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Urinary cytokines as markers of latent inflammation in children with chronic pyelonephritis and anorectal malformations☆

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Summary

Introduction
Anorectal malformations (ARMs) comprise a range of defects in the development of the lowest portion of the intestinal tract that are often associated with anomalies of the urinary tract. We hypothesize that ARMs may specifically predispose the patients to prolonged urinary tract infection (UTI) and transition from a state of active (clinically apparent) inflammation to a state of latent inflammation following antibiotic treatment. Yet diagnosis of latent inflammation in the urinary tract is problematic.

Objective
The aim was to investigate the urinary levels of proinflammatory (IL-1β, IL-6, IL-8, and MCP-1), anti-inflammatory (IL-10), and proangiogenic (VEGF) cytokines in the clinical course of chronic pyelonephritis (CP) as potential biomarkers of latent inflammation in the urinary tract in children with ARM.

Patients and methods
A total of 34 children (age range 4—120 months) with CP in the active phase of inflammation were divided into two groups: CP with ARM group included 20 patients and CP without ARM group included 14 patients. The control group included 20 healthy children similar by age and gender. Urine samples were collected at the time of enrollment, 5—7 days after institution of antibiotic treatment, and 1.5 months after enrollment. Cytokine concentrations were measured by ELISA.

Results
Upon enrollment, we detected increased urinary levels of IL-10 and MCP-1 and normal levels of IL-1β, IL-6, IL-8, and VEGF in CP with ARM patients as well as normal levels of all of these cytokines in CP without ARM patients. After 5—7 days of antibiotic treatment, despite significant clinical and laboratory improvement observed in both patient groups, we documented a prominent increase in the urinary concentrations of all measured cytokines indicating ongoing inflammation in the urinary tract. Following 1.5 months of enrollment, in CP without ARM patients, IL-8 and MCP-1 were increased, IL-1, IL-6, and VEGF were close to control, and IL-10 was below the control level, indicating partial resolution of the inflammatory process. In contrast, in CP with ARM patients, IL-1β, IL-6, IL-8, MCP-1, and VEGF were increased suggesting persistent inflammation in the urinary tract (Table).

Conclusion
Based on the urinary cytokine profile, we conclude that presence of ARM may be associated with transition from active to latent inflammation in the urinary tract after antibiotic treatment for UTI. Follow-up monitoring of the urinary cytokines may provide a better assessment of inflammatory activity in the urinary tract in children with combined urological and anorectal pathologies.

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Table Urinary cytokine concentrations in healthy controls and chronic pyelonephritis patients 1.5 months after enrollment.

Cytokine	Control	CP with ARM		CP without ARM		
	Median (IQR)	Median (IQR)	*p* vs. control	Median (IQR)	*p* vs. control	*p* vs. CP with ARM
IL-1β	3.7 (1.2—7.4)	33.2 (19.4—55.9)	<0.001	4.0 (3.2—5.7)	0.375	<0.001
IL-6	2.4 (1.5—3.5)	52.5 (29.3—109.1)	<0.001	4.9 (4.2—7.4)	0.218	0.047
IL-8	6.1 (4.9—9.5)	389.7 (371.4—400.4)	<0.001	236.3 (197.6—326.9)	0.017	0.196
IL-10	6.4 (5.5—9.7)	6.9 (3.5—18.1)	0.375	1.7 (1.2—3.1)	0.011	0.007
VEGF	166 (142—238)	733 (597—792)	<0.001	312 (218—384)	0.255	0.041
MCP-1	122 (107—154)	425 (354—509)	<0.001	367 (329—392)	0.002	0.375

Note. Concentrations are given in pg/mL. *P* values were calculated using the Kruskal—Wallis test followed by the post hoc Dann test and adjusted for multiple comparisons. CP = chronic pyelonephritis; ARM = anorectal malformation; IQR = interquartile range.

