Table 3 Correlations between the ratios of post- to pre-CsA administration for five gene products

Gene products	Correlation	Gene products			
		GRP94	eIF2α	Bcl-2	GADD153
GRP78	ρ	0.78	0.90	0.87	0.28
	P	<0.001	<0.001	<0.001	0.27
GRP94	ρ		0.71	0.73	0.09
	P		0.0015	<0.001	0.72
eIF2α	ρ			0.86	0.45
	P			<0.001	0.073
Bcl-2	ρ				0.19
	P				0.46

ρ, Spearman rank correlation coefficient (Spearman's rho)

Immunofluorescence staining for GRP78 and GRP94 was performed on renal biopsy tissue of three patients, all of which showed similar results. Representative images of immunofluorescence staining for GRP78 and GRP94 in renal biopsy tissues pre- and post-CsA administration from a patient are shown in Fig. 2. The expressions of GRP78 and GRP94 were remarkably increased in tubuli of renal biopsy specimens post-CsA administration compared with those pre-CsA administration. In the renal biopsy tissues of the patients examined in this study, routine electron microscopic studies displayed no sign of apoptosis.

Discussion

The molecular events occurring in renal cells as a result of CsA administration are not well understood, especially in humans. Improved management of CsA treatment has resulted in a decreased incidence of nephrotoxicity in clinical practice [13, 14], but it is important to determine the molecular events due to CsA for future prevention of nephrotoxicity.

In our study we examined the molecular events due to CsA in clinical practice, not in non-clinical experimental conditions. Therefore, although the number of patients (N=17) was low, our data provide valuable information. In a recently published study based on our clinical practice, we reported that based on monthly measured C_0 or C_2 levels of CsA, CsA doses could be adjusted for well-designed targets and that this approach appeared to reduce CsA nephrotoxicity [13, 14].

To reduce selection bias for patients, we attempted to include all patients with similar treatment conditions during the study period. Proteinuria itself induces ER stress [19–21]. Previous studies have demonstrated that the tubulointerstitial ER stress response may occur due to an increase in filtered proteins and that ER stress may be proapoptotic. Therefore, we selected patients for our study who showed no signs of proteinuria in their pre- and post-CsA renal biopsies.

Even under clinical conditions, our study demonstrated CsA-induced molecular events. Our data showed that the expressions of GRP78, GRP94, eIF2α, and Bcl-2 were significantly upregulated, while GADD153 expression was unchanged post-CsA therapy. These data suggest that CsA induced an UPR due to ER stress, but did not cause apoptosis when administered in low doses. A balance between ER stress and the UPR, where apoptosis does not occur in CsA administration, appears to be important.

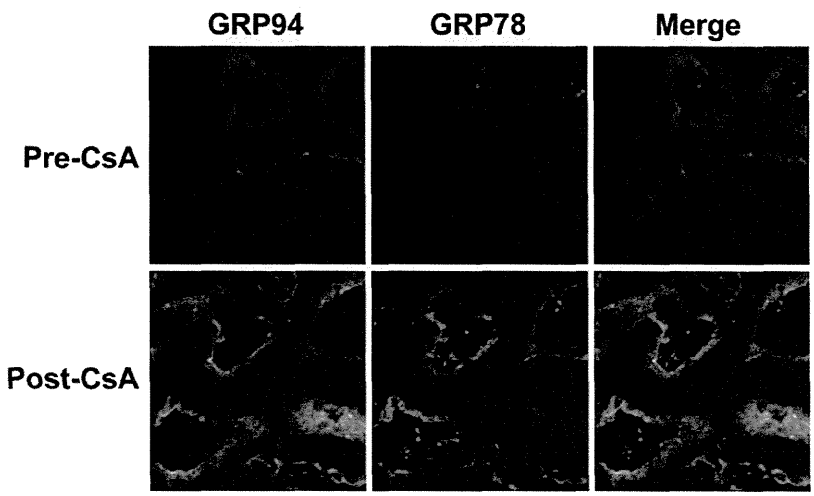

Fig. 2 Representative images of immunofluorescence staining for GRP78 and GRP94 in renal biopsy tissues pre- and post-cyclosporine (CsA) administration from a patient with frequently relapsing nephrotic syndrome (original magnification, ×400)

As shown in Fig. 1, molecular changes in GRP78, GRP94, eIF2α, and Bcl-2 ranged from no change to maximum changes, indicating that ER stress was not observed in some patients. The distribution/variation of the data may reflect inter-individual differences in the efficiency of CsA action, as well as the effect of various uncontrollable factors in clinical practice. We attempted to identify the relationships between trends of ER stress-related molecules and clinical characteristics, such as age, monitoring methods (C_0 or C_2), blood concentrations of CsA, and the presence of relapse during CsA administration. However, we did not find any such relationships (data not shown), possibly due to the low number of patients in our study. However, other possibilities should be considered. The ER stress-related molecular changes observed in our study were events that occurred prior to any changes in the clinical parameters within the framework of low-dose conditions. Low-dose CsA administration may be one of the reasons for a lack of relationship between the clinical data and molecular events. Strictly controlled CsA administration resulted in narrow ranges of CsA concentrations in our study (Table 1). As a result, since we did not observe CsA nephrotoxicity in any of the 17 patients, we did not examine ER stress-related molecules in patients with CsA nephrotoxicity. This is a weak point of this study. We cannot claim that ER stress causes apoptosis with CsA nephrotoxicity based on the data of our study. It would be interesting to investigate the same ER stress-related molecules in kidney specimens with CsA nephrotoxicity. Further studies are required to investigate these specimens.

Several possible limitations should be considered with respect to study. First, the number of patients with protein data was small. Second, there is the possibility of data modification by steroids at pre-CsA administration. All 17 patients in our study were given prednisolone at biopsy pre-CsA administration. However, the finding of pre-CsA data distributed at approximately 1.0 relative to the control with no medication supports the reliability of our data. It should also be noted that the mechanisms of CsA nephrotoxicity are multifactorial, i.e., not only ER stress, but include other mechanisms, such as mitochondrial injury [2].

It has been shown that ER stress is implicated in congenital nephrotic syndrome and drug-induced nephrotic syndrome [8, 21]. Mutations in the cytoskeletal protein, α-actinin-4, lead to a familial form of focal segmental glomerulosclerosis (FSGS). Mice that develop FSGS with an α-actinin-4 K256E transgene in podocytes demonstrate glomerular ER stress, including the upregulation of ER chaperones, phosphorylation of eIF2α, and the induction of GADD153 [22]. CsA may stabilize the podocyte [23] and thus changes in protein expression after this treatment may not be due to CsA effects at all, but rather reflect glomerular mechanisms instead.

In conclusion, the results of our study demonstrate that the UPR due to ER stress induced by CsA may act in a defensive manner and that less apoptosis occurs under low-dose conditions. This finding may be important for the rationale for administering CsA.

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BRIEF REPORT

Atypical *Pneumocystis jiroveci* pneumonia with multiple nodular granulomas after rituximab for refractory nephrotic syndrome

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Abstract
Background Rituximab, an anti-CD20 antibody that targets B cells, is a promising agent against steroid-dependent and steroid-resistant nephrotic syndrome in children.
Case-Diagnosis/Treatment We report a 3-year-old boy who presented with atypical *Pneumocystis jiroveci* pneumonia (PCP) following administration of rituximab for refractory nephrotic syndrome. He had received cyclosporine and daily prednisolone for over 1 year. Following rituximab therapy, a hazy shadow was observed on his chest X-ray. Chest-computed tomography revealed multiple nodular lesions in bilateral lungs, although his clinical symptoms were subtle. PCR analysis demonstrated the presence of *Pneumocystis* DNA in his bronchoalveolar lavage. Lung wedge resection of the nodular lesion exhibited granulomas containing a few cysts of *P. jiroveci* that primarily consisted of T cells and histiocytes and lacked B cells. A deficiency of B cells following rituximab treatment suggests a dramatic effect on the immune response and, therefore, could result in granulomatous PCP. Nodular granulomatous lesions of PCP comprise an emerging concept previously reported in adults with hematological disease, bone marrow transplant, or treatment with rituximab. We report the first pediatric case of nodular PCP. Granulomatous PCP can be life-threatening. Moreover, bronchoalveolar lavage often fails to demonstrate the presence of *P. jiroveci* DNA. Wedge biopsy is warranted for definitive diagnosis. Our patient fully recovered with sulfamethoxazole/trimethoprim treatment because of early detection.
Conclusions The indication of rituximab for refractory nephrotic syndrome has increased recently. Therefore, recognition of the risk of atypical PCP is important. Our findings suggest that PCP prophylaxis should be considered following rituximab therapy.

