Interleukin (IL)-6 and IL-8 in Children with Febrile Urinary Tract Infection and Asymptomatic Bacteriuria

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Urine and serum interleukin (IL)-6 and IL-8 responses were higher in children with febrile urinary tract infection \( (n = 61) \) than in those with asymptomatic bacteriuria \( (n = 39) \). By univariate analysis, cytokine levels were related to age, sex, reflux, renal scarring, urine leukocytes, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and bacterial properties (P fimbriae but not hemolysin). Multivariate modeling showed that urine IL-6 responses were higher in girls than boys, increased with age, and were positively associated with CRP, ESR, serum IL-6, and urine leukocyte counts. The urine IL-8 response was not influenced by age, but it was influenced by P fimbriae and was associated with ESR, CRP, urine leukocytes, and female sex. The results show that cytokine responses to urinary tract infection vary with the severity of infection and that cytokine activation is influenced by a variety of host and bacterial variables.

The symptoms and signs of urinary tract infection (UTI) depend on the host response to the infecting bacterial strain [1]. Local and systemic inflammatory changes cause fever and the symptoms that characterize acute pyelonephritis. In patients with asymptomatic bacteriuria (ABU), the bacteria do not generate a host response of sufficient magnitude to cause symptoms. *Escherichia coli* strains that cause acute pyelonephritis or ABU are known to differ in virulence [2], but the molecular basis for the difference in host response and in clinical appearance is not well understood.

Cytokines, such as interleukin (IL)-6 and IL-8, are activated in patients with UTI [3-12]. It is speculated that cytokines are mediators of the host responses to UTI and that a difference in magnitude or quality (or both) of the cytokine response among patients with acute pyelonephritis and ABU underlies the difference in clinical presentation [5]. The aims of the present study were to compare the IL-6 and IL-8 responses with UTI among children with acute pyelonephritis and ABU and to analyze the influence of bacterial and host parameters on the cytokine response using univariate and multivariate techniques.

**Patients and Methods**

The study included 61 children from a prospective study of febrile UTI [13] and 39 children with ABU from a screening study [14]. To be included in the febrile group, children (2 months to 6 years old) had to be experiencing their first known symptomatic UTI episode, have a fever of at least 38.5°C within 24 h of diagnosis, and have bacteriuria, as determined by culture of urine obtained by suprapubic bladder aspiration (any growth), by uniform growth of at least 10^5 bacteria/mL in 2 consecutive urine samples, or by growth of at least 10^5 bacteria/mL in 1 urine sample and a positive nitrite test. Urine samples for cytokine analysis were available from 48 febrile children, and serum samples were available from 38. ABU was detected by screening 3581 children at 2 weeks, 3 months, and 10 months of age and was confirmed by culture of urine obtained by suprapubic bladder aspiration. Samples for cytokine analysis were available from 39 children with ABU (9 girls and 30 boys).

Reflux was detected by cystourethrography [15] in 18 children with acute pyelonephritis (4 with grade 1, 10 with grade 2, and 4 with grade 3 reflux on a 5-grade scale) and in 4 others with ABU (2 with grade 1 and 2 with grade 2). Renal scarring was detected by urography [16] in 2 children with acute pyelonephritis at the time of diagnosis and in another 7 children at follow-up (new scarring). No scarring was detected in the ABU group at inclusion or follow-up.

*E. coli* were isolated from children in 59 febrile episodes; the remaining 2 children were infected with *Klebsiella* and *Enterococ-
Table 1. Cytokine responses and host and bacterial variables in children with UTI.

<table>
<thead>
<tr>
<th></th>
<th>Asymptomatic bacteriuria</th>
<th>Febrile UTI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine IL-6 (U/mL), n</td>
<td>39</td>
<td>48</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>serum IL-6 (U/mL), n</td>
<td>0 (0)</td>
<td>28 (0–760)</td>
<td></td>
</tr>
<tr>
<td>Urine IL-8 (pg/mL), n</td>
<td>37</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>serum IL-8 (pg/mL), n</td>
<td>0 (0–380)</td>
<td>88 (0–3870)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>age (years)</td>
<td>0.2 (0.1–0.9)</td>
<td>0.9 (0.2–5.8)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Girls/boys</td>
<td>9/30</td>
<td>44/17</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>reflux (+/-)</td>
<td>4/33</td>
<td>18/43</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>New scarring (+/-)</td>
<td>0/28</td>
<td>7/51</td>
<td></td>
</tr>
<tr>
<td>Urine leukocytes (cells/µL)</td>
<td>22 (0–4000)</td>
<td>700 (11–10,000)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>1 (1–21)</td>
<td>100 (1–200)</td>
<td></td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>9 (1–40)</td>
<td>45 (2–72)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>No. of Escherichia coli</td>
<td>32</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>P fimbriae (+/-)</td>
<td>9/23</td>
<td>46/13</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Hemolysin (+/-)</td>
<td>13/19</td>
<td>36/23</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Except as indicated by +/- (present/absent) and for girls/boys, data are median (range); CRP, C-reactive protein; ESR, erythrocyte sedimentation rate.

