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

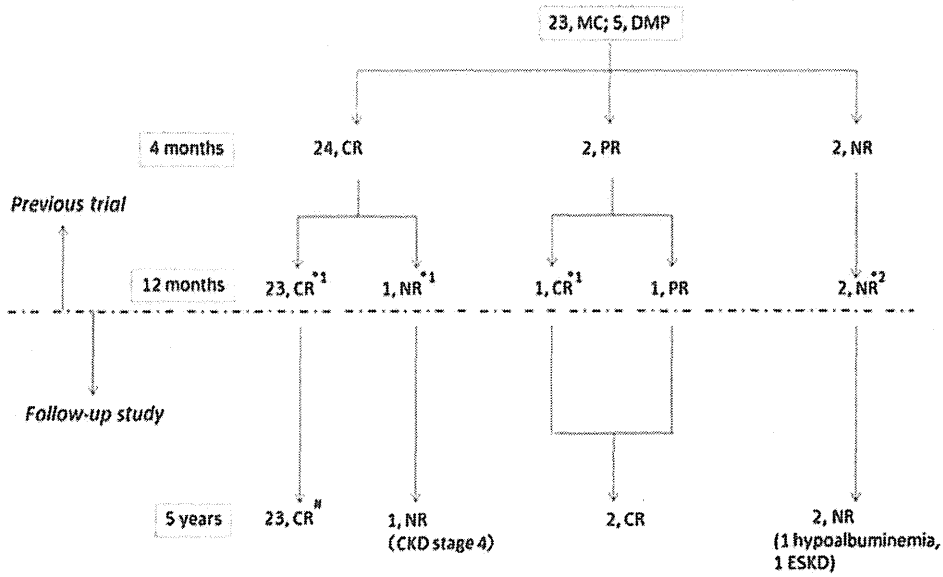

Fig. 1 Outcome at 4 months, 12 months, and 5 years in the 28 patients with MC/DMP at enrollment in the initial study. MC, minimal change; DMP, diffuse mesangial proliferation; CR, complete remission; PR, partial remission; NR, non-remission; CKD, chronic kidney disease; ESKD, end-stage kidney disease.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

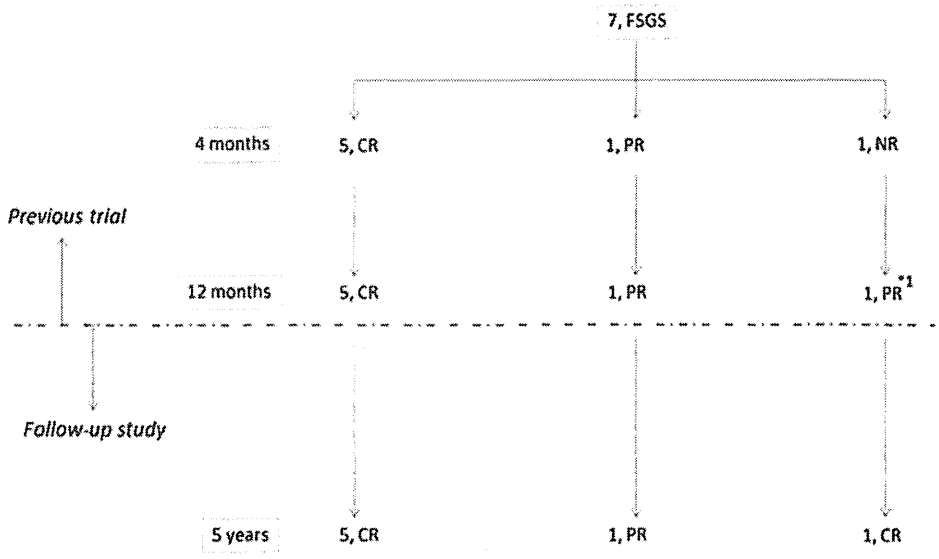

Fig. 2 Outcome at 4 months, 12 months, and 5 years in the seven patients with FSGS at enrollment in the initial study. FSGS, focal segmental glomerulosclerosis; CR, complete remission; PR, partial remission; NR, non-remission. *number of patients receiving off-protocol treatment

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.

Acknowledgments This study was supported in part by the Kidney Foundation, Japan. The results presented in this paper have not been published previously in whole or part, except in abstract form at the 43rd Annual American Society of Nephrology Conference.

The authors would like to thank Drs. Goto M (Tokyo), Hamada R (Tokyo), Harada T (Kanagawa), Hasegawa K (Tokyo), Hataya H (Tokyo), Hayakawa H (Niigata), Ikeda M (Tokyo), Kaito H (Hyogo), Kamei K (Tokyo), Kitayama H (Shizuoka), Kobayashi A (Hyogo), Kodama S (Fukuoka), Miura K (Tokyo), Nakamura T (Kanagawa), Nozu K (Hyogo), Ochiai R (Tokyo), Sakai T (Tokyo), Sekine T (Tokyo), Suzuki R (Tokyo), Tanaka R (Hyogo), Wada N (Shizuoka), Wakaki H (Tokyo), and Yoshidome K (Kagoshima) of the Japanese Study Group of Renal Disease in Children for their contributions to the study.

Conflict of interest statement Norishige Yoshikawa has received research grants from Novartis, Japan.

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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.

Clin J Am Soc Nephrol 8: 756–762, 2013. doi: 10.2215/CJN.09010912

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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

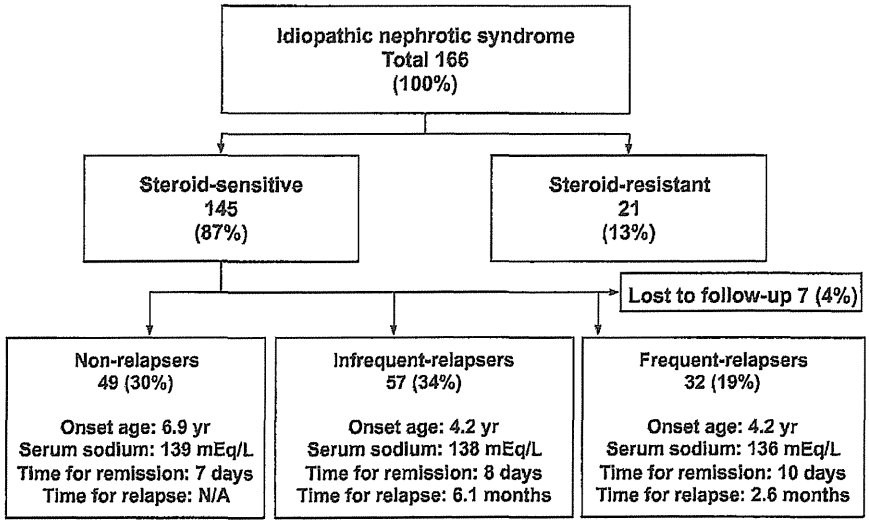

Figure 1. | Status of 166 children with nephrotic syndrome 2 years from the start of initial treatment. Data are shown as median if applicable. N/A, not applicable; time for relapse, time from start of initial treatment to first relapse; time for remission, time from start of initial treatment to disappearance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

