

# Evaluation and Comparison of Urinary Cytokines for the Diagnosis of Acute Pyelonephritis

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## Abstract

**Background:** The accurate diagnosis and management of febrile urinary tract infection (UTI) is a clinical challenge in the absence of specific clinical and laboratory findings in infants and young children.

**Objectives:** The aim of this study was to identify and compare the diagnostic and therapeutic implications of recently introduced cytokines for the diagnosis of acute pyelonephritis (APN).

**Methods:** This multicenter prospective study was performed on 37 (female/male = 6.5:1) children with symptomatic culture-proven APN and 37 (female/male = 1.6:1) age-matched febrile children without UTIs as the control group. Urine samples were obtained before antibiotic treatment in both groups and 3 - 4 days after treatment in the UTI group, and evaluated for interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, IL-10, tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), monocyte chemoattractant protein-1 (MCP-1), and vascular endothelial growth factor (VEGF) using an ELISA immunoassay kit.

**Results:** Mean urinary IL-1 $\alpha$ , IL-4, IL-6, and IL-8 concentrations significantly increased in the acute phase of APN compared to the control group, and decreased following antibiotic treatment.

**Conclusions:** We recommend routine urinalysis and urine culture for the diagnosis of children with APN. Urinary IL-4 was a relatively good cytokine for the prediction and treatment-monitoring of children with acute febrile UTI.

**Keywords:** Pyelonephritis, Cytokine, Urinary Cytokine, Interleukin, Urinary Tract Infection

## 1. Background

Urinary tract infection (UTI) is the most common serious bacterial infection in febrile infants and young children, second to otitis media and pharyngitis (1). The early diagnosis of acute febrile UTI is based on clinical manifestations and a few laboratory tests with limited accuracy. Urine culture testing is the gold standard for the diagnosis of UTI, but requires 2 - 3 days for the identification of the responsible organism, with false-positive and false-negative results. In addition, urinalysis and inflammatory markers, such as fever, white blood cell (WBC) count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP), have limited sensitivity and specificity for the diagnosis of acute pyelonephritis (APN) (2-4). The DMSA renal scan is another option for the diagnosis of APN, with positive findings in 50% of patients with a febrile UTI, and it has been recently recommended for the evaluation of renal damage 3 - 6 months following an acute febrile UTI (5). According to these limitations, new sensitive and noninvasive diagnostic tests, including cytokines, have been introduced for the

accurate diagnosis of febrile UTI (3, 6).

## 2. Objectives

The aim of this study was to identify and compare the sensitivity, specificity, and accuracy of urinary ILs to suggest the most valuable test for the diagnosis of APN, compared to conventional diagnostic markers.

## 3. Methods

This prospective cross-sectional study was conducted over a 1-year period from 2014 - 2015 on 74 children. It was approved by the institutional ethics committee, and informed consent was obtained from the parents. The study protocol conforms to the ethical guidelines of the 1975 declaration of Helsinki.

A total of 37 children with APN were compared with 37 children in an age-matched control group with other bacterial infections, such as pneumonia, meningitis, arthritis,

and otitis, with a fever of  $> 38.5^{\circ}\text{C}$  and increased WBC, ESR, and CRP. Patients with congenital anomalies of the urinary tract (CAKUT), neurogenic bladder, immunodeficiency, renal dysfunction, or recent antibiotic treatment were excluded from the study.

Urine samples were collected by suprapubic aspiration or urethral catheterization during early infancy and by the midstream technique in older children. APN was defined as a UTI (any growth from suprapubic aspiration samples, growth of  $> 10^5$  CFU/mL from midstream or urine-bag collections, or  $> 10^4$  from urethral catheterizations) associated with a fever of  $> 38.5^{\circ}\text{C}$ , leukocytosis (WBC  $> 13,000/\text{mm}^3$ ), increased ESR, pyuria, and positive CRP. Pyuria was defined as  $> 5$  WBC/hpf of voided urine on culture.

DMSA scans were performed on patients with ultrasonographic abnormal scarred kidneys or on ill patients with suspected APN within the first 5-7 days of admission. DMSA scans showed pyelonephritic changes as focal or diffuse areas of decreased cortical uptake, with the preservation of renal contour in 60% of patients.

