Table 1. Patient characteristics according to CKD stage

	All patients	Stage 3	Stage 4	Stage 5	P-value*
n	447	315	107	25	
Age (years)	8.6 ± 4.5	8.6 ± 4.6	8.4 ± 4.2	9.9 ± 4.5	0.321
Sex, male/female (*n*)	272/175	192/123	67/40	13/12	0.618
Serum creatinine (mg/dL)	1.6 ± 1.2	1.1 ± 0.4	2.2 ± 0.8	5.3 ± 2.0	<0.001
Height (cm)	119.6 ± 27.8	120.5 ± 28.1	117.1 ± 26.9	118.1 ± 28.9	0.547
Height (SD)	−1.5 ± 1.8	−1.3 ± 1.5	−1.8 ± 2.1	−2.8 ± 3.2	<0.001
BUN (mg/dL)	35.5 ± 18.7	28.3 ± 9.7	48.4 ± 18.1	74.9 ± 31.5	<0.001
Cystatin-C (mg/L)	2.1 ± 0.8	1.9 ± 0.5	3.1 ± 1.0	4.1 ± 0.9	<0.001
eGFR abbreviated (mL/min/1.73 m^2)[a]	39.6 ± 15.9	47.3 ± 11.4	22.6 ± 5.3	10.4 ± 3.3	<0.001
eGFR complete (mL/min/1.73 m^2) [b]	39.9 ± 12.4	43.9 ± 10.0	24.7 ± 5.2	13.5 ± 4.0	<0.001

Values are means ± standard deviation. CKD, chronic kidney disease; SDS, standard deviation score; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate.
[a]Abbreviated Schwartz equation [19], eGFR = 41.3 [height (m)/SCr (mg/dL)].
[b]Complete Schwartz equation [19], eGFR = 39.1 [height (m)/Scr (mg/dL)]$^{0.516}$[1.8/cystatin C (mg/L)]$^{0.294}$ × [30/BUN (mg/dL)]$^{0.169}$ [1.099]^{male} [height (m)/1.4]$^{0.188}$.
*P-values were determined by analysis of variance for all variables except sex, which was analyzed by the χ^2 test.

considered as an event. The day on which SCr was measured that was closest to 1 April 2010 was used as the starting point (i.e. T = 0 years). Cox's proportional hazard regression model was used to identify possible predictors of CKD progression by calculating hazard ratios (HRs) with 95% confidence intervals (CIs). All statistical analyses were carried out using SAS system version 9 (SAS Institute, Inc., Cary, NC, USA).

RESULTS

Patient characteristics

The characteristics of the patients, as of 1 April 2010, are summarized in Table 1. Of the 447 children in this cohort, 405 were of Asian ethnicity and 3 were of another ethnicity; ethnicity was not reported by the institution for the remaining 39 children.

As would be expected, SCr, blood urea nitrogen and cystatin C levels increased significantly with increasing CKD stage, consistent with reductions in eGFR, as determined with the abbreviated and complete Schwartz equations [19]. Children with Stage 5 CKD tended to be older than children with Stage 3/4 CKD.

Progression to ESKD and renal replacement therapy

Table 2 shows the patient outcomes during this survey. Overall, 52 patients progressed to ESKD during the follow-up period [median follow-up period (interquartile range) 1.49 years (1.16–1.64 years); Stage 3, *n* = 9; Stage 4, *n* = 29; Stage 5, *n* = 14]. Of these, 1/9 patients in Stage 3, 21/29 patients in Stage 4 and 8/14 in Stage 5 had CAKUT. Five deaths (sepsis in two; acute encephalitis, graft versus host disease and acute heart failure and pulmonary edema caused by advanced uremia in one each) occurred during the study period, of which four occurred before and one occurred after progression to ESKD. The detailed characteristics of patients with progression to ESKD or who died are presented in Table 3. The Kaplan–Meier analysis for the time to ESKD or death (included as an event) is presented in Figure 1. Among 429/447 children with available data, the survival rates at 1 year were 98.3, 80.0 and 40.9% in children with Stage 3, 4 and 5 CKD,

Table 2. Outcomes and renal replacement therapies according to CKD stage

	All patients	Stage 3	Stage 4	Stage 5
n	447	315	107	25
Data not provided by the participating institution	18	11	4	3
Death before progression to ESKD	4	1	2	1
ESKD	52[a]	9	29[a]	14
Renal replacement therapies				
PD	27	6	15	6
Preemptive kidney transplantation	16	1	11	4
Kidney transplantation after PD	3	0	1	2
HD	4[a]	2	1[a]	1
PD after HD	2	0	1	1
Change in CKD stage (excluding death before progressing to ESKD)				
To Stage 2		43	1	0
To Stage 3		210	6	0
To Stage 4		40	56	1
To Stage 5 (5D)		10 (9)	38 (29)	20 (14)

CKD, chronic kidney disease; ESKD, end-stage kidney disease; PD, peritoneal dialysis; HD, hemodialysis.
[a]Includes one death.

respectively. The Kaplan–Meier plot and survival rates were almost identical when deaths were censored instead of being included as an event; the survival rates at 1 year were 98.3, 80.9 and 43.1% in children with Stage 3, 4 and 5 CKD, respectively.

The most common chronic renal replacement therapy in children with ESKD was peritoneal dialysis, which was used in 27 children, followed by preemptive kidney transplantation in 16 patients (Table 2).

