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Urine Interleukin-8 as a Marker of Vesicoureteral Reflux in Infants

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ABSTRACT

OBJECTIVE. Vesicoureteral reflux (VUR) is a common finding in children presenting with urinary tract infection (UTI) and prenatally diagnosed urinary tract dilatation and in relatives of index patients. Children with VUR are at risk for ongoing renal damage with subsequent infections. Detecting VUR and renal scarring currently depends on imaging modalities with associated problems of radiation, invasiveness, and expense. Noninvasive methods would greatly facilitate diagnosis and would also help in identifying relatives of index cases who should be screened. Interleukin-8 (IL-8) is produced by epithelial cells of the renal tract in response to inflammatory stimuli and has been shown to increase during acute UTI. The objective of this study was to assess the urine levels of IL-8 as a noninvasive marker of VUR in infants in the absence of a recent UTI episode.

METHODS. We evaluated urine concentrations of IL-8 in 59 infants aged 1 month to 2 years. All infants were free of UTI for a minimum of 3 weeks before IL-8 evaluation. Infants were divided into 3 groups: group A, subjects with proven VUR (24 infants aged 0.15–1.95 years, median 0.43); group B, subjects with a history of UTI but negative investigation for VUR (14 infants aged 0.32–1.95 years, median 0.57); and group C, subjects without any history of acute or chronic condition that might impair renal function (21 infants aged 0.08–1.92 years, median 0.33). IL-8 concentrations were determined by a commercially available quantitative enzyme-linked immunosorbent assay. To avoid dilution effects, urinary levels of IL-8 were expressed as the ratio of cytokine-to-urinary creatinine.

RESULTS. Results were presented as medians and ranges. The Kruskal-Wallis test, the Mann-Whitney rank sum *U* test, and the Spearman rank order correlation test were performed for the univariate analysis. Two-tailed *P* values were calculated and the conventional level of significance *P* < .05 was applied in all cases. Infants in groups A and B had been free of UTI for a period of 3 to 52 weeks (median, 5.0 weeks) and 3 to 78 weeks (median, 4.5 weeks), respectively, before IL-8 determination. No significant difference was noted in the length of the UTI-free period between groups A and B (*P* = .469). Urine creatinine concentrations did not differ

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Key Words

diagnostic tests, interleukin-8, reflux nephropathy, urinary tract infection, vesicoureteral reflux

Abbreviations

VUR—vesicoureteral reflux
UTI—urinary tract infection
VCUG—voiding cystourethrography
DMSA—dimercaptosuccinic acid scintigraphy

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among groups A, B, and C (medians 1.15, 2.25, and 1.15 $\mu\text{mol/mL}$, respectively; $P = .080$). The median urine IL-8/creatinine concentrations ($\text{pg}/\mu\text{mol}$) were 40.5 (range, 2.04–3874) in group A, 1.91 (range, 0.001–386) in group B, and 2.47 (range, 0.002–55.6) in group C. Urine IL-8/creatinine concentrations were significantly higher in group A than both in group B ($P = .0003$) and in group C ($P < .0001$). No significant difference was observed between groups B and C ($P = .749$). In group A, no significant correlation was shown between IL-8/creatinine concentrations and the presence of renal parenchymal damage ($P = .506$), reflux grade ($P = .770$), or time from UTI ($P = .155$). A receiver-operator characteristic curve was constructed by plotting the sensitivity versus the specificity for different cutoff concentrations of IL-8/creatinine. With a cutoff concentration of urinary IL-8/creatinine at 5 $\text{pg}/\mu\text{mol}$, the sensitivity of this marker in diagnosing VUR was 88%, the specificity 69%, the positive prognostic value 66%, and the negative prognostic value 89%. In higher cutoff concentrations, specificity of the marker increased but sensitivity rapidly decreased.