E. coli species, respectively. E. coli caused 32 of the episodes in children with ABU; 2 were infected with Proteus species, 1 with Staphylococcus aureus, 3 with Klebsiella species, and 1 with Enterococcus species. P fimbriae were identified by the ability of the bacteria to induce P blood group–dependent mannose-resistant agglutination of human erythrocytes [17, 18]. Hemolysin production was assessed in nutrient agar with 5% washed horse erythrocytes.

The host response to UTI was analyzed in blood and urine samples obtained at study entry. Erythrocyte sedimentation rate (ESR, millimeters per hour) and C-reactive protein (CRP, milligrams per liter) were quantitated. Leukocytes in uncentrifuged urine were counted using a Fuchs-Rosenthal chamber. The IL-6 activity in serum and urine samples was determined using the B9 bioassay with neutralizing anti-IL-6 antibodies [19]. Urine IL-6 levels were confirmed by ELISA, based on the M16 monoclonal anti-human IL-6 antibody, with polyclonal antibodies for detection [20]. Serum IL-6 levels were confirmed using the Medgenix test (Medgenix Diagnostics, Fleurus, Belgium). IL-8 levels in serum and urine were determined by ELISA (reagents were provided by M. Ceska, Sandoz, Vienna) [21]. IL-6 levels ≥20 U/mL and IL-8 levels ≥30 pg/mL were considered positive (responders) [4, 6].

The savage score method [22] and Fisher’s exact test were used for univariate comparisons. Spearman’s rank correlation test was used to compute univariate correlations. Principal component analysis [23] and partial least squares to latent structures [24] were used for multivariate analysis.

Results

Children with febrile UTI had higher IL-6 levels in urine and serum and higher urine IL-8 levels than the children with ABU (table 1). A urine IL-6 response (≥20 U/mL) occurred in 30 (63%) of 48 children with febrile UTI and in 0 of 39 with ABU (P < .01). Elevated urine IL-8 levels (≥30 pg/mL) were found in 13 (76%) of 17 children with febrile UTI compared with 11 (30%) of 37 children with ABU (P < .01). There was a serum IL-6 response in 19 of 38 children with febrile UTI, but only 1 (3%) of 30 with ABU (P < .01) had a response. Five (19%) of 26 children with ABU had a serum IL-8 response. Serum for analysis of IL-8 was not available from subjects in the febrile group.

The patients with ABU differed from those with febrile UTI by age, sex, reflux, inflammatory response, and properties of the infecting E. coli strain (table 1). By univariate analysis, these variables all had a significant association with the cytokine response. In the combined febrile and ABU groups, urine IL-6 levels increased with age (P < .001). The median urine IL-6 levels were significantly higher in girls than in boys (P < .001) and in children with reflux (P < .001) or new renal scarring (P < .05) than in those with radiologically normal urinary tracts. Of 49 children infected with P fimbriated E. coli, 23 (47%) had elevated urine IL-6 levels compared with 6 (20%) of 30 children infected with other E. coli strains (P < .01). There was a correlation of urine IL-6 levels to urine leukocytes, CRP, and ESRs (all P < .001).

In the febrile group, the urine IL-6 levels increased with age (P < .01) and were higher in girls (P < .01) and in children with reflux (P < .01) than in boys or children without reflux, respectively. In the ABU group, serum IL-6 was correlated to CRP (P < .01).

Univariate analysis of the combined study group showed that serum IL-6 levels increased with age (P < .01). The median
Figure 1. A, 3-dimensional projection of febrile UTI (filled symbols) and asymptomatic bacteriuria (ABU); (open symbols) based on 12 study variables: age, sex, reflux, renal scarring, C-reactive protein (CRP), erythrocyte sedimentation rate (SR), leukocytes, P fimbriae, hemolysin, serum IL-6, urine IL-6, and urine IL-8. Fever was diagnostic criterion and therefore excluded from analysis. B, 2-dimensional, variable-oriented projection of combined febrile UTI and ABU groups. UIL8 = urine IL-8, PFM = P fimbriae, HEMO = hemolysin, ULEU = urine leukocytes, SIL6 = serum IL-6, UIL6 = urine IL-6, SCAR = renal scar. t[1] to t[3] are first 3 principal components, that is, t[1] is combination of variables that explains largest part of variance, t[2], orthogonal to t[1], is another combination of variables that explains largest part of residual variance, and so on. Loading scores p[1] and p[2] are coefficients with which variables are combined to form principal components t[1] and [2].

serum IL-6 levels were higher in girls than in boys (P < .01) and higher in children with reflux (P < .01) or in those with new scarring (P < .01) than in those without radiologic abnormalities. There was a correlation between serum IL-6 levels and urine leukocytes (P < .01), ESRs (P < .05), and CRP (P < .001). In the febrile group, serum IL-6 levels were elevated in children with new scarring (P < .05) and reflux (P < .01) but showed no association with the other study variables.

Univariate analysis of the combined study groups showed that the median urine IL-8 levels were higher in girls than in boys (P < .05) and higher in children infected with P fimbriated E. coli (P < .01) than with other E. coli strains. The urine IL-8 levels showed a correlation to urine leukocytes (P < .01), CRP (P < .01), and ESRs (P < .01). Urine IL-8 showed a poor association with the study variables when the febrile and ABU groups were analyzed separately.

