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Urinary monocyte chemotactic protein 1 as a predictive marker of steroid responsiveness in children with idiopathic nephrotic syndrome

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Abstract

Objective: We previously reported that macrophages contribute to the pathogenesis of refractory nephrotic syndrome (NS). To elucidate the mechanism behind macrophage accumulation and to identify a predictive biomarker of steroid responsiveness, we compared differences in cytokine and chemokine levels in serum and urine between steroid-sensitive (SSNS) and steroid-resistant (SRNS) children with NS.

Methods: Eighteen children with NS (7.1±4.3 years; male-to-female ratio, 12:6) were divided into an SSNS group (n=10) and an SRNS group (n=8) according to their clinical course. Serum and urinary samples were collected at the time of onset and remission. Samples from age-matched healthy children were used as controls (n=15). Cytokines and chemokines were measured using a cytometric bead array kit.

Results: Clinical findings and laboratory data at sampling were comparable between the SRNS and SSNS groups. Serum cytokines and chemokines did not significantly differ at the time of onset between remission and control groups. In contrast, at onset, several urinary chemokines were significantly elevated in children with NS (IP-10, MCP-1, MIG, RANTES; all $p<0.01$). Urinary MCP-1 levels were significantly elevated in the SRNS group compared with the SSNS group ($p<0.01$).

Conclusions: Chemokines might be associated with the pathogenesis of NS. Increased urinary excretion of MCP-1 in children with SRNS is a potential predictive biomarker of steroid responsiveness in idiopathic NS. Further histological studies, including macrophage accumulation, are required to determine the mechanisms of steroid resistance in refractory NS.

Keywords: Biomarkers, Macrophages, Monocyte chemotactic protein-1, Steroid-resistant nephrotic syndrome, Urinary chemokines

Introduction

Idiopathic nephrotic syndrome (NS) is one of the most common kidney diseases in children and is characterized by heavy proteinuria, hypoalbuminemia, edema, and hypercholesterolemia. Most cases of idiopathic NS are steroid-sensitive; however, some cases become steroid-resistant during their clinical course.

Although the pathogenesis and pathophysiology of idiopathic NS is not clearly understood, it is reported that the immune system, including activation of lymphocytes, plays an important role.[1,2] An association among several cytokines and chemokines with idiopathic NS has been reported[2–4]; however, there is little information regarding urinary cytokine and chemokine levels in idiopathic NS.

Histological reports suggest that macrophages play an important role in the pathogenesis of focal segmental glomerulosclerosis, a form of steroid-resistant NS.[5,6] We have also previously reported that macrophages contribute to the pathogenesis of refractory pediatric NS.[7]

To elucidate the mechanism behind macrophage accumulation and to identify a predictive biomarker of steroid responsiveness, we compared differences in cytokine and chemokine levels in serum and urine between steroid-sensitive NS (SSNS) and steroid-resistant NS (SRNS) children.

Methods

Patients

Eighteen patients with INS, who were admitted to the Fujita Health University Hospital or Toyokawa City Hospital between 2013 and 2015, were included in this study (Table 1). Patients were divided into a steroid-sensitive group (n=10) and a steroid-resistant group (n=8). SSNS was defined as patients who were in remission within 4 weeks of steroid therapy. SRNS was defined as patients who were not in remission within 4 weeks of steroid therapy, and were treated with high-dose intravenous methylprednisolone and/or cyclosporine resulting in remission. Serum and urine samples were collected at the time of onset (initial onset or relapse) and remission, and stored at –70°C for subsequent measurement of cytokines and chemokines. Control serum and urine samples were collected from age-matched

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Table 1 Patients' profiles

	SSNS Group	SRNS Group	Control	P-value
Number per group	10	8	15	—
Male:Female	6:4	6:2	6:9	ns
Age at initial onset (years)	5.8±3.6	5.1±4.1	—	ns
Age at sampling time of onset (years)	6.2±3.6	9.8±5.3	6.7±2.3	ns
Time from starting therapy to remission (days)	7.1±2.3	75.6±76.8	—	P<0.05
Time from proteinuria disappearance to sampling at remission (days)	5.9±6.3	5.6±4.1	—	ns
Proteinuria (g/g Cr)	16.8±19.1	17.5±18.3	—	ns
Serum albumin (g/dL)	1.7±1.1	2.2±1.0	—	ns
Serum total cholesterol (mg/dL)	429±144	385±140	—	ns
Serum creatinine (mg/dL)	0.29±0.14	0.35±0.17	—	ns
eGFR (mL/min/1.73 m^2)	177±44.3	174±62.6	—	ns
Selectivity index at onset	0.12±0.07	0.09±0.10		ns
PSL at sampling (mg/kg/day)				
Onset	0.0±0.0	0.9±0.6	—	P<0.001
Remission	1.5±0.6	1.2±0.4		ns
Patients underwent renal biopsy	3	8	—	
Minimal change	3	7	—	
Mesangial proliferative nephritis	0	1		

