Cytokines and chemokines in systemic and urinary tract infection by Escherichia coli
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Chapter 2

Interleukin-8, but not interleukin-6, is elevated in urine of postoperative patients with urinary tract infection.

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Abstract

**Background:** Urine of patients with urinary tract infection (UTI) contains high levels of IL-6 and IL-8. However, knowledge of the kinetics of their release in urine is limited. **Aim:** To compare the appearance of IL-6 and IL-8 in urine after uncomplicated surgery and surgery complicated by UTI.

**Patients and Methods:** Urine IL-6 and IL-8 were prospectively measured in surgical patients with a urinary catheter, who did (N=10) or did not (N=20) develop UTI. Statistics by oneway ANOVA and Mann-Whitney test.

**Results:** Although urine IL-6 increased in the two to four days preceding the bacteriological documentation of UTI, a similar increase was observed in patients who did not develop UTI. Urine IL-8 was elevated on the day UTI was diagnosed, while remaining low in controls. **Conclusion:** In postoperative UTI, urine IL-8 is a better marker for the early host response than urine IL-6.
**Introduction**

Hospital-acquired urinary tract infections (UTIs) give rise to approximately 500,000 cases a year in the United States alone. The presence of a urethral catheter is associated with 80 to 95 per cent of cases [1]. Besides bacterial factors, such as the presence of fimbriae and the production of hemolysin, host factors are considered to play an important role in the pathogenesis of UTI [2]. In particular, the inflammatory response mounted in the urinary tract may be involved in host defense against ascending UTI.

Interleukin(IL-)6 is an immunoregulatory cytokine with a wide spectrum of biological activities [3]. IL-8 is a prototypic member of the CXC chemokine family, which primarily targets neutrophils, cells commonly found in urine from patients with UTI [4]. Elevated levels of IL-6 and IL-8 have been found in urine of patients with asymptomatic bacteruria [5] and acute pyelonephritis [6, 7]. Despite mounting evidence that both cytokines play a role in these conditions, knowledge of the kinetics of their release in urine directly before and after the development of UTI and of their potential value as early markers for UTI is limited. Therefore, in the present study we prospectively followed a large group of surgical patients who received a urinary catheter (and thus were at risk for UTI), and compared sequentially measured urinary IL-6 and IL-8 concentrations in patients who did and those who did not develop UTI.

**Patients and Methods**

*Study design*

Patients older than 18 years of age, who were about to undergo major abdominal surgery, were eligible for this study. Written informed consent was obtained from all study participants and the study was approved by the ethics and research committees of the Academic Medical Center. Exclusion criteria were: serum creatinine levels higher than 110 µmol/L (men) or higher than 95 µmol/L (women), bacteriuria or any other urological disease, a urinary catheter in situ or incontinence. Patients randomly received either a suprapubical or a transurethral catheter. Catheterization was performed under general anesthesia (directly before
Chapter 2

surgery) and the catheter was connected to a closed collection system. Urine (midstream portion) was collected before catheterization and 48 hours after the catheter was removed. When the catheter was in situ, urine was collected every other day directly from the urinary bladder. Urine sediments were examined and urine cultures were performed. The rest of the sample was centrifuged at 2000 RPM for 7 min and the supernatant was stored at -20 °C until assays were performed. Criteria for the diagnosis UTI were the following: at least one of the clinical symptoms of UTI such as fever or flank pain, a positive urine sediment (> 100 cells/mm³) and a positive urine culture (>10⁵ colony forming units and < 3 bacterial species per ml urine). Each patient who developed UTI (UTI+patients) was matched for duration of catheterization with two other patients who did not develop UTI (UTI+controls).

Assays

IL-6 (PharMingen, San Diego, CA) and IL-8 (CLB, Amsterdam, the Netherlands) were measured by ELISA according to the instructions of the manufacturers. Detection limits of the assays were 16 pg/ml (IL-6) and 5 pg/ml (IL-8). Urine concentrations are expressed per mmol creatinine in order to correct for dilution of urine.

