and pneumonia: before and after treatment

Six patients with UTI and four patients with pneumonia had a follow-up examination after 3 days' antibiotic treatment, at which time all of these patients became afebrile with improved general condition. As shown in Fig. 1, in patients with UTI and pneumonia alike, levels of IL-6 fell into normal range on Day 3 (3.4 ± 1.0 pg/ml for UTI; 7.5 ± 2.1 pg/ml for pneumonia), while serum CRP levels, though significantly decreased compared to pretreatment levels ($P < 0.01$ for both groups), remained elevated on Day 3 (66.2 ± 15.6 pg/ml for UTI; 64.0 ± 29.1 pg/ml for pneumonia, normal range < 10 mg/l). Levels of TNF-alpha on Day 3 also remained elevated despite clinical improvement (22.7 ± 4.2 pg/ml for UTI; 21.5 ± 1.7 pg/ml for pneumonia).

DISCUSSION

In this study, we evaluated measurements of IL-1 beta, IL-6, and TNF-alpha as diagnostic tools in children who presented with fever for at least 4 days, in comparison with CRP. We have found that: 1) serum IL-1-beta was undetectable, while the pattern of TNF-alpha was erratic in our patients; 2) children with UTI and pneumonia had significantly higher IL-6 and CRP compared to those with probable viral syndrome; 3) determination of the IL-6 concentration may improve the specificity for detecting bacterial infection by using CRP alone; 4) IL-6 levels fell to normal range, while CRP levels were still elevated after three days' antibiotic therapy in children with UTI and pneumonia.

Serum IL-6 levels measured in our patients with UTI and pneumonia, though elevated, were low compared with those reported in neutropenic children with bacterial infection.[10] This finding may be related to the very short half life of IL-6, as reported by Castalli *et al.*[12] Our patients had been febrile for more than 4 days before blood was drawn for IL-6 determination, at which time IL-6 might have declined significantly. Therefore, even minor increases of IL-6 levels may have clinical significance, while CRP levels, which usually peaked later than IL-6,[10] were greatly elevated in our patients with UTI and pneumonia, probably due to a longer half life of 4-7 hours, as reported by Young *et al.*[13]

Deodar *et al.*[3] reported that CRP values in most viral infections are lower than 40 mg/l. Putto *et al.*[1]