Keywords Nephrotic syndrome · Rituximab · *Pneumocystis jiroveci* · Granulomatous · Granulomatous *Pneumocystis jiroveci* pneumonia

Abbreviations

PCP	*Pneumocystis jiroveci* pneumonia
RTX	Rituximab
NS	Nephrotic syndrome
FRNS	Frequently relapsing nephrotic syndrome
SDNS	Steroid-dependent nephrotic syndrome
SRNS	Steroid-resistant nephrotic syndrome

Introduction

Treatment of refractory childhood nephrotic syndromes, such as frequently relapsing nephrotic syndrome/steroid-dependent (FRNS/SDNS) and steroid-resistant nephrotic syndrome (SRNS), remains a challenge. Although various

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immunosuppressive agents are effective, a substantial number of children are intractable. Recently, rituximab (RTX), a monoclonal antibody that targets the B cell specific antigen CD20, has been demonstrated to be effective for FRNS/SDNS and SRNS in children [1–3]. RTX is relatively well tolerated; however, occasionally severe or life-threatening adverse events occur, including progressive multifocal leukoencephalopathy [4], interstitial pneumonia [5], and ulcerative colitis [6]. Moreover, a decreased number of B cells potentially induces opportunistic infection and deterioration of infection. *Pneumocystis jiroveci*, formerly known as *Pneumocystis carinii*, pneumonia (PCP) is a rare but serious cause of mortality in patients with acquired immunodeficiency syndrome (AIDS), as well as in immunocompromised hosts. RTX increases susceptibility to PCP. Previous cases of PCP following rituximab treatment have been reported for various indications, such as B cell lymphoma [7–9], rheumatoid arthritis [10], Wegener's granulomatosis [11], autoimmune hemolytic anemia [12, 13], pure red cell aplasia [14], pemphigus [15], and acute rejection of kidney transplants [16, 17]. PCP usually presents as a bilaterally diffuse and fairly symmetric interstitial pattern on chest X-ray and as patchy ground-glass opacities on high-resolution computed tomography (hrCT). However, immunocompromised hosts who exhibit hematological malignancy with or without bone marrow transplants, chemotherapy including RTX, and AIDS infrequently present with atypical PCP with multiple nodular granulomatous lesions [18–20]. We report the first pediatric case of nodular granulomatous PCP in which the patient was treated with a single dose of 375 mg/m^2 of RTX against SDNS. RTX potentially increases the risk of granulomatous PCP. Written informed consent for publication of this information was obtained from the child's family.

Case report

The patient is a 3-year-old boy with SDNS. He had been healthy before he presented with NS at 2 years of age. At primary onset, he was initially treated with 25 mg/day (60 mg/m^2/day) of daily prednisolone (PSL) for 4 weeks. NS was in remission 10 days after initiation of PSL. PSL was then reduced to 16 mg/day (40 mg/m^2) on alternate days, but NS had relapsed soon after PSL reduction. He was treated with 25 mg of daily PSL again. Proteinuria resolved in 7 days. However, as PSL was reduced to 20 mg/day on alternate days, he experienced a second relapse. Thereafter, we stopped reducing PSL by administration on alternate days. Nevertheless, his NS repeatedly relapsed whenever daily PLS was reduced to less than 15 mg. Cyclosporine, losartan and mizoribine were added after 5, 8, and 9 months after diagnosis, respectively. Renal biopsy was performed 5 months after the onset of NS.

One year after onset, at his fifth relapse, the patient was treated with daily PSL 18 mg/day (32 mg/kg/m2), cyclosporine 50 mg/day (3.3 mg/kg/day), and losartan 15 mg/day (1 mg/kg/day). He was then treated with daily PSL 25 mg/day (1.8 mg/kg/day) for 4 weeks. The dose of PSL was slightly less than that used previously, because he was intolerant to the previous full dose of PSL owing to a mood disorder. After 4 weeks, his urinary protein/creatinine ratio was still 6, and serum albumin level was 2.5 g/dl. Additionally, he suffered from serious steroid-related toxicities, including growth retardation, obesity, and bilateral glaucoma. He was transferred to our institute because of SRNS.

After admission, he was treated with one course of methylprednisolone pulse therapy (30 mg/kg/day, three consecutive days). Four days after this therapy, his NS completely remitted. Thereafter, we administered 375 mg/m^2 of RTX 2 days after the remission, and we added two more courses of methylprednisolone pulse therapy for reduction of steroids and prevention of further relapses. The patient did not have a reaction to RTX infusion, and was finally discharged. After RTX infusion, daily PSL 1 mg/kg/day was continued for 1 month, and it was reduced to 0.25 and 0.5 mg/kg/day every other day. Cyclosporine was continued, and its 2-h blood concentration was maintained between 400 and 600 ng/dl. However, his NS relapsed 50 days after RTX infusion, and he was admitted to our clinic again. Upon admission, he experienced no symptoms. His temperature was 36.3 °C, heart rate was 110/min and blood pressure was 100/50 mmHg. However, his respiratory rate was slightly fast (30/min). Oxygen saturation remained above 95 % while the patient was awake, but dropped to 92 % during sleep. Other physical examinations did not exhibit any remarkable findings. However, a chest radiograph exhibited patchy and solid infiltrates spread over both lungs (Fig. 1a). Chest helical computed tomography (CT) revealed multiple solid opacities in bilateral lobes.

A peripheral blood examination showed the following: hemoglobin, 13.0 g/dl; platelets, 34.6×10^4/μl; and white blood cells, 6.1×10^4/μl with 72.9 % neutrophils, 18.2 % lymphocytes, 5.2 % monocytes, and 1.5 % eosinophils. The biochemistry profile indicated normal liver and renal function. Lactate dehydrogenase was elevated to 500 IU/l and the isoenzyme patterns suggested that the origin was the lung. The CRP level was 1.0 mg/dl, and erythrocyte sedimentation rate was 44.0 mm/1 h. Serum creatinine and blood urea nitrogen were 0.15 and 11.6 mg/dl, respectively, and serum total protein and albumin were 5.1 and 3.5 g/dl, respectively. Urinary protein was 208.5 mg/dl, and the urinary protein/creatinine ratio was 2.1. The amount of CD19- and CD20-positive B cells in peripheral blood was zero (before RTX infusion, they were 552 and 604/μl, respectively). β-d-Glucan was elevated to 322 pg/ml (normal: <20 pg/ml). Cytomegalovirus antigenemia, Epstein–Barr virus genome, and QuantiFERON (test for tuberculosis)

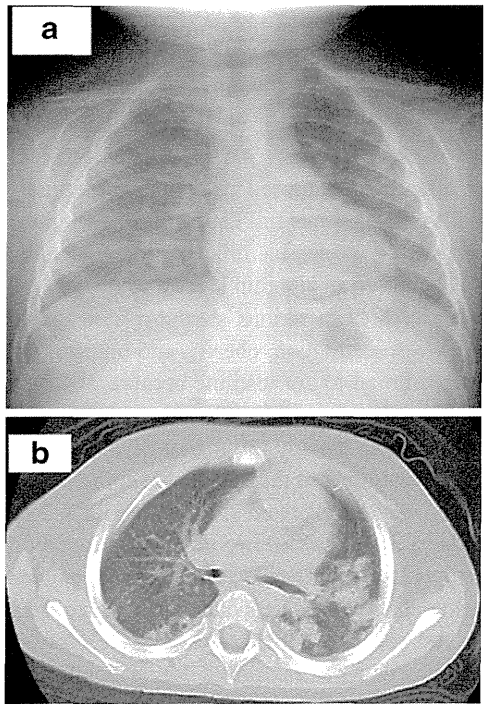

Fig. 1 Chest X-ray and high-resolution computed tomography (CT) scan of the lung. **a** Chest X-ray shows patchy and solid infiltrates spread over both lungs on admission. **b** A high-resolution CT scan of the chest 3 weeks after admission shows exacerbation of solid opacities in both lobes

results were all negative. Urinalysis exhibited mild albuminuria (urinary protein: 208 mg/dl, urinary creatinine: 99 mg/dl). Because of marked elevation of β-d-glucan, infection with fungus or *P. jiroveci* was suspected. We performed bronchoalveolar lavage (BAL) under general anesthesia on day 3. After bronchoscopy, the patient was treated with oral TMP-SMX for *Pneumocystis* at a dose of TMP 5 mg/kg every 6 h and voriconazole for fungus, including *Aspergillus* 15 mg/kg/day. PSL was maintained at 10 mg/day to prevent a withdrawal reaction, and cyclosporine was halted. Microscopy was negative but polymerase chain reaction analysis revealed *Pneumocystis* DNA. No other pathogens were detected in his blood culture and BAL. We stopped voriconazole and continued a full dose of TMP-SMX for 3 weeks followed by a prophylactic dose of TMP (5 mg/kg/day). Although CD19- and CD20-positive cells in peripheral blood recovered 8 months after RTX, he was treated with prophylactic TMP for 10 months because he was still being treated with MMF.

Although the patient's oxygen saturation improved, and β-d-glucan returned to a normal range, lactate dehydrogenase levels remained elevated. Another chest hrCT scan 3 weeks from the initiation of TMP-SMX demonstrated a further increase in the number and size of the pulmonary nodules (Fig. 1b). To rule out any other causes that were overlooked, the patient underwent open thoracic wedge resection of the nodule lesion. A biopsy sample showed necrotic granulomas containing a few cysts of *P. jiroveci* and Ziehl-Neelsen staining was negative (Fig. 2a/e). Immunohistochemistry revealed that the granulomas primarily consisted of CD3-positive T cells and CD68-positive histiocytes; however, there was an absence of B cells (Fig. 2b, c, d). His respiratory status remained stable, and lactate dehydrogenase soon returned to the normal range. A chest hrCT on the 52nd day exhibited an apparent decrease in nodular lesions. The patient's NS also spontaneously remitted along with the recovery of pulmonary findings.