During the follow-up period, 40 and 10 of 315 children with Stage 3 CKD progressed to Stage 4 and Stage 5 (Stage 5D in 9/10 patients) CKD, respectively, while 38/107 patients with Stage 4 CKD progressed to Stage 5 (Stage 5D in 29/38 patients).

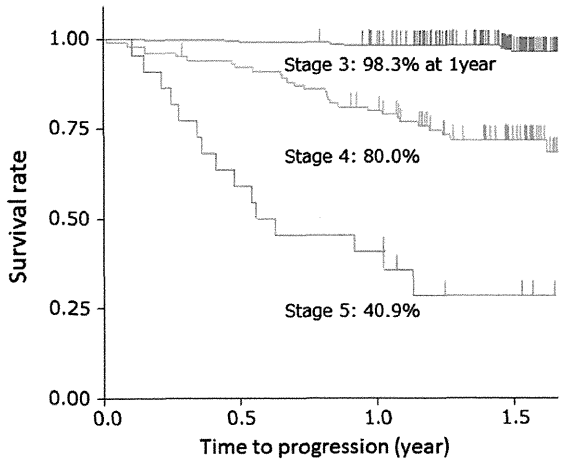
Factors associated with CKD progression

CKD progression was defined as ESKD or death occurring during follow-up. Table 4 shows the factors that were

Table 3. Characteristics of patients who progressed to ESKD or who died

CKD stage in 2010[a]	Age in 2010 (years)	Sex	Primary etiology	Method of detecting CKD	Recognizable syndrome
Deaths					
3	3.6	Male	Unknown	Urinary tract infection	Down syndrome
4	3.4	Male	Cortical necrosis (perinatal period)	Blood analysis in the neonatal period, asphyxia, neonatal shock	—
4	0.7	Male	CAKUT without obstructions	Fetal ultrasonography/ultrasonography in the neonatal period	—
4 Deaths after ESKD	8.3	Male	Drug induced	Detected during the management of other diseases(e.g. heart disease)	—
5	13.5	Female	CAKUT without obstructions	Failure to thrive, weight loss and general fatigue	—
Progression to ESKD					
3 ($n = 9$)	9.8 ± 4.9	6 males 3 females	CAKUT without obstructions (1); chronic glomerulonephritis (2); congenital nephrotic syndrome (1); focal segmental glomerulosclerosis (2); nephronophthisis (1); other inherited kidney damage (2)	Analysis by chance (4); annual urinalysis at school (3); blood analysis in the neonatal period, asphyxia, neonatal shock (1); fetal ultrasonography/ultrasonography in the neonatal period (1)	Bardet–Beadle syndrome (1); Lowe syndrome (1)
4 ($n = 28$)	9.5 ± 4.7	15 males 13 females	CAKUT with obstructions (4); CAKUT without obstructions (17); congenital nephrotic syndrome (1); hemolytic uremic syndrome (1); nephronophthisis (3); neurogenic bladder (1); other inherited kidney damage (1)	Analysis by chance (6); annual urinalysis at school (2); blood analysis in the neonatal period, asphyxia, neonatal shock (4); dysuria, including neurogenic bladder and nocturia (1); failure to thrive, weight loss and general fatigue (3); fetal ultrasonography/ultrasonography in the neonatal period (6); symptoms of glomerulonephritis (edema, oliguria or gross hematuria (1); unknown (1); urinalysis at 3 years (2); urinary tract infection (2))	15q syndrome (1); chromosomal anomalies (1); Ellis–van Creveld syndrome (1); Prune belly syndrome (1); renal coloboma syndrome (1)
5 ($n = 14$)	9.9 ± 1.2	9 males 5 females	CAKUT with obstructions (1); CAKUT without obstructions (7); cortical necrosis (perinatal period) (3); nephronophthisis (1); polycystic kidney disease (2)	Analysis by chance (2); annual urinalysis at school (2); blood analysis in the neonatal period, asphyxia, neonatal shock (1); failure to thrive, weight loss and general fatigue (2); fetal ultrasonography/ultrasonography in the neonatal period (5); unknown (1); urinary tract infection (1)	—

CAKUT, congenital anomalies of the kidney and urinary tract; CKD, chronic kidney disease; ESKD, end-stage kidney disease. Values in parentheses indicate the number of patients. Age is shown as the mean ± SD.
[a]Data are presented for individual patients (deaths) or groups by CKD stage (alive).



Number of participants remaining

Stage 3	315	296	287	178
Stage 4	107	92	78	41
Stage 5	25	13	9	3

FIGURE 1. Kaplan–Meier plot showing time to ESKD according to CKD stage. $T = 0$ years was defined as the day on which serum creatinine was measured that was closest to 1 April 2010. The 1-year survival rates are shown for each stage.

Table 4. Risk factors for ESKD (Cox regression model)

Variable	HR	95% CI	P-value
Sex			
Female	1.56	0.67–3.62	0.306
Male	1.00	—	—
Age			
Age <2 years (versus 2 years to the start of puberty)[a]	9.06	2.29–35.84	0.002
After puberty (versus 2 years to the start of puberty)[a]	4.88	1.85–12.85	0.001
Recognizable syndrome[b]	2.54	0.75–8.58	0.133
CKD stage			
CKD Stage 4 (versus Stage 3)	11.12	4.22–29.28	<0.001
CKD Stage 5 (versus Stage 3)	26.95	7.71–94.17	<0.001
CAKUT	0.60	0.25–1.47	0.261
Preterm delivery (<37 weeks)	1.33	0.50–3.53	0.562
Heavy proteinuria[c]	7.56	3.22–17.77	<0.001
Hypertension[d]	0.53	0.19–1.46	0.219
Use of antihypertensive drugs	1.08	0.42–2.75	0.874

ESKD, end-stage kidney disease; HR, hazard ratio; CI, confidence interval; CKD, chronic kidney disease; CAKUT, congenital anomalies of the kidney and urinary tract.