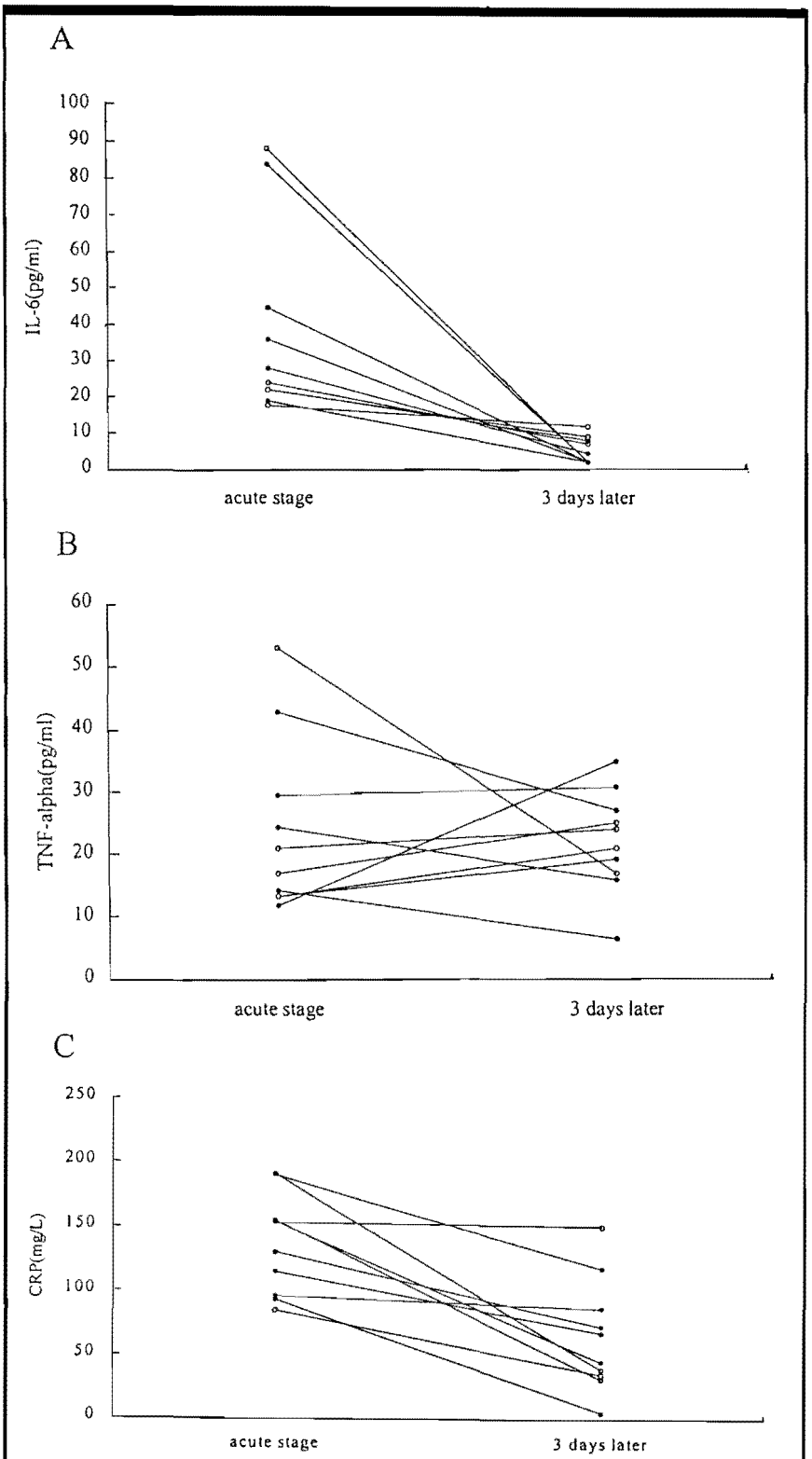

Fig. 1 Serum concentrations of A: interleukin-6 (IL-6); B: tumor necrosis factor (TNF)-alpha, and C: C-reactive protein (CRP) before and three days after antibiotic therapy in six children with urinary tract infections (solid dot), and four children with pneumonia (open dot).

indicated that a value of CRP > 40 mg/l detected 79% of bacterial infections with 90% specificity in children with febrile illness. However, we found a much lower specificity (48%) for detecting bacterial infections using a cut-off value of CRP 40 mg/l, which can be improved greatly by using IL-6 20 pg/ml as a cut-off point (specificity 86.9%). Ten (45.5%) of our 22 patients with probable viral syndrome had CRP levels greater than 40 mg/l, whose IL-6 levels remained low. Therefore, serum IL-6 measurement may improve the specificity of CRP for evaluating children with prolonged fever who have no clinical clue for bacterial infection. Low levels of IL-6 could be reassuring and may obviate the need for empirical antibiotic therapy in these patients.

We found that even though CRP levels in patients with UTI and pneumonia decreased in response to antibiotic treatment, the values remained abnormally elevated on Day 3. On the contrary, IL-6 in these patients became undetectable. CRP has been shown to be a useful marker for guiding duration of antibiotic therapy in suspected neonatal bacterial infection.[14] Likewise, determination of IL-6 may be useful in evaluating the effectiveness of antibiotic treatment, and persistent high levels of IL-6 could indicate the need for treatment modification. Further controlled study using IL-6 values as a basis for therapeutic decision is needed.

In conclusion, of the three pro-inflammatory cytokines, IL-6 seems superior as a diagnostic tool for infectious disease in children with prolonged febrile infection, while measurement of IL-1 beta and TNF-alpha is of limited use. Our pre-

liminary findings that IL-6 might improve the specificity of CRP for detecting bacterial infections, encourage further studies involving more children with community-acquired infection.

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