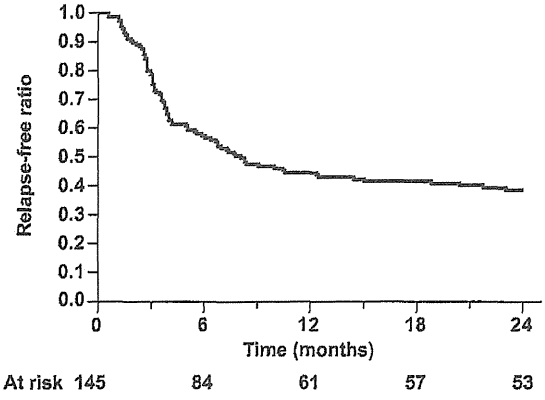

Figure 2. | Kaplan–Meier analysis of relapse-free ratio in children with steroid-sensitive nephrotic syndrome.

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=$16.56–2.06\times10^{184}$). 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.

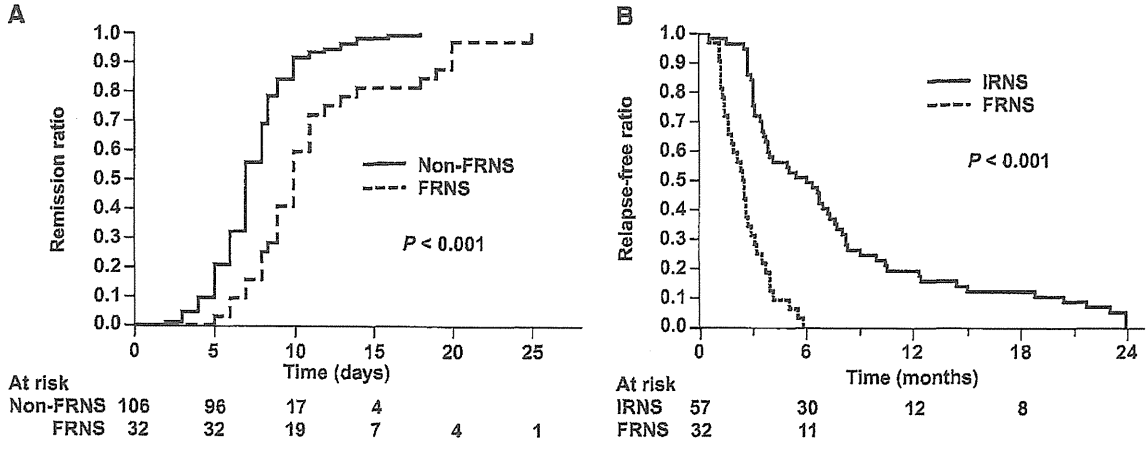

Figure 3. | Initial remission ratio and relapse-free ratio in nephrotic syndrome. Kaplan–Meier analysis of time for initial remission (A) and time for first relapse (B). *P* values are from log-rank test. FRNS, frequent-relapsing nephrotic syndrome; IRNS, infrequent-relapsing nephrotic syndrome.

Table 4. Cox regression model of factors associated with frequently relapsing nephrotic syndrome at 2 years after initial therapy (n=138)

Factor	Unadjusted			Adjusted		
	HR	95% CI	P	HR	95% CI	P
Male (versus female)	1.32	0.63–3.02	0.47	1.41	0.64–3.34	0.40
Onset age (<5 to ≥5 yr)	2.69	1.31–5.94	0.007	1.50	0.71–3.39	0.29
Serum sodium (<135 to ≥135 mEq/L)	2.54	1.15–5.22	0.02	1.34	0.59–2.83	0.47
Time from start of initial treatment to disappearance of proteinuria (≥9 to <9 d)	6.65	3.17–15.19	<0.001	3.09	1.42–7.27	0.004
Time from start of initial treatment to first relapse (<6 to ≥6 mo)	8.66×10^{6}	29.46–N/A	<0.001	5.09×10^{6}	$16.56–2.06\times10^{184}$	<0.001

HR, hazard ratio; CI, confidence interval; N/A, not available.

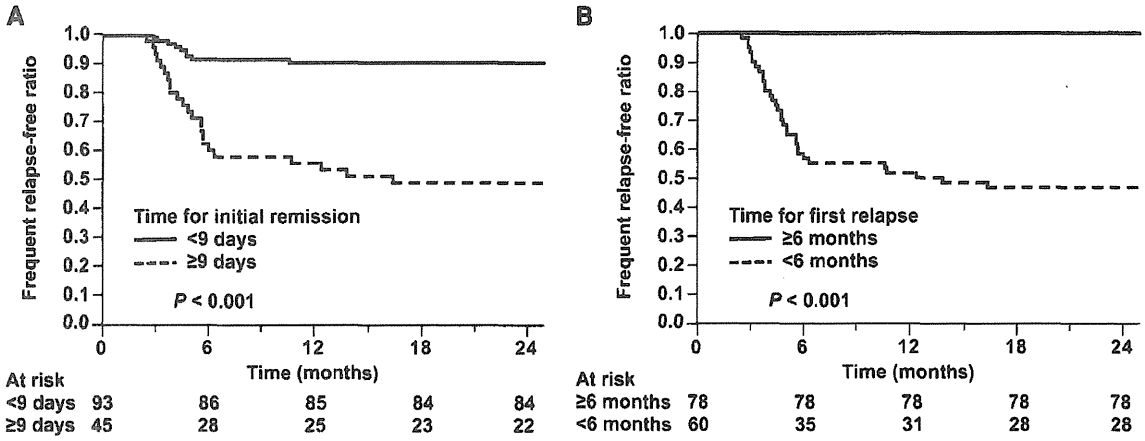

Figure 4. | Frequent relapse-free ratio stratified by times for initial remission and first relapse. Frequent relapse related with time to initial response (A) and time from start of initial treatment to first relapse (B). P values are from log-rank test.

better than the ratio reported by the ISKDC (Table 3). These findings provide a rationale for reconsidering the ISKDC regimen.