[a]Age at the start of puberty was defined as 10.8 years for males and 10.0 years for females [23].

[b]Recognizable syndromes included Down syndrome, Kabuki syndrome, Townes–Brocks syndrome, VATER association, prune belly syndrome, Wolf–Hirschhorn syndrome and branchio-oto-renal syndrome, among others.

[c]Urine protein/creatinine ratio >2.0 g/g urine creatinine.

[d]Systolic blood pressure >95th percentile.

independently associated with CKD progression, as determined using Cox's proportional hazards model. As shown in this table, CKD stage and heavy proteinuria were significantly associated with disease progression. Age of <2 years and age at or above the start of puberty were significantly associated with increased risk of disease progression. In contrast, sex, the presence of a recognizable syndrome, disease (CAKUT or other disease), preterm delivery (<37 weeks), hypertension (systolic blood pressure >95th percentile) [24] and the use of antihypertensive drugs were not associated with disease progression. The results did not change when we included the duration of disease instead of age or eGFR calculated using the abbreviated Schwartz equation instead of CKD stage, or if deaths were censored instead of being included as an event (data not shown).

DISCUSSION

The main findings of this prospective cohort study in Japanese children with CKD Stages 3–5 are that (i) the prognosis of CKD in children is poor, as disease progression to a higher CKD stage or ESKD occurred in a sizeable number of children, particularly those with advanced (Stages 4/5) CKD, and (ii) advanced CKD stage and heavy proteinuria were independently associated with progression to ESKD. Age of <2 years and age at or above the start of puberty (≥10.8 years in males and ≥10.0 years in females) were also significantly associated with increased risk of disease progression. To our knowledge, this is the first nationwide, prospective cohort study of children with pre-dialysis CKD to examine the risk for progression to ESKD in Asia.

The present results are broadly consistent with those reported elsewhere, showing the poor outcomes of CKD in children [1, 3–6, 11, 12, 14–16, 25]. In a retrospective analysis of 176 children with dysplastic kidneys and ≥5 years of follow-up, Gonzalez Celedon *et al.* [1] reported that there was an early improvement in renal function, which lasted until ~3.2 years of age, and was followed thereafter by maintained or deteriorating renal function, particularly after 7 and 11 years of age. They reported that hypertension, albuminuria, number of febrile urinary tract infections, eGFR at onset and puberty were significantly associated with disease progression. Sanna-Cherchi *et al.* [26] reported that the prognosis of CAKUT was also poor, as 58/312 patients required dialysis by 30 years of age. Elevated SCr and proteinuria were associated with worse outcomes, as were specific disorders (solitary kidney, posterior urethral valves and vesicoureteral reflux). In the present study, a sizeable proportion (12.5%) of children progressed from Stage 3 to 5 CKD to ESKD during the follow-up period (median 1.49 years). In addition, children with advanced stage CKD (4/5) are at particularly high risk of progressing to ESKD, irrespective of the primary etiologies of CKD. Furthermore, as in the study by Sanna-Cherchi *et al* [26], we found that proteinuria was a risk factor for progression to ESKD. We also found that age <2 years and age at or above the start of puberty were significantly associated with increased risk of progressing to ESKD relative to the risk in patients aged 2 to the start of puberty (10.8 years in males and 10.0 years in females). These results may reflect the risk of disease progression in very young patients with severe congenital complications and that disease progression may be more pronounced in puberty.

The CKD in Children cohort study in the USA [5, 6], as well as studies performed in France [15], Sweden [14], Italy [11] and Australia/New Zealand [25], consistently reported that many children with CKD ultimately require renal replacement

therapies. However, renal transplantation was reported to achieve better long-term outcomes and reduce the mortality rate compared with dialysis in children with ESKD [25]. Although the most common modality (51.9%) of renal replacement therapies was peritoneal dialysis in our cohort, ~30% of children with ESKD received preemptive kidney transplantation, reflecting the current trends in Japan. The superiority and clinical benefits of preemptive kidney transplantation relative to dialysis should be confirmed in future studies.

The present study and the studies described above have consistently shown that heavy proteinuria is independently associated with CKD progression. Prior studies have also indicated that antihypertensive drugs, particularly angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin receptor blockers (ARBs), help to delay or prevent the progression to ESKD in children [27, 28]. These drugs not only lower blood pressure, but also have antiproteinuric, antifibrotic and anti-inflammatory properties. In the present study, 28.4 and 28.2% of patients were prescribed an ARB or ACEI, respectively, and 7.2% were prescribed a calcium channel blocker [9]. In contrast, the use of an antihypertensive drug and hypertension *per se* were not associated with progression to CKD in our cohort study. In the ItalKid project, also an observational study, the use of an ACEI did not significantly modify the progressive course of hypodysplastic nephropathy in children [29]. Therefore, in children with CKD, the effects of antihypertensive drugs, particularly ACEIs and ARBs, on modifying disease progression shown in adults need to be verified in future studies. We are now conducting a randomized controlled trial to prospectively examine the renoprotective effects of ARBs to address this issue (UMIN ID: UMIN000006917, http://indice.umin.ac.jp).

