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Citation for the published paper:

Otto, Gisela and Burdick, Marie and Strieter, Robert and Godaly, Gabriela

"Chemokine response to febrile urinary tract infection."

*Kidney Int.* 2005 Jul;68(1):62-70.

<http://dx.doi.org/10.1111/j.1523-1755.2005.00381.x>

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## **Chemokine Response to Febrile Urinary Tract Infection**

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**Short title:** Chemokine responses in febrile UTI

**Acknowledgments:** This study was supported by grants from the Swedish Medical Research Association; the Medical Faculty, University of Lund; the Swedish Medical Research Council (grant 7934 and grant 14578); the Royal Physiographic Society of Lund; and, the Österlund and Crafoord foundations. G Godaly is a recipient of The Swedish Society for Medical Research stipend.

## ABSTRACT

Chemokine response to febrile urinary tract infection (UTI).

*Background.* Mucosal CXC chemokines recruit inflammatory cells to the infected urinary tract. The chemokine response repertoire of the urinary tract and the relationship to disease severity have not been examined, however.

*Methods.* This study quantified CXC (CXCL1, CXCL3, CXCL5, CXCL8, CXCL9, CXCL10) and CC (CCL2, CCL4, CCL5) chemokines in sequential urine samples obtained from 50 patients with febrile UTI during 24 hours after diagnosis.

*Results.* All patients had elevated chemokine levels, but bacteremic infections caused higher CXCL1, CXCL3, CXCL5, CXCL8, and CCL2 responses. CCL2 and CXCL8 levels were higher in patients with acute pyelonephritis symptoms and CCL2, CXCL3, CCL4, CXCL5 and CXCL10 were significantly correlated to CRP and temperature. Women and men showed different chemokine responses.

*Conclusion.* Febrile UTIs are accompanied by a complex chemokine response. The response magnitude reflects disease severity, and the repertoire is influenced by gender and underlying disease.

Keywords: febrile UTI, chemokines, inflammation, patients, urine

## INTRODUCTION

The diverse manifestations of urinary tract infection (UTI) reflect the quality, localisation and magnitude of the inflammatory response to pathogenic bacteria [1]. Acute pyelonephritis is accompanied by local symptoms from the upper urinary tract like flank pain or costo-vertebral angle tenderness and by fever and malaise reflecting the systemic involvement. The temperature or circulating acute-phase reactants like C-reactive protein (CRP) are used to quantify the systemic inflammatory response, but there is no tradition of measuring local host response parameters in urine, even though the local repertoire of inflammatory mediators reflects both the site and severity of infection.

Chemokines are small chemotactic proteins of 8-10 kDa, that selectively target and activate specific cell populations and attract them to inflamed tissue sites [2]. They are divided into four subgroups (CXC, CC, C and CX<sub>3</sub>C), based on the arrangement of the first two of four conserved cysteine residues. The CXC family is further subdivided according to the sequence glutamic acid-leucine-arginine (ELR) near the N terminus immediately preceding the first cysteine residue. ELR containing CXC chemokines are potent neutrophil activators and include CXCL1, CXCL3, CXCL5, and CXCL8 (previously referred to as GRO $\alpha$ , GRO $\gamma$ , ENA-78 and IL-8). CXC chemokines lacking the ELR motif, CXCL9 and CXCL10 (previously referred to as Mig and IP-10), have a weak if any neutrophil-activating activity, but attract and activate T cells and NK cells. CC chemokines like CCL2 (previously referred to as

MCP-1), CCL4 (previously referred to as MIP-1 $\beta$ ) and CCL5 (previously referred to as RANTES) attract mostly monocytes and T-cells, but also neutrophils [3, 4].