Discussion

This is the first childhood case of granulomatous PCP. Additionally, this is the first case of PCP following treatment with rituximab against SDNS. PCP is a serious cause of mortality in immunocompromised patients, affecting up to 50 % of patients. Our patient was treated with a high dose of daily PSL, cyclosporine, and mizoribine for an extended period. Additional rituximab potentially strengthens immunosuppression and increases the susceptibility to PCP. However, our patient's clinical and radiological findings substantially differed from typical PCP, and we had to rule out viral or fungal infection, tuberculosis, and rituximab-induced interstitial pneumonia.

Accumulating evidence suggests that, under some circumstances, RTX may significantly increase the risk of PCP. Recently, cyclophosphamide, doxorubicin, vincristine and prednisone with rituximab (R-CHOP) against B-cell lymphoma was shown to increase the prevalence of PCP compared with classical CHOP (identical treatment without RTX). Additionally, PCP after rituximab has been previously reported in patients with rheumatoid arthritis, Wegener's granulomatosis, autoimmune hemolytic anemia, pure red cell aplasia, pemphigus, and acute rejection of kidney transplants. Therefore, B cell depletion by RTX may increase the risk of PCP. They suggested the necessity of PCP prophylaxis after RTX therapy.

The host's defense against *Pneumocystis* is thought to be critically dependent on CD4+ helper T cells. The incidence of PCP in human immunodeficiency virus (HIV) infected patients is elevated when the level of circulating CD4 cells falls below 200/μl. However, B cells potentially play a significant role in protection against *Pneumocystis*. Transgenic B-cell functionally deficient mice, in which B cells do not express major histocompatibility complex class 2 antigens (and thus were unable to act as antigen-presenting cells), are susceptible to *Pneumocystis carinii* (formerly the species *Murina*). These mice fail to clear the *Pneumocystis* infection, most likely because of the inefficient generation of protective CD4+ memory and effector T cells in the lungs [21]. The

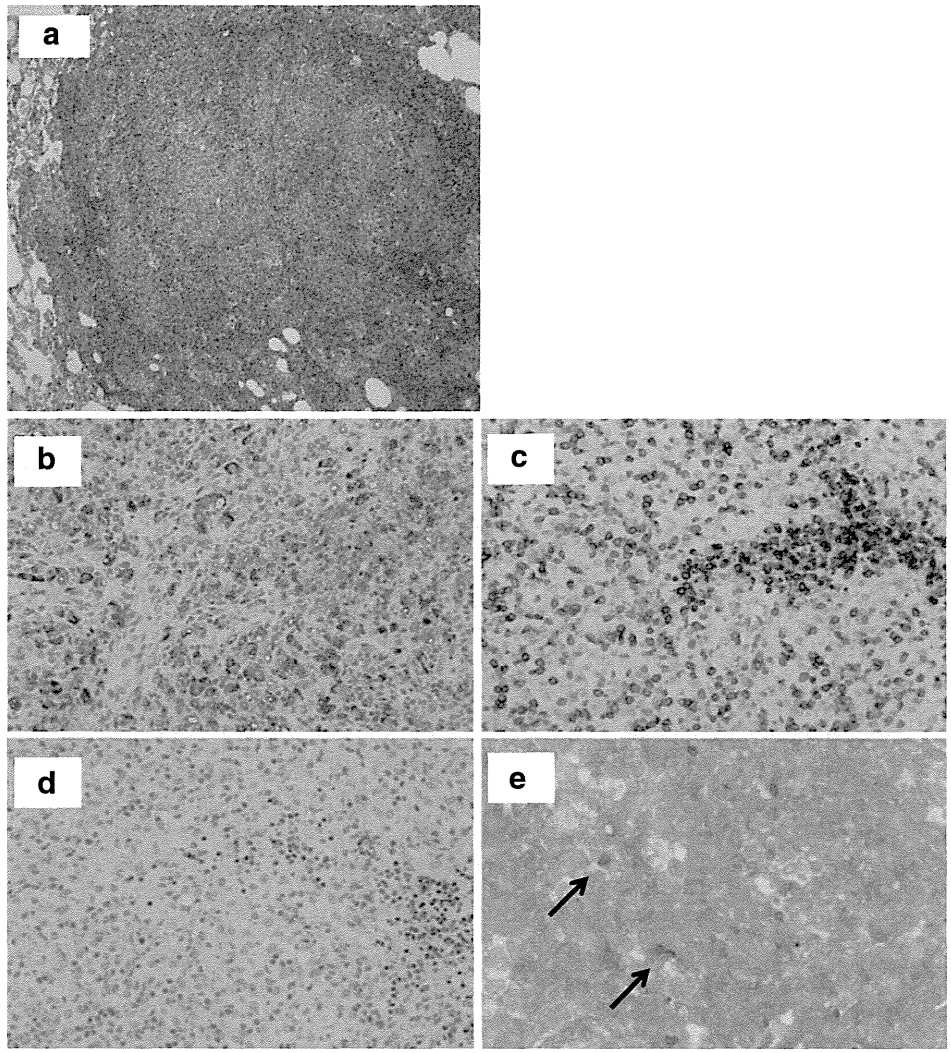

Fig. 2 Pathological findings of lung biopsy. The biopsy sample shows necrotic granulomas (**a**, hematoxylin-eosin stain, magnification ×100), consistent with CD3-positive T cells (**b**) and CD68-positive histiocytes (**c**) (positive cells were stained brown), but without CD20-positive B cells (**d**). Several cysts of *P. jiroveci* were identified inside of the granulomas by Grocott's stain (**e**, *arrows*)

deficiency of B cells potentially results in attenuated immunoprotection against *P. jiroveci*.

Our patient was likely infected with *P. jiroveci*. However, he developed granulomatous PCP after RTX treatment; therefore, RTX potentially contributed to the development of granulomatous PCP. A defect in B cells induces high susceptibility to *P. jiroveci*, as well as development of granulomatous PCP. Granulomatous PCP is an emerging concept. Granulomatous PCP has been previously reported in up to 4–5 % of PCP patients. A total of 35 adult patients have previously exhibited granulomatous PCP. They suffered from AIDS, hematological neoplasms, and solid malignancy, but none of these patients had NS. Interestingly, three of them were treated with RTX prior to the development of PCP [19, 20]. B cell function appears to be impaired in most of these patients, as in our patient. In our patient, immunohistochemical staining revealed that granulomas primarily consisted of T cells and histiocytes without B cells. B cells may be essential for the clearance of *P. jiroveci*, and macrophages may substitute for the clearance of *P. jiroveci* by forming granulomas. In fact, we found several cysts of *P. jiroveci* inside the granulomas. B cell deficiency following RTX potentially modifies the immune response to *P. jiroveci* and induces a granulomatous reaction. Furthermore, Totet et al. reported that granuloma formation is not related to any specific genotype of *P. jiroveci* [22, 23].

Although our patient exhibited no symptoms and his general condition was well on admission, the mortality rate can be 35–50 % in patients without HIV compared with 10–20 % in those with HIV [24, 25]. The diagnosis of conventional PCP is traditionally performed by BAL. However, in the case of granulomatous PCP, BAL frequently fails to detect *P. jiroveci* [18]. An open lung biopsy is required to generate the correct diagnosis if BAL fails to detect *P. jiroveci*.

Our case study suggests that RTX may modulate T cell immunity and increase susceptibility to PCP. Furthermore, RTX treatment may cause a granulomatous pattern and make the correct diagnosis of PCP difficult. Indication of rituximab

for refractory NS has been recently expanding. Therefore, physicians should be aware of the risk of PCP and its atypical manifestations under combined immunosuppressive therapy, including RTX. Prophylaxis for PCP should be considered after RTX against refractory NS.

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ORIGINAL ARTICLE

Prospective 5-year follow-up of cyclosporine treatment in children with steroid-resistant nephrosis

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Abstract
Background Cyclosporine has improved remission rates in children with steroid-resistant nephrotic syndrome (SRNS). However, little prospective long-term follow-up data is available.
Methods We prospectively followed and analyzed 5-year outcomes of all 35 patients enrolled in our previous prospective multicenter trial with cyclosporine and steroids in children with SRNS. At enrollment, 23 cases were classified as minimal change (MC), five as diffuse mesangial proliferation (DMP), and seven as focal segmental glomerulosclerosis (FSGS).
Results Renal survival at 5 years (median 7.7 years) was 94.3 %. Patient status was complete remission (CR) in 31 (88.6 %) (MC/DMP, 25; FSGS, 6); partial remission in one (FSGS); and non-remission in three (MC/DMP), including chronic kidney disease and end-stage kidney disease in one each. Among 31 patients with CR, 22 (71.0 %) were receiving treatment with immunosuppressants at 5 years, including cyclosporine in 19, and seven of these 22 continued to show frequent relapse. Response to cyclosporine at 4 months predicted 5-year outcome in 31 of 35 patients.
Conclusions Although SRNS treatment with cyclosporine provides high renal survival and remission rates, many children require ongoing immunosuppression. Management has advanced from the prevention of end-stage kidney disease to the long-term maintenance of remission and management of relapse after induction therapy.