We found that the times from the start of initial treatment to the disappearance of proteinuria differed significantly among our three SSNS subgroups, being significantly longer in FR than non-FR (10 versus 7 days) groups. A time of ≥9 days was significant for FRNS in both unadjusted and adjusted analyses. The adjusted HR for an initial response time ≥9 days compared with <9 days was 3.09 (95% CI=1.42–7.27, P=0.004). These findings suggest that the time from the start of initial treatment to the disappearance of proteinuria may predict whether a patient will develop FRNS. Patients with an initial response time ≥9 days should, therefore, be considered for more intensive treatments, such as a long course of corticosteroids. This time cutoff may also be useful for selecting potential FRs for clinical trials. Interestingly, an initial remission time ≥9 days (odds ratio=3.00, 95% CI=1.20–7.90, P=0.02, n=123) was previously shown to be a significant predictor of steroid dependency in a logistic model (19). Another study, however, reported no correlation between time to initial response and frequency of relapse in 218 SSNS patients who showed minimal change during the 2 years after initial response (8). The reason for these discrepancies is unclear, although they may have been because of differences in the ethnic/racial characteristics of the included patients.

Both unadjusted and adjusted analyses showed that the time from start of initial treatment to first relapse was a significant predictor of FRNS. The adjusted odds ratio for the time from initiation of treatment to first relapse <6 months was 5.09×10^{6} (95% CI=$16.56–2.06\times10^{184}$, P<0.001). These findings suggest that patients who relapse within 6 months after initial remission be considered for more intensive treatment. Based on this finding, an RCT has been designed to examine the efficacy and safety of mizoribine, one of immunosuppressants, to prevent FRNS (UMIN000005103).

A limitation may be the possibility of influence of difference in treatment for each relapse between the original ISKDC regimen and our regimen, although one

of the ISKDC RCTs showed that there was no significant difference in the number of relapses during the follow-up period of 6 months between the original ISKDC regimen and the prolonged regimen similar to our regimen (20). Generally, renal biopsies are not indicated at onset when patients fulfill the inclusion criteria of this study. Therefore, renal biopsies were not required for the study. Another limitation is that our study did not include a validation cohort. Therefore, data in the current study should be validated in a separate cohort.

Although we did not examine biochemical parameters such as lipoprotein(a), there is a report suggesting the predictive value of it in nephrotic status (21).

In conclusion, despite this study being prospective and observational in nature rather than a controlled study, our findings suggest the validity of the ISKDC regimen in the treatment of patients with idiopathic NS. Our data also indicated that an initial remission time ≥9 days and first relapse within 6 months were significant risks for the development of FRNS. These findings may also be useful in selecting potential FRs for clinical trials.

Acknowledgments

The authors thank the patients and Drs. Akioka Y (Tokyo), Araki Y (Hokkaido), Awazu M (Tokyo), Baba M (Kanagawa), Furuse A (Kumamoto), Fujinaga S (Saitama), Goto M (Tokyo), Hamada R (Tokyo), Hamahira K (Hyogo), Hamasaki Y (Tokyo), Harada T (Kanagawa), Hatae K (Fukuoka), Hattori M (Tokyo), Hattori S (Kumamoto), Hayashida S (Kumamoto), Higashida K (Yamanashi), Hiramatsu M (Oita), Hiramoto R (Chiba), Igarashi T (Tokyo), Ikeda M (Tokyo), Ikeda T (Kumamoto), Ishihara Y (Kanagawa), Ito S (Kanagawa), Kagami S (Tokushima), Kaku Y (Fukuoka), Kameda A (Hyogo), Kamei K (Tokyo), Kamezaki K (Fukuoka), Kamimaki I (Tochigi), Kamitsuji H (Nara), Kitagawa K (Hyogo), Kobayashi Y (Tochigi), Kodama S (Kagoshima), Konomoto T (Miyazaki), Kosaka T (Tokyo), Maeda E (Gunma), Matsuyama T (Tokyo), Minato T (Hyogo), Mishiku Y (Kanagawa), Miyamoto H (Hyogo), Morita M (Tochigi), Nakanishi N (Osaka), Niimura F (Kanagawa), Nishino M (Osaka), Nozu K (Hyogo), Ochiai R (Tokyo), Ota K (Hyogo), Otsuka Y (Saga), Owada Y (Tochigi), Sako M (Tokyo), Sato T (Saga), So H (Kagoshima), Suehiro F (Hyogo), Suzuki T (Tokyo), Tamura K (Ibaraki), Tanaka R (Hyogo), Tanaka Y (Tokyo), Tsuchida S (Akita), Ushijima T (Kumamoto), Wakaki H (Tokyo), Yamaoka K (Osaka), Yan K (Tokyo), Yoshidome K (Kagoshima), and Yoshiya K (Hyogo) of the Japanese Study Group of Renal Disease in Children for their contributions.

This study was supported in part by the Kidney Foundation, Japan.

Parts of this study were presented at the 37th Annual Meeting of the American Society of Nephrology, October 30, 2004, St. Louis, Missouri, and published in abstract form (Nakanishi *et al.*, *J Am Soc Nephrol*, 15: 358A, 2004).

Disclosures

None.

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Received: September 3, 2012 **Accepted:** December 21, 2012

Published online ahead of print. Publication date available at www.cjasn.org.

CJASN ePress. Published on December 5, 2013 as doi: 10.2215/CJN.13071212

Article

Cyclosporine C_2 Monitoring for the Treatment of Frequently Relapsing Nephrotic Syndrome in Children: A Multicenter Randomized Phase II Trial

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Summary

Background and objectives An open-label, multicenter, randomized phase II trial was conducted from July 1, 2005 to March 29, 2011 to compare two protocols for treating children with frequently relapsing nephrotic syndrome using microemulsified cyclosporine.

Design, setting, participants, & measurements Ninety-three children with frequently relapsing nephrotic syndrome were randomly assigned to group A (n=46) or group B (n=47). In both groups, the 2-hour postdose cyclosporine level was monitored. For group A, the cyclosporine target was set to 600–700 ng/ml for the first 6 months and 450–550 ng/ml for the next 18 months; for group B, it was set to 450–550 ng/ml for the first 6 months and 300–400 ng/ml for the next 18 months. The primary end point was the sustained remission rate. At the end of the study, if there was no difference in safety profile between the two groups and the sustained remission rate in group A was superior to group B with a decision threshold of 8%, then the regimen for group A would be determined the better treatment.