UTIs elicit a mucosal chemokine response [5-8]. Epidemiologic studies of symptomatic infections showed elevated urine and serum CXCL8 levels in patients with febrile UTI [5, 7, 9-15], and both CXC and CC chemokines were detected in patients with urosepsis [12, 15, 16]. In paediatric populations, the urinary tract chemokine response was shown to be specific for febrile UTI, as elevated urine CXCL8 levels were detected in children with febrile UTI but not in those with febrile infection of unknown origin [11]. The first chemotactic signal emanates from uro-epithelial cells which are efficient chemokine producers [17, 18] (for review see [19]). Uropathogenic *E. coli* stimulate a chemokine response through different TLR4 dependent signalling pathways in the epithelial cells [20-22]. P fimbriae are one essential virulence factor, as shown by the rapid CXCL8 response to deliberate intravesical inoculation of the human urinary tract with P fimbriated *E. coli* [23, 24] and by the lack of chemokine production during long-term asymptomatic carriage of the non-fimbriated strain. The chemokine response is essential for the anti-bacterial defence of the urinary tract, and especially the CXC chemokines are crucial to recruit inflammatory cells to infected sites within the urinary tract [12, 18, 25-27].

The earlier studies have shown that virulent strains trigger a urinary tract chemokine response in patients with symptomatic UTI, but have not addressed how the chemokine repertoire reflects disease severity. This study investigated the

chemokine response in patients with febrile UTI, the effect of bacteremia and the relationship to disease severity.

## **METHODS**

### **Patients**

Fifty patients with community-acquired febrile UTI were included in this study [28]. The patients were 18 – 85 years old, with febrile UTI requiring hospitalization and parenteral antibiotic therapy. All patients had significant bacteriuria at admission, a temperature  $\geq 38^{\circ}$  C, and either focal symptoms from the urinary tract, a positive nitrite test at admission, increased leukocyte counts in urine, or instrumentation of the urinary tract/acute urinary retention preceding the onset of fever. The patients were hospitalized and treated with parenteral ceftazidime [28]. Patients with bacteremic febrile UTI had at least one positive blood culture with the same bacterial species in blood and urine. Symptoms, signs and medical history were registered by standardized questionnaire. The patients assigned to the compromised group had diabetes, chronic lymphocytic leukemia, systemic lupus erythematoses, alcoholism, Morbus Crohn, cancer, treatment with corticosteroids or urinary tract abnormalities. Patients with urinary tract abnormalities had prostatic hyperplasia, operations of the urogenital tract, renal stones, prolapse of the uterus, urinary tract devices, indwelling urinary catheter, intermitted catheterization or an artificial urinary sphincter. Focal symptoms from the upper urinary tract included flank pain, costo-vertebral angle tenderness and symptoms from the lower urinary tract included dysuria, frequency and suprapubic pain.

The study was approved by the research ethics committee of the Medical Faculty, at the University of Lund.

## **Bacterial cultures**

Freshly voided urine samples were semi-quantitatively cultured. Blood samples for aerobic and anaerobic culture were taken twice at inclusion [29]. Significant bacteriuria was defined by growth of a single bacterial species at  $\geq 10^4$  cfu/mL in women and  $\geq 10^3$  cfu/mL in men.

## **Host response parameters**

Blood and urine samples were obtained at inclusion, and at 6 - 8, 12 - 14 and 24 hours after the onset of antibiotic therapy. Serum and urine samples were immediately frozen at  $-70^\circ$  C. C-reactive protein (CRP) was quantified at inclusion, after 12, 24 and 48 hours, and on day 3. Total white blood cell counts (WBC), neutrophil counts (N), erythrocyte sedimentation rate (ESR), and orosomuroid were analyzed at inclusion and on day three.

Urine leukocytes were microscopically counted in centrifuged urine. Pyuria was defined as  $\geq 5$  leukocytes/microscopic field nts, and abundant pyuria as  $\geq 30$  leukocytes/microscopic field.

## **Chemokine assays**

Antigenic CXCL1, CXCL3, CXCL5, CXCL8, CXCL9, CXCL10, CCL2, CCL4, and CCL5 were quantified using a modification of the double ligand method previously described [30, 31]. Briefly, flat bottomed 96 well microtiter plates were coated with 50  $\mu$ l/well of the appropriate polyclonal antibodies for 24 hrs at  $4^\circ$ C. Samples were added, followed by incubation for 1 hr at  $37^\circ$ C. Biotinylated polyclonal rabbit anti-

human CXCL1, CXCL3, CXCL5, CXCL8, CXCL9, CXCL10, CCL2, CCL4, and CCL5 antibodies were added (50  $\mu$ l/well), and incubated for 45 min at 37°C followed by Streptavidin-peroxidase conjugate for 30 min at 37°C. Chromogenic substrate was added, and the reaction was terminated with 3 M H<sub>2</sub>SO<sub>4</sub> (50  $\mu$ l/well). Plates were read at 490 nm in an automated microplate reader (Bio-Tek Instruments, Inc., Winooski, VT, USA). The ELISA detects cytokine concentrations >10 pg/ml. The chemokine concentrations were determined in urine samples obtained at inclusion, and 6, 12 and 24 hours after onset of antibiotic therapy. The 24 hours peak value was the highest concentration recorded during the first 24 hours after onset of antibiotic therapy.