Keywords Steroid resistance · Nephrotic syndrome · Pediatric nephrology · Cyclosporine · Long-term outcome

Introduction

The use of cyclosporine has revolutionized the treatment of steroid-resistant nephrotic syndrome (SRNS) in children, and outcomes have gradually improved over the last

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15 years. In particular, Waldo et al. [1] and Ehrich et al. [2, 3] achieved remission rates of around 80 %, and the recent Cochrane review [4] confirmed the adoption of cyclosporine as the first-line treatment. Most studies of the safety and efficacy of cyclosporine to date have been retrospective, however, and the few prospective studies that have appeared have been short-term ones. No prospective long-term data on the safety and efficacy of cyclosporine for children with SRNS have yet appeared.

Our previous prospective multicenter trial in children with SRNS also obtained high renal survival and remission rates with cyclosporine and steroid therapy. This study was limited to 12-month protocol treatment [5], however, and therefore provided no prospective information on long-term outcome in these patients. The availability of prospective long-term data would confirm these 12-month findings, and validate the fundamental shift in treatment of this disease from the prevention of end-stage kidney disease (ESKD) to the long-term maintenance of remission and management of relapse after induction therapy.

Here, to better understand the long-term safety and efficacy of cyclosporine in children with SRNS, we evaluated outcomes in patients of our previous prospective study at 5 years after enrollment.

Methods

Previous trial

The present study analyzed all 35 patients enrolled in the previous trial, consisting of a consecutive series of 21 boys and 14 girls with a median age of 2.7 years at enrollment between January 2001 and December 2007. Entry criteria are described in the previous report [5]. Nephrotic syndrome was diagnosed based on a urinary protein/creatinine ratio of ≥ 1.8 mg/mg and serum albumin level of ≤ 2.5 g/dl. SRNS was diagnosed if remission was not achieved (serum albumin level ≤ 2.5 g/dl) after treatment with 2 mg/kg prednisolone daily in three divided doses for 4 weeks. Renal biopsy in all patients at enrollment showed minimal change (MC) in 23, diffuse mesangial proliferation (DMP) in five, and focal segmental glomerulosclerosis (FSGS) in seven. All patients received 12-month treatment with cyclosporine at a trough level of 120–150 ng/ml for 3 months, followed by 80–100 ng/ml for 9 months; and prednisolone at 1 mg/kg/day for 4 weeks, followed by 1 mg/kg every second day for months 2–12. Those with FSGS additionally received methylprednisolone pulse therapy (MPT) at 30 mg/kg on 3 consecutive days in weeks 1, 2, 5, 9, and 13. For patients with MC/DMP who did not achieve remission within 4 months, treatment was restarted with the regimen of the FSGS group (cyclosporine, prednisolone, and MPT). Patients with FSGS who did not achieve remission within 4 months were given off-protocol treatment selected at the discretion of the physician. Patients who progressed to SRNS or frequently relapsing nephrotic syndrome (FRNS) after once achieving remission also received off-protocol treatment.

Follow-up study

Treatment of nephrotic syndrome after expiration of the 12-month protocol treatment was at the discretion of the attending physician. However, we recommended that patients who experienced relapse of SRNS receive the previous protocol treatment again, and that while those who had progression to FRNS received cyclosporine therapy [6] or cyclophosphamide. Treatment with cyclosporine was conducted under mainly trough and occasionally C2 control.

Five-year follow-up data were available for all 35 patients, including analysis of outcomes, treatment for nephrotic syndrome, and adverse events. Outcome at year 5 was classified as complete remission (CR), partial remission (PR), or non-remission. CR was defined as negative or trace proteinuria (dipstick method or urinary protein/creatinine ratio ≤ 0.20 mg/mg) on urinalysis and a serum albumin level of > 2.5 g/dl. CR was also considered to include cases of steroid-sensitive nephrotic syndrome, including cases with no relapse, infrequent relapse, and frequent relapse, with the latter defined as three relapses within any 6-month period or four or more relapses within any 12-month period. PR was defined as a serum albumin level > 2.5 g/dl but persistent proteinuria (dipstick method +1 or greater; or urinary protein/creatinine ratio > 0.20 mg/mg) on urinalysis. Stage 3 or greater chronic kidney disease (CKD) was defined as estimated GFR (eGFR) < 60 ml/min/1.73 m^2, and ESKD as the requirement for dialysis or kidney transplantation.

Data were obtained from the participating institutions ($n=14$) for analysis at 1-year intervals, and included body weight and height, blood pressure, blood analysis (complete blood cell count, blood chemistry), urine tests (urinalysis, quantitative proteinuria), and eGFR as determined by the Schwartz equation [7]. Hypertension was defined as the need for antihypertensive medication, including angiotensin-converting enzyme (ACE) inhibitors or angiotensin-receptor blockers (ARB), except when given for renoprotective purposes. Growth was evaluated from height measured at the most recent examination, and expressed as the height standard deviation score. The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the primary investigator's institution. Informed consent was obtained from the parents before the start of study procedures. This study has been registered in a public trials registry, the University

Hospital Medical Information Network (UMIN, ID C000000009 http://www.umin.ac.jp/ctr/index.htm.)

Results

Five-year follow-up data were available for all 35 patients in the previous study (21 boys and 14 girls; median age 7.7 years [range 6.4–20.1]), including the five of 28 patients enrolled with MC/DMP and one of seven with FSGS who received off-protocol treatment. Follow-up in two of these 35 patients was for 4 years 3 months, due to movement of away from the study area in one and illness of the attending physician in one, but these patients were included in the analysis.

Overall renal survival rate at 5 years was 94.3 % [33/35; 95 % confidence interval (CI), 80.8–99.3], with one patient each developing CKD and ESKD (Figs. 1 and 2). Of the 28 MC/DMP patients, 24 were CR at 4 months, of whom 23 were CR and one was non-remission at 12 months and CKD stage 4 (eGFR 19.6 ml/min/1.73 m^2) at 5 years (Fig. 1). Of the remaining four, two were PR at 4 months, of whom one each was CR and PR at 12 months and both were CR at 5 years; and the two who were non-remission at 4 months remained at this classification at 12 months and 5 years (one hypoalbuminemia, one ESKD). Of the seven with FSGS at enrollment, five were CR and one was PR at all three time points, while one was classified as non-remission at 4 months, PR at 12 months and CR at 5 years (Fig. 2). Thus, of the patients entering with MC/DMP, outcome at 5 years was the same or better than that at 4 months in 27 of 28; and likewise the same or better in all seven entering with FSGS.

Table 1 shows disease status and immunosuppressant use in the 31 patients (31/35; 95 % CI, 73.3–96.8) classified as CR at 5 years. Of these 31, immunosuppressant therapy was started for the control of frequent relapse and continued to maintain remission in 22 (71 %; 95 % CI, 52.0–85.8; MC/DMP 18, FSGS four), among whom seven (MC/DMP six, FSGS one) were FRNS at 5 years despite immunosuppression. By type of treatment at 5 years, 12 patients were treated with cyclosporine, seven with cyclosporine and mizoribine, one with cyclophosphamide, and two with mizoribine only. Further, the one patient with PR (FSGS) at 5 years was also treated with cyclosporine. Thus, a total of 23 patients with CR or PR were continuing to receive immunosuppressant therapy at 5 years.