SSNS: steroid-sensitive nephrotic syndrome; SRNS: steroid-resistant nephrotic syndrome; eGFR: estimated glomerular filtration rate; PSL: prednisolone.

healthy (non-renal/inflammatory disease) children (n=15).

The study was conducted in accordance with the principles contained within the Declaration of Helsinki and approved by the institutional review board of Fujita Health University. Written informed consent for the use of samples for research purposes was obtained from the parents of all patients.

Cytokine analysis

The quantification of six cytokines, including interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), interleukin (IL)-2, IL-4, IL-6, and IL-10, and five chemokines, IL-8, regulated on activation, normal T-cell expressed and secreted (RANTES), monokine induced by interferon-γ (MIG), monocyte chemotactic protein-1 (MCP-1), and interferon inducible protein-10 (IP-10), in serum and urine were determined using a cytometric bead array kit (BD Biosciences, San Jose, CA, USA). Assays were performed according to the manufacturer's instructions. Serum and urine biomarkers from the two patient groups were compared (onset *vs.* remission, onset *vs.* control, and remission *vs.* control). Additionally, serum and urine biomarkers from patients with SSNS and SRNS were compared.

Statistical analysis

Demographic factors and clinical data of patients with SSNS and SRNS were compared using the Fisher's exact test or Mann–Whitney U test, as appropriate. One-way analysis of variance (ANOVA) with post-hoc analysis using the Tukey's multiple-comparison test was used for comparisons among the three groups. Pearson's single and Spearman's correlation coefficients were used for correlation analysis of parametric and non-parametric data, respectively. Statistical analyses were performed using GraphPad 6.0 (GraphPad Software, Inc., La Jolla, CA, USA). Data are expressed as median and interquartile range (25–75th percentile). A P-value <0.05 was considered statistically significant.

Results

Patient profiles

There were no significant differences in age and gender among SSNS, SRNS, and control groups. Laboratory data (urine protein, serum albumin, total cholesterol, creatinine levels, estimated glomerular filtration rate, and selectivity index) were also compatible between SRNS and SSNS groups. The dose of prednisolone at sampling was significantly higher in the SRNS group compared with the SSNS group at onset, although there was no difference between the groups at remission. The time from proteinuria disappearance to sampling at remission was not different between the SSNS and SRNS groups. Eleven patients (SSNS=3, SRNS=8) underwent renal biopsies for their unfavorable clinical course; which showed minimal change in 10 patients (SSNS=3, SRNS=7), and mesangial proliferative glomerulonephritis in one SRNS patient. The three children in the SSNS group underwent a biopsy because of their frequent relapse.

Comparison of serum or urinary cytokine and chemokine levels at onset and at remission

Most cytokines (IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-10) were scarcely detected in the serum of NS children and the control group (data not shown). Several chemokines were elevated in the serum, although there were no differences at the time of onset and remission in NS children and the control group, except for RANTES. The serum RANTES level was significantly lower in NS children compared with the control group, although there was no difference in NS children between onset and remission (Figure 1). In contrast, several urinary chemokines (IP-10, MCP-1, MIG, and RANTES) were significantly higher in NS children at onset compared with at remission and compared with the control group (Figure 1).

Comparison of serum or urinary cytokine and chemokine levels between SSNS and SRNS groups