CONCLUSIONS. We present evidence that urine IL-8 concentrations remain elevated in infants with VUR even in the absence of UTI and that a cutoff of 5 $\text{pg}/\mu\text{mol}$ IL-8/creatinine is of high sensitivity and adequate specificity for diagnosing VUR. Elevated urine IL-8 levels in VUR and renal scarring have already been reported; however, the present study is, to our knowledge, the first to confirm significant differences between infants with VUR and infants with a history of UTI alone and healthy controls, and to suggest a reliable cutoff concentration for diagnosing VUR. Our findings additionally suggest that inflammatory process in VUR is ongoing even after UTI has resolved, pointing against the currently held belief that sterile reflux cannot harm kidneys. The chronic inflammatory cell infiltrate associated with reflux nephropathy rather than VUR itself might offer an explanation for the secretion of IL-8, which may well be independent of reflux grade. Using urine IL-8 for diagnosing VUR is not free of limitations, because IL-8 may be elevated as a result of urinary tract manipulation or undetected UTI. In addition, this study focused on infants and not in older children with longstanding VUR. Increased urine IL-8 concentrations after UTI has resolved is a promising noninvasive marker for an initial screening for VUR in infancy with high sensitivity and adequate specificity.

VESICoureteral reflux (VUR) is a common finding in children presenting with urinary tract infection (UTI) and prenatally diagnosed urinary tract dilatation and in relatives of index patients.^{1,2} Children with VUR are believed to be at risk for ongoing renal damage with

subsequent infections, resulting in hypertension and reduced renal function.¹ VUR provides access for both infection and transmission of bladder pressure to the kidney; however, the progress from VUR and UTI to reflux nephropathy, renal parenchymal damage, and renal scarring has not been thoroughly elucidated.^{2–4} Detecting VUR and renal scarring currently depends on imaging modalities with associated problems of radiation, invasiveness, and expense.^{1,4,5} Noninvasive methods would greatly facilitate diagnosis and would also help in identifying relatives of index cases who should be screened.⁶

Cytokines are well known to modulate the inflammatory response in UTI and renal damage, but less is known on their role after acute infection has resolved.^{4,7–12} In an attempt to identify noninvasive markers of VUR, we evaluated urine levels of interleukin-8 (IL-8) in children with VUR in the absence of a recent UTI episode. IL-8, a proinflammatory mediator and a major chemoattractant for neutrophils, is produced by epithelial cells of the renal tract in response to inflammatory stimuli and has been shown to increase during UTI.^{6,9–10,12–17}

METHODS

Subjects

We evaluated IL-8 levels in the urine of 59 infants aged 1 month to 2 years. All infants selected for the study were free of UTI for a minimum of 3 weeks before IL-8 evaluation, as determined by clinical findings, normal blood white cell count, erythrocyte sedimentation rate, or C-reactive protein and urine microscopy and culture.^{8–10} Bacteriuria was determined by culture of urine obtained by suprapubic bladder aspiration (any growth), transurethral catheterization (growth of at least 10^4 bacteria/mL), or uniform growth of at least 10^5 bacteria/mL in 2 consecutive urine samples.^{1,10} Infants were divided into 3 groups: group A, subjects with proven VUR (24 infants aged 0.15–1.95 years, median 0.43); group B, subjects with a history of UTI but negative investigation for VUR (14 infants aged 0.32–1.95 years, median 0.57); and group C, subjects without any history of acute or chronic condition that might impair renal function (21 infants aged 0.08–1.92 years, median 0.33). The study was approved by the Hospital Research Committee.

Investigative Protocol

Patients in groups A and B were studied while undergoing urinary tract evaluation after documented UTI. Imaging protocol in general followed the guidelines of the American Academy of Pediatrics.¹ Urinary tract ultrasonography was performed within a week from acute infection to determine kidney size and outline, and to indicate any dilatation or anomalies. The presence and grade of vesicoureteral reflux were determined by voiding cystourethrography (VCUG), which was performed 6

to 8 weeks after infection. Reflux was graded I–V according to the International Reflux Study Committee.^{1,18} The grade of reflux was cumulated if bilateral. The presence and grade of renal parenchymal abnormalities were determined by technetium-99m-labeled dimercaptosuccinic acid scintigraphy (DMSA), which was performed 3 to 6 months after infection. Renal parenchymal damage was defined as focal or multifocal perfusion defects or as split renal uptake of <45%. Cortical scarring was defined as a defect in the normal kidney outline.^{1,19} Group C included infants followed up for nonurinary tract problems. These controls did not have a history of UTI or a known underlying condition that might impair renal function.

