Evaluation of Inflammatory and Renal-Injury Markers in Women Treated with Antibiotics for Acute Pyelonephritis Caused by Escherichia coli

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The evolution and the relationship between inflammatory and renal-injury markers in women with acute uncomplicated pyelonephritis under antimicrobial therapy were investigated in a prospective study. Markers were measured before and after 6 and 24 h after the intravenous administration of 1 g of ceftriaxone. Before treatment, the median levels of all markers except the serum creatinine levels were high. Twenty-four hours after the onset of antibiotic treatment, the C-reactive protein (CRP) level continued to be high, while the serum interleukin-6 (IL-6) and IL-8 levels and the urine IL-6, IL-8, albumin, and immunoglobulin G (IgG) levels decreased significantly. In contrast, serum creatinine and tumor necrosis factor alpha levels and urine N-acetyl-b-glucosaminidase, a2-microglobulin, and b2-microglobulin levels did not change over time. There was a significant correlation between IL-6 and IL-8 levels and urine albumin and IgG levels (urine albumin and IgG levels are glomerular and urinary tract-injury markers) as well as between serum CRP levels and the levels of the tubular-injury markers. In women with acute pyelonephritis, appropriate antibiotic treatment rapidly decreases serum IL-6 levels and urine IL-6 and IL-8 levels, which correlate well with urine albumin and IgG levels.

Although acute pyelonephritis (APN) causes less morbidity in adults than in the pediatric population, in which APN may lead to renal scars and altered renal function and/or arterial hypertension (3), little is known about the magnitude of renal damage caused by APN in adults or its relationship to the inflammatory process. This is interesting because the antibiotics used to treat infections caused by gram-negative bacteria may exert a proinflammatory effect via endotoxin release, and this effect can be stronger or weaker, depending on the class of antibiotic used (21, 22). Hence, in APN and other more severe infections caused by gram-negative bacteria, it is conceivable that an antibiotic-induced systemic response and/or organ injury mediated by inflammation may occur (16, 22). This could have implications in adult patients with a high risk of renal insufficiency, such as patients with one kidney, renal transplant recipients, or patients with advanced cirrhosis.

Elevations of urine and serum cytokine levels have been observed in patients with different forms of urinary tract infection (UTIs), especially those with upper UTIs (11, 15). Interleukin-6 (IL-6) is an endogenous pyrogen and an activator of acute-phase reactants and lymphocytes (14). In patients with febrile UTIs, IL-6 reflects the degree of inflammatory response in the urinary tract (19). IL-8 is a chemoattractant for neutrophils (1), and in patients with UTIs it has been related to the degree of pyuria and renal scarring (10). A relationship between the degree of inflammation in APN and renal dysfunction and scarring has been reported previously (10, 12, 24). Urinary albumin and immunoglobulin G (IgG) are considered markers of glomerular dysfunction (2, 23). N-Acetyl-b-glucosaminidase (NAG) is a renal hydrolytic enzyme located primarily in the lysosomal fraction of the renal tubular cell, and in the event of proximal renal tubular damage, its concentration in urine increases (20). Urinary a1-microglobulin and b2-microglobulin have been proposed to be markers of proximal tubular dysfunction (4, 5). Nearly all a1- and b2-microglobulins filtered by the glomerulus are reabsorbed by the proximal tubule; increased urinary excretion of these proteins denotes malfunction of the proximal portion of the renal tubule (7). It has been shown that in the absence of nephrotic compounds or previous renal diseases, the urine a1-microglobulin level is a useful marker of tubular damage in patients with APN (5). In fact, patients with APN have elevated urine a1-microglobulin levels, whereas patients with cystitis do not (6).

In order to establish the evolution of inflammatory- and renal-injury markers (RIMs) in patients with APN receiving antibiotic therapy, as well as the possible correlation between them, we conducted an observational study with women with uncomplicated APN treated with ceftriaxone.