With regard to non-remission, only three (8.6 %) of 35 patients were in non-remission at 5 years, 1 each with ESKD, CKD stage 4, and hypoalbuminemia. All were originally classified with MC/DMP and had received off-protocol treatment (Fig. 1). The patient with ESKD was classified as DMP at enrollment at age 11.9 years, and as non-remission at both 4 and 12 months of treatment. Treatment was changed at 4 months by the addition of MPT to cyclosporine owing to this non-remission (albumin persistently below 2.5 g/dl and heavy proteinuria). Despite this, she progressed to ESKD at 24 months, at which time she was classified as FSGS. A subsequent graft from her mother at age 18 years was lost due to relapse of FSGS, and she is currently on dialysis. The second non-remission patient with CKD originally had MC at enrollment at age 8.2 years, and was categorized as CR at 4 months. However, she had a relapse at 6 months, at which time she was classified as steroid-sensitive nephrotic syndrome, and again at 9 months, when she was

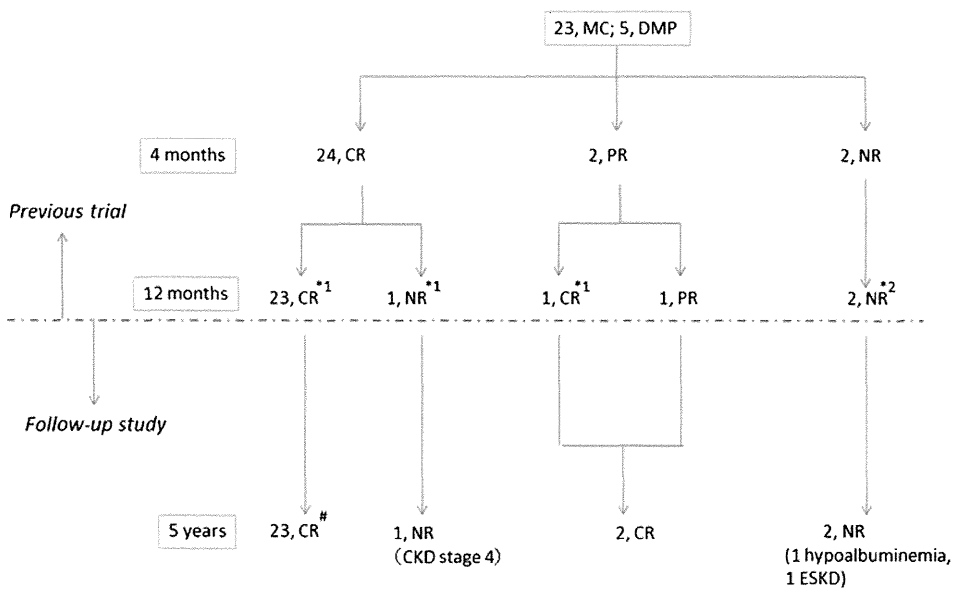

Fig. 1 Outcome at 4 months, 12 months, and 5 years in the 28 patients with MC/DMP at enrollment in the initial study. MC, minimal change; DMP, diffuse mesangial proliferation; CR, complete remission; PR, partial remission; NR, non-remission; CKD, chronic kidney disease; ESKD, end-stage kidney disease. *number of patients receiving off-protocol treatment; # follow-up was 4.3 years in two patients

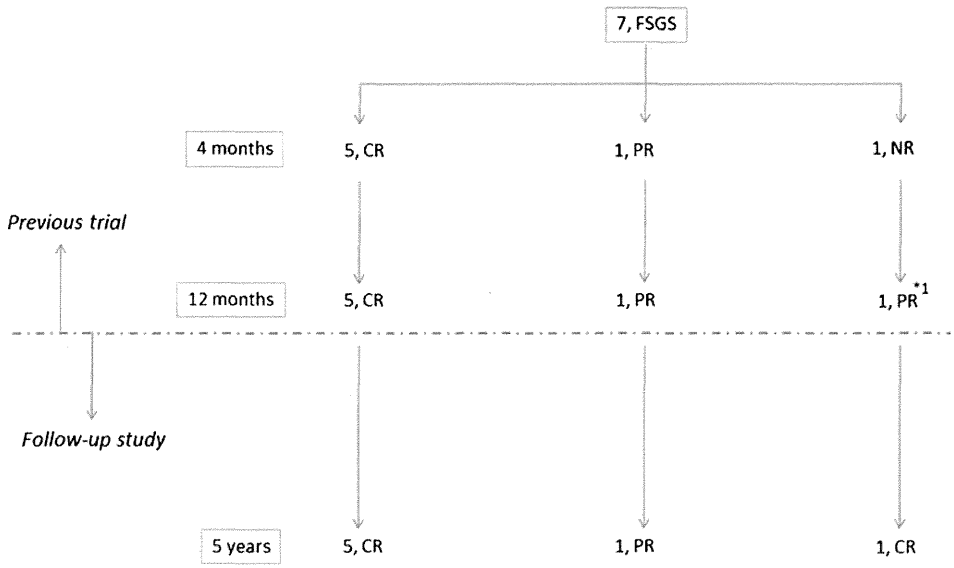

Fig. 2 Outcome at 4 months, 12 months, and 5 years in the seven patients with FSGS at enrollment in the initial study. FSGS, focal segmental glomerulosclerosis; CR, complete remission; PR, partial remission; NR, non-remission. *number of patients receiving off-protocol treatment

classified as SRNS. She remained refractory to subsequent treatment with several immunosuppressants, mainly cyclosporine, and worsened to CKD stage 4 (eGFR 19.6 ml/min/1.73 m^2) at 5 years. The third non-remission patient had hypoalbuminemia at 5 years. However, this patient showed a somewhat irregular response: he was originally classified as MC on enrollment at age 2.7 years, and at 4 months as non-remission. He then went off protocol, but remained in non-remission at 12 months and 5 years, at which time he showed continuing low serum albumin versus slightly elevated but not nephrotic-range proteinuria (urinary protein/creatinine ratio 0.2–0.3 mg/mg) without edema for which no cause could be found, and he currently continues without apparent problems under conservative treatment.

Table 1 Administration of immunosuppressants at year 5* by pathological diagnosis at enrollment

Immunosuppressant therapy	Status	MC/DMP	FSGS	Total
(+)	Non-relapse	6	1	7
	Infrequent relapse	6	2	8
	Frequent relapse	6	1	7
	Subtotal	18	4	22
(−)	Non-relapse	5	2	7
	Infrequent relapse	2	0	2
	Frequent relapse	0	0	0
	Subtotal	7	2	9
Total		25	6	31

MC minimal change; *DMP* diffuse mesangial proliferation; *FSGS* focal segmental glomerulosclerosis

*Follow-up was 4.3 years in two patients

Adverse events

Five-year follow-up confirmed that the safety of the protocol treatment was generally good. The most common adverse events attributable to immunosuppressant therapy at 5 years were hypertension in eight, low mineral bone density in six, and cataracts, trichosis, and gingival hypertrophy in two patients each (Table 2). All cases were mild and were manageable with treatment. Mean standard deviation score for body height at the end of 5 years' follow-up was −0.33± 1.26, with four patients below −2.0. Mean body mass index at this time was 18.3±3.4, with two patients over 25, whereas none were over 30. Further, mean eGFR (excluding two patients with CKD stage 4/ESKD) was 130.4±21.6 ml/min/1.73 m^2, with no patients <90 ml/min/1.73 m^2.

Table 2 Adverse events at year 5* by pathological diagnosis at enrollment

Adverse event	MC/DMP (*n*=28) *n* (%)	FSGS (*n*=7) *n* (%)	Total (*n*=35) *n* (%)
Hypertension	6 (21.4)	2 (28.6)	8 (22.9)
Low mineral bone density	6 (21.4)	0 (0.0)	6 (17.1)
Cataracts	2 (7.1)	0 (0.0)	2 (5.7)
Hypertrichosis	2 (7.1)	0 (0.0)	2 (5.7)
Gingival hypertrophy	1 (3.6)	1 (14.3)	2 (5.7)
Gastric pain	1 (3.6)	0 (0.0)	1 (2.9)
Photosensitivity	0 (0.0)	1 (14.3)	1 (2.9)
Electroencephalogram abnormality	1 (3.6)	0 (0.0)	1 (2.9)
Hyperlipidemia	1 (3.6)	0 (0.0)	1 (2.9)

MC minimal change; *DMP* diffuse mesangial proliferation; *FSGS* focal segmental glomerulosclerosis

*Follow-up was 4.3 years in two patients

Discussion

In this 5-year prospective follow-up study in a cohort of children with SRNS, we found that protocol treatment with cyclosporine and steroid therapy given early after diagnosis for 1 year and followed with appropriate ongoing maintenance therapy provided a favorable long-term outcome. In most cases, outcome could be predicted at the early time of 4 months of treatment. Renal survival rate was 94.3 %, and the incidence of adverse effects was acceptable. However, relapse was frequent and the incidence of immunosuppressant dependency was high. This is the first prospective study to confirm the major shift in the treatment of these patients from the prevention of ESKD to management of relapse and long-term administration of immunosuppressants.

More specifically, 25 of the 28 patients enrolled with MC/DMP were CR at 5 years, and only three were non-remission. All seven patients who enrolled with FSGS were in remission at 5 years, with six classified as CR and one as PR. Thus, 31 (88.6 %) of the original 35 patients were CR at 5 years. Allowing for differences in both disease definition and treatment, this rate compares well with the 40–60 % rates in the three previous studies we are aware of in children with SRNS [8–10]. Safety in the present study was good, with the most common adverse event being hypertension (in eight). However, all adverse events were mild and manageable. Together with the high renal survival, these results support the favorable outcome and safety of treatment with cyclosporine and steroid in these patients.