Serum cytokine and chemokine levels did not significantly

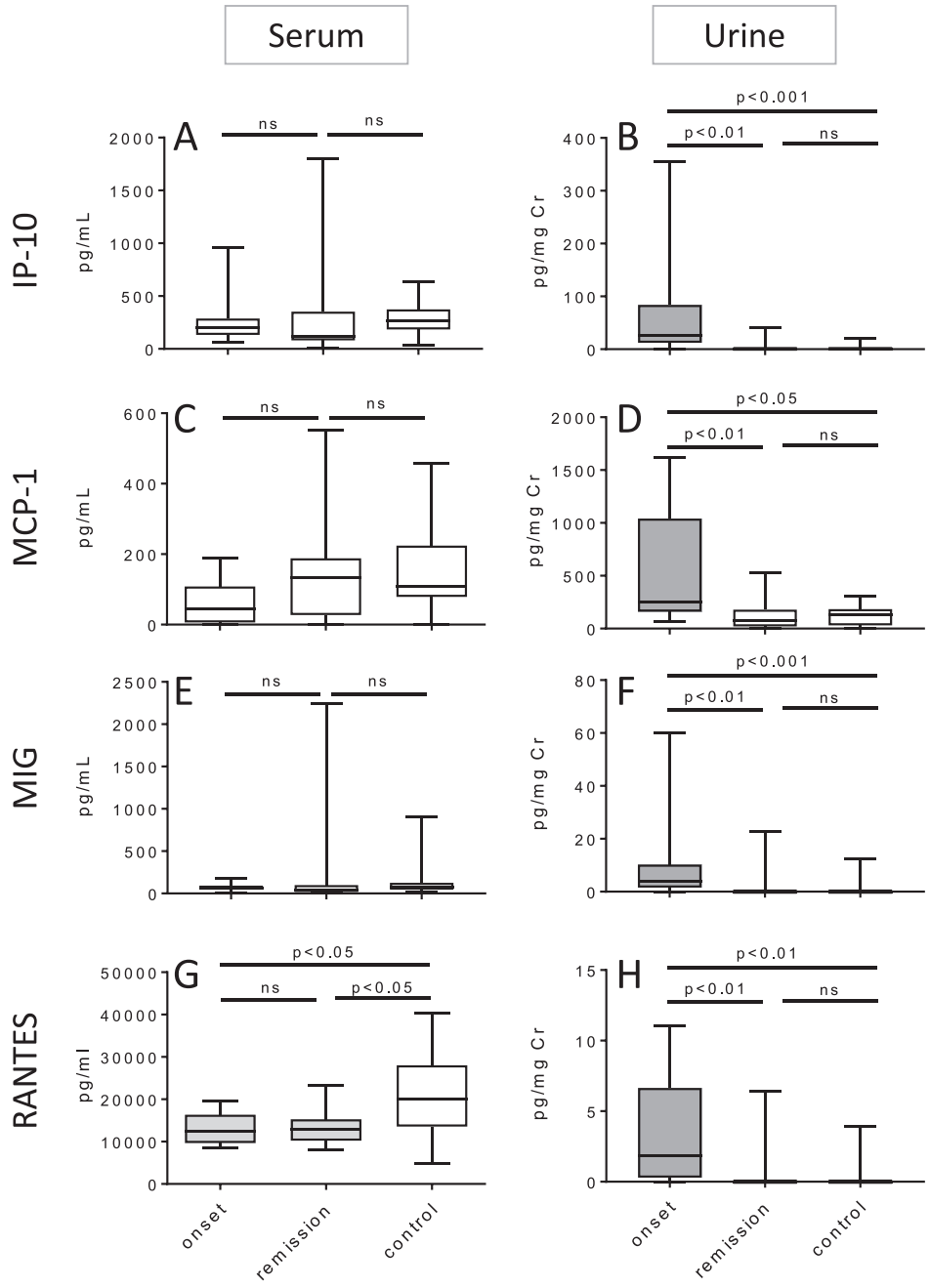

Figure 1 Comparison of serum (A, C, E, G) or urinary (B, D, F, H) chemokine levels among children with nephrotic syndrome at onset, at remission, and normal children (control).

differ among the SSNS, SRNS, and control groups, except for RANTES; which was significantly lower in the NS group (in both the SSNS and SRNS groups) compared with the control group. In contrast, several chemokines, including IP-10, MCP-1, MIG, and RANTES, and IL-6 were elevated in urine samples from children with NS at onset. Among these chemokines, the urinary MCP-1 level was significantly elevated in the SRNS group (Figure 2). These urinary cytokine and chemokine levels at remission did not significantly differ between the SSNS and SRNS groups.

Relationships among serum and urinary chemokines and cytokines

There was no relationship among serum chemokines and urinary chemokines or protein levels (data not shown). For urinary cytokines and chemokines, the urinary MCP-1 level significantly correlated with urinary IL-6 and IP-10 levels, but not with the other cytokines or chemokines (Figure 3).