MATERIALS AND METHODS

Women with uncomplicated APN, defined as fever (axillary temperature, >37.9°C), flank pain, and pyuria and a culture of urine positive for a uropathogen (>100,000 CFU/ml) in the absence of urological abnormalities, were ran-
TABLE 1. Levels of inflammation markers at different stages of the study and significance level by Wilcoxon signed-rank test

| Inflammation marker and time of evaluation | Median (IQR) | P (Wilcoxon test) |
|-------------------------------------------|--------------|-------------------|
| Serum CRP (mg/dl)                         |              |                   |
| Before                                    | 11.1 (5.9, 14.9) | 0.213            |
| 6 h                                       | 13.4 (8.5, 15.6) | 0.484            |
| 24 h                                      | 12.4 (6.8, 16.6) | 0.064            |
| Serum TNF-α (pg/ml)                       |              |                   |
| Before                                    | 35 (23, 77)  | 0.995             |
| 6 h                                       | 45 (29, 56)   | 0.057             |
| 24 h                                      | 34 (26, 48)   | 0.430             |
| Serum IL-6 (pg/ml)                        |              |                   |
| Before                                    | 97 (43, 152)  | 0.975             |
| 6 h                                       | 81 (42, 159)  | 0.005             |
| 24 h                                      | 44 (23, 90)   | 0.035             |
| Urine IL-6 (pg/ml)                        |              |                   |
| Before                                    | 81 (36, 207)  | 0.394             |
| 6 h                                       | 80 (22, 146)  | 0.014             |
| 24 h                                      | 17 (10, 42)   | 0.014             |
| Urine IL-8 (pg/ml)                        |              |                   |
| Before                                    | 433 (139, 828) | 0.975          |
| 6 h                                       | 353 (136, 660) | 0.001           |
| 24 h                                      | 59 (12, 133)  | 0.002             |

* Before, 6 h, and 24 h indicate before antibiotic administration and 6 and 24 h after antibiotic administration, respectively. Reference values: CRP, 0.1 to 0.8 mg/dl; TNF-α, <15 pg/ml; serum IL-6, <5 pg/ml; urine IL-6, <5 pg/ml; urine IL-8, <126 pg/ml.

a For each marker, the first entry is for the comparison of the values for before and 6 h after antibiotic administration, the second entry is for the comparison of the values for 6 and 24 h after antibiotic administration, and the third entry is for the comparison of the values for 24 h after and before antibiotic administration.

RESULTS

Twenty-two women with uncomplicated APN were studied. The mean (standard deviation) age was 49.4 (21.8) years. For
all but one of the patients the urine culture yielded *Escherichia coli*. Blood cultures were positive for four patients (18%), with *E. coli* growing in all of them. Patients were afebrile in a mean (standard deviation) of 1.64 (0.84) days after the onset of antibiotic therapy.

The median (interquartile range [IQR]) levels of inflammation markers in serum and urine before administration of the first dose of 1 g of ceftriaxone and 6 and 24 h later and the significance levels determined by the Wilcoxon signed-rank test are shown in Table 1. At the baseline, the levels of all inflammation markers were 2 to 19 times higher than the reference levels. Serum IL-6, urine IL-6, and urine IL-8 concentrations decreased significantly 24 h after antibiotic administration, with no changes at 6 h. Serum CRP and TNF-α levels did not change significantly and remained high during the first 6 and 24 h (Table 1).

Median urine RIM levels at the baseline and 6 and 24 h after the administration of 1 g of ceftriaxone and the significance level determined by the Wilcoxon signed-rank test are shown in Table 2. With the exception of urine NAG and serum creatinine levels, RIM levels were high at the baseline. After the onset of antibiotic therapy, the median α₁-microglobulin and β₂-microglobulin levels in urine at 6 h and the median NAG levels in urine at 24 h were higher than the baseline levels; however, the comparisons of the distributions were not statistically significant. Urine IgG levels decreased significantly after 24 h, with no change at 6 h, while urine albumin levels decreased significantly after 6 and 24 h from the onset of antibiotic treatment. Creatinine levels did not change significantly during the study period (Table 2).

