ORIGINAL ARTICLE

Cytokines in urine in elderly subjects with acute cystitis and asymptomatic bacteriuria

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Abstract

Objective. Searching for useful diagnostic tools to discriminate between asymptomatic bacteriuria (ASB) and acute cystitis, this study compared urinary levels of cytokines/chemokines and leukocyte esterase in three groups of elderly subjects; those with acute cystitis, those with ASB, and those without bacteriuria. **Design.** Comparative laboratory. **Setting.** Primary care. **Subjects.** A total of 16 patients with acute cystitis, 24 subjects with ASB, and 20 controls without bacteriuria, all of whom were aged 80 or over. **Main outcome measures.** Urinary levels of IL-1 β , TNF- α , IL-12, IL-18, CXCL1 (GRO- α), CXCL8 (IL-8), CCL2 (MCP-1), IL-6, IL-10, and leukocyte esterase. **Results.** Urinary levels of CXCL1, CXCL8, and IL-6 were significantly higher in acute cystitis patients than in the ASB group. The sensitivities and specificities for CXCL8, IL-6, and leukocyte esterase to discriminate between acute cystitis and ASB were 63% (95% CI 36–84) and 96% (95% CI 77–100) (cut-off >285 pg/mg creatinine), 81% (95% CI 54–95) and 96% (95% CI 77–100) (cut-off >30 pg/mg creatinine), and 88% (95% CI 60–98) and 79% (95% CI 57–92) (cut-off >2, on a scale of 0–4), respectively. **Conclusions.** The results indicate that measurement of urinary cytokines, and also leukocyte esterase, when using a cut-off value >2, could be useful in clinical practice to discriminate between symptomatic and asymptomatic urinary tract infections in the elderly. A combination of IL-6 and leukocyte esterase could be even more useful. This needs to be evaluated in prospective studies on the diagnosis and treatment of urinary tract infections in an elderly population.

Key Words: Aged, bacteriuria, chemokines, cytokines, CXCL8, family practice, interleukin-6, leukocyte esterase, urinary tract infections

Urinary tract infections (UTIs), and predominantly acute cystitis, are common in the elderly. In this age group in Sweden, they account for nearly half of the antibiotic prescriptions [1]. Asymptomatic bacteriuria (ASB) is also common in the elderly, 19% in women and 9% in men in a Swedish community-living population aged ≥ 80 years [2]. In institutional living the prevalence of ASB in women is 30–50% [3–5].

There is convincing evidence that, except in pregnant women, ASB is a harmless condition and should not be treated [6]. However, it is often

difficult to differentiate between acute cystitis and ASB in daily practice [7]. This may be due to patients' difficulties in communicating their symptoms because of cognitive impairments or when the elderly patient presents diffuse systemic symptoms without apparent focal urinary signs. Then a finding of bacteriuria may be difficult to interpret, and may lead to a failure to detect the true causes of the symptoms as well as to overuse of antibiotics.

In a UTI, both immune cells and various other cell types such as endothelial and uro-epithelial cells participate in the host response. Cytokines

It is often difficult to discriminate between acute cystitis and asymptomatic bacteriuria (ASB) in the cognitively impaired, or when elderly patients present diffuse symptoms.

- A urinary dipstick test for leukocyte esterase may be helpful in separating acute cystitis from ASB.
- Testing for urinary IL-6 may improve diagnosis, especially when combined with testing for urinary leukocyte esterase.
- More accurate diagnosing may contribute to a reduction of antibiotic overuse.

play an important role in the regulation of the local inflammatory response to infections [8–19].

Although ASB is asymptomatic by definition, it is not merely a colonization of bacteria in the urinary tract. ASB can be regarded as an asymptomatic infection, since ASB is associated with leukocyturia, elevated urinary cytokines, and an elevated urinary antibody to uropathogens [20,21]. However, the levels of cytokines/chemokines in the urine of elderly people with regard to ASB and acute cystitis have not yet been fully investigated.

The aim of this exploratory study was to investigate the levels of cytokines/chemokines and leukocyte esterase in the urine of elderly patients with acute cystitis as compared with individuals with ASB and without bacteriuria, searching for possible diagnostic tools to discriminate between ASB and acute cystitis.