Discussion

In this study, we found that several serum and/or urinary chemokines were elevated in children with NS, suggesting involvement of immune cells in the development of NS. These chemokines, that increased in urine only at onset (IP-10, MCP-1, MIG, and RANTES), would be involved in the development and/or recurrence of NS. It is possible that the relatively high dose of prednisolone given to patients in the SRNS group at

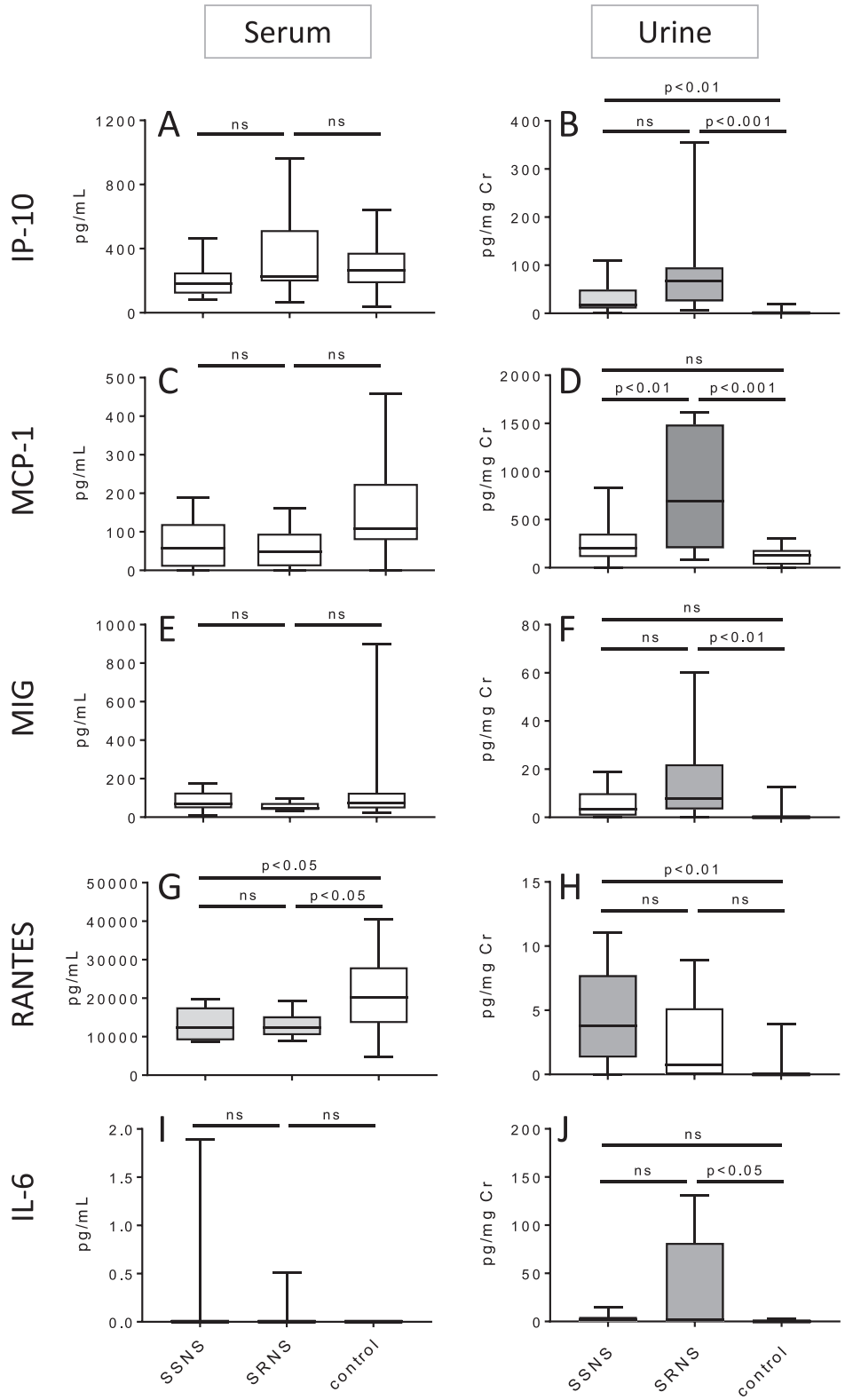

Figure 2 Comparison of serum (A, C, E, G, I) or urinary (B, D, F, H, J) chemokine levels among steroid-sensitive nephrotic syndrome (SSNS), steroid-resistant nephrotic syndrome (SRNS), and normal control groups.

onset might have affected the urinary MCP-1 level. However, there were no significant differences in doses of prednisolone nor in urinary MCP-1 levels between the groups at remission despite the cumulative dose of prednisolone being significantly higher in the SRNS group. This suggests that elevated urinary MCP-1 in the SRNS group at onset was not due to prednisolone treatment but may have been due to the underlying pathological differences between the groups. Although, similar findings have been