Regarding the prognosis of SRNS, our findings suggest that response to treatment at 4 months may predict status at 5 years. Of the 28 patients enrolled with MC/DMP, 25 of 26 classified as CR or PR at 4 months were CR at 5 years, with only one worsening to non-remission (CKD stage 4); while of the seven enrolled with FSGS, all six with CR or PR at 4 months remained at CR or PR at 5 years. These findings suggest that outcome at month 4 of treatment allows a reasonable prediction of outcome at 5 years, and accordingly that those responding poorly at 4 months should be considered for other treatment. This suggestion is consistent with the finding of Niaudet et al. [11]. At our institution, we currently treat these non-responders using pulse therapy with methylprednisolone [12], while others have reported combination or replacement with mycophenolate mofetil (MMF) [13, 14], apheresis [15], or rituximab [16]. Interestingly, a recent study found that the addition of rituximab to induction therapy with prednisolone and calcineurin inhibitors showed no decrease in proteinuria, albeit that observation time was relatively short [17]. The three 4-month non-responders in the present study did not differ from the other patients in any regard at either onset or enrollment. Treatment of these refractory cases awaits the development of novel therapies.

Importantly, the majority of children required ongoing immunosuppressant therapy following the initial 12-month treatment with cyclosporine. Of the 31 of 35 who were CR at 5 years, 22 (71.0 %) were receiving immunosuppressants, of whom seven were still classified as FRNS. Moreover, the one PR patient at 5 years was also receiving immunosuppressants at this time. Further, of the 35 patients enrolled, only one received no further immunosuppressants following initial treatment (2 years) (data not shown). Hymes et al. reported a remission rate of 14 of 18 patients (78 %) at 12 months, of whom nine subsequently were FRNS after the cessation of cyclosporine [18]. Other papers have reported similar findings [19–22]. Very recently, Gellermann and colleagues reported the use of MMF in maintenance therapy after induction with cyclosporine, and reported that no patients relapsed, albeit that patient population was relatively small (n=18) [23]. These relapse rates notwithstanding, however, renal survival in patients who respond to induction therapy with immunosuppressants is generally high [24], and patients achieving complete remission have good renal survival [20, 25, 26], thus supporting the current consideration of cyclosporine as able to increase the number of children who achieve complete remission in patients with SRNS [1–4, 11, 27]. In this regard, the Cochrane review [4] and the KDIGO guideline [28] both describe calcineurin inhibitors as being first-line treatment for SRNS.

Our 5-year renal survival rate of 94.3 % contrasts with that of previous retrospective studies at this time point, with Cattran et al. reporting a rate of 73 % in 38 children [20]; Mekahli reporting 75 % in 78 [19]; and Paik reporting 84 % in 92 [25]. Several factors may explain these differences. First, treatment in the present patients was started early and initially with cyclosporine and steroids, with a mean duration from the onset of nephrotic syndrome to the start of protocol treatment of 3.4 months [5]. This contrasts with these other studies, which started later and with cyclophosphamide or other agents. Second, our patients were young, with a mean age at enrollment of 2.7 years. Mekhali suggested that older age (>10 years) at onset was a predictor of worse outcome [19]. Third, another difference may have been with regard to ethnicity. A poor outcome in African American patients has been suggested [20, 29, 30], with Roberti and Vyas for example reporting a renal survival of 60 % in ten patients with FSGS receiving tacrolimus, of whom seven were African American, albeit that mean age was older than the present patients [10]. Overall, these findings suggest that when SRNS is diagnosed, treatment should be started early and with a relatively strong regimen based around cyclosporine. Further, these differences may explain the ability to predict 5-year outcome at the relatively early 4 months in our patients, as mentioned above.

Of interest, outcome in children with FSGS at enrollment was as good as that in children with MC/DMP. Namely, 25 (89 %; CR 25, PR 0) of 28 enrolled with MC/DMP were categorized in remission at 5 years versus seven (100 %; CR 6, PR 1) of seven with FSGS. Given that the number of patients was low and treatment was not randomized, our findings give no indication as to the efficacy of MPT in FSGS. Confirmation of this treatment awaits a randomized prospective clinical trial. Further, again allowing for the low number of patients, it is interesting that all three non-remission cases were originally MC/DMP, which may suggest that initial biopsy findings are not a good indicator of outcome.

Several strengths of the study warrant mention. First, the study was conducted under a prospective multicenter design. Second, follow-up was relatively long, and follow-up rate was high. Several limitations also warrant mention. First, the ratio of patients with FSGS in this follow-up study was relatively low. This was attributable to chance arising under the protocol of our previous study. Second, although continuation of protocol treatment following the initial 1 year of treatment was recommended, treatment was at the discretion of the attending physician. Third, we did not perform genetic analyses. However, none of our patients were indicated for this at the time of diagnosis.

In conclusion, we found that initial treatment with cyclosporine and steroid in children with SRNS provides a favorable long-term outcome, with acceptable adverse effects. Status at 4 months of treatment provided reasonably good predictability of status at 5 years. While immunosuppressant dependency after cyclosporine treatment remains of concern, these results indicate a substantial change in treatment away from the prevention of renal failure to the long-term maintenance of remission and management of relapse after induction therapy. Further investigation of strategies for immunosuppressant dependency and the development of alternative treatments for refractory cases are required.

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Article

Two-Year Outcome of the ISKDC Regimen and Frequent-Relapsing Risk in Children with Idiopathic Nephrotic Syndrome

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Summary

Background and objectives Early identification of frequently relapsing children with idiopathic nephrotic syndrome is desirable.

Design, setting, participants, & measurements The relapse status and clinical data of patients previously registered (January of 1993 to December of 2001) in a multicenter prospective study of the International Study of Kidney Disease in Children regimen were analyzed for risk of frequent relapsers over a 2-year follow-up period.

Results Of 166 children with nephrotic syndrome (113 boys and 53 girls; median age=5.1 years), 145 (87.3%, median age=5.5 years) children were steroid-sensitive, and 21 (12.7%, median age=2.9 years) children were steroid-resistant. Of 145 children with steroid-sensitive nephrotic syndrome, 32 (22.1%, median age=4.2 years) children experienced frequent relapses over 2 years. The time to initial response was significantly longer (10 versus 7 days, $P<0.001$, log-rank test) in the 32 frequent relapsers than in the 106 nonfrequent relapsers. The time from start of initial treatment to first relapse was significantly shorter (2.6 versus 6.1 months, $P<0.001$, log-rank test) in the 32 frequent relapsers than in the 57 infrequent relapsers. In a Cox regression model, the time to initial response ≥9 days and the duration from start of initial treatment to first relapse <6 months were significant predictors of frequent relapses (unadjusted and adjusted).

Conclusions Initial remission time ≥9 days and first relapse within 6 months were associated with frequent relapses. These findings may also be useful also in selecting potential frequent relapsers for clinical trials.

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Introduction

The standard initial treatment for children with idiopathic nephrotic syndrome (NS), proposed by the International Study of Kidney Disease in Children (ISKDC), consists of an 8-week regimen of corticosteroids (1,2). Although more than 80% of children with idiopathic NS are steroid-sensitive (SS), about 60% of these patients experience relapses. Moreover, a considerable number have frequent relapses and develop corticosteroid toxicities after repeated treatments (1,2). Although some controlled studies (3–6) and a meta-analysis (7) have shown that long-course corticosteroid regimens result in a longer sustained remission of the disease than the ISKDC regimen, the most appropriate treatment of idiopathic NS has not been determined. There have been no recent large-scale reports of the outcome of the ISKDC regimen in patients with idiopathic NS. Early identification of frequently relapsing (FR) NS is desirable. We, therefore, assessed the 2-year outcomes and risks of FRNS after initial therapy based on the ISKDC regimen in children with idiopathic NS.

Materials and Methods

Patients

The study protocol was based on the Declaration of Helsinki and approved by the regional research ethics vetting boards (Wakayama Medical University #799). We analyzed data from children with idiopathic NS who had been in the control group (prednisolone alone) of a randomized control trial (RCT) of the Japanese Study Group of Renal Disease in Children testing Sairei-to, a Chinese herbal medicine, in patients with idiopathic NS to obtain basic data for new RCTs. Detailed information on these RCTs is available on the website http://www.wan.jp/jsrdc (Japanese version only available) and http://www.umin.ac.jp/ctr (UMIN000000747 and UMIN000005103). Between January of 1993 and December of 2001, children newly diagnosed with idiopathic NS at 46 hospitals in Japan were entered into the study. The criteria for NS were in accordance with ISKDC (8) and included (*1*) heavy proteinuria, ≥40 mg/h per meter^2, (*2*) hypoalbuminemia, ≤2.5 g/dl, and (*3*) age at diagnosis, ≥1 and <16 years. In addition, none of these children had hematuria (<20

erythrocytes/high-power field), hypertension, hypocomplementemia, or renal insufficiency, and none had received immunosuppressive therapy. Patients/parents were instructed to dip the urine daily and record results.

Treatment Regimen

Initially, all patients received 2.0 mg/kg per day prednisolone (maximum of 80 mg) in three divided doses for 4 weeks followed by 1.3 mg/kg per 2 days in a single dose for 4 weeks. Treatment for each relapse consisted of 2.0 mg/kg per day prednisolone for 4 weeks followed by tapering to 2.0 mg/kg per 2 days in a single dose for 2 weeks, 1.0 mg/kg per 2 days for 2 weeks, and 0.5 mg/kg/2 days for 2 weeks. Prednisolone doses were calculated from the standard body weight per body length.