Material and methods

Patients with acute cystitis

Sixteen consecutive patients ≥ 80 years old (15 women and one man, median age 85 years), diagnosed with an acute cystitis at Britsarvets primary healthcare centre in Falun, Sweden, between March and September 2005, were included. The diagnosis required new onset of dysuria, frequency, and/or urgency and significant bacterial growth (in two subjects the urine was not cultured but tested positive for nitrite).

Subjects with ASB

Twenty-four individuals with ASB (22 women and two men, median age 86 years) were recruited from a population-based study of ASB in the community-dwelling elderly [2]. They all had growth of $\geq 10^8$ CFU/l of *Escherichia coli* in two consecutive urine samples, obtained within 1–2

weeks, without new onset of dysuria, frequency, and/or urgency.

Subjects without bacteriuria

Twenty controls (15 women and five men, median age 84 years) with one negative urinary culture and no symptoms of UTI were recruited from the same population-based study mentioned above [2].

Urine samples

Urine cytokine detection. All urine samples were stored at -20°C until tested. After thawing, they were centrifuged at 3000 rpm for 10 min at 4°C before analyses. Quantitative determination of IL- 1β , TNF- α , IL-12, CXCL8, IL-6, and IL-10 urine concentrations were performed using a human Cytometric Bead Array Inflammation kit (CBA; Becton Dickinson Biosciences, Stockholm, Sweden), analysed by flow cytometry (FACS Canto, Becton Dickinson), according to the manufacturer's instructions. The levels of CXCL1 and CCL2 were analysed using human Quantikine[®] ELISA (R&D Systems, Abingdon, UK). CCL2 was analysed according to the manufacturer's instructions. There was no protocol for urine samples for CXCL1, and therefore spike, recovery, and linearity experiments were performed to validate the assays, as recommended by the manufacturer.

Urine creatinine detection. Urine creatinine concentration was measured using a modified Jaffe reaction (ADVIA 1650, Bayer, Stockholm, Sweden), and the ratios of cytokine/creatinine (pg/mg) were calculated to normalize sample results for dilution.

Urine leukocyte esterase detection. Urinary leukocyte esterase was analysed semi-quantitatively using Clinitek 50 (Bayer) on a urinary dipstick (Multistix[®] 5, Bayer), showing the leukocyte esterase concentration on a scale from 0 to 4.

Statistics

The software used was the SPSS package for Windows, Rel. 14.0.0, 2005. Urine cytokine concentrations in the acute cystitis and ASB groups and negative controls were compared using the Mann–Whitney U-test. P-values < 0.05 were considered significant. Yates's correction was used when calculating confidence intervals for sensitivity and specificity.

Table I. Urinary levels of tested cytokines in patients with acute cystitis, ASB, and negative controls.

	Acute cystitis		ASB		Negative controls
	(n = 16)		(n = 24)		(n = 20)
	Median (range)	P-value	Median (range)	P-value	Median (range)
IL-1 β	97.1 (0–11 500)	0.47	62.7 (15.8–153)	0.72	57.5 (25.9–186)
TNF- α	5.8 (0–33.9)	0.087	11.0 (2.4–20.0)	0.043	6.8 (2.8–21.1)
IL-12	17.8 (0–53.2)	0.28	22.1 (5.3–67.8)	<0.001	6.3 (0–31.9)
IL-18	4.1 (1.1–16.8)	0.61	5.0 (0.9–27.8)	0.045	8.1 (1.8–35.4)
CXCL1 (GRO- α)	222 (0–>6000)	0.011	38.0 (0–783)	<0.001	0.0 (0–33.8)
CXCL8 (IL-8)	313 (137–550)	<0.001	69.6 (6.5–809)	<0.001	14.5 (2.5–50.7)
CCL2 (MCP-1)	340 (144–621)	0.29	267 (72.6–591)	0.82	243 (117–22 800)
IL-6	54.7 (10.7–443)	<0.001	14.4 (7.1–37.4)	0.046	11.7 (5.6–69.1)
IL-10	12.5 (1.4–31.3)	0.35	14.6 (5.3–31.6)	0.002	9.2 (1.7–16.6)

Note: Concentrations are in pg/mg creatinine.