### **Statistics**

The Mann-Whitney U test, the Wilcoxon's signed ranks test for paired data, the Fisher's exact test and the Pearson's correlation test were used. *P* values <0.05 were considered statistically significant (two-tailed).

## **RESULTS**

### **Patients**

Patients with febrile UTI were enrolled in the study [28]. Twenty-four patients with bacteremia and 26 non-bacteremic patients were matched according to gender, age (+/- 10 years) and date of inclusion in the study. *E. coli* were found in 47 patients and *Citrobacter freundii*, *Klebsiella pneumoniae*, or *Proteus Mirabilis* in three patients. Pyuria was detected in 89 % of the patients, and in 72 % pyuria was abundant. The host background variables, symptoms and the acute phase response to infection are described in Table 1. The bacteremic patients had higher temperatures at inclusion (median 39.9° vs. 39.2°C; P<0.01) and higher CRP concentrations from 12 hours after the onset of therapy, but there was no difference in host background variables or focal symptoms between the bacteremic and non-bacteremic groups. The study population included 27 women and 23 men with median ages of 49 and 73 years, respectively (P <0.01). Forty-one per cent of the women and 96 % of the men were assigned to the compromised group with other illness, and urinary tract abnormalities occurred in 15 % of the women and in 96 % of the men.

### **Urine chemokine repertoire**

Urine chemokine concentrations were measured at inclusion and during the first 24 hours after the onset of therapy (Figure 1). 78 % of the patients had elevated levels of all nine measured chemokines. At inclusion, CXCL8 responses were detected in all patients, and CXCL1 and CXCL3 responses were detected in 98 %, CXCL9, CXCL10

and CCL2 in 96 %, CXCL5 in 71 %, CCL5 in 65 %, CCL4 in 59 %. CXCL8 and CXCL10 levels were higher than the remaining chemokines at all time points. The chemokine concentrations declined after the onset of antibiotic therapy except CCL4, which lacked therapy-related kinetics (Figure 2).

There was a marked difference in the chemokine repertoire between men and women with febrile UTI. The women had higher CCL2 responses than the men at all times (Figure 4). They also had higher concentrations of CXCL1 at 6 and 24 h and higher peak 24 h values.

#### **Chemokine response and disease severity.**

The chemokine response magnitude varied with the disease severity. Bacteremia was accompanied by higher urine chemokine concentrations than non-bacteremic infections (Figure 3). CCL2 was higher throughout the 24 h period, CXCL3 during the first 12 h, CXCL5 from 6 h, and CXCL1 and CXCL8 at 12 h. The level of the other chemokines did not differ between patients with or without bacteremia, however, and there was no difference in the chemokine repertoire between the bacteremic and the non-bacteremic groups.

The frequency of focal urinary tract symptoms is shown in Table 1, and the chemokine response in relation to symptoms in Table 2. 26 patients had clinical signs of acute pyelonephritis, such as flank pain and costovertebral angle tenderness. 14 patients had symptoms only from the lower urinary tract, such as dysuria, frequency

and urgency. The CCL2 concentrations were higher in the patients with acute pyelonephritis symptoms as compared to the group with symptoms only from the lower urinary tract ( $P < 0.01$ ). The CXCL8 concentrations behaved in a similar way at some time points. The remaining chemokines did not vary in relation to focal symptoms.

The chemokine response was examined as a function of the systemic host response in each patient. Significant positive correlations to the maximum body temperature were observed for CCL2, CCL4, CXCL3, CXCL5, and CXCL10. The CCL2, CCL4, CXCL3 and CXCL10 concentrations were positively correlated to fever duration after the start of antibiotic therapy (Table 3). CCL2, CCL4, CXCL3, CXCL5, CXCL9 and CXCL10 were positively correlated to CRP (Table 4). In patients without bacteremia, the CXCL8 and CCL5 concentrations were significantly correlated to the WBC counts in peripheral blood (Table 5).