Interleukin-8 Assay

All children had a urine analysis for the usual investigation workup and residual samples were used for this study. Urine was collected by suprapubic aspiration or by urine specimen bags and was not centrifuged. Samples were stored in a deep freezer at -70°C and thawed to room temperature before cytokine analysis. The urinary concentrations of IL-8 were determined by a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). IL-8 commercial kits were obtained from R&D systems (Abingdon, U.K.). All measurements were performed in duplicate wells and the results were read in an automated microplate reader. According to the manufacturer, the lower detection limit of the assay was typically <10 pg/mL for IL-8. To compare results from different children and avoid dilution effects, urinary levels of cytokines were expressed as the ratio of cytokine-to-urinary creatinine (pg/ μmol creatinine).^{4,8,13} Urine creatinine was determined by a standard laboratory photometric assay (Olympus Diagnostica, Hamburg, Germany).

Statistical Analysis

Results were presented as medians and ranges. The Kruskal-Wallis test, the Mann-Whitney rank sum *U* test, and the Spearman rank order correlation test were performed for the univariate analysis. Two-tailed *P* values were calculated and the conventional level of significance $P < .05$ was applied in all cases.

RESULTS

Infants in groups A and B had been free of UTI for a period of 3 to 52 (median, 5.0) weeks and 3 to 78 (median, 4.5) weeks, respectively, before IL-8 determination. No significant difference was noted in the length of the UTI-free period between groups A and B ($P = .469$). Among the 24 infants in group A, 23 had been diagnosed with UTI before VUR confirmation; in a 3-month-old girl, VUR had been prenatally diagnosed, and no episodes of UTI were documented thereafter. In group A, reflux occurred on the right side in 8 cases, on

the left side in 7 cases, and bilaterally in 9 cases. Renal parenchymal damage was further confirmed by DMSA in 17 infants.

Urine creatinine concentrations did not differ among groups A, B, and C (medians 1.15, 2.25, and 1.15 $\mu\text{mol}/\text{mL}$, respectively; $P = .080$ by Kruskal-Wallis test). The median urine IL-8/creatinine concentrations (pg/ μmol) were 40.5 (range, 2.04–3874) in group A, 1.91 (range, 0.001–386) in group B, and 2.47 (range, 0.002–55.6) in group C (Fig 1). Urine IL-8/creatinine concentrations were significantly higher in group A than both in group B ($P = .0003$) and in group C ($P < .0001$). No significant difference was observed between groups B and C ($P = .749$). In group A, no significant correlation was shown between IL-8 concentrations and presence of renal parenchymal damage ($P = .506$), reflux grade (Spearman $r = 0.063$, $P = .770$), or time from UTI (Spearman $r = -0.307$, $P = .155$). In addition, no correlation was found when analysis focused on the 9 infants in group A with the highest IL-8 concentrations (Fig 1).

A receiver-operator characteristic (ROC) curve was constructed by plotting the sensitivity versus the specificity for different cutoff concentrations of IL-8/creatinine (Fig 2). The curve showed that with a cutoff concentration of urinary IL-8/creatinine at 5 pg/ μmol , the sensitivity of this marker in diagnosing VUR was 88% and the specificity 69%; at the same cutoff point, the

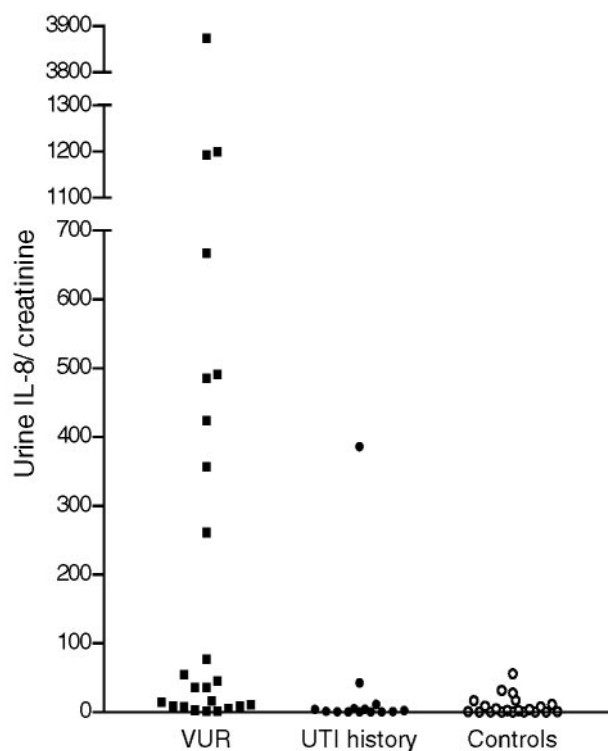


FIGURE 1
Scatter graph demonstrating urine IL-8/creatinine concentrations (pg/ μmol) in infants with proven vesicoureteral reflux, in infants with a history of urinary tract infection only but no vesicoureteral reflux, and in infants with no history of urinary disease.