Results

Urinary levels of tested cytokines and chemokines, corrected for urine creatinine, are presented in Table I, where the levels in patients with acute cystitis, ASB, and negative controls are compared. We found significantly higher levels of urine CXCL1, CXCL8, and IL-6 in subjects with ASB than in negative controls, and even higher levels in acute cystitis.

Comparisons without correction for urine creatinine revealed similar significant differences among the three groups regarding CXCL1 and CXCL8. For IL-6 there were higher urine levels in acute cystitis than in ASB, while there was no difference when the ASB group was compared with negative controls.

Figure 1 shows the distribution of CXCL1, CXCL8, CCL2, and IL-6 in the three different groups, visualizing the interquartile range and median values. In CXCL1 there were non-detectable urinary levels in most negative controls and occasionally in the ASB and acute cystitis groups, while there were detectable levels of CXCL8, CCL2, and IL-6 in all subjects.

Table II shows values for sensitivity and specificity in discriminating between acute cystitis and asymptomatic bacteriuria. Values for leukocyte esterase in acute cystitis and asymptomatic bacteriuria are presented in Figure 2, where they are plotted together with IL-6, illustrating diagnostic possibilities. Combined test with leukocyte esterase >2 and IL-6 >30 pg/mg gives a specificity of 100% and a sensitivity of 69% (95% CI

Figure 1. Distributions of urinary CXCL1, CXCL8, CCL2, and IL-6 in patients with acute cystitis, ASB, and negative controls. Concentrations are in pg/mg creatinine (log. scale).

Table II. Sensitivity and specificity with 95% confidence intervals (CI) for urinary cytokines (cut-off values in pg/mg creatinine) and leukocyte esterase when discriminating between acute cystitis and ASB.

	Sensitivity		Specificity	
	(%)	(95% CI)	(%)	(95% CI)
CXCL1 (>150pg/mg)	69	(42–88)	79	(57–92)
CXCL8 (>285 pg/mg)	63	(36–84)	96	(77–100)
CXCL8 (>135 pg/mg)	100	¹	71	(49–87)
IL-6 (>30 pg/mg)	81	(54–95)	96	(77–100)
Leukocyte esterase (>2)	88	(60–98)	79	(57–92)

Note: ¹CI could not be calculated because there were no cases with cystitis and a negative test.

42–88) in discriminating between acute cystitis and asymptomatic bacteriuria. Among the negative controls ($n=20$), 16 had no urinary leukocyte esterase, 3 had level 1, and 1 had level 2. None of the controls had a level higher than 2.

Discussion

This study revealed higher urinary levels of CXCL1, CXCL8, and IL-6 in patients with acute cystitis than in patients with ASB.

To make the results more reliable, we tried to clearly define the groups, making sure that only those subjects with distinct symptoms of infection from the lower urinary tract were included in the acute cystitis group. However, this was a small, exploratory study and the results need to be confirmed, preferably in a more clinical context.

Correction for urinary creatinine was carried out to diminish the influence of differences in diuresis, in order to create a more accurate image of real cytokine exudation into the urine. However, the fact that the differences in urinary levels of CXCL1, CXCL8, and IL-6 between the acute cystitis and

ASB groups remained when testing without correction for creatinine indicates that measurement of urine cytokine levels, without this correction, could be possible in clinical practice.

In our study, the inclusion criteria for subjects with ASB were limited to those with growth of *E. coli* while in the acute cystitis group we found a variety of the common uropathogenic bacteria. Nevertheless, we think it is possible to compare urinary cytokine levels, as it has been shown that at least CXCL8 is elevated in UTI, independent of the causative bacteria [13,16]. CXCL8 has also been shown to be highly stable in urine as a function of time and temperature [16].

Except for higher levels of TNF- α and IL-12, and lower levels of IL-18 in the ASB group as compared with negative controls, we found no differences between the groups with regard to the pro-inflammatory cytokines IL-1 β , TNF- α , IL-12, and IL-18, and this is in accordance with the findings of others [13,22,23].