Table 3 shows the levels of significance of the correlation between each inflammation marker and each RIM at the time points studied. A relation between CRP levels and the levels of tubular-injury markers was found at the three time points: an increment in the CRP level is related to an increment in the tubular-injury marker level. The same kind of relation was found between CRP and urine albumin levels at 6 h and 24 h after the onset of treatment. There was no correlation between TNF-α levels and the levels of any of the RIMs studied except for urine IgG levels at 6 h. Serum and urine IL-6 levels and urine IL-8 levels correlated well with urine albumin and IgG levels at the three time points studied and with urine α₁-microglobulin, β₂-microglobulin, and NAG levels at 24 h. Serum IL-6 levels also correlated with urine NAG levels at the baseline, with β₂-microglobulin levels at 6 h, and with α₁-microglobulin levels at the three time points studied (Table 3).

### DISCUSSION

In the present study we analyzed the local and systemic inflammatory responses in patients with APN as well as the associated renal damage before and after the onset of effective antimicrobial therapy. The levels of the proinflammatory cytokines and CRP were elevated at the baseline. However, during the first 24 h of appropriate treatment a different evolutionary pattern emerged. On the one hand, no changes in TNF-α and CRP levels were detected throughout the study period; on the other hand, there was a significant decrease in IL-6 and IL-8 levels after the first 6-h period, during which they were stable. High levels of proinflammatory cytokines have previously been described in patients with upper UTIs (11); however, studies describing the evolving pattern of inflammation in adults with APN during antibiotic treatment are scarce. The rapid decline in serum IL-6 and urine IL-6 and IL-8 levels after the onset of antimicrobial therapy in this study suggests that in women with APN effective antimicrobial therapy significantly reduces the inflammatory process in 24 h. This has recently been reported in children with APN (13). The different patterns of CRP and interleukins can be explained in part by the longer half-life of CRP than those of the interleukins (8, 21) and because in patients with septic conditions the production of proinflammatory cytokines is down-regulated (21). We do not have a satisfactory explanation for the lack of a decline in TNF-α levels, but a similar evolutionary profile, despite significant decreases in the levels of other proinflammatory interleukins, has been described previously (21).

One interesting finding of our study is that the urine albumin and IgG levels followed the evolution of the IL-6 and IL-8 levels, respectively, with a significant correlation at the three time points of evaluation used in the study. Although urine IgG and albumin levels can be elevated by the urinary tract inflammatory response itself and could be considered markers of generalized urinary tract inflammation, they can also reflect glomerular damage (2, 23). The correlation between cytokine levels and renal and urinary tract injury may reflect in part the underlying link between bacterial infection, inflammation, and organ injury. In fact, in patients with renal scarring urine IL-8 levels are significantly higher than those in controls (10). Moreover, exogenous administration of cytokines has induced
TABLE 3—Continued

Table: Spearman’s rho value (P*r*)

| Serum IL-6 | Urine IL-6 | Urine IL-8 |
|------------|------------|------------|
| Basal | 6 h | 24 h | Basal | 6 h | 24 h | Basal | 6 h | 24 h |
| 0.03 (0.899) | −0.10 (0.645) | −0.20 (0.499) | −0.39 (0.079) | −0.39 (0.073) | −0.18 (0.456) | −0.18 (0.450) | 0.12 (0.618) | 0.15 (0.559) |
| 0.46 (0.029) | 0.24 (0.277) | 0.52 (0.022) | 0.32 (0.151) | 0.24 (0.281) | 0.54 (0.017) | 0.42 (0.066) | 0.40 (0.076) | 0.71 (<0.001) |
| 0.49 (0.021) | 0.45 (0.034) | 0.56 (0.012) | 0.07 (0.749) | 0.31 (0.165) | 0.49 (0.038) | 0.13 (0.596) | 0.31 (0.171) | 0.81 (<0.001) |
| 0.29 (0.195) | 0.46 (0.030) | 0.59 (0.009) | 0.20 (0.394) | 0.29 (0.189) | 0.54 (0.018) | 0.24 (0.313) | 0.39 (0.082) | 0.65 (0.003) |
| 0.60 (0.003) | 0.72 (<0.001) | 0.77 (<0.001) | 0.66 (0.001) | 0.77 (<0.001) | 0.75 (<0.001) | 0.59 (0.006) | 0.54 (0.011) | 0.80 (<0.001) |
| 0.46 (0.030) | 0.44 (0.039) | 0.39 (0.097) | 0.80 (<0.001) | 0.68 (<0.001) | 0.45 (0.053) | 0.58 (0.008) | 0.48 (0.029) | 0.56 (0.016) |