Results Eight children from an ineligible institution, where cyclosporine levels were not measured, were excluded from all analyses. At 24 months, the sustained remission rate was nonsignificantly higher in group A (n=43) than group B (n=42; 64.4% versus 50.0%; hazard ratio, 0.57; 95% confidence interval, 0.29 to 1.11; P=0.09), and the progression-free survival rate was significantly higher (88.1% versus 68.4%; hazard ratio, 0.33; 95% confidence interval, 0.12 to 0.94; P=0.03). The relapse rate was significantly lower in group A than group B (0.41 versus 0.95 times/person-year; hazard ratio, 0.43; 95% confidence interval, 0.19 to 0.84; P=0.02). The rate and severity of adverse events were similar in both treatment groups.

Conclusion The sustained remission rate was not significantly different between the two treatment groups, but the regimen with the higher 2-hour postdose cyclosporine level target improved progression-free survival and reduced the relapse rate.

Clin J Am Soc Nephrol 9: ●●●–●●●, 2014. doi: 10.2215/CJN.13071212

Introduction

Cyclosporine has been found to be effective for the treatment of frequently relapsing nephrotic syndrome (FRNS) or steroid-dependent nephrotic syndrome (SDNS) in children (1–6). Kidney Disease Improving Global Outcomes Clinical Practice Guideline for Glomerulonephritis recommends that cyclosporine or tacrolimus be given as corticosteroid-sparing agents for FRNS children (7). However, tacrolimus is still off label for FRNS in Japan. Therefore, the development of more effective and safer regimens with cyclosporine for FRNS children is important.

A protocol for treating children with FRNS using Sandimmune, an older formulation of cyclosporine, was previously established in Japan (8). In patients who received Sandimmune in a dose that maintained the whole-blood trough level (C_0) at 80–100 ng/ml for the first 6 months and 60–80 ng/ml for the next 18 months, the estimated sustained remission rate (SRR) was 57% at month 24, and mild chronic cyclosporine nephrotoxicity was found in 20% of patients who underwent renal biopsy after 24 months of treatment.

In 2000, a newer formulation of microemulsified cyclosporine (mCyA; Neoral Novartis, Basel, Switzerland) was introduced in Japan. We previously examined whether treatment with mCyA, titrated by C_0 monitoring with the C_0 target set to the same concentrations mentioned above, was effective and safe in children with FRNS the Japanese Study Group of Renal Disease in Children 07 (the JSRDC07) trial (9). In the JSRDC07 trial, the estimated SRR at month 24 was 58.1%, and mild chronic cyclosporine nephrotoxicity was detected in only 8.6% of patients. Based on these results, the Japanese Society for Pediatric Nephrology

(JSPN) recommended mCyA titrated by C_0 monitoring, in which the C_0 target was set to 80–100 ng/ml for the first 6 months and 60–80 ng/ml for the next 18 months, as the standard treatment with mCyA for children with FRNS.

Because cyclosporine is stably absorbed after administration of mCyA, the dose of mCyA can be titrated based on the area under the concentration time curve during the first 4 hours after treatment (AUC_{0-4}) in children who receive kidney transplants (10). It has been reported that the best single-point predictor of AUC_{0-4} is the 2-hour postdose cyclosporine level (C_2) and that C_2 management of mCyA treatment is effective and safe in pediatric kidney transplant recipients (11). One of the clinical benefits of C_2 monitoring, shown in the majority of studies on transplantation, is a reduction in mean cyclosporine dose, which may reduce the rate of adverse effects of cyclosporine, including chronic cyclosporine nephrotoxicity (12). Several reports described the efficacy and/or safety of mCyA treatment with C_2 monitoring, mainly with single daily dose, in children with FRNS (13–20). However, there were few prospective studies to determine appropriate C_2 target with two divided oral doses of mCyA in children with FRNS. In addition, it is not known whether C_2 monitoring or C_0 monitoring is better in children with FRNS.

To address these questions, we first needed to decide on an appropriate treatment protocol for C_2 monitoring in children with FRNS. Therefore, we conducted an open-label, multicenter, randomized phase II controlled trial designed to select a better treatment for FRNS in children by comparing two target cyclosporine C_2 levels (the Japanese Study Group of Kidney Disease in Children 03 [JSKDC03] trial; University Hospital Medical Information Network–Clinical Trials Registry: C000000008).

Materials and Methods

Patients

The study was approved by the institutional review board at each center and complied with the Declaration of Helsinki. Written assent was obtained from patients when they were old enough to understand, and written informed consent was obtained from all of their parents.

Patients were registered from 14 centers in Japan (Supplemental Table 1) and randomized to the higher (group A) or lower target C_2 group (group B) between July 1, 2005 and January 9, 2009. To be included in the study, patients needed to (1) have FRNS, (2) be 1–18 years old, and (3) have renal biopsy findings showing minor glomerular abnormalities, diffuse mesangial proliferation, or FSGS within 12 months before enrollment. Patients were excluded from the study if they had been treated with cyclosporine, were pregnant, or had (1) a history of steroid resistance, (2) a creatinine clearance rate of ≤60 ml/min per 1.73 m^2, (3) active infections, (4) secondary nephrotic syndrome, (5) poorly controlled hypertension, or (6) severe liver dysfunction. The last patient visit was on March 29, 2011.

The definitions of nephrotic syndrome (21,22) are as follows. Nephrotic syndrome was defined as urine protein-to-creatinine ratio≥1.8 or above and serum albumin≤2.5g/dl. Remission was defined as negative protein on urine dipstick test or urine protein-to-creatinine ratio<0.2 for 3 consecutive days. Relapse was defined as protein≥2+ on urine dipstick test for 3 consecutive days. FRNS was defined as two or more relapses within 6 months after initial remission or four or more relapses within any 12-month period. SDNS was defined as relapse occurring two times consecutively during the reduction of the prednisolone dosage or within 2 weeks after its discontinuation. Steroid-resistant nephrotic syndrome (SRNS) was defined as the daily administration of prednisolone at 60 mg/m^2 per day that does not lead to remission within 4 weeks.

Trial Design

The JSKDC03 was an open-label, multicenter, prospective, randomized phase II controlled trial. We adopted the selection design proposed by Simon *et al.* (23) and generalized by Sargent *et al.* (24), which is frequently used for the development of antibacterial and anticancer agents, for the comparison of the C_2 monitoring of mCyA in phase II trial setting. The selection design has been used to choose which regimen should be further tested in a phase III trial, typically in limited number of patients. Randomized phase II design does not bring a confirmatory result; however, it has the advantage of being able to evaluate with a uniform evaluation criteria.