The strengths of this study are that the cohort was representative of children with CKD throughout Japan, as the information was obtained from ~80% of the institutions that manage children with CKD at the time of establishment of the cohort, and the follow-up rate of this cohort was 96%.

Some limitations also warrant mention. We classified CKD using reference SCr levels determined enzymatically in Japanese children. These diagnostic criteria have not been validated globally and so the criteria may not be appropriate for other populations, particularly non-Asian children. However, as described in our prior report [9], this approach was necessary because of potential limitations of using the Schwartz equation in Japanese children or for screening purposes, where SCr is available, but height is not. The duration of follow-up, ~1.5 years, is also relatively short in the context of CKD progression. The pubertal stage of patients was not assessed in this study. Therefore, to estimate the effects of puberty on disease progression, we stratified the patients according to the mean age of Japanese children at the start of puberty (10.8 years in males and 10.0 years in females [23]) in lieu of the actual pubertal stage.

In conclusion, this nationwide, prospective cohort study showed that 12.5% of children with pre-dialysis CKD (stages 3–5) ultimately progressed to ESKD in the follow-up period (median 1.49 years). In particular, children with Stage 4 or 5 were at very high risk of progression to ESKD. Heavy proteinuria was also significantly associated with progression to ESKD. A longer follow-up of this cohort is currently underway to explore outcomes of these children beyond adolescence and into adulthood.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

ACKNOWLEDGEMENTS

This work was supported by 'Research on rare and intractable diseases, Health and Labour Sciences Research Grants' from the Ministry of Health, Labour and Welfare, Japan. The authors would like to thank Drs Takuhito Nagai (Aichi), Kenichi Satomura (Osaka), Takuji Yamada (Aichi), Midori Awazu (Tokyo), Hiroshi Asanuma (Tokyo), Hideo Nakai (Tochigi), Toshiyuki Ohta (Hiroshima), Kazumoto Iijima (Hyogo), Takeshi Matsuyama (Tokyo), Mayumi Sako (Tokyo), Hidefumi Nakamura (Tokyo), Shuichiro Fujinaga (Saitama), Hirotsugu Kitayama (Shizuoka), Naoya Fujita (Shizuoka), Masataka Hisano (Chiba), Daishi Hirano (Tokyo), Yuko Akioka (Tokyo), Hiroshi Hataya (Tokyo), Shunsuke Shinozuka (Tokyo), Ryoko Harada (Tokyo), Hiroyuki Satoh (Tokyo), Takashi Sekine (Tokyo), Yoshinobu Nagaoka (Hokkaido), Yoshimitsu Gotoh (Aichi), Yohei Ikezumi (Niigata), Takeshi Yamada (Niigata), and Akira Matsunaga (Yamagata) of the Pediatric CKD Study Group in Japan for their contributions to the study. The authors also would like to thank the institutions listed in the Supplementary data, Table for their participation in surveys, and Mr Masaaki Kurihara, Ms Chie Matsuda and Ms Naomi Miyamoto of the Japan Clinical Research Support Unit (Tokyo) for their help with data management.

CONFLICT OF INTEREST STATEMENT

The results presented in this paper have not been published previously in whole or part, except in abstract format. Kenji Ishikura has received lecture fees and travel expenses from Novartis Pharma and Asahi Kasei Pharma. Osamu Uemura has received lecture 370 fees and travel expenses from Asahi Kasei Pharma and Siemens Group in Japan. Yuko Hamasaki has received research grants from Novartis Pharma, and lecture fees from Novartis Pharma, Astellas Pharma, and Pfizer Japan. Ryojiro Tanaka has received lecture fees from Pfizer Japan. Koichi Na-375 kanishi has received lecture fees from Novartis Pharma, Asahi Kasei Pharma, and Astellas Pharma. Masataka Honda has received lecture fees from Novartis Pharma and Asahi Kasei Pharma.

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Received for publication: 4.8.2013; Accepted in revised form: 7.1.2014

Pediatr Nephrol
DOI 10.1007/s00467-014-2765-z

ORIGINAL ARTICLE

Rituximab treatment combined with methylprednisolone pulse therapy and immunosuppressants for childhood steroid-resistant nephrotic syndrome

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Received: 13 September 2013 / Revised: 19 December 2013 / Accepted: 13 January 2014

Abstract
Background Calcineurin inhibitors (CIs) with/without intravenous methylprednisolone pulse therapy (MPT) constitute the standard treatment for childhood-onset, steroid-resistant nephrotic syndrome (SRNS). However, some patients fail to achieve remission. We treated SRNS patients resistant to CIs and MPT with additional rituximab combined with MPT and immunosuppressive agents.
Methods Ten patients (aged 2–14 years) with CI- and MPT-resistant SRNS were enrolled. Patients were administered rituximab (1–4 doses; 375 mg/m^2) followed by MPT (30 mg/kg/day of methylprednisolone for 3 consecutive days) once every 2–4 weeks until complete remission (CR). We analyzed clinical outcome and safety.
Results Six patients received a single dose of rituximab, 2 received two doses, and 2 received four doses. Seven patients achieved CR, 1 achieved partial remission, and 2 showed no response. Although 2 patients with no response progressed to end-stage renal failure, 7 patients with CR preserved normal renal function without proteinuria at the last observation. There were two serious adverse events.
Conclusions Additional rituximab combined with conventional MPT and immunosuppressive agents is a promising option for overcoming refractory SRNS. Aggressive B cell suppression by rituximab may ameliorate resistance to conventional treatments and a cocktail of other immunosuppressive agents, such as CIs, MMF, mizoribine, may be beneficial. However, as intense immunosuppression may cause serious adverse events, further evaluation is necessary.