Systemic inflammatory markers, such as WBC, ESR, and CRP levels, were evaluated at the time of presentation. Urine samples were obtained before antibiotic treatment in both groups and 48-72 hours after treatment in the UTI group, and frozen at  $-80^{\circ}\text{C}$  until examination. Urinary concentrations of interleukins (IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, and IL-10), tumor necrosis factor- $\alpha$  (TNF $\alpha$ ), monocyte chemoattractant protein-1 (MCP-1), and vascular endothelial growth factor (VEGF) were analyzed with a newly introduced commercially available ELISA kit (Randox laboratories, UK) according to the manufacturer's instructions. The results were expressed as ng/mL.

All tests were analyzed using SPSS for Windows (version 13.0, SPSS Inc., Chicago, IL, USA). Continuous variables were compared with independent-sample t-tests and ANOVA, and the categorical variables were compared with Fisher's exact or chi-square tests. P values of  $< 0.05$  were considered statistically significant.

#### 4. Results

A total of 37 patients (female/male = 6.5/1) with UTIs and 37 patients (female/male = 3.6/1) with non-UTI febrile infections were enrolled in this study. The mean age of the UTI group was  $39.01 \pm 27.99$  months, compared to  $44.05 \pm 32.10$  months in the control group ( $P = 0.697$ ). There was no significant difference in terms of fever, WBC, and ESR between the two groups. However, qualitative serum CRP was significantly higher in the UTI group compared to the control group ( $P = 0.001$ ). *Escherichia coli* was the most often encountered microorganism, isolated in more than 85% of

patients, followed by *Klebsiella*, *Enterococcus*, and *Proteus* in the majority of patients. None of our patients had positive blood cultures.

The mean levels of urinary IL-1 $\alpha$  ( $2.62 \pm 4.70$ ,  $P = 0.005$ ), IL-4 ( $2.31 \pm 0.56$ ,  $P = 0.000$ ), IL-6 ( $29.94 \pm 63.78$ ,  $P = 0.015$ ), and IL-8 ( $419.93 \pm 608.91$ ,  $P = 0.001$ ) were significantly higher in the UTI group than in the control group. IL-1 $\alpha$  ( $0.98 \pm 2.40$ ), IL-4 ( $2.19 \pm 0.55$ ), IL-6 ( $5.68 \pm 11.52$ ), and IL-8 ( $173.82 \pm 431.98$ ) were decreased 3-4 days following antibiotic treatment (Table 1). IL-1 $\beta$  ( $P = 0.001$ ,  $r = 0.421$ ) and IL-4 ( $P = 0.000$ ,  $r = 0.434$ ) had significant correlations with serum CRP. Moreover, patients with abnormal 99mTc-DMSA scans had significantly higher urinary IL-6 ( $P = 0.014$ ) and MCP-1 concentrations ( $P = 0.001$ ) (Table 2).

#### 5. Discussion

Both mucosal and systemic inflammatory mediators, including cytokines and chemokines, are activated in the early phase of bacterial infections, with proinflammatory and immunoregulatory functions (7-9). They are involved in B cell and T cell proliferation and neutrophil chemoattraction, along with secretion of CRP, immunoglobulins, and acute-phase proteins (2, 6, 8, 10).

Clinical manifestations of APN depend on the production of different cytokines, and measurement of urinary ILs seems to be useful for the rapid diagnosis and localization of inflammation within the urinary tract system. Increased urinary IL-1, IL-4, IL-6, and IL-8 concentrations have been reported in children with acute febrile UTIs compared to the healthy children and those with lower UTIs, asymptomatic bacteriuria, and non-renal febrile infections (2, 11-15). Similarly, urinary IL-1, IL-4, IL-6, and IL-8 increased during the acute phase of infection compared to non-renal febrile infections. Despite previous reports of increased urinary IL-2 and IL-10 concentration in patients with APN (14, 16). We did not find significant increases of these two biomarkers.

Previous studies showed rapid elimination of urinary cytokines after the initiation of antibiotic treatment. Therefore, the determination of urinary ILs is helpful for the evaluation of treatment response in APN, and persistently elevated urinary IL concentrations indicate treatment modifications (6, 9, 17). Accordingly, increased urinary IL levels decreased following antibiotic treatment and were considered for therapeutic monitoring in our patients.

A significant correlation has been reported between cytokine response and the severity of infection (9, 11). IL-1 $\beta$  was significantly correlated with CRP and WBC in a study by Sheu et al. (2). Urinary IL-1 and IL-4 were independently correlated with serum CRP in our study. However, Renata

**Table 1.** Comparison of Variables in Children With and Without UTI (all Variables are Measured in ng/mL and Expressed as mean  $\pm$  SD).