The purpose of this trial was to select a better treatment for FRNS in children by comparing two target cyclosporine C_2 levels: a higher target C_2 (group A) and a lower target C_2 (group B). A statistically significant difference in primary end point between the two groups was not required in this trial. The criteria for selection were as follows: when there was no difference in safety profile between the two groups and the SRR at 24 months in group A was superior to the SRR in group B with a decision threshold of 8%, the regimen for group A was selected as the better treatment for FRNS. Otherwise, the regimen for group B was selected. The decision threshold of 8% was set before the start of the study based on a consensus reached by pediatric nephrologists in the JSKDC.

The total sample size was determined as 100. Randomization of the patients into two groups was performed in a 1:1 ratio with a dynamic balancing method. A prestudy calculation of sample size and the method of randomization are described in detail in Supplemental Appendix.

Experimental Intervention

Within 7 days after randomization, treatment with mCyA commenced. mCyA was administered orally at least 15 minutes before meals and started at a dose of 3–4 mg/kg body wt divided into two equal doses. We adjusted each dose of mCyA to the target C_2 ranges by increasing or decreasing it by 20%–30%.

The total duration of mCyA treatment was 24 months. Group A received mCyA in a dose producing a whole-blood C_2 level between 600 and 700 ng/ml for the first 6 months and between 450 and 550 ng/ml for the next 18 months. Group B received mCyA in a dose producing a whole-blood C_2 level between 450 and 550 ng/ml for the first 6 months and between 300 and 400 ng/ml for the next 18 months.

How to determine the target C_2 levels and corticosteroid treatment at the relapse during the study is described in Supplemental Appendix. No patients received corticosteroids as a maintenance therapy. Measurement of cyclosporine

concentrations and other variables is also described in Supplemental Appendix.

After 24 months of treatment, the dose of mCyA was tapered off within 3 months, and all patients were scheduled to undergo renal biopsies.

The use of immunosuppressive agents, except for prednisolone and mCyA, was prohibited during the trial. The experimental intervention was stopped if (*1*) patients developed FRNS, SDNS, or SRNS after the start of mCyA treatment, (*2*) patients and/or their parents required the intervention to be stopped, (*3*) patients developed severe adverse events that required intervention to be stopped, (*4*) the primary investigator or the institutional review board at each center decided to stop the trial, or (*5*) patients were not followed up.

End Points

The primary end point was relapse-free survival based on the period of time until the first relapse. There were two secondary end points. One end point was the probability of progression-free survival based on the time until the progression to FRNS, SDNS, or SRNS. The other end point was the relapse rate, which was calculated by dividing the total number of relapses by the total duration of observations for all patients combined.

We also evaluated the rate and severity of development of chronic cyclosporine nephrotoxicity and other adverse events that occurred during the trial. A pathologist on our team (M.N.) evaluated the development of chronic cyclosporine nephrotoxicity, which was defined as cyclosporine-associated arteriolopathy and/or cyclosporine-induced tubulointerstitial lesions showing characteristic striped tubulointerstitial lesions.

Statistical Analyses

Statistical analyses were performed on an intention-to-treat basis. Individuals that did not complete 24 months of the study were still included in the analysis and counted as events. The Kaplan–Meier method was used to estimate the SRR at 24 months after randomization based on the relapse-free survival. The Cox proportional hazard model was used to estimate the hazard ratio and its 95% confidence interval (95% CI) between the groups. These methods and the log-rank test were also used to analyze progression-free survival. The unequal variance *t* test was used to compare the distributions of the average of C_2 and AUC_{0-4}. Fisher's exact test was used to assess the statistical significance of comparisons at the patient level. All statistical analyses were conducted using SAS 9.1 software (SAS Institute, Cary, NC).

Adverse events corresponding to defined classes were tabulated first for 2 years.

Results

Patients

Between April of 2005 and March of 2009, 94 children with minimal change nephrotic syndrome, diagnosed based on pathologic analysis, were registered. One patient was later found to be ineligible because of not meeting the definition of FRNS; therefore, 93 patients were randomly assigned to two treatment groups (group A, n=46; group B, n=47). However, eight patients (three patients in group A; five patients in group B) were from an institution deemed ineligible, because C_2 levels were not measured; thus, these patients were excluded from all analyses. Twenty-five patients discontinued the treatment regimen before the end

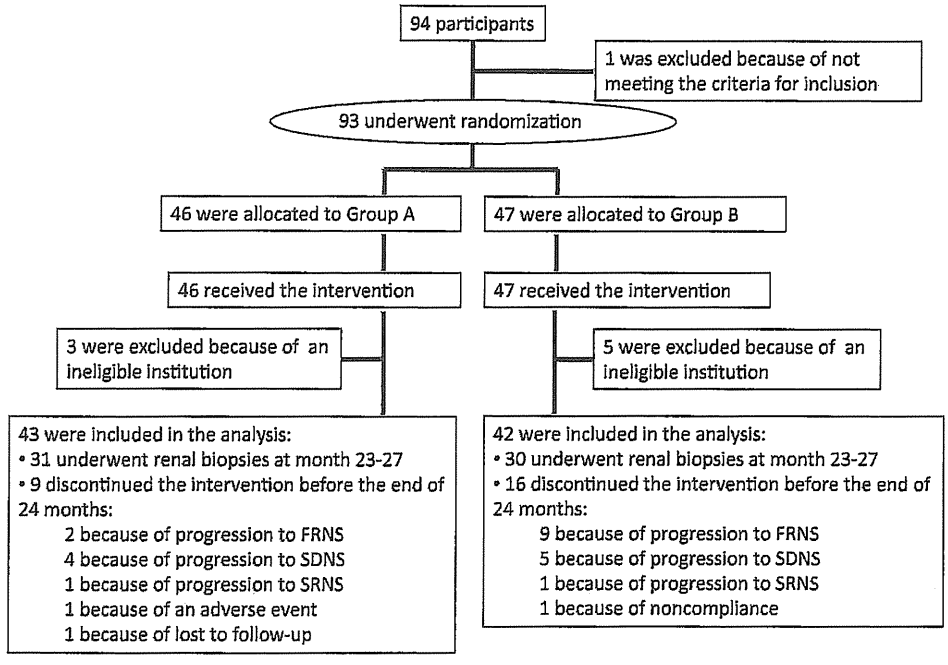

Figure 1. | Flow diagram of the patients. FRNS, frequently relapsing nephrotic syndrome; SDNS, steroid-dependent nephrotic syndrome; SRNS, steroid-resistant nephrotic syndrome.

of the 2-year study period but were included in the analysis for their time in the study. Eleven patients (two patients in group A; nine patients in group B) discontinued treatment because of progression to FRNS. Nine patients (four patients in group A; five patients in group B) discontinued treatment because of progression to SDNS. Two patients (one patient in group A; one patient in group B) discontinued treatment because of progression to SRNS. One patient (group A) discontinued treatment because of an adverse event, one patient (group A) discontinued treatment because of loss to follow-up, and one patient (group B) discontinued treatment because of noncompliance (Figure 1).