Clinical Definitions

Remission and relapse were defined in accordance with the guidelines of the ISKDC (8). Response was defined as a reduction in the rate of urinary protein excretion to <4 mg/h per meter^2 (dipstick zero to trace with early-morning urine) for 3 consecutive days. SSNS was defined as a response during the initial 8-week prednisolone regimen, and steroid-resistant NS (SRNS) was defined as a failure to respond during the initial 8-week therapy. Relapse was defined as a reappearance of proteinuria≥40 mg/h per meter^2 (dipstick≥2+ with early-morning urine) for 3 consecutive days. FRNS was defined as more than or equal to two relapses of NS within 6 months of the initial episode or more than or equal to four relapses within any 12-month period. Renal insufficiency was defined as an estimated GFR calculated using the Schwartz Equation (9) of <60 ml/min per 1.73 m^2.

Statistical Analyses

All statistical analyses were performed using JMP9.0 software (SAS Institute Inc., Cary, NC). The clinical characteristics of patient groups were compared using Fisher's exact test. Continuous variables were compared using the Wilcoxon test. Extended Fisher's exact test and the Kruskal–Wallis test with posthoc Steel–Dwass test were used to compare differences among the three subpopulations of patients with SSNS. Time-course events of patients with SSNS were analyzed using the Kaplan–Meier method and the log-rank test. A Cox regression model was used to identify factors associated with the risk of FRNS (10). Factors related to FRNS were selected based on clinical importance. A two-tailed P value<0.05 was regarded as significant.

Results

Clinical Course, Onset Age, and Sex Ratio

A total of 166 children with idiopathic NS, 113 boys and 53 girls (2.1:1), satisfied the inclusion criteria; their conditions 2 years after the start of initial treatment are shown in Figure 1. Of these 166 children, 145 (87%) children, 98 boys and 47 girls (2.1:1), had SSNS, and 21 (13%) children, 15 boys and 6 girls (2.5:1), had SRNS. There was an overall male preponderance in both groups but no significant difference in sex ratio (P=0.81). Similarly, there were no differences in sex ratio when the SSNS group was subdivided into children with no relapse (31 boys and 18 girls), infrequent relapse (39 boys and 18 girls), and FR (23 boys and 9 girls) (P=0.89). The clinical characteristics of these patients are summarized in Table 1. Median onset age was similar in the SSNS and SRNS groups (P=0.18), but it differed significantly among the three SSNS subgroups (P=0.04), between FRs and nonrelapsers (P=0.03), and between FRs and non- and infrequent relapsers (P=0.05). There was no significant difference in median onset age between males and females in the SSNS and SRNS groups and three SSNS subgroups.

There was no significant difference in median of proteinuria, estimated GFR, total protein, albumin, and total

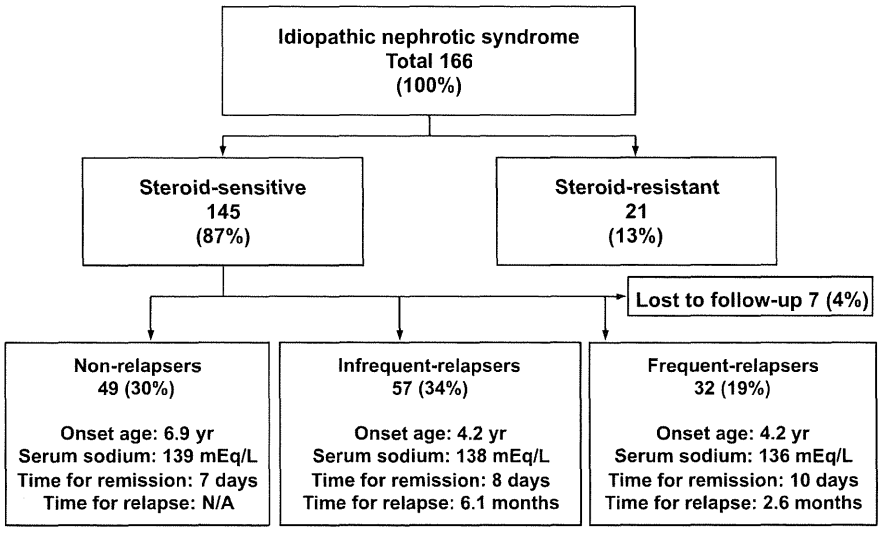

Figure 1. | Status of 166 children with nephrotic syndrome 2 years from the start of initial treatment. Data are shown as median if applicable. N/A, not applicable; time for relapse, time from start of initial treatment to first relapse; time for remission, time from start of initial treatment to disappearance of proteinuria.

Table 1. Clinical data and status of 166 children with nephrotic syndrome

Characteristic	Total (n=166)	Steroid-Sensitive (n=145)	Nonfrequent Relapser (n=106)	Nonrelapser (n=49)	Infrequent Relapser (n=57)	Frequent Relapser (n=32)	Steroid-Resistant (n=21)	P[a]
Sex (male/female)	113/53	98/47	70/36	31/18	39/18	23/9	15/6	0.81; 0.67
Onset age (yr)	5.1 (3.2–9.5)	5.5 (3.5–9.5)	6.0 (3.7–9.5)	6.9 (4.3–9.2)	4.2 (3.2–9.8)	4.2 (2.6–7.7)	2.9 (2.0–10.7)	0.18; 0.05
Proteinuria (g/d per meter^2)	4.2 (2.7–7.2)	4.1 (2.7–7.0)	3.8 (2.5–6.5)	3.4 (2.7–6.4)	4.0 (2.5–6.9)	5.6 (3.0–7.8)	6.9 (2.8–15.5)	0.13; 0.12
Estimated GFR (ml/min per 1.73 m^2)	111 (95–124)	111 (95–124)	111 (96–125)	115 (97–128)	110 (95–119)	109 (91–124)	109 (96–132)	0.93; 0.35
Total protein (g/dl)	4.1 (3.8–4.4)	4.1 (3.8–4.4)	4.1 (3.8–4.5)	4.1 (3.8–4.6)	4.1 (3.7–4.5)	4.0 (3.7–4.3)	4.1 (3.6–4.6)	0.91; 0.10
Albumin (g/dl)	1.6 (1.4–2.0)	1.6 (1.4–2.0)	1.6 (1.4–2.0)	1.7 (1.5–2.1)	1.6 (1.4–1.9)	1.6 (1.3–1.9)	1.7 (1.3–2.3)	0.63; 0.24
Serum sodium (mEq/L)	138 (135–140)	138 (135–140)	138 (136–140)	139 (137–140)	138 (135–140)	136 (133–139)	137 (134–140)	0.36; 0.008
Total cholesterol (mg/dl)	430 (346–505)	429 (353–505)	440 (363–505)	398 (333–486)	452 (405–511)	399 (327–491)	452 (304–506)	0.83; 0.25
Time from start of initial treatment to disappearance of proteinuria (d)	N/A	8 (6–10)	7 (6–8)	7 (5–8)	8 (7–9)	10 (8–13)	N/A	<0.001[b]
Time from start of initial treatment to first relapse (mo)	N/A	N/A	N/A	N/A	6.1 (3.2–9.6)	2.6 (1.5–3.5)	N/A	<0.001[c]

Data value median (25%–75%) except sex. N/A, not applicable.

[a]P values are steroid-sensitive versus steroid-resistant in left position and nonfrequent relapser versus frequent relapser in right position.

[b]P values are nonfrequent relapser versus frequent relapser.

[c]P values are infrequent relapser versus frequent relapse.

cholesterol at onset among subgroups. Serum sodium was significant lower in the FR than the nonfrequent relapser group (P=0.008) (Table 1).

Detailed data of onset age are shown in Table 2. Approximately 50% of children with SSNS experienced onset from 2 to less than 6 years of age. The distributions of onset age differed significantly between the SSNS and SRNS groups (P=0.04). Children with SRNS were younger at onset, with 24% presenting with disease before 2 years of age. Two of five SRNS patients with onset age <2 years showed minor glomerular abnormality and FSGS. A renal biopsy was not done in one of five patients. There were no differences in the distributions of onset age between boys and girls in the SSNS (P=0.25) and SRNS (P=0.99) groups and in the entire cohort with NS (P=0.15). Of 145 patients in the SSNS group, 32 (22%) children, 23 boys and 9 girls (2.6:1), developed FRNS over the 2-year follow-up period, with 22 (69%) of these children being ≤5 years at onset.

Kaplan–Meier analysis showed that 57%, 44%, 41%, and 39% of the patients in the SSNS group remained relapse-free at 6, 12, 18, and 24 months, respectively, from the start of initial treatment (Figure 2 and Table 3). All patients in the FRNS group had a first relapse within 6 months of the start of initial treatment; 24 (75%) of 32 patients with FRNS showed steroid-dependent NS during the 2-year study period.