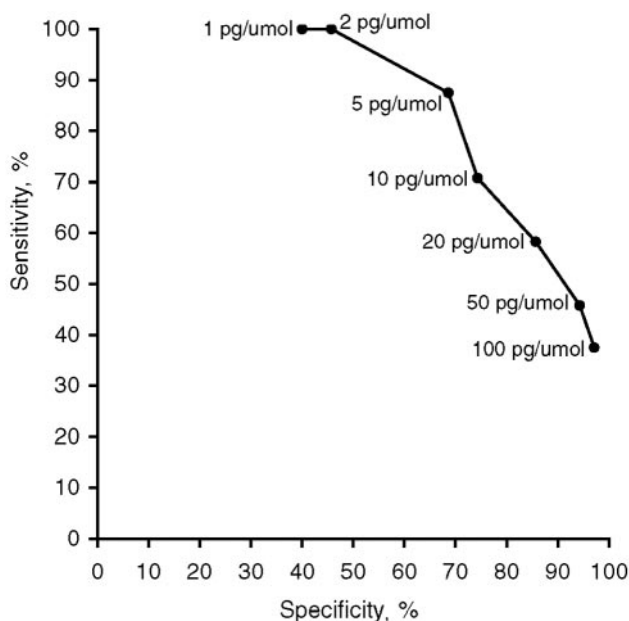


FIGURE 2 Sensitivity and specificity of urine IL-8/creatinine (pg/ μ mol) for diagnosing vesicoureteral reflux at different cut off values.

positive prognostic value was 66% and the negative prognostic value was 89%. In higher cutoff concentrations, specificity of the marker increased but sensitivity rapidly decreased. Thus, at the cutoffs of both 100 and 200 pg/ μ mol IL-8/creatinine, specificity was 97% and sensitivity 37.5%.

CONCLUSIONS

We present evidence that urine IL-8 levels remain elevated in infants with VUR even in the absence of a UTI. Elevation of urine IL-8 has been demonstrated in acute inflammatory renal disorders, including UTI,^{4,7,9,10,12} and a cutoff of 200 pg/mL has been proposed as a marker for diagnosing UTI.¹² Urine IL-8 secretion in UTI is quickly reduced after treatment, a finding confirmed in the group B of our study. Our findings suggest that the cutoff of 5 pg/ μ mol IL-8/creatinine is of high sensitivity and adequate specificity for diagnosing VUR. Higher cutoffs were associated with higher specificity but very low sensitivity. Studies focusing on serum IL-8 in patients with VUR have been inconclusive for such an association.^{10,16} Significantly elevated urine IL-8 levels in VUR and renal scarring have already been shown^{7,12}; however, the present study is, to our knowledge, the first to confirm significant differences between infants with VUR and infants with a history of UTI alone and healthy controls and to suggest a reliable cutoff concentration for diagnosing VUR.

Our findings additionally suggest that inflammatory process in VUR is ongoing even after UTI has resolved, pointing against the currently held belief that sterile reflux cannot harm kidneys.² The increase of IL-8 could

not be explained by the residual inflammation caused by UTI, because this increase was not noted in infants with UTI alone after UTI had resolved. Furthermore, it is of interest that among all the subjects of this study, the second highest IL-8 concentration was observed in a UTI-free infant with prenatally diagnosed VUR and normal DMSA. The chronic inflammatory cell infiltrate associated with reflux nephropathy rather than VUR itself⁴ might offer an explanation for the elevated levels of IL-8, which may well be independent of reflux grade.

The present study focused on young children, because this age group is at higher risk for developing renal damage.¹ Current imaging modalities, including VCUg and DMSA present with considerable limitations as screening tools, because they are associated with radiation and invasiveness.^{4,5} Ultrasound is a useful noninvasive tool for the definition of gross urinary tract anatomy; however, the modality's low sensitivity to detect reflux, which often is a dynamic condition, poses certain limitations to its use as a screening method.^{1,6,20} Using urine IL-8 seems to be a promising diagnostic marker for VUR. This marker is not free of limitations, because IL-8 may be elevated as a result of urinary tract manipulation, vaginitis, or balanitis,¹² or as a result of an undetected UTI. The latter scenario might explain the elevated IL-8 concentration in an infant in the non-VUR group B (Fig. 1). Nevertheless, determining urine IL-8 levels after UTI has resolved may provide substantial help as a screening test in evaluating high-risk patients for VUR and siblings of patients diagnosed with VUR.