Characteristics of the patients are shown in Table 1. There was no clinically important difference between the two treatment groups.

Table 1. Characteristics of the patients

Variables	Group A (n=43)	Group B (n=42)
Men	32 (74.4)	31 (73.8)
Age at entry (yr)	7.0±4.3	7.1±3.7
1–5	25 (59.5)	19 (45.2)
6–10	8 (19.1)	14 (33.3)
11–13	6 (14.3)	5 (11.9)
14–18	4 (9.3)	4 (9.5)
Minimal change subtype of NS	43 (100.0)	42 (100.0)
Duration of NS (mo)	18.9±35.5	12.7±15.9
History of SDNS	26 (60.5)	26 (61.9)
Previous treatment with immunosuppressive agent(s)	8 (18.6)	10 (23.8)
Mizoribine	6 (14.0)	9 (21.4)
Cyclophosphamide	1 (2.3)	1 (2.4)
Chlorambucil	1 (2.3)	0 (0)
Total protein (g/dl)	5.9±0.6	5.8±0.7
Albumin (g/dl)	3.4±0.7	3.3±0.7
BUN (mg/dl)	11.5±4.0	12.8±3.4
Creatinine (mg/dl)	0.3±0.1	0.4±0.1
Study baseline eGFR (ml/min per 1.73 m^2)	122.3±30.6	116.5±21.4

Values are n (%) or mean±SD. NS, nephrotic syndrome; SDNS, steroid-dependent nephrotic syndrome; eGFR, estimated GFR.

C_2 and AUC_{0-4} Levels of Cyclosporine

The mean C_2 levels during the first 6 months, the mean C_2 levels during the next 18 months, and the AUC_{0-4} levels at 3 and 9 months after randomization were all significantly higher in group A than group B (P<0.001 in all cases) (Table 2). The distribution of exact mean C_2 levels and actual doses of mCyA received by patients in the two groups are shown in Supplemental Tables 2 and 3, respectively.

Efficacy

The primary end point, relapse-free survival, is shown in Figure 2. The estimated SRR 24 months after randomization was 64.4% (95% CI, 48.0% to 76.8%) in group A and 50.0% (95% CI, 34.2% to 63.9%) in group B. The SRR in group A was 14.4% higher than the SRR in group B, which was larger than the decision threshold of 8%; 27 of 43 patients in group A and 21 of 42 patients in group B had not experienced any relapse by the end of 24 months after randomization. The hazard ratio for relapse was 0.57 (95% CI, 0.29 to 1.11; P=0.09). The relapse rates in groups A and B were 0.41 and 0.95/person-year, respectively. The ratio of the two relapse rates was 0.43 (95% CI, 0.19 to 0.84; P=0.02) (Table 3).

The estimated progression-free survival rate at 24 months was 88.1% in group A and 68.4% in group B; seven patients in group A showed progression (two patients to FRNS, four patients to SDNS, and one patient to SRNS), whereas 15 patients in group B showed progression (nine patients to FRNS, five patients to SDNS, and one patient to SRNS). The hazard ratio for progression was 0.33 (95% CI, 0.12 to 0.94; P=0.03) (Figure 3).

Safety

The medians (25th and 75th percentiles) of estimated GFRs before mCyA treatment and at month 24 were 119.0 (106.4–130.9) and 116.0 (106.9–129.0) in group A and 114.0 (102.4–125.0) and 121.3 (109.9–134.3) in group B, respectively. There was no difference between the two groups; 61 patients (31 patients in group A; 30 patients in group B) underwent renal biopsies: 60 patients during months 23–27 and one patient at month 31. Two patients in group A (6.5%) and zero patients in group B developed mild to moderate chronic cyclosporine nephrotoxicity (Supplemental Table 4). This difference in the rate of development of chronic cyclosporine nephrotoxicity was not statistically significant.

Table 2. Mean 2-hour postdose cyclosporine levels and areas under the concentration time curve during the first 4 hours after treatment with cyclosporine

Cyclosporine	Group A (Mean±SD)	Group B (Mean±SD)	P Value
C_2 (ng/ml)			
Months 1–6	566.4±86.9 (n=43)	472.7±73.7 (n=42)	<0.001
Months 7–24	489.5±56.4 (n=40)	382.2±86.8 (n=37)	<0.001
AUC_{0-4} (ng·h/ml)			
Month 3	1944.7±487.9 (n=39)	1554.7±462.8 (n=40)	<0.001
Month 9	1704.7±545.2 (n=36)	1316.6±366.0 (n=34)	<0.001

C_2, 2-hour postdose cyclosporine level; AUC_{0-4}, area under the concentration time curve during the first 4 hours after treatment with cyclosporine.

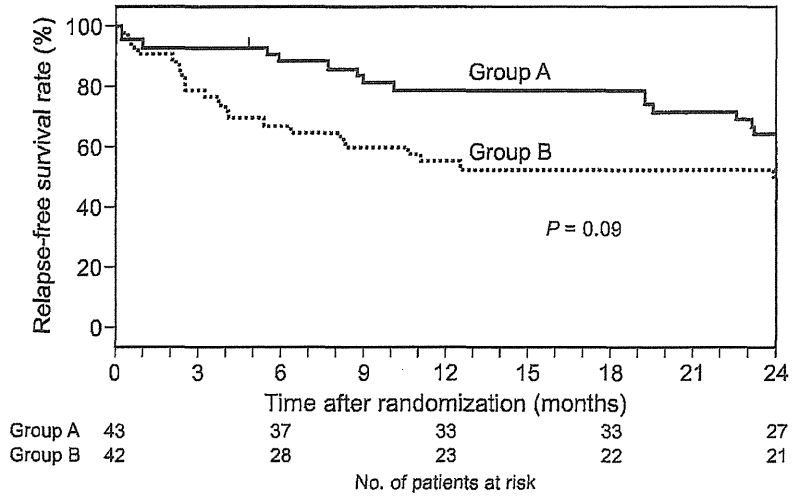

Figure 2. | Relapse-free survival probability (Kaplan–Meier curves).