glomerular injury and enhanced renal damage in animals with glomerulonephritis (18). Thus, more renal damage occurs with a stronger inflammatory response. In this regard, it is striking that in our study, 24 h after the onset of effective antimicrobial therapy, IL-6 and IL-8 levels as well as the levels of glomerular and urinary tract-injury markers (urinary albumin and IgG) decreased significantly and nearly reached normal levels. This finding strengthens the importance of starting antimicrobial therapy as early as possible in patients with APN in order to prevent or limit renal injury. The early institution of antibiotic therapy has been shown in previous experiments with animals to mitigate the extent of renal scarring (9). On the other hand, because different antibiotics can induce a more intense or a less intense proinflammatory response (22), it is conceivable that the magnitude of organ injury could depend on the particular antibiotic used. In this regard, a previous study found a nonsignificant increase in proinflammatory cytokine levels 4 h after the administration of a selective PBP 3 binding antibiotic (ceftriaxone) which was not observed after treatment with a specific PBP 2 inhibitor (imipenem) in patients with sepsis caused by gram-negative bacteria (21). In the present study we again found that the behavior of TNF-α was different from those of IL-6 and IL-8 6 h after the onset of treatment with ceftriaxone, another beta-lactam selective for PBP 3. At that time point, serum TNF-α levels tended to be higher than those at the baseline, whereas the interleukin levels were reduced. Although differences in sampling times (4 versus 6 h) can partly justify these discrepancies, we believe that the available data altogether cast some doubts about the real magnitude of the proinflammatory effect attributable to beta-lactams selective for PBP 3 in patients with APN. However, as in previous studies differences between antibiotics were detected (21). This is an area in which additional comparative studies are clearly needed. Another application of the findings of this study is complicated UTIs. It would be valuable to know if a lack of reduction in the levels of these markers of inflammation and renal dysfunction signify an unresolved infection, such as a renal abscess or an infection caused by a drug-resistant microorganism.

The levels of the tubular-injury markers α1-microglobulin and β2-microglobulin were elevated before the onset of antibiotic treatment and remained high during the first 24 h, with no significant changes. NAG levels were not elevated at the first two times of evaluation, but they were increased at 24 h. The elevations in the levels of the tubular-injury markers could be related to glomerular damage. It is known that patients with albuminuria also have elevated levels of α1- and β2-microglobulin excretion but that this situation improves as the albuminuria resolves (17). In our study, however, the levels of the tubular-injury markers did not change, despite the decrease in albuminuria, and correlated well with CRP levels at the three time points. Our findings indicate that in APN patients, tubular damage persists longer than glomerular injury, even if the infection is being controlled. We can only speculate that inflammation-mediated glomerular alteration seems functional in nature and hence improves as the level of inflammation decreases, whereas tubular injury could be structural, as the elevation of the levels of NAG (an intracellular enzyme) suggests. The persistence of tubular dysfunction after injury has previously been shown in patients with other pathologies, such as acute urine retention, in which it may linger for more than 6 months after the obstruction is relieved (17). The importance and consequences of this finding for adults with APN remains to be elucidated. Moreover, urine α1- and β2-microglobulin levels were somewhat higher at 6 h than before the onset of therapy, although not significantly. This could be related to the proinflammatory effect of the antibiotic used (21), although the small change observed together with the lack of a correlation of the tubular-injury marker levels with the levels of TNF-α (the only cytokine which showed some increment shortly after the start of treatment) does not support this hypothesis.

From a clinical point of view, our findings indicate the need for further studies aimed at investigating the effects of different classes of antibiotics on renal inflammation and injury when they are used to treat APN. Until this issue is clarified, our findings point to the importance of starting antimicrobial therapy as soon as possible in patients with pyelonephritis to promptly reduce the inflammatory response and, eventually, prevent or limit organ damage.

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