Time from the Start of Initial Treatment to Disappearance of Proteinuria in the SSNS Group

The median (25%–75%) times from the start of initial treatment to the disappearance of proteinuria in the SSNS group and its subgroups are shown in Table 1. This time was significantly longer in the FR (10 [8–13] days) than the nonrelapser (7 [5–8] days) and infrequent relapser (8 [7–9] days) subgroups (P<0.001 each). The time differed significantly between the FR and non-FR (nonrelapser and infrequent relapser) subgroups (10 [8–13] versus 7 [6–8] days, P<0.001). In contrast, the difference between the nonrelapser and infrequent relapser subgroups was not significant (P=0.07). Kaplan–Meier method and log-rank test also showed that the initial response time was significantly longer in the FR than the non-FR (Figure 3A). These findings suggest that the ease of proteinuria disappearance differed in the FRNS and non-FRNS groups. There were no significant differences between males and females in the SSNS group and its subgroups.

Time from the Start of Initial Treatment to First Relapse in Patients with SSNS

The median (25%–75%) time from the start of initial treatment to first relapse was significantly shorter in the FR than the infrequent relapser subgroups (2.6 [1.5–3.5] versus 6.1 [3.2–9.6] months, P<0.001) (Table 1). Kaplan–Meier method and log-rank test also showed that the first relapse was significantly earlier in the FR than the infrequent relapser (Figure 3B). There was no significant difference between males and females in either group.

Factors Associated with FRNS

Table 4 presents the results of Cox regression analyses of factors associated with FRNS. A time of initial response ≥9 days and time from start of initial treatment to first relapse <6 months were significant in both unadjusted (P<0.001 in both) and adjusted (P=0.004 and <0.001, respectively) analyses. The adjusted hazard ratio (HR) for an initial response time ≥9 days compared with <9 days was 3.09 (95% confidence interval [CI]=1.42–7.27). The adjusted

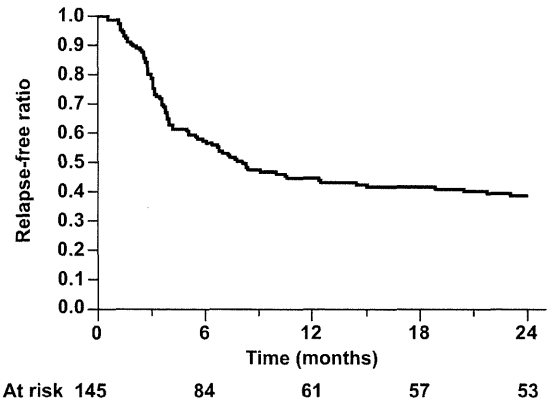

Figure 2. | Kaplan–Meier analysis of relapse-free ratio in children with steroid-sensitive nephrotic syndrome.

Table 2. Subdivision of patients by age at the onset of idiopathic nephrotic syndrome

Status	Onset Age (yr)				
	≥1 to <2	≥2 to <6	≥6 to <10	≥10 to <14	≥14 to <16
Total					
Total (n=166)	13 (8%)	76 (46%)	40 (24%)	28 (17%)	9 (5%)
Male (n=113)	8 (7%)	54 (48%)	24 (21%)	18 (16%)	9 (8%)
Female (n=53)	5 (9%)	22 (42%)	16 (30%)	10 (19%)	0 (0%)
Steroid-sensitive					
Total (n=145)	8 (6%)	69 (48%)	37 (26%)	24 (17%)	7 (5%)
Male (n=98)	5 (5%)	49 (50%)	22 (22%)	15 (15%)	7 (7%)
Female (n=47)	3 (6%)	20 (43%)	15 (32%)	9 (19%)	0 (0%)
Steroid-resistant					
Total (n=21)	5 (24%)	7 (33%)	3 (14%)	4 (19%)	2 (10%)
Male (n=15)	3 (20%)	5 (33%)	2 (13%)	3 (20%)	2 (13%)
Female (n=6)	2 (33%)	2 (33%)	1 (17%)	1 (17%)	0 (0%)

HR for time from start of initial treatment to first relapse <6 months was 5.09×10^{6} (95% CI=16.56–2.06×10^{184}). Kaplan–Meier method and log-rank test also showed that initial remission time ≥9 days and first relapse within 6 months were associated with frequent relapsing (Figure 4). These findings suggest that an initial response within 9 days and an early first relapse, especially within 6 months, may be significant risk factors for the development of FRNS.

Discussion

Prolonged initial steroid treatment for more than 3 months has been reported to decrease the risks of relapse in pediatric patients with SSNS (7,11,12). However, even with new corticosteroid regimens, 80%–90% of children with SSNS have relapses, with nearly 50% relapsing frequently (13). Therefore, the initial approach to the treatment of SSNS will likely vary considerably (14).

We have analyzed 2-year outcomes in children with primary NS after initial therapy based on the ISKDC regimen. Large-scale reports describing the outcomes of the ISKDC regimen in children with idiopathic NS have been published, but none have been published recently (2,15). Therefore, our study may provide valuable data on children with idiopathic NS, although they were from over 10 years ago.

Our results suggest that the incidence of FRNS in children with idiopathic NS was not as high as previously reported. We found that the incidence of FRNS was 19% among all children with NS (32 of 166) and 22% among children with SSNS (32 of 145) 2 years after initial treatment. In comparison, a previous study reported that the incidence of FRNS 6 months after initial treatment with prednisone was 28% among children with NS (102 of 363) and 31% among children with SSNS (102 of 334) (2). The Arbeitsgemeinschaft für Pädiatrische Nephrologie study reported that 12 of 37 (32%) patients with SSNS using the ISKDC regimen were FRNS 6 months after initial treatment (4), and a Cochrane meta-analysis found that 110 of 289 (38%) patients using the ISKDC regimen were FRNS (7), a significantly higher percentage than we observed ($P<0.001$, Fisher's exact test). Relatively small-scale studies from Japan included in the Cochrane analysis showing that the rates of FRNS were high, 43% (13 of 30) in 2000 (16) and 52% (15 of 29) in 1988 (3), suggesting that the differences in relapse rates were not caused by race or study period.

Of our 166 patients with NS, 21 (13%) patients had SRNS. SRNS generally depends on histology. Similarly, the ISKDC reported that 22% (103 of 471) had SRNS, with 7% (25 of 363) having minimal change (17). In addition, the sex ratios and onset age distributions among our patients were similar to those values reported previously (2,15,18).

The relapse-free ratio after initial treatment in our SSNS was intermediate between the standard and long Arbeitsgemeinschaft für Pädiatrische Nephrologie regimens and

Table 3. Relapse-free rates after a standard or long regimen in patients with steroid-sensitive nephrotic syndrome

Study Group	Time (mo)			
	6 (%)	12 (%)	18 (%)	24 (%)
This study[a] (standard, n=145)	57	44	41	39
ISKDC[b] (MCNS; standard, n=218)	45			28
APN[c] (long 12 wk, n=34)	76	62		49
APN[c] (standard, n=37)	51	35		19

ISKDC, International Study of Kidney Disease in Children; MCNS, minimal change nephrotic syndrome; APN, Arbeitsgemeinschaft für Pädiatrische Nephrologie.
[a]Data analyzed by the Kaplan–Meier method.
[b]From reference 8.
[c]From reference 16.

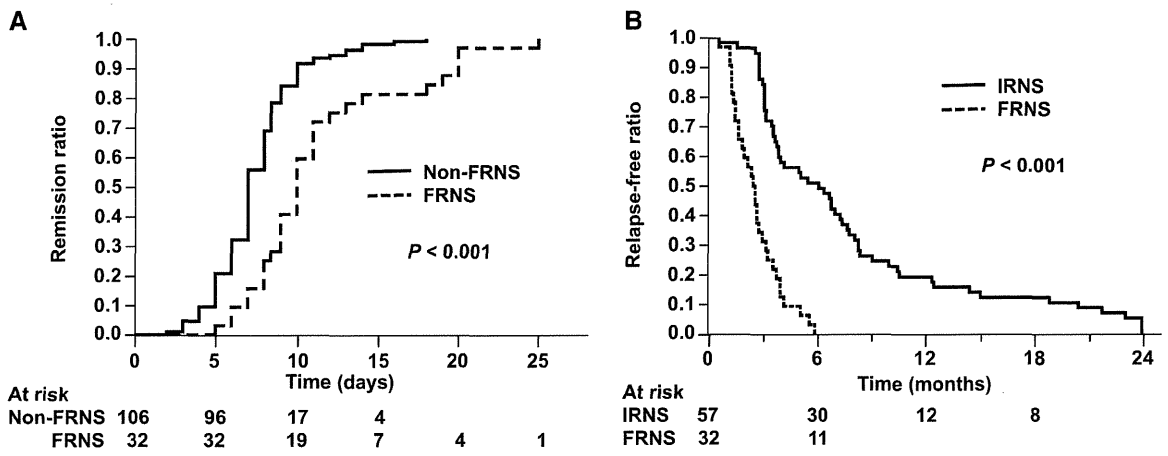

Figure 3. | Initial remission ratio and relapse-free ratio in nephrotic syndrome. Kaplan–Meier analysis of time for initial remission (A) and time for first relapse (B). *P* values are from log-rank test. FRNS, frequent-relapsing nephrotic syndrome; IRNS, infrequent-relapsing nephrotic syndrome.