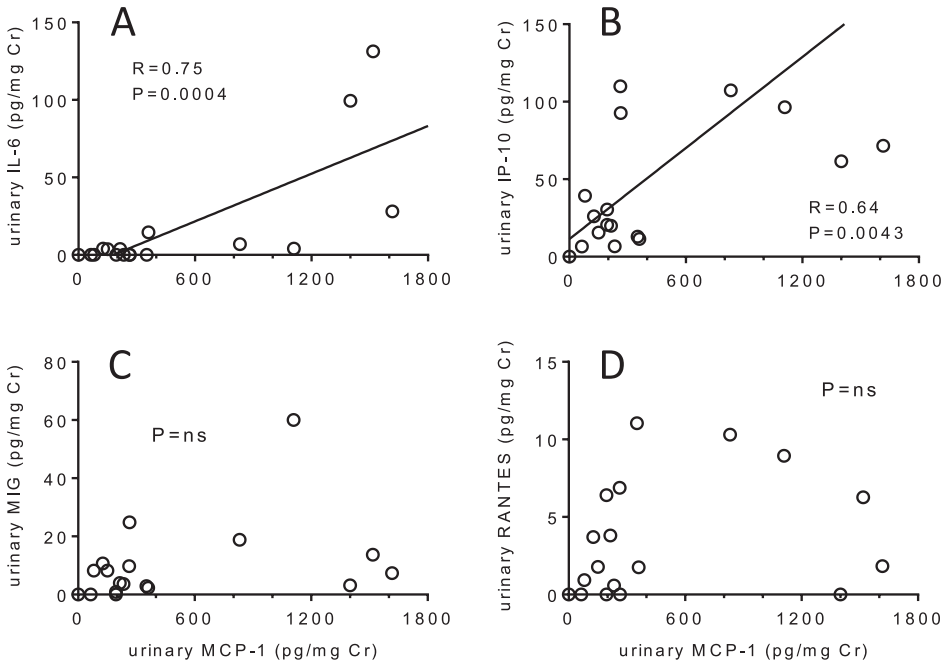

Figure 3 The relationship between urinary MCP-1 and (A) urinary IL-6, IP-10 (B), MIG (C), and RANTES (D).

reported in previous studies,[2,8] our finding that urinary MCP-1 was elevated significantly in the SRNS group, but not in the SSNS group, suggests that urinary MCP-1 could be a predictive biomarker of steroid responsiveness in INS.

MCP-1 is a chemokine that recruits monocytes/macrophages into foci of active inflammation.[9] Many reports indicate the important roles of macrophages in the pathogenesis of focal segmental glomerulosclerosis; one of the important causes of SRNS.[10–12] We have also reported that macrophages play an important role in the pathogenesis of SRNS, showing a significant increase in glomerular macrophage accumulation in biopsies from children with SRNS compared with those with SSNS.[7] These results suggest that MCP-1 could be involved in the mechanism for steroid-resistance via recruitment of monocytes/macrophages into kidney tissue.

Another important finding was that MCP-1 increased only in urine and not in serum, and there was no relationship between serum and urinary MCP-1 levels (data not shown). This suggests that urinary MCP-1 might be kidney tissue-derived and not leakage of serum-derived MCP-1. Although we did not perform immunohistochemistry for MCP-1 using biopsied tissue, it has been reported that glomerular cells, such as mesangial cells, could produce MCP-1 in response to various stimuli.[13–16] These findings support our idea that urinary MCP-1 was glomerular cell-derived, and might contribute to the recruitment of macrophages into glomeruli.

The role of glomerular macrophages in SRNS and the mechanism responsible for the contribution of macrophages to steroid-resistance have not been elucidated. The significant association between urinary MCP-1 and IL-6, or IP-10 in the current study gives rise to the hypothesis that macrophages recruited into glomeruli by the stimulation of MCP-1 could produce IL-6 or IP-10, which then contributes to tissue injury or further recruitment of immune cells.[17–21]

The major limitations of this study are the limited sample size and the potential lack of validity of the histological assessments. Histological assessments for MCP-1 expression and macrophage accumulation should provide informative data, including the source of MCP-1 in the kidney, and the role of MCP-1 on kidney tissue in SRNS.

In conclusion, we found that increased urinary excretion of MCP-1 in children with SRNS was a potential predictive biomarker of steroid responsiveness in INS. MCP-1 may play a vital role in the pathogenesis of steroid resistance. Further histological studies are required to determine the mechanisms behind steroid resistance in refractory NS.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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