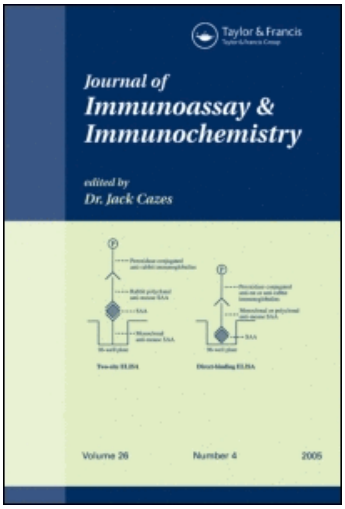


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# Urinary Evaluation of the Balance Between the Soluble Interferon-Gamma Receptor (IFN-γR1) and the Interleukin-4 Receptor (IL-4Rα) in Children with Vesicoureteral Reflux

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**Abstract:** We planned to investigate the urinary soluble cytokine receptor profile in patients with vesico-ureteric reflux (VUR). The urine levels of soluble interferon-γ receptor R1 (sIFN-γR) and soluble interleukin-4 receptor α (sIL-4R) were measured using an ELISA technique. The urine levels of sIFN-γR in the patients with VUR were significantly higher than those in the healthy controls ($p < 0.001$). On the other hand, although the urine sIL-4R levels in the patients with VUR were also higher than those in the controls, there were no significant differences between them. The urinary soluble receptor levels did not correlate with the clinical severity of VUR. These results suggest that there may be an immunological basis to VUR complicatedly.

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**Keywords:** Cytokine balance, Reflux nephropathy, Soluble interferon-gamma receptor (sIFN-$\gamma$R1), Soluble interleukin 4 receptor (sIL-4R$\alpha$), Urine, Vesicoureteral reflux (VUR)

## INTRODUCTION

Vesicoureteral reflux (VUR) is a common finding in children presenting with urinary tract infection (UTI) and those that have been prenatally diagnosed urinary tract dilatation and in relatives of index patients.[1,2] Children with VUR are believed to be at risk of ongoing renal damage and 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]



Cytokines are small regulatory peptides with diverse functions. They exert their actions on target cells via specific receptors. A number of cytokine receptors not only have membrane-bound forms but also soluble counterparts (soluble cytokine receptors) in body fluids such as serum and urine, which are formed either by proteolytic cleavage of the membrane-anchored receptor (sIL-2R, sIL-1R, sTNF-R) or by alternative splicing of mRNA (sIL-4R, sIL-5R, sIL-7R, sG-CSF R).[5,6] The soluble cytokine receptors retain their ligand binding capacity and act as agonists, antagonists of cytokine signaling[6–8] or carrier proteins of the ligand to target cells.[5,6,9] Thus, soluble cytokine receptors might modulate immune reactions.

The balance between the soluble Th1 type cytokine receptor (IL-2 receptor, IFN-$\gamma$ receptor) and the soluble Th2 type cytokine receptor (IL-4 receptor) in serum and urine showed considerable variation according to the disease state of diarrhea positive hemolytic uremic syndrome (D + HUS) in our previous study.[10–12] However, less is known about the balance of soluble cytokine receptors in body fluids involved in other diseases. We suggested that the simultaneous evaluation of multiple kinds of soluble cytokine receptors may provide useful information on the state of other kidney and urologic diseases.

In the present study, we evaluated the urine levels of the soluble interferon-$\gamma$ receptor R1 (sIFN-$\gamma$R1, a Th1 type cytokine receptor) and the sIL-4R$\alpha$ chain (Th2 type cytokine receptor) in children with VUR in the absence of a recent UTI episode.

## EXPERIMENTAL

### Subjects

The study Group 1 consisted of 20 VUR children with a median age of 1.6 years (range: 8 months-5.0 years) and included 14 boys and 6 girls. There were 15 (75%) children with unilateral reflux and 5 (25%) with bilateral reflux. Eight (40%) of the children with VUR had developed renal scars. VUR was diagnosed based on voiding cystourethrography (VCUG) and a 5-grade scale taken from the International Reflux Study in Children (IRSC).[13] Moreover, all the children underwent ultrasonographic examination of their urinary system and, if necessary, dimercaptosuccinic acid (DMSA) renoscintigraphy. VCUG was performed when an abnormal ultrasonographic image was produced. Examinations in the study group were performed at least 4 weeks after the UTI, but in most children, this period was longer than 3 months. The children had no signs of UTI, and the acute phase indicators were negative. The control Group 2 included 20 healthy children (11 boys, 9 girls) with a median age of 6.0 (range 2.0–6.0) years and a negative familiar history of urinary tract abnormalities and a negative history of UTI. They were not treated with any drugs at the point of examination. Urine samples were centrifuged at 2000 $\times$g for 10 min to remove insoluble material and immediately frozen and stored at $-70^{\circ}$C until use. The purpose, procedure, and benefits of our project were explained to the participants or their parents, and we obtained their informed consent.