Table 3. Relapse rates

Treatment Group	Total Number of Relapses	Duration of Observation (d)	Relapse Rate (per person-yr)	Ratio of Relapse Rates (95% Confidence Interval)	*P* Value
Group A	34	30,259	0.41	0.43(0.19 to 0.84)	0.0
Group B	66	25,490	0.95		

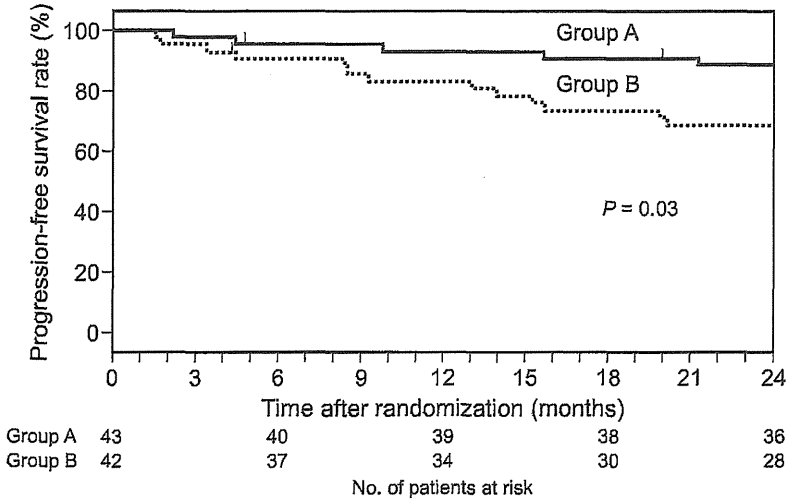

Figure 3. | Progression-free survival probability (Kaplan–Meier curves).

A summary of other adverse events reported during the trial is shown in Table 4. We report cumulative events that occurred within 24 months after randomization, because this time point is when all participants had had an equal opportunity to have an event. The rate and severity of adverse events were similar in both treatment groups. Three patients in group A and two patients in group B had grade III adverse events requiring hospitalization, including one patient in group A who discontinued protocol treatment because of posterior reversible leukoencephalopathy syndrome (25) (month 20), which recovered completely after discontinuation of the protocol treatment. Two of the patients in group A and both of the patients in group B subsequently recovered and restarted protocol treatment as recommended by a physician (Table 4).

Table 4. Summary of adverse events that occurred within 24 months after randomization

Event	Group A (n=43) n (%)	Group B (n=42) n (%)
Grade 3 adverse events		
Pneumonia[a]	3[b,c,d] (7.0)	1[e] (2.4)
Encephalopathy[a]	1[c] (2.3)	1[f] (2.4)
Posterior reversible encephalopathy syndrome[a]	1[b] (2.3)	0
Pneumomediastinum[g]	1[c] (2.3)	0
Grade 1 or 2 adverse events		
Infection[a]	15 (34.9)	13 (31.0)
Asthma[a]	3 (7.0)	1 (2.4)
Edema[a]	1 (2.3)	2 (4.8)
Moon face[a]	3 (7.0)	4 (9.5)
Centripetal obesity[a]	2 (4.7)	1 (2.4)
Hypertrichosis[a]	23 (53.5)	20 (47.6)
Acne[a]	4 (9.3)	2 (4.8)
Cutaneous striae[a]	0	1 (2.4)
Hypertension[g]	7 (16.3)	5 (11.9)
Gingival hyperplasia[g]	4 (9.3)	7 (16.7)
Gastrointestinal event[g]	2 (4.7)	0
Dermatological event[g]	5 (11.6)	3 (7.1)
Neuropsychiatric event[g]	4 (9.3)	3 (7.1)
Pain[g]	0	3 (7.1)
Cataract[g]	2 (4.7)	0
Glaucoma[g]	1 (2.3)	0
Chronic sinusitis[g]	0	1 (2.4)
Cough[g]	1 (2.3)	0
Hyperglycemia[g]	2 (4.7)	2 (4.8)
Hyperkalemia[g]	1 (2.3)	1 (2.4)
Hyperbilirubinemia[g]	2 (4.7)	3 (7.1)
Hyperuricemia[g]	1 (2.3)	1 (2.4)
High-serum glutamic oxaloacetic transaminase[g]	1 (2.3)	3 (7.1)
High-serum glutamic pyruvic transaminase[g]	2 (4.7)	1 (2.4)
High amylase[g]	1 (2.3)	0
High serum creatinine phosphokinase[g]	1 (2.3)	0
Low GFR[g]	1 (2.3)	0
Others[g]	1 (2.3)	3 (7.1)

[a]Multiple reports were recorded for these adverse events.
[b]One patient in group A had pneumonia at month 11 and recovered after 7 days without discontinuing protocol treatment. The same patient had posterior reversible encephalopathy syndrome at month 20, and protocol treatment was discontinued. He recovered completely after 10 days.
[c]One patient in group A had pneumonia, encephalopathy, and pneumomediastinum after influenza infection at month 5 and recovered after 7 days. Protocol treatment was restarted after the recovery.
[d]One patient in group A had pneumonia at month 21 and recovered after 12 days without discontinuing protocol treatment.
[e]One patient in group B had pneumonia at month 5 and recovered after 7 days without discontinuing protocol treatment.
[f]One patient in group B had encephalopathy after rotavirus infection at month 1 and recovered after 7 days. Protocol treatment was restarted after the recovery.
[g]Only the first occurrence of these adverse events was recorded.

Discussion

This study is the first to attempt to select better C_2 levels of cyclosporine in the form of mCyA for FRNS in children. The SRR in group A was 14.4% higher than the SRR in group B, which was larger than the decision threshold of 8%. Also, there was no difference between the two groups with respect to the frequency and severity of adverse events. Therefore, we considered that the C_2 monitoring regimen for group A, in which the target C_2 level was 600–700 ng/ml for the first 6 months and 450–550 ng/ml for the next 18 months, was better than the regimen for group B, in which the target C_2 level was 450–550 ng/ml for the first 6 months and 300–400 ng/ml for the next 18 months. Referencing the report by Ushijima *et al.* (26) on the pharmacokinetic profile of Japanese nephrotic syndrome children treated with mCyA, the mean C_0 levels for months 7–24 in group A might have ranged from 60 to 80 ng/ml, which was lower than the levels in the previous studies (7).