### **Chemokine responses and other illness**

Thirty-three patients suffered from diseases that might compromise their resistance to UTI (Table 1). Twenty patients were assigned to a group with urinary tract abnormalities (1 woman, 19 men) and 13 to a group with more complex medical illness (10 women, 3 men). The remaining 17 patients were healthy except for the febrile UTI episode (16 women, 1 man). The CXCL1 and CCL2 concentrations were higher in the patients without compromising conditions (Figure 4). The CXCL3 and

CXCL9 concentrations, in contrast, were higher in the patients with complex medical illness.

### **Summary analysis of the chemokine response in relation to disease severity**

CCL2 responses were higher in patients with bacteremia and in patients with clinical signs of acute pyelonephritis and were positively correlated to fever and CRP. CCL2 was generally high in women, most of whom had normal urinary tracts, but CCL2 was low in men, most of whom had urinary tract abnormalities.

The CXCL1 and CXCL3 responses resembled CCL2 in that they were higher in bacteremic patients, and positively correlated to fever and CRP. CXCL1 was higher in women than in men while CXCL3 was higher in patients with complicating diseases.

CCL4 and CXCL10 were positively correlated to fever and CRP, but were not specifically elevated in the bacteremic group. CXCL9 was positively correlated to CRP. CCL5 showed a positive correlation to WBC in patients without bacteremia, but no other association CCL5 to the other study variables was detected.

CXCL8 was high in the bacteremic patients but low in patients with lower urinary tract symptoms. In addition, CXCL8 was positively correlated to the peripheral blood WBC count in the patients without bacteremia.

## DISCUSSION

This study investigated the local chemokine response repertoire and the magnitude of the chemokine response in patients with febrile UTI. Nine CXC and CC chemokines were elevated at the time of diagnosis. CXCL8, CXCL1, CCL2, CXCL3 and CXCL5 were higher in patients with bacteremia and/or symptoms of acute pyelonephritis, and were significantly correlated to fever and CRP in individual patients. We conclude that febrile UTIs elicit a complex chemokine response, and that the response magnitude reflects the disease severity.

The first wave of chemokines in urine originates from the epithelial cells that form the mucosal barrier [6, 32]. Pathogenic bacteria adhere to the luminal surface of these cells, as they infect the bladder or the renal pelvis, and stimulate the production of cytokines and chemokines [6, 33]. Human uroepithelial cell lines have been shown to secrete several CXC and CC chemokines following *in vitro* challenge with uropathogenic *E. coli* (G. Godaly, manuscript in preparation). In this study, the chemokine response in patients with febrile UTI was shown to agree with the epithelial response repertoire *in vitro*. The results support the hypothesis that many of the secreted chemokines in urine are of epithelial origin.

The urine chemokine concentrations were higher in patients with culture verified bacteremia and the response magnitude varied with parameters of systemic disease severity. The chemokines thus resembled the urine IL-6 response, which is higher in bacteremic than in non-bacteremic patients and shows strong positive correlations to

CRP and temperature [28, 34]. The IL-6 response may be functionally important as IL-6 can trigger the acute phase reaction and act as an endogenous pyrogen. In the present study, the bacteremic patients had higher levels of CCL2 and the neutrophil-activating CXC chemokines, but they did not develop a different urine chemokine repertoire. The temperature, fever duration and CRP levels in the entire group were positively correlated to CCL2, CCL4, CXCL3, CXCL5, CXCL9, and CXCL10, but while the chemokines did all correlate at some time point, the timing of correlation differed. Two aspects of pathogenesis may contribute to these results. The higher response may reflect the virulence of the P fimbriated *E. coli* strains that cause bacteremia, and their ability to activate a strong mucosal inflammatory response [20, 35]. In addition, the bacteremic infection is likely to activate a larger number of cell types, as the bacteria cross the epithelial barrier, traverse the submucosa and invade local blood vessels. Mesangial, endothelial, interstitial cells and renal fibroblasts produce both CXC and CC chemokines [36, 37], and recruited inflammatory cells [6] contribute to the later waves of chemokine response. However, systemic LPS injections in healthy volunteers did not cause a significant increase in urine chemokine levels, despite a transient increase in systemic CCL2, CCL4, CXCL1, CXCL5, CXCL8, and CXCL10 concentrations, suggesting that chemokines are not simply filtered from blood to urine [12, 16]. Furthermore, we did not detect a different chemokine repertoire in the bacteremic group, despite the potential for activation of numerous cell types. We propose that the mucosa remains the main effect source of chemokines in bacteremia and that the higher chemokine response is due mainly to increased mucosal inflammation.