### Measurement of the Soluble Cytokine Receptor Level

The urine sIFN-$\gamma$R1 and sIL-4R$\alpha$ levels were measured with an enzyme-linked immunosorbent assay (ELISA) that we described in our previous study.[10]

The concentrations of urine sIFN-$\gamma$R and sIL-4R were corrected for urine creatinine (Cre) content and expressed in ng/mg Cre.

## RESULTS

### Urinary sIFN-$\gamma$R and sIL-4R Levels in Patients with VUR

The urine sIFN-$\gamma$R levels were significantly higher in the patients with VUR (Group 1: median: 0.609 ng/mg creatinine) than in the healthy controls (Group 2: median: 0.052 ng/mg creatinine) (Table 1). On the other hand, although the urine sIL-4R levels in the patients with VUR

**Table 1.** sIFN-$\gamma$R and sIL-4R levels in urine in children with vesicoureteral reflux (VUR)

| Groups | Number | Median (IQR) sIFN-$\gamma$R (ng/mg Cre) | sIL-4R (ng/mg Cre) | sIFN-$\gamma$R/ sIL-4R ratio |
|---|---|---|---|---|
| VUR (Group 1) | 20 | 0.609 (1.043) | 5.963 (8.589) | 0.006 (0.023) |
| Control (Group 2) | 20 | 0.052 (0.101) | 3.653 (4.223) | 0.011 (0.048) |
| p1 | | $p < 0.001$ | $p = 0.2134$ | $p = 0.4171$ |
| Unilateral VUR | 15 | 0.571 (1.273) | 6.264 (10.568) | 0.004 (0.045) |
| Bilateral VUR | 5 | 0.648 (0.488) | 2.769 (4.898) | 0.007 (0.010) |
| p2 | | $p = 0.8958$ | $p = 0.1761$ | $p = 0.4581$ |
| VUR with scarring | 8 | 0.414 (1.122) | 3.014 (5.232) | 0.006 (0.040) |
| VUR without scarring | 12 | 0.648 (0.980) | 6.132 (16.793) | 0.005 (0.021) |
| p3 | | $p = 0.8774$ | $p = 0.3159$ | $p = 0.4171$ |

p1 – a comparison of Group 1 with Group 2, p2 – a comparison of unilateral VUR with bilateral VUR, p3 – a comparison between VUR with and without renal scarring. The Mann-Whitney U rank sum test was used to investigate significant differences. IQR: inter-quartile range, Cre: urine creatinine.



(Group 1: median: 5.963 ng/mg creatinine) were also higher than those of the controls (Group 2: median: 3.653 ng/mg creatinine), there were no significant differences between them (Table 1).

No statistically significant differences were observed in the urine sIFN-$\gamma$R and sIL-4R levels between the children with unilateral and bilateral VUR (Table 1). Moreover, no statistically significant differences were observed in the urine sIFN-$\gamma$R and sIL-4R levels between the VUR cases with scarring and those without scarring (Table 1). There were no significant differences in the ratio of urine sIFN-$\gamma$R and sIL-4R levels (the so called "soluble cytokine receptor balance") in any of the comparisons performed.

A statistical analysis of the results according to the VUR grade of individual patients is shown in Figure 1. Spearman's correlation rank coefficients (two-tailed) were used to evaluate the relationship between VUR grade and urinary soluble cytokine receptor levels. There was no significant statistical correlation between urine soluble cytokine receptor levels and VUR grade (sIFN-$\gamma$R vs VUR grade: $p = 0.5429$, sIL-4R vs VUR grade: $p = 0.5904$).

## DISCUSSION

VUR is recognized to be an important predecessor of reflux nephropathy because renal scarring is observed in 30% of refluxing kidneys following an initial episode of urinary sepsis.[14,15] The characteristic morphological