REFERENCES

1. American Academy of Pediatrics. Committee on Quality Improvement. Subcommittee on Urinary Tract Infection. Practice parameter: the diagnosis, treatment, and evaluation of the initial urinary tract infection in febrile infants and young children. *Pediatrics*. 1999;103:843–852
2. Smellie JM. Vesico-ureteric reflux. *Acta Paediatr*. 1999;88:1182–1183
3. Hellerstein S. Long-term consequences of urinary tract infections. *Curr Opin Pediatr*. 2000;12:125–128
4. Ninan GK, Jutley RS, Eremin O. Urinary cytokines as markers of reflux nephropathy. *J Urol*. 1999;162:1739–1742
5. Kramer SA. The role of newer modalities in the diagnosis of vesicoureteral reflux. *J Urol*. 1996;155:683–684
6. Hollowell JG. Screening siblings for vesicoureteral reflux. *J Urol*. 2003;168:2138–2141
7. Haraoka M, Senoh K, Ogata N, Furukawa M, Matsumoto T, Kumazawa J. Elevated interleukin-8 levels in the urine of children with renal scarring and/or vesicoureteral reflux. *J Urol*. 1996;155:678–680
8. Tullus K, Escobar-Billing R, Fituri O, et al. Interleukin-1 alpha and interleukin-1 receptor antagonist in the urine of children with acute pyelonephritis and relation to renal scarring. *Acta Paediatr*. 1996;85:158–162
9. Tullus K, Fituri O, Linne T, et al. Urine interleukin-6 and interleukin-8 in children with acute pyelonephritis, in relation to DMSA scintigraphy in the acute phase and at 1-year follow-up. *Pediatr Radiol*. 1994;24:513–515

10. Benson M, Jodal U, Agace W, et al. Interleukin (IL)-6 and IL-8 in children with febrile urinary tract infection and asymptomatic bacteriuria. *J Infect Dis.* 1996;174:1080–1084
11. Jacobson SH, Lu Y, Brauner A. Tumour necrosis factor soluble receptors I and II and interleukin-1 receptor antagonist in acute pyelonephritis in relation to bacterial virulence-associated traits and renal function. *Nephrol Dial Transplant.* 1996;11:2209–2214
12. Rao WH, Evans GS, Finn A. The significance of interleukin 8 in urine *Arch Dis Child.* 2001;85:256–262
13. Kassir K, Vargas-Shiraishi O, Zaldivar F, Berman M, Singh J, Arrieta A. Cytokine profiles of pediatric patients treated with antibiotics for pyelonephritis: potential therapeutic impact. *Clin Diagn Lab Immunol.* 2001;8:1060–1063
14. Roilides E, Papachristou F, Gioulekas E, et al. Increased urine interleukin-6 concentrations correlate with pyelonephritic changes on 99mTC-dimercaptosuccinic acid scans in neonates with urinary tract infections. *J Infect Dis.* 1999;180:904–907
15. Krzemien G, Roszkowska-Blaim M, Kostro I, et al. Urinary levels of interleukin-6 and interleukin-8 in children with urinary tract infections to age 2. *Med Sci Monit.* 2004;10:CR593–597
16. Jutley RS, Youngson GG, Eremin O, Ninan GK. Serum cytokine profile in reflux nephropathy. *Pediatr Surg Int.* 2000;16:64–68
17. Godaly G, Bergsten G, Hang L, et al. Neutrophil recruitment, chemokine receptors, and resistance to mucosal infection. *J Leukoc Biol.* 2001;69:899–906
18. Lebowitz RL, Olbing H, Parkkulainen KV, Smellie JM, Tamminen-Mobius TE. International system of radiographic grading of vesicoureteral reflux. International Reflux Study of Children. *Pediatr Radiol.* 1985;15:105–109
19. Christian MT, McColl JH, MacKenzie JR, Beattie TJ. Risk assessment of renal cortical scarring with urinary tract infection by clinical features and ultrasonography. *Arch Dis Child.* 2000;82:376–380
20. Hoberman A, Charron M, Hickey RW, et al. Imaging studies after a first febrile urinary tract infection in young children. *N Engl J Med.* 2003;348:195–202

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