The data matrix with 12 variables (age, sex, reflux, new scarring, urine leukocytes, urine IL-6, urine IL-8, serum IL-6, CRP, ESR, P fimbriae, hemolysin) was reassessed using principal component analysis (figure 1A). The children with ABU formed a tight cluster, well separated from the febrile group. In contrast, two subsets were distinguished in the febrile group; a two-dimensional, variable-oriented principal component analysis was used to resolve the variables characteristic of the two clusters (figure 1B). One cluster was characterized by infection with P fimbriated and hemolysin-positive E. coli, by elevated levels of urine IL-8 and leukocytes, and elevated ESRs. The other cluster was characterized by the absence of these bacterial parameters, elevated urine and serum IL-6 levels, reflux, scarring, and older age. CRP did not further contribute to the separation of these groups.

Partial least squares-to-latent structures analysis on the combined data set (ABU and febrile UTI) showed a significant association between urine IL-6 and CRP, female sex, ESR, serum IL-6, age, and urine leukocytes. A model with these variables explained 47% of the variation in urine IL-6. Urine IL-6 in the febrile group was influenced by female sex, reflux, serum IL-6, and age, explaining 32% of the variation. In the combined data set, urine IL-8 showed an association with ESR, P fimbriae, CRP, urine leukocytes, and female sex, but these variables explained only 13% of the variation in urine IL-8. Reflux was negatively correlated with urine IL-8. No significant models for urine IL-8 were found when the ABU and febrile groups were analyzed separately. Analysis of variables influencing serum IL-6 and serum IL-8 levels gave no significant models in any of the groups.

Discussion

Infections of the urinary tract activate local and systemic cytokine responses [3–12]. This study demonstrated that the
serum IL-6, urine IL-6, and urine IL-8 levels were higher in children with febrile UTI than in those with ABU. The clear correlation between cytokine levels and disease severity suggested that the cytokine response provides a partial explanation for the link between bacterial infection, inflammation, and the severity of UTI.

Children with ABU and febrile UTI differ in age, sex, reflux, and renal scarring [25]. In addition, there are differences in virulence of the causative E. coli strains and in the responses to infection (measured as fever, CRP, ESR, and leukocytes in blood and urine). In the present study, we observed that the cytokine responses followed the background variables associated with acute pyelonephritis. IL-6 increased with age and was higher in girls and children with reflux or renal scarring than in boys or children with radiologically normal urinary tracts. These results show that the same host variables are associated with increased cytokine responses and acute pyelonephritis. We speculate that persons with a tendency to respond with high cytokine levels are more likely to develop acute pyelonephritis. Conversely, the ABU group may include persons who are low cytokine responders and who consequently remain asymptomatic despite the presence of bacteria in the urinary tract.

Both IL-6 and IL-8 are important early mediators of inflammation. IL-6 is an endogenous pyrogen, activator of acute-phase reactants, including CRP, and a maturation factor for mucosal lymphocytes. IL-8 is a chemoattractant for neutrophils. Release of cytokines from the site of infection precedes the onset of fever, acute-phase responses, and neutrophil responses [6]. Despite the difference in kinetics of the responses, we had expected to find higher IL-6 and IL-8 levels in patients with fever, acute-phase reactants, or marked urine neutrophil responses. Univariate and multivariate analysis of the combined febrile and ABU groups showed that urine IL-6, serum IL-6, and urine IL-8 levels were significantly related to CRP, ESRs, and leukocyte counts. These associations did not remain significant when the children with febrile UTI or ABU were analyzed separately, suggesting that the differences were valid for the groups but not for individual patients.

We observed several differences between the IL-6 and IL-8 host response patterns. IL-6 but not IL-8 varied with age, renal scarring, and reflux. Principal component analysis suggested that the febrile UTI group could be separated into two subsets according to the two-dimensional, variable-oriented projection. One subset consisted of younger P fimbriated E. coli–infected children with high IL-8 and leukocyte levels and high ESRs. This subset probably contains children who have uncomplicated febrile UTI, experience single episodes of infection caused by bacteria of high virulence, are treated, and rarely go on to develop renal scarring. The neutrophil-dominated inflammatory response in such patients may even contribute to the elimination of bacteria from the urinary tract [6]. The second subset of children was older, had elevated IL-6 levels, vesicoureteric reflux, renal scarring, and were infected with P fimbriab-negative and nonhemolysin-producing bacteria more often than the first group. This subset may contain the patients with vesicoureteric reflux who develop recurrent acute pyelonephritis and run a higher risk to develop renal scarring.

This study shows that cytokine responses to UTI vary with the severity of infection and that cytokine activation is influenced by a variety of host and bacterial variables. Taken together, these results indicate that cytokine measurement can be used to further discriminate between patients with symptomatic UTI and ABU and to define patient groups for further study.

Acknowledgment

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References

14. Wettergren B, Jodal U, Jonasson G. Epidemiology of bacteriuria during