Keywords Rituximab · Steroid-resistant nephrotic syndrome · B cell · Methylprednisolone pulse therapy · Immunosuppressive agents

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Introduction

The treatment of idiopathic steroid-resistant nephrotic syndrome (SRNS) remains challenging. Recently, calcineurin inhibitors (CIs) with or without intravenous methylprednisolone pulse therapy (MPT) have been used as the first line of treatment for childhood-onset SRNS [1, 2], and renal prognosis has greatly improved. However, some patients fail to achieve remission with this conventional treatment. A large number of patients with SRNS progress to end-stage renal failure if remission is not achieved.

Rituximab is a monoclonal antibody directed against the cell surface antigen CD20 expressed on B lymphocytes (B cells). The efficacy of rituximab has been proven in childhood steroid-dependent nephrotic syndrome (SDNS) in terms of prevention of relapses [3–11]. By contrast, the benefit of rituximab for patients with SRNS has not been proven [5, 6, 12–19]. A global investigation conducted by a British group reported that the response rate of rituximab treatment was 44 % in children with SRNS compared with 82 % for those with SDNS [5]. A multicenter study in India and United States also reported that a beneficial effect of rituximab treatment was observed in only 48 % of children with SRNS compared

Published online: 06 February 2014

with 83 % of those with SDNS [6]. A recent randomized controlled trial in Italy did not support the clinical efficacy of additional rituximab for SRNS [12]. Therefore, the efficacy of rituximab treatment against SRNS is still controversial.

Our research group previously reported two girls with focal segmental glomerulosclerosis (FSGS) [20] and one boy with collapsing FSGS [21] who successfully achieved complete remission (CR) using rituximab and MPT. All 3 patients received MPT and 2 of them continued with immunosuppressive agents after rituximab treatment. We believe that additional rituximab may weaken disease activity, and patients of SRNS may acquire improved sensitivity to conventional treatments, such as MPT and immunosuppressive agents. Therefore, we treated SRNS patients who were resistant to CIs and MPT with rituximab combined with MPT and immunosuppressive agents. In the current study, we retrospectively reviewed our strategy of this combination treatment and analyzed its efficacy and safety.

Patients and methods

Patients with SRNS who continued suffering from nephrotic range proteinuria (serum albumin level <3 g/dl and urinary protein creatinine ratio ≥2 g/gCr), despite combination therapy with CIs and MPT, were included in the study. SRNS was defined as a lack of remission despite therapy with prednisolone at 2 mg/kg/day or 60 mg/m^2 of the body surface area (maximum, 80 mg/day) for 4 weeks. This study protocol was based on the Declaration of Helsinki and approval for the off-label use of rituximab was obtained from the ethics committee of our center (#645). All patients' parents gave their written informed consent.

Rituximab was administered in one to four doses of 375 mg/m^2 of the body surface area (maximum of 500 mg). When we planned to treat with more than a single dose, we administered one dose per week repeatedly. To minimize infusion reactions, patients received intravenous methylprednisolone (1–1.5 mg/kg), oral acetaminophen (10 mg/kg, maximum of 300 mg), and chlorpheniramine maleate (d; 0.04 mg/kg, maximum of 2 mg) 30 min prior to rituximab infusions. All patients were admitted to our center and were monitored for at least 24 h after rituximab infusion for observation of infusion reactions. MPT (30 mg/kg/day of intravenous methylprednisolone for 3 consecutive days, maximum of 1 g/day) was given after rituximab treatment once every 2 to 4 weeks repeatedly until CR. Immunosuppressive agents used at the time of rituximab treatment (CIs with or without antimetabolic agents) and oral prednisolone were continued. Regarding antimetabolic agents, mizoribine was switched to mycophenolate mofetil in some patients as the latter is more effective.

Levels of serum creatinine and serum albumin, as well as the urinary protein to creatinine ratio, were monitored at least once a month. CD19+ B cell counts using flow cytometry were also monitored once a month until recovery. B cell depletion was defined as CD19+ count <1 % of total lymphocyte count and B cell recovery was defined as CD19+ cell count ≥1 %. Estimated glomerular filtration rate was defined according to creatinine-based equations of Japanese children (2–12 years, 0.35×height [cm]/serum creatinine [s-Cr, mg/dl]; boys 13–18 years, 0.45×height/s-Cr; girls 13–18 years, 0.35×height/s-Cr) [22]. Clinical efficacy was categorized into CR (urinary protein creatinine ratio <0.2 g/gCr), partial remission (PR, urinary protein creatinine ratio ≥0.2 and <1.0 g/gCr), and no response (NR, urinary protein creatinine ratio ≥1.0 g/gCr). We also evaluated the long-term outcome and adverse events (infusion reactions and late-onset adverse events).