Variable	Pre-Treatment	Post-Treatment	Control	P-Value
IL-1 $\alpha$	2.62 $\pm$ 4.70	0.98 $\pm$ 2.40	0.31 $\pm$ 0.47	0.005
IL-1 $\beta$	11.33 $\pm$ 26.77	12.36 $\pm$ 63.92	0.77 $\pm$ 0.77	0.393
IL-2	0.96 $\pm$ 1.28	1.68 $\pm$ 1.42	1.13 $\pm$ 1.32	0.068
IL-4	2.31 $\pm$ 0.56	2.19 $\pm$ 0.55	1.52 $\pm$ 0.35	0.000
IL-6	29.94 $\pm$ 63.78	5.68 $\pm$ 11.52	7.86 $\pm$ 16.54	0.015
IL-8	419.93 $\pm$ 608.91	173.82 $\pm$ 431.98	20.33 $\pm$ 16.63	0.001
IL-10	1.03 $\pm$ 0.20	1.05 $\pm$ 0.29	1.13 $\pm$ 0.50	0.491
TNF- $\alpha$	1.19 $\pm$ 0.38	1.24 $\pm$ 0.37	1.23 $\pm$ 0.43	0.844
MCP-1	162.3 $\pm$ 260.7	69.60 $\pm$ 83.51	191.8 $\pm$ 407.9	0.168
VEGF	81.17 $\pm$ 68.08	70.58 $\pm$ 50.32	92.98 $\pm$ 407.9	0.688

**Table 2.** Correlation of Urinary Biomarkers With Systemic Inflammatory Response, Pyuria, and DMSA Scan in Patients With UTIs (all Variables are Expressed as P-Values and Measured in ng/mL).

Variable	WBC	ESR	CRP	Urine WBC	DMSA Scan
IL-1 $\alpha$	0.864	0.616	0.050	0.462	0.995
IL-1 $\beta$	0.474	0.608	0.001	0.304	0.36
IL-2	0.706	0.567	0.967	0.580	0.395
IL-4	0.699	0.303	0.000	0.723	0.578
IL-6	0.850	0.125	0.70	0.063	0.014
IL-8	0.790	0.384	0.057	0.012	0.905
IL-10	0.550	0.809	0.752	0.389	0.115
TNF- $\alpha$	0.409	0.921	0.877	0.974	0.179
MCP-1	0.654	0.705	0.741	0.061	0.001
VEGF	0.271	0.134	0.798	0.054	0.645

found no correlation between ILs and systemic inflammatory markers in febrile UTI (10). Urinary IL-6 increased in accordance with DMSA parenchymal defects in the acute phase of pyelonephritis in our patients, in line with previous studies (18).

TNF- $\alpha$  was the other evaluated inflammatory mediator of serious bacterial infections in this study, as it is a rapid and reliable test for the diagnosis of upper UTIs (3, 19) and is decreased following antibiotic treatment (20). However, TNF- $\alpha$  levels showed no significant changes in Lin's study (18) which is similar to our results.

MCP-1 is a component of proinflammatory chemotactic cytokines, called chemokines, involved in immune and inflammatory responses. Increased MCP-1 levels were detected in patients with UTIs at admission, at least in part locally produced, and were decreased with specific treatment in a study by Olszyna et al. (21). Similarly, urinary MCP-

1 increased during the acute phase of febrile UTIs and decreased following antibiotic treatment in our patients, although with a non-significant correlation. We showed that a positive correlation between MCP-1 and DMSA renal scans was the most reliable indicator of acute parenchymal infections.

Our cytokine kit included VEGF, or vascular permeability factor (VPF), which is a glycosylated protein with vasodilator, angiogenetic, and inflammatory effects. However, this showed no significant change in our patients and was not considered an accurate index for the diagnosis of febrile UTI.

In conclusion, urinary IL-4 was a relatively sensitive and accurate biomarker for the differentiation of APN from other infectious disorders in the present study. However, simple and inexpensive tests, such as urinalysis and urine cultures, are still recommended for the diagnosis of chil-

dren with APN. Urinary IL-4 is suggested as a useful test for the identification of APN in highly suspicious patients with false-positive or false-negative urine results, to prevent unnecessary radiologic evaluations and long-term follow-up.

## Footnotes

**Authors' Contribution:** Azar Nickavar performed study design and conception, data collection, writing of the manuscript, and supervision of the study; Baranak Safaeian performed data collection and organized the questionnaire and funding support; Ehsan Valavi organized the questionnaire and performed data collection.

**Conflict of interest:** The authors declare no conflict of interest.

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