Introduction

Anorectal malformations (ARMs) comprise a wide range of defects in the development of the lowest portion of the intestinal tract. Defects vary from minor and easily cured to complex, which are often associated with anomalies of the urinary tract [1—3]. Anatomical, histological, and functional abnormalities in the lower intestinal and urinary tracts (e.g., the presence of pathological recto-urinary communications, impaired colonic motility and tone, alterations of the intestinal mucosa, etc.) facilitate translocation of potentially pathogenic gut microflora to the urinary system [4—6]. Additionally, ARMs are commonly associated with urinary tract obstruction and stasis that makes these patients particularly vulnerable to urinary tract infection (UTI) [3,7]. Recent studies have shown that UTI is an important risk factor of renal scarring. Even a single episode of acute pyelonephritis leads to renal scarring and deterioration of renal function in 10—65% cases despite timely institution of antimicrobial therapy [8—11]. We hypothesize that ARMs may specifically predispose the patients to prolonged UTI characterized by transition from a state of active (clinically apparent) inflammation to a state of latent inflammation following antibiotic treatment. Yet, because of poor clinical manifestations, diagnosis of latent inflammation in the urinary tract is problematic indicating the need for elaboration of new sensitive and specific diagnostic screening tests.

Numerous studies have shown that the measurement of serum and urinary levels of cytokines in patients with acute pyelonephritis seems to be a useful tool to predict the risk of renal scarring in the follow-up [10,12,13]. Less attention was paid to the analysis of urinary cytokine levels after obvious clinical improvement of a patient following the institution of antibiotic therapy. Analysis of urinary cytokine concentrations after resolution of clinical signs and symptoms of acute pyelonephritis would help in diagnosing the latent inflammatory process, monitoring the efficacy of treatment approach, and prediction of renal fibrosis. To our knowledge, this is the first study aimed to investigate the urinary levels of proinflammatory (interleukin [IL]-1β, IL-6, IL-8, and monocyte chemoattractant protein [MCP]-1), anti-inflammatory (IL-10), and proangiogenic (vascular endothelial growth factor [VEGF]) cytokines in the clinical course of chronic pyelonephritis (CP) as potential biomarkers of latent inflammation in the urinary tract in children with ARM.

Materials and methods

The study was approved by the local research ethics committee. All parents were provided information regarding the research in a plain language. The children were included in the study after their parents agreed and signed the informed consent form.

Patients

A total of 34 children (age range 4—120 months) with congenital anomalies of the urinary tract and CP in the phase of active inflammation were prospectively enrolled in this study during a period of 2 years. The active phase of CP was diagnosed according to the following criteria: (a) a fever of $\geq$38 °C (axillary temperature), (b) abdominal or flank pain in children $\geq$ 36 months of age (irritability or poor feeding in children $<$ 36 months of age), (c) pyuria (defined as $\geq$ 10 white blood cells [WBCs] per high-power field), (d) a positive urine culture (growth of a single pathogen at 10^5 cfu/mL in a midstream clean-catch urine sample obtained from toilet-trained children or growth of a single pathogen at 10^4 cfu/mL in a sample obtained via urinary catheterization). The patients were divided into two groups: group A included 20 CP patients (12 boys and 8 girls) with ARM; and group B included 14 CP patients (9 boys and 5 girls) without ARM. All patients were treated empirically with broad-spectrum antibiotics, and the regimen was later adjusted according to the results of antibiotic susceptibility testing of the urine bacteria isolates. Patients who had received prior outpatient antibiotic treatment, had any other identifiable source of infection, or suffered systemic diseases were excluded from the study. The control group included 20 healthy children similar by age and gender without a history of UTI or any congenital anomaly who were periodically monitored for their development and growth.

Imaging studies

All patients underwent renal and bladder ultrasound examination for the detection of urinary tract anomalies within 48 h of admission and voiding cystourethrography for the detection of vesicoureteral reflux (VUR) 7—10 days after completion of the antibiotic treatment and obtaining a negative urine culture result. The presence of VUR was