Results

Clinical characteristics of the patients

Ten patients met the inclusion criteria (Table 1). Patients 1 and 3 have been previously reported [20, 21]. Genetic analysis was performed in patients 6, 9, and 10, and no *NPHS2* or *WT1* mutations were found. Except for CIs and MPT, several immunosuppressive agents, including cyclophosphamide, mizoribine, and mycophenolate mofetil, were administered in most of the patients. CIs and antimetabolic agents (mizoribine or mycophenolate mofetil) were used in combination. Plasma exchange was performed in 4 patients and low density lipoprotein apheresis was given in 1 patient. Three patients suffered from impaired renal function at the time of rituximab treatment.

Results of the treatment

Six patients received a single dose of rituximab, 2 received two doses, and 2 received four doses (Table 2). All of the patients achieved B cell depletion. All of the patients received MPT after rituximab treatment and continued CIs. Unfortunately, cyclosporine was discontinued in patient 7 because she suffered from severe pneumonia due to influenza virus after rituximab treatment. Regarding antimetabolic agents, 3 patients (5, 9, and 10) continued with mycophenolate mofetil, and 3 patients (4, 6, and 7) switched from mizoribine to mycophenolate mofetil after rituximab treatment. Two patients (2 and 8) continued with mizoribine and 2 patients (1 and 3) did not use antimetabolic agents. These last 4 patients did not use mycophenolate mofetil as they were mainly followed up by other hospitals. Seven patients (70 %) achieved CR, 1 patient achieved PR, and 2 patients showed NR. Although remission was achieved after B cell recovery in

Table 1 Clinical characteristics of patients

Patient	Age (years)	Gender	Renal biopsy	Diagnosis of SRNS	History of remission	Duration of disease (months)	Duration of proteinuria (months)	Data at rituximab treatment	Previous treatment serum albumin (g/dl)	eGFR (ml/min/1.73 m^2)	Urinary protein creatinine ratio (g/gCr)
1	11	Female	FSGS	At onset	No	11	11	MPT, PSL, CsA, PE	2.1	72.0	8.7
2	14	Male	FSGS	6 years after onset	Yes	121	11	MPT, PSL, CsA, CPM, MZR, PE, LDL-A	1.6	166.6	20.6[a]
3	2	Male	FSGS	At onset	No	4	4	MPT, PSL, CsA, PE	1.9	126.9	284.1[a]
4	12	Female	MGA	At onset	No	23	23	MPT, PSL, CsA, MZR	2.7	173.0	2.6
5	5	Male	FSGS	At onset	Yes	33	12	MPT, PSL, CsA, MZR, MMF	2.0	62.8	5.1
6	2	Female	FSGS	At onset	No	3	3	MPT, PSL, CsA, MZR, PE	2.0	136.0	140.5[a]
7	7	Female	FSGS	6 years after onset	Yes	63	6	MPT, PSL, CsA, MZR	1.4	155.8	29.3
8	14	Male	FSGS	At onset	No	63	63	MPT, PSL, CsA, MZR	2.9	45.0	7.7
9	10	Female	MGA	At onset	Yes	61	41	MPT, PSL, CsA, Tac, CPM, MZR, MMF	2.4	112.4	3.4
10	2	Female	DMP	At onset	No	10	10	MPT, PSL, CsA, MMF	1.9	170.9	4.3
	8.5[b]	Male=4 patients Female=6 patients	FSGS=7 patients MGA=2 patients DMP=1 patient	At onset=8 patients	Yes=4 patients No=6 patients	28[b]	11[b]	MPT=10, PSL=10, CsA=10, Tac=1, MMF=3, MZR=7, CPM=2, PE=4, LDL-A=1	2.0[b]	131.5[b]	8.2[b]

eGFR estimated glomerular filtration ratio, *SRNS* steroid-resistant nephrotic syndrome, *FSGS* focal segmental glomerulosclerosis, *MGA* minor glomerular abnormalities, *DMP* diffuse mesangial proliferation, *MPT* methylprednisolone pulse therapy, *PSL* prednisolone, *CsA* cyclosporin A, *Tac* tacrolimus, *CPM* cyclophosphamide, *MZR* mizoribine, *MMF* mycophenolate mofetil, *PE* plasma exchange, *LDL-A* low density lipoprotein apheresis

[a] Three patients (patient 2, 3, and 6) were receiving daily intravenous albumin infusion to control edema when urine samples were collected

[b] Values represent median

Table 2 Results of the treatment

Patient	Number of doses of rituximab[a]	Number of courses of MPT[b] until CR	Oral medication after rituximab treatment	Outcome	Time until 2+ on the dipstick test (days)	Time until CR (days)	B cell recovery after rituximab treatment (days)
1	2	1	PSL, CsA	CR	56	379	288
2	4	(4)[c]	PSL, CsA, MZR	NR	–	–	NA[f]
3	4	2	PSL, CsA	CR	30	150	69
4	1	1	PSL, CsA, MMF	CR	29	41	NA[f]
5	2	26[d]	PSL, CsA, MMF	PR	42	–	175
6	1	6	PSL, CsA, MMF	CR	15	156	156
7	1	1	PSL, MMF	CR	8	55	118
8	1	(1)[c]	PSL, CsA, MZR	NR	–	–	NA[f]
9	1	5	PSL, Tac, MMF	CR	4	91	231
10	1	3	PSL, CsA, MMF	CR	5	75	115
	1 dose=6 patients 2 doses=2 patients 4 doses=2 patients	2.5[e]	PSL=10 patients CsA=9, Tac=1 patient MMF=6, MZR=2 patient	CR=7 patients PR=1 patient NR=2 patients	22[e]	91[e]	174[e]