CXC-chemokines play an important role in neutrophil activation [14], and urinary levels of CXCL8 correlate with the level of leukocyturia [13,16,24]. There is evidence that in UTI these chemokines are produced locally in the urinary tract [13,24,25]. We found gradually increasing urinary levels of CXCL1 and CXCL8 going from negative controls to ASB and on to acute cystitis. Our findings are in accordance with previous reports on younger populations [9,18,25,26].

IL-6 is a pro-inflammatory and regulatory cytokine, stimulating the production of acute phase reactants such as CRP and the development of fever. We found distinctly higher urinary levels of IL-6 in acute cystitis than in ASB patients, and somewhat higher levels again in ASB patients than in negative controls. This difference, comparing UTI with ASB, has also been found in children [9]. Urine IL-6 was elevated in children and adults with UTI [13,17,23,27], and urinary levels of IL-6 in UTI reflected disease severity [17,19,28].

Figure 2. Urinary IL-6 (pg/mg creatinine) plotted against urinary leukocyte esterase in subjects with acute cystitis and ASB. Cut-off values (leukocyte esterase: >2, IL-6: >30pg/mg) indicated as bold lines.

With the exception of an early study by Nicolle [15] this is, to our knowledge, the first study evaluating urinary cytokine response in relation to bacteriuria in an elderly population. ASB is highly prevalent in the elderly, 20% in community-living women aged 80 and over [2], up to 50% in an institutionalised elderly population [3,20], and the cytokine response found in our study fits into a model where ASB in this population can be regarded as an infection and not just bacterial colonization. Symptoms in UTI are caused by the inflammatory response. However, the bacteria in ASB may be less virulent and cause a milder inflammatory response [29]. As cytokines/chemokines are early mediators of this inflammatory response, they are likely to be found in lower levels in patients with ASB than symptomatic UTI.

Predictive values are more useful in clinical practice than sensitivity and specificity but imply knowledge about the prevalence in the population, and thus our data do not permit such calculations. However, when the testing is bound to subjects with bacteriuria there will be a relatively high prevalence of acute cystitis and consequently the above specified values for sensitivity and specificity (see Table II) will result in a high positive predictive value as far as to discriminate between acute cystitis and ASB in subjects with diffuse symptomatology. Other researchers have proposed the measurement of cytokines in diagnosing febrile UTI in younger patients, with cut-off values similar to those used in our study [17–19].

Leukocytes in the urine are common in the elderly. In a Finnish study, the prevalence was 47% in a population aged 85 years or over, 77% in those with bacteriuria, and 37% in those without [30]. Others have found leukocyturia in 90% of subjects with ASB [20], but these studies do not usually distinguish among different levels of leukocyturia. Although previous studies indicate that an increase in the number of leukocytes in the urine is a non-specific finding, our results may demonstrate that with a higher cut-off value (>2) measurement of leukocyte esterase could help in distinguishing between symptomatic UTI and ASB. Further, a combination of urinary IL-6 and leukocyte esterase (see Figure 2) might increase clinical accuracy.

In conclusion, in the light of increasing rates of antibiotic resistance and high rates of antibiotic prescriptions for UTI in elderly people, it is essential to increase diagnostic accuracy to avoid overuse of antibiotics in this age group. In daily practice the first urinary test performed is often a dipstick test measuring leukocyte esterase. This study indicates that measuring leukocyte esterase and applying a higher cut-off value than is usually used may be a

first step to improve the diagnostic process. In addition, a combination of leukocyte esterase and IL-6 in a dipstick in the future might be still more useful. However, the results need to be confirmed, preferably in a larger, prospective study on UTI in the elderly, evaluating the use of urinary IL-6 and leukocyte esterase in the diagnostic process.

Acknowledgements

The study was approved by the Regional Ethical Review Board of Uppsala, Sweden. Financial support for this study was provided by the county council of Dalarna, Sweden and STRAMA (Swedish Strategic Programme for the Rational Use of Anti-microbial Agents). Thanks are also offered to Marita Skarstedt for excellent technical assistance.

Conflicts of interests: None.

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