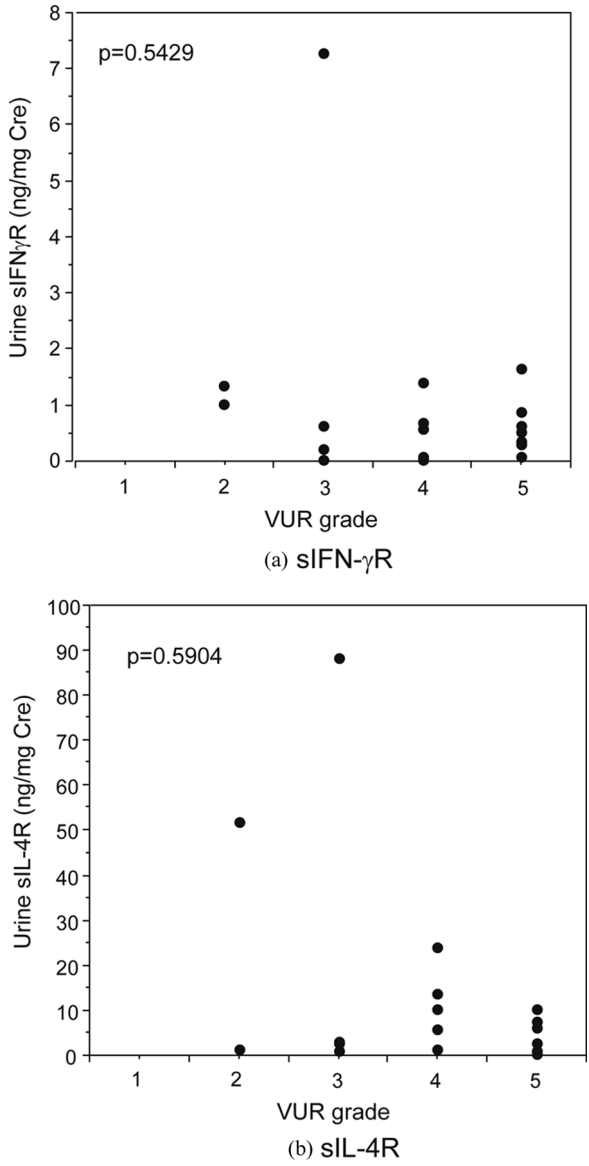


**Figure 1.** The correlation between urinary soluble cytokine receptors levels and VUR grade. Graphs representing the urine sIFN-γR levels (a) and sIL-4R levels (b) of 20 patients with VUR. The closed circles indicate the urinary soluble cytokine receptor levels of individual patients. Each patient was assigned according to the 5-grade scale of the International Reflux Study in Children (IRSC).[13] Spearman's correlation test was applied to evaluate the possible correlation between urinary soluble cytokine receptors levels and VUR grade.

feature in kidneys with reflux nephropathy is a prominent mononuclear inflammatory cell infiltration.[16] Therefore, we considered that the pathogenesis of VUR might involve in the immunological responses. Increased levels of several cytokines such as IL-6, tumor necrosis factor $\alpha$ (TNF-$\alpha$),[17] and IL-8[18] have been reported in the body fluids of patients with VUR in the absence of a recent UTI episode.

A Th1/Th2 cytokine imbalance has been observed in a variety of pathological conditions.[19,20] Soluble cytokine receptors might be concerned in the regulation of Th1/Th2 balance. We reported that the soluble cytokine receptor balance in both serum and urine shifts with disease states in patients with HUS.[10–12] In the present study, to investigate the balance of urinary soluble cytokine receptors in patients with VUR and renal scarring, we measured both the sIFN-$\gamma$ receptor R1 (Th1 type cytokine receptor) and sIL-4R$\alpha$ (Th2 type receptor) levels in urine.

As far as we know, this is the first study that has described the urine levels of sIFN-$\gamma$R and sIL-4R in patients with VUR. In the present study, both the urine sIFN-$\gamma$R and sIL-4R levels in children with VUR (Group 1) were higher than those in the control group (Group 2) (Table 1). The high levels of both urine soluble cytokine receptors are probably the consequence of inflammation and tissue damage as might be expected in pyelonephritis associated VUR. The alteration in urine of both types of soluble receptors was independent of VUR grading (Figure 1), the unilateral and bilateral types of VUR (Table 1), and the presence of scars (Table 1). Thus, urinary soluble receptors levels are not correlated with the clinical severity of VUR. The chronic inflammatory cell infiltration associated with reflux nephropathy might offer an explanation for the secretion of the soluble receptors.

The balance between sIFN-$\gamma$R and sIL-4R (the sIFN-$\gamma$R/sIL-4R ratio) in the patients with VUR did not shift (Table 1). The results suggest that both types of the soluble receptors were elevated in patients with VUR. The sIL-4R level was increased in the bronchoalveolar lavage from patients with asthma, which is a Th2 dominant disease, compared with the nonatopic controls.[21] Under culture conditions, the release of sIL-4R was paralleled by the secretion of IL-4 by T cells.[9] Although few reports regarding the relationship between the serum sIL-4R level and disease status have been published, the elevated sIL-4R level might be related to Th2 dominant diseases. On the other hand, little information is available on sIFN-$\gamma$R. Thus, the pathological significance of sIFN-$\gamma$R remains unclear. The pathological significance of the soluble cytokine receptor balance in body fluids needs to be studied further.

Our results indicate that the changes in the urine Th1 type cytokine-soluble receptor (sIFN-$\gamma$R) levels depend on the onset of VUR. To examine patients with VUR, the urine soluble cytokine receptor balance might provide a more feasible method of measurement than serum values.

To present solid evidence, further studies, including model animal experiments using the $Pax2^{1Neu+/-}$ mouse, which develops VUR spontaneously,[22] are needed to clarify the physiological and clinical significance of the balance of soluble cytokine receptors.

## CONCLUSION

We determined the soluble cytokine receptor balance between sIFN-$\gamma$R and sIL-4R in patients with VUR. We have documented that the urine sIFN-$\gamma$R levels in patients with VUR in the absence of a recent UTI episode were significantly higher than those in the control. However, the urinary sIFN-$\gamma$R levels did not correlate with the clinical severity of VUR. To present solid evidence, further detailed studies with larger patient groups should be conducted to clarify the soluble cytokine receptor balance system involved in VUR.



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