Statistical analysis

Data are given as mean and SE. Concentrations of IL-6 and IL-8 were analyzed in time by oneway ANOVA. Comparisons between different patient groups were done by Mann-Whitney U test. α was set at 0.05. Suprapubical and transurethral catheter groups yielded similar results (data not shown) and were combined.

Results

Patients and controls.

One hundred and sixty five patients undergoing major abdominal surgery took part in this study. 10 patients (60 ± 5 years), 3 men and 7 women developed UTI (UTI+patients) 6 ± 1 days after catheterization. Escherichia
**IL-6 and IL-8 in UTI complicating surgery**

coli was cultured from urine of all 10 patients. Of UTI-controls, matched for the duration of catheterization, 8 were male and 12 female (68 ± 3 years).

**IL-6 and IL-8 concentrations**

Before surgery, urine IL-6 and IL-8 concentrations were either low or undetectable in both UTI-controls and UTI+patients. In UTI+patients, the highest IL-6 and IL-8 concentrations were measured in urine collected on the day UTI was documented (Fig. 1). IL-6 levels increased in the two to four days preceding UTI; however, a similar increase in urinary IL-6 was also found during this time period in UTI-controls, who were matched for the duration of catheterization (nonsignificant for the difference between UTI+patients and UTI-controls). In contrast, urinary IL-8 was increased in

**Figure 1.** Mean and SEM IL-6 (upper panel) and IL-8 (lower panel) concentrations in urine measured on the day UTI was diagnosed (inf) and 2, 4 and 6 days before infection, from 10 postoperative patients with a urinary catheter who developed UTI (closed circles). 20 patients, whose urine remained sterile, served as controls (open circles). The increase in urine IL-6 was similar in two groups. IL-8 levels were significantly higher in patients, who developed UTI (P<0.01).
UTI+patients only on the day the urine culture became positive, while such a rise was not observed in UTI-controls (P < 0.01). These data suggest that IL-6, but not IL-8, is released in urine of postoperative patients who have a urinary catheter in place for a number of days, irrespective of the presence of UTI. Indeed, data obtained from UTI-controls relative to the duration of catheterization, revealed a gradual rise in urine IL-6 concentrations from day 4 onwards (P = 0.005 for change in time), while during the same time period urine IL-8 levels remained unchanged (Fig. 2).

**Figure 2.** Mean ± SEM IL-6 (black triangles) and IL-8 (white triangles) concentrations in urine of 20 patients with a urinary catheter who did not develop UTI (UTI-controls) following uncomplicated surgery. Urine IL-6 increased after surgery (P=0.005), while IL-8 levels remained unaltered.

**Discussion**

To our knowledge, this is the first study investigating the release of IL-6 and IL-8 in urine from patients prior to bacteriologic documentation of UTI. The limited data available concerning the kinetics of the release of these cytokines in UTI have been obtained from follow-up of patients after the diagnosis had already been established [8]. Our findings are in line with
earlier reports showing that both abdominal surgery in humans [9] and traumatic catheterization in mice [10] are followed by elevated levels of IL-6 in urine. Deliberate colonization of the human urinary tract with *E. coli* resulted in an intermittent elevation of urine IL-6 in spite of the continuous presence of bacteria [8]. Our study extended these observations by demonstrating that in the clinical setting of early postoperative UTI, there was no significant increase in urine IL-6 prior to or on the day of diagnosis, when compared to postoperative patients who did not develop UTI.

Jacobson et al. found that, in comparison with IL-6, urine IL-8 was elevated in UTI more consistently and for a more prolonged period of time [11]. We showed that in patients who did not develop UTI, urine IL-8, unlike IL-6, remained low after surgery. Together these data support the notion that contrary to IL-6, the increase in urinary IL-8 observed on the day of diagnosis can be attributed to UTI itself.

Furthermore, our results suggest, that in postoperative patients who develop UTI, IL-8 but not IL-6, marks the early phase of the local host response to the infection.
Chapter 2

References