The magnitude and repertoire of chemokines differed between patients with symptoms of acute pyelonephritis or from the lower urinary tract. Especially CCL2 was higher in patients with pyelonephritis symptoms than in the remaining group. Elevated CCL2 levels have previously been observed in patients with inflammatory kidney diseases [37] and after LPS perfusion of isolated kidneys [36], suggesting that CCL2 reflects renal involvement. This may indicate that the patients in this study with high concentrations of CCL2 and CXCL8 but without focal symptoms actually had renal involvement. The lack of focal symptoms may reflect their age, since these patients were older than patients with focal pyelonephritis symptoms (median age 79 versus 50 years), and since acute pyelonephritis symptoms are less common in older patients [38]. Patients with lower urinary tract symptoms, on the other hand, did not have a unique chemokine response repertoire, suggesting that the bladder contributes less strongly than the kidneys to the local chemokine response.

The marked difference in chemokine response repertoire between men and women was unexpected and may reflect gender *per se* or a difference in UTI pathogenesis between men and women. The majority of female patients had structurally normal urinary tracts, and did not suffer from other diseases that might predispose to UTI. Their acute symptoms were typical of acute pyelonephritis, and they were infected with P fimbriated *E. coli* strains that stimulate CCL2 production [39]. Most of the men, in contrast, had urinary tract abnormalities allowing less virulent bacteria to cause febrile UTI. A parallel study has shown that many men were infected with

strains lacking P fimbriae [39], and these strains are poor CCL2 inducers *in vitro* (G. Godaly, manuscript in preparation). The frequency of immunosuppressive therapy or diseases did not differ between men and women. We propose that these differences in chemokine repertoire between men and women may result from differences in disease pathogenesis.

The broad chemokine response repertoire provides an interesting basis to speculate about the different cell types that may be recruited to the infected urinary tract. Neutrophils are the key effector cells of the innate defence, but other cell types may be involved in enhancing or preventing the tissue damage that accompanies chronic infection. Several neutrophil-activating CXC chemokines were elevated in the present study. CXCL8 has been shown to direct the movement of neutrophils across the renal pelvic epithelium and into the urine [25-27, 40]. Deletion of ELR+ CXC chemokine function in IL-8 receptor (IL-8R)  $-/-$  mice caused subepithelial neutrophil accumulation in kidneys and later the mice developed renal scarring [41]. Other CXC chemokines have potent neutrophil-activating functions, but their contribution to neutrophil recruitment in the urinary tract has not been investigated. Neutralizing CXCL1 and CXCL5 antibodies have been shown to partially inhibit the neutrophil chemotactic activity of urine from patients with urosepsis, suggesting that these responses deserve further study [12]. Lymphocytes are recruited into the kidney during the later stages of infection, and plasma cells are abundant in the cellular infiltrate during the development of renal scarring [42] but specific immunity is not involved in the clearance of acute urinary tract infection [43]. CCL2, CCL4, and

CCL5 might signal the recruitment of monocytes, macrophages and different lymphocyte populations, CXCL9 and CXCL10 may attract activated T cells into the kidneys and CCL5 might be essential for the activation of mucosal mast cells.

In conclusion, febrile UTIs are accompanied by a broad and diverse chemokine response that reflects disease severity. It is important to consider if the chemokine response adds a new level of resolution that might be of use in the diagnosis of UTI. Urine CXCL8 may be proposed as a general marker of UTI, useful for example in patients where it may be difficult to distinguish febrile UTI from fever of unknown origin with or without bacteruria. Urine CCL2 may serve as an indicator of disease severity, and may confirm kidney involvement, which is common in otherwise healthy women with febrile UTI. Future studies, including the evaluation of chemokine responses in afebrile UTI patients are needed however, to address the value of chemokines as diagnostic tools in UTI.