MPT methylprednisolone pulse therapy, *PSL* prednisolone, *CsA* cyclosporin A, *Tac* tacrolimus, *MZR* mizoribine, *MMF* mycophenolate mofetil, *PE* plasma exchange, *CR* complete remission, *PR* partial remission, *NR* no response

[a] Single dose means 375 mg/m^2

[b] One course of MPT means 30 mg/kg/day of intravenous methylprednisolone for 3 consecutive days

[c] MPT was discontinued in patients 2 and 8 as they progressed to end-stage renal failure

[d] Patient 5 was followed by another hospital because of too much MPT

[e] Values represent the median

[f] Data not available. We could not obtain any data from the time of B cell recovery in 3 patients as they were followed by other hospitals

2 patients (1 and 3), the median time to reach 2+ on the dipstick test was 22 days and all patients with CR or PR showed decreased proteinuria during B cell depletion.

Long-term prognosis

Three of the 7 patients with CR experienced subsequent relapses after recovery of B cells and all of them received additional rituximab treatment (Table 3). Three of the other 4 patients received additional rituximab just after B cell recovery for prevention of relapse, and have successfully maintained remission. Prednisolone was successfully discontinued in all the patients who achieved CR, except for patient 9, although 3 of them were re-treated with steroids for subsequent relapses. Two patients with NR progressed to end-stage renal failure, 1 patient with PR had mild renal insufficiency, and 7 patients with CR preserved normal renal function without proteinuria (urinary protein creatinine ratio<0.2 g/g) at the last observation.

Adverse events after treatment

Rituximab was infused a total of 27 times, including additional treatment, and infusion reactions were observed for 11 infusions (41 %). In total, 18 symptoms were observed (cough, n=4; respiratory disturbance, n=3; hypoxemia, n=3; abdominal pain, n=2; rash, n=2; wheezing, n=1; sore throat, n=1; nausea, n=1; hypertension, n=1), but no serious adverse events were observed. Two patients showed late-onset adverse events. Patient 3 developed agranulocytosis 56 days after the fifth rituximab infusion concomitant with upper respiratory infection. He was admitted to our center and received antibiotics and granulocyte colony-stimulating factor, resulting in prompt recovery. Patient 7 suffered from severe pneumonia from the influenza H1N1 virus. She required artificial respiration transiently, resulting in full recovery with oseltamivir.

Discussion

To date, there have been nine reports with a total of 72 pediatric patients with SRNS treated with rituximab published in the literature [5, 6, 13–19] (Table 4). A total of 34 patients (47 %) experienced a beneficial effect from rituximab (CR, n=21; PR, n=13); and more than half of them showed NR. Because of these poor results in previous reports, the efficacy of rituximab for SRNS has not been established. However, there have been few reports on the combination

Table 3 Long-term outcome

Patient	Number of relapses	Number of additional rituximab doses	Data at the last observation					
			Medication at the last observation	Serum albumin (g/dl)	eGFR (ml/min/ 1.73 m^2)	Urinary protein creatinine ratio	Time from the first rituximab dose (months)	Time from the last rituximab dose (months)	Time from the last MPT (months)
1/CR	3	2	MMF, ARB	3.9	105.8	0.11	88	21	21
2/NR	0	0			ESRF[b]		74	74	62
3/CR	6	3	CsA, MMF, ACE-I	4.5	87.4	0.09	65	8	8
4/CR	0	0	MMF	3.7	113.7	0.14	39	39	38
5/PR	0	0	CsA, MMF, ACE-I, ARB	3.6	74.4	0.65	37	37	8
6/CR	5	1	CsA, MMF, ACE-I	4.3	114.8	0.00	32	16	28
7/CR	0	1	MMF	4.6	120.6	0.05	32	27	31
8/NR	0	0			ESRF[b]		23	23	22
9/CR	0	1	Tac, MMF, PSL, ACE-I	3.8	93.7	0.08	13	3	10
10/CR	0	1	CsA, MMF, Saireito	4.0	154.9	0.00	12	7	11
				4.0[a]	109.8[a]	0.09[a]	34.5[a]	22[a]	21.5[a]

eGFR estimated glomerular filtration ratio, *ESRF* end-stage renal failure, *MPT* methylprednisolone pulse therapy, *PSL* prednisolone, *CsA* cyclosporin A, *Tac* tacrolimus, *MMF* mycophenolate mofetil, *ACE-I* angiotensin converting enzyme inhibitor, *ARB* angiotensin receptor blocker

[a] Values represent the median

[b] Two patients of ESRF (patients 2 and 8) were excluded from the data at the last observation

treatment of MPT and immunosuppressant agents after rituximab treatment.