We found that the rate of relapse of nephrotic syndrome was significantly lower in group A than group B patients. This finding agrees with a previous finding that FRNS patients with higher C_2 levels at month 1 tend to have lower relapse rates during cyclosporine treatment (9).

In the previous studies of mCyA treatment by C_2 monitoring for childhood FRNS, the mean relapse rates varied from 0.2 to 1.5 per year under the mean C_2 levels, which ranged from 497.8 to 729.0 ng/ml (13,16,18,20). The relapse rate in group A in the present study (0.41/person-year) was not inferior to the relapse rates in previous studies. Therefore, we considered that the regimen with C_2 target for group A is acceptable for the treatment for childhood FRNS. However, it remains to be elucidated whether the regimen is also acceptable for other populations, because most of C_2 monitoring studies for childhood FRNS were carried out in Japan.

Several grade III adverse events were reported in both groups in this trial. However, all patients with those severe adverse events recovered completely, and most patients restarted protocol treatment. Therefore, we considered adverse events in this trial acceptable. In the present study, two patients (4.7%) in group A developed mild to moderate chronic cyclosporine nephrotoxicity, and zero patients in group B developed this condition. Although the reason is unclear, the prevalence of chronic cyclosporine nephrotoxicity in the present study was much lower than the prevalence in a previous study (discussed in Supplemental Appendix) (15), suggesting that the regimens used in the present study were safe with respect to the development of this condition. The two patients who developed cyclosporine nephrotoxicity both had 9-month AUC levels that seemed to be notably higher than the mean for group A (Supplemental Table 4). However, it is premature to make a conclusion that the higher 9-month AUC levels were responsible for the nephrotoxicity, because the number of patients who developed chronic cyclosporine nephrotoxicity was very low.

One limitation of our study is that, at one particular center, C_2 levels were not measured in most patients. Because we had defined the full analysis set as registered patients whose treatments were correctly started in the protocol, the steering committee considered that center to be ineligible and decided that all eight patients at the center should be excluded from the full analysis set.

Another limitation is that the mean C_2 levels during the first 6 months in group A did not reach the target range, suggesting that it is difficult to control C_2 levels in children, especially when the C_2 target is relatively high. We speculate that a slight difference in dose of mCyA may induce a relatively large difference in C_2 concentrations in children when the C_2 target is relatively high. Nevertheless, the mean C_2 levels in group A were significantly higher than the mean C_2 levels in group B throughout the trial. In addition, the levels of AUC_{0-4} at months 3 and 9 were significantly higher in group A than group B. We, therefore, conclude that patients in both groups were treated in accordance with the protocol. Additional discussion on the target C_2 levels for phase III trials is in Supplemental Appendix.

It is still controversial whether C_2 or C_0 monitoring is better for renal transplant recipients (10,11,27–34). It is also unclear whether C_2 or C_0 monitoring is better for children with FRNS treated with mCyA. Although our study shows that C_2 monitoring with the target C_2 set for group A is promising, phase III trials are required to compare the efficacy and safety of the regimen with the efficacy and safety of the JSPN-recommended C_0 monitoring protocol.

Acknowledgments

We thank all our patients and their families who participated in the Japanese Study Group of Kidney Disease in Children 03.

This study was supported by Ministry of Health, Labour and Welfare, Japan Grant H15-shouni-002.

This study was partly presented at Kidney Week 2011 on November 12, 2011, in Philadelphia, PA, and published in part in abstract form (Iijima *et al.*, *J Am Soc Nephrol* 22: 6B, 2011).

Physicians who participated in the Japanese Study Group of Kidney Disease in Children 03 are listed in Supplemental Appendix.

Disclosures

K. Iijima received grants from Pfizer Japan, Inc.; Kyowa Hakko Kirion, Co. Ltd.; Abbot Japan Co. Ltd.; Takeda Pharmaceutical Co. Ltd.; Asahi Kasei Pharma Corporation; Astellas Pharma, Inc.; Terumo Medical Care K.K.; Chugai Pharmaceutical Co. Ltd.; Benesis (currently, Japan Blood Product Organization); Dainippon Sumitomo Pharma Co. Ltd.; Genzyme Japan K.K.; Novartis Pharma K.K.; Mizutori Clinic; AbbVie LLC; and Janssen Pharmaceutical K.K.; and lecture fees from Novartis Pharma K.K.; Asahi Kasei Pharma Corporation; Baxter Limited; Sanofi K.K.; Pfizer Japan, Inc.; Meiji Seika Pharma Co. Ltd.; Taisho Toyama Pharmaceutical Co. Ltd.; Kyorin Pharmaceutical Co. Ltd.; Kyowa Hakko Kirion, Co. Ltd.; Dainippon Sumitomo Pharma Co. Ltd.; Astellas Pharma, Inc.; Chugai Pharmaceutical Co. Ltd.; and Kowa Pharmaceutical Co. Ltd. S.I. received lecture fees from Asahi Kasei Pharma Corporation, Novartis Pharma K.K., and Chugai Pharmaceutical Co. Ltd. R.T. received a lecture fee from Pfizer Japan, Inc. K. Ishikura received a lecture fee from Novartis Pharma K.K. Y. Ohashi received a lecture fee from Chugai Pharmaceutical Co. Ltd. N.Y. received grants from Astellas Pharma, Inc.; Dainippon Sumitomo Pharma Co. Ltd.; Teijin Pharma Limited; Bayer Yakuhin Ltd; Novartis Pharma K.K.; Kyowa Hakko Kirin Co. Ltd.; Benesis; GlaxoSmithKline K.K.; Asahi Kasei Pharma Corporation; Kyorin Pharmaceutical Company Limited; Otsuka Pharmaceutical Co. Ltd.; Nippon Shinyaku Co. Ltd.; Japan Blood Products Organization; and Maruho Co. Ltd.; and lecture fees from Novartis Pharma K.K.; Dainippon Sumitomo Pharma Co. Ltd.; Kyorin Pharmaceutical Company Limited; Asahi Kasei Pharma Corporation; Astellas Pharma, Inc.; Chugai Pharmaceutical Co. Ltd.; Merck & Co., Inc.; Boehringer Ingelheim; Ono Pharmaceutical Co. Ltd; and Genzyme Japan K.K. The other authors have no conflicts of interest to declare.

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Received: December 25, 2012 **Accepted:** September 25, 2013

Published online ahead of print. Publication date available at www.cjasn.org.

This article contains supplemental material online at http://cjasn.asnjournals.org/lookup/suppl/doi:10.2215/CJN.13071212/-/DCSupplemental.