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**Table 1.** Host background variables, symptoms, host response parameters, and urine culture data at inclusion.

	FebrileUTI	
	Non-bacteremic	Bacteremic
Host background variables		
Men/women, no.	13/13	10/14
Age, years, median (range)	64 (23-82)	69 (29-85)
Complicating disease <sup>a</sup> , no. (%)	16 (62.0)	17 (71.0)
History of other urinary tract disease <sup>b</sup> , no. (%)	15 (58.0)	11 (46)
Use of urinary tract devices <sup>c</sup> , no.	2	5
Symptoms		
Dysuria, frequency, urgency, no. (%)	17 (70.8)	13 (54.2)
Flank pain, costovertebral angle tenderness, no. (%)	13 (50.0)	13 (54.0)
Hypotension, no.	1	2
Host response parameters, median (range)		
Temperature, °C	39.2 (38.0-40.6)	39.9 (38.2-41.0)
Fever duration before enrollment, days	2.0 (0.2-4.0)	1.0 (0.25-8.0)
C-reactive protein, mg/L	96 (12-280)	148 (30-344)
Erythrocyte sedimentation rate, mm/h	54 (6-81)	50 (10-87)
White blood cell count, x10 <sup>9</sup> /L	11.5 (3.7-19)	13 (4.4-23) <sup>d</sup>
Urine culture data, no. (%)		
<i>Escherichia coli</i>	26 (100)	21 (87.5)
Non- <i>E. coli</i>	0 (0)	3 (12.5) <sup>e</sup>

<sup>a</sup> Diabetes mellitus, chronic lymphocytic leukemia, treatment with corticosteroids, systemic lupus erythematoses, ethylism, Morbus Crohn, cancer with or without urinary tract abnormalities, **prostatic hyperplasia**, operations of the urogenital tract, renal stones, prolapse of the uterus, urinary tract devices, multiple sclerosis with bladder dysfunction, cancer of the prostate.

<sup>b</sup> Prostatic hyperplasia, operations of the urogenital tract, renal stone, prolapse of the uterus, urinary tract devices.

<sup>c</sup> Indwelling urinary catheter (n=4; 3 ≥ 4 weeks), intermitted catheterization, artificial urinary sphincter.

<sup>d</sup> The WBC count of one patient with chronic lymphatic leukemia is not included (96.0 x 10<sup>9</sup>)

<sup>e</sup> *K. pneumoniae* (1), *Citrobacter freundii* (1), *Proteus mirabilis* (1).

**Table 2.** Urine chemokine responses in relation to focal urinary tract symptoms in febrile UTI.

<b>Patient group</b>	<b>Chemokine concentration, ng/ml, median (range)</b>	
	<b>CXCL8</b>	<b>CCL2</b>
<b>Clinical signs of pyelonephritis<sup>a</sup></b>	1.45 (0.31-7.70)	0.52 (0.11-2.30)
<b>Lower urinary tract symptoms<sup>b</sup></b>	0.88 (0.20-4.10)	0.10** (0.01-1.50)
<b>No focal UTI symptoms</b>	8.90* (0.360-651)	0.52* (0.14-19.2)

NOTE The comparison is based on the maximum chemokine concentrations in each patient. No differences were found in CXCL1, CXCL3, CXCL5, CXCL9, CXCL10, CCL4 and CCL5.

<sup>a</sup> Flank pain, costovertebral angle tenderness, with or without lower urinary tract symptoms.

<sup>b</sup> Dysuria, frequency, urgency, but no pyelonephritis symptoms.

\*\* P< 0.01 in comparison with Clinical signs of pyelonephritis (the Mann-Whitney U test).

\* P<0.05 in comparison with lower urinary tract symptoms (the Mann-Whitney U test).

**Table 3.** Correlation analysis of temperature and fever duration to urine chemokines

Chemokine, hours	Coefficient of correlation, r		
	Temperature at inclusion	Temperature day 1	Fever duration
<b>CXCL3</b> at inclusion	0.33**	0.09	0.31**
peak 24 h	0.26*	0.12	0.39**
<b>CXCL5</b> at inclusion	0.10	0.11	0.10
peak 24 h	0.26*	0.16	0.15
<b>CXCL10<sup>a</sup></b> at inclusion	0.24*	0.29**	0.32**
peak 24 h	0.01	0.17	0.22
<b>CCL2<sup>b</sup></b> at inclusion	0.12	0.21	0.14
peak 24 h	0.23	0.40**	0.24*
<b>CCL4</b> at inclusion	-0.01	0.11	0.30**
peak 24 h	0.13	0.28*	0.40**

NOTE Only chemokines with significant positive correlations are included in the table. There was no positive correlation of CXCL1, CXCL8, CXCL9 and CCL5 to temperature.

<sup>a</sup> Maximum correlation was CXCL10 at 24 h to temperature day 1 ( $r=0.39^{**}$ ) and to fever duration ( $r=0.42^{**}$ ).

<sup>b</sup> Maximum correlation was CCL2 at 12 h to temperature day 1 ( $r=0.40^{**}$ ) and to fever duration ( $r=0.34^{**}$ ).

\*  $P < 0.05$ , one-tailed significans (The Pearson's correlation test)

\*\*  $P < 0.05$ , two-tailed significans (The Pearson's correlation test)

**Table 4.** Correlation analysis of CRP and chemokines.

Chemokine, hours	Coefficient of correlation, r	
	CRP at inclusion	CRP max
<b>CXCL3</b> at inclusion	0.36**	0.27*
peak 24 h	0.25*	0.46**
<b>CXCL5</b> at inclusion	0.36**	0.43**
peak 24 h	0.14	0.04
<b>CXCL9</b> at inclusion	0	-0.02
peak 24 h	0.28*	0.31**
<b>CXCL10</b> at inclusion	0.14	0.26*
peak 24 h	0.15	0.12
<b>CCL2</b> at inclusion	0.16	0.36**
peak 24 h	0.25*	0.43**
<b>CCL4</b> at inclusion	0.41**	0.36**
peak 24 h	0.40**	0.43**

NOTE Only chemokines with significant correlations are included in the table.

There was no positive correlation of CXCL1, CXCL8 and CCL5 to CRP.

\*  $P < 0.05$ , one-tailed significance (The Pearson's correlation test)

\*\*  $P < 0.05$ , two-tailed significance (The Pearson's correlation test)

**Table 5.** Correlation analysis of chemokines to WBC counts

Chemokine	hours	WBC in the non-bacteremic group
		Coefficient of correlation, r
<b>CXCL8</b>	at inclusion	0.29
	at 6 h	0.45**
	peak 24 h	0.35*
<b>CCL5</b>	at inclusion	0.45**
	at 6 h	0.37*
	peak 24 h	0.25

Note. Only chemokines with significant positive correlations are included.

Results for CXCL3 CXCL5, CXCL9 CXCL10, CCL2 and CCL4 were negative.

\*  $P < 0.05$ , one-tailed significance (The Pearson's correlation test)

\*\*  $P < 0.05$ , two-tailed significance (The Pearson's correlation test)

## FIGURE LEGENDS

**Figure 1.** Urine chemokine concentrations in 50 patients with febrile UTI. Samples were obtained at inclusion and during the first 24 hours after the onset of antibiotic therapy. The peak 24 hour values represent the highest concentrations in each patient during this period. Dots represent values from individual patients and the horizontal bars the median in each group. Chemokines are not usually detected in urine from uninfected and otherwise healthy individuals.

**Figure 2.** Decline in urine chemokine concentrations after onset of therapy.

Values are in percent of the concentration at inclusion and group means at 6 and 24 hours are shown (\* $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ , Wilcoxon's signed ranks test for paired data). CXCL1 (×), CXCL3 (\*), CXCL5(◆), CXCL8 (■), CXCL9 (▲), CXCL10 (●), CCL2 (l), CCL4 (ll), and CCL5 (lll).

**Figure 3.** Chemokine response and bacteremia.

Chemokines that were higher in the bacteremic than in nonbacteremic patients at 12 hours after onset of therapy are shown. (Mann-Whitney U-test; \*  $P < 0.05$ , \*\*  $P < 0.01$ ).

**Figure 4.** Chemokine concentrations in relation to host background variables.

A. Complicating disease.

B. CXCL1 and CCL2 in relation to gender.

(Mann-Whitney U-test; \*  $P < 0.05$ , \*\*  $P < 0.01$ ).