According to our experience [20, 21], we believe that rituximab alone is insufficient for the achievement of

Table 4 Previous reports about rituximab treatment in childhood (age≤20 years) SRNS

Reference	Number of patients	Age	Renal histology	Dose of rituximab (mg/m^2)	Clinical efficacy
Bagga et al. [13]	5	2.8–16.0 years	MGA 2	375×4	CR 4
			FSGS 3		PR 1
Suri et al. [14]	1	11 months	FSGS	375×4	CR
Peters et al. [15]	1	15 years	FSGS	1,000×2	CR
Fernandez-Fresnedo et al. [16]	2	19 years, 19 years	FSGS 2	375×4	NR 2
Chaumais et al. [17]	1	9 years	Not reported	375×4	Dead because of PF
Prytula et al. [5]	27	18 months to 11 years	MGA 11	375×1-5	CR 6
			FSGS 11		PR 6
			MesP 3 IgMN 1		NR 15
			No biopsy 1		
Gulati et al. [6]	30	2–20 years	MGA 15	375×4	CR 7
			FSGS 15		PR 6
					NR 17
Kari et al. [18]	4	8–11 years	MGA 1	375×1	CR 1
			FSGS 2 IgMN 1		NR 3
Hirano et al. [19]	1	2 years	MGA	375×2	CR

We excluded our two case reports previously published [20, 21], the national survey in Japan, which included our cases [11], and the randomized controlled trial [12] in which we could not judge clinical efficacy (CR, PR or NR)

MGA minor glomerular abnormalities; *FSGS* focal segmental glomerulosclerosis; *MesP* mesangial proliferation; *IgMN* IgM nephropathy; *PF* pulmonary fibrosis

remission against refractory SRNS. Therefore, we performed rituximab treatment, followed by MPT and CIs with or without antimetabolic agents. Our protocol is unique because of the multiple drug therapy, including rituximab, and we believe that this combination can ameliorate the condition of SRNS. Patients with CI- and MPT-resistant SRNS are at an extremely high risk of developing end-stage renal failure. However 70 % of them were able to achieve CR, and we believe that our approach can prevent progression to end-stage renal failure. The median time to achieve CR was 91 days, which is too long to be a direct effect of rituximab, and 2 patients achieved CR after B cell recovery. However, the median time to reach urinary protein creatinine ratio <1.0 was 22 days and in all patients with CR or PR proteinuria decreased during B cell depletion. Because rituximab treatment is an ultimate B cell suppressive therapy, more aggressive B cell suppression may be pivotal in the treatment of refractory SRNS. In 3 of the 7 patients with CR, proteinuria started to decrease 2 weeks after rituximab treatment. Recently, it was reported that rituximab directly binds sphingomyelin phosphodiesterase acid-like 3b in podocytes and preserves its expression, resulting in prevention of apoptosis in podocytes and the amelioration of proteinuria [23]. The rapidity of the decrease in proteinuria in these 4 patients could be partly explained by this direct effect of rituximab on podocytes. This mechanism is still on an experimental basis and the precise mechanism of rituximab is still unknown. However, we believe that rituximab may weaken disease activity and improve the sensitivity to MPT and immunosuppressive agents.

Infusion reactions were observed in approximately 40 % of infusions, but they were comparatively mild. Two patients experienced severe late-onset adverse events (agranulocytosis and severe pneumonia). Although no patients experienced life-threatening adverse events, we need to be aware of this possibility, because adding rituximab to MPT and immunosuppressive agents may cause excessive immunosuppression. In Japan, the standard treatment for childhood-onset SRNS is CIs with or without MPT, and most patients with SRNS can achieve remission with this treatment. Therefore, rituximab should not be used as the first-line treatment for SRNS.

Our study has several limitations. First, our study was a retrospective, single-center observational study and the number of patients was very low. To confirm the effectiveness and adverse events of our treatment, we need to evaluate them with more patients in a multicenter prospective study. Second, because the protocol for MPT and immunosuppressive agents after rituximab treatment was not rigorously determined, each patient was treated with immunosuppressive agents differently. Third, the ethnicity of our patients was Japanese; therefore, the efficacy of rituximab needs to be proven in other ethnicities. Fourth, genetic analysis was performed in only 3 patients who achieved CR. There remains the possibility that other patients, especially non-responders, might have genetic abnormalities that are usually refractory to immunosuppressive treatment. We consider that if we were to screen the genetic background of all patients, and administer rituximab only in patients without genetic abnormalities, the treatment results might improve. Fifth, our median observation period was 35 months, which is relatively short and a longer follow-up is necessary to evaluate the long-term outcome. However, once patients acquire CR, they usually become steroid-sensitive. Thus, we believe that the renal outcomes of 7 patients with CR may well be good.

In conclusion, additional rituximab treatment combined with conventional MPT and immunosuppressive agents is a promising option for overcoming CI- and MPT-resistant SRNS. We hypothesize that rituximab might weaken disease activity, and that patients might acquire improved sensitivity to conventional treatments. However, because our study was retrospective and the number of patients was limited, we are planning a larger prospective study to confirm the efficacy of additional rituximab therapy combined with MPT and immunosuppressive agents in patients with intractable SRNS who have had genetic background screening.

Acknowledgements The authors thank Drs Y. Akioka (Tokyo), Y. Kaku (Fukuoka), W. Simabukuro (Fukuoka), N. Tanaka (Okayama), H. Nagasako (Kagoshima), and M. Hattori (Tokyo) for their contributions to this study.

Conflict of interest None.

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Clin Exp Nephrol
DOI 10.1007/s10157-014-0941-x

ORIGINAL ARTICLE

The problem of transition from pediatric to adult healthcare in patients with steroid-sensitive nephrotic syndrome (SSNS): a survey of the experts

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Received: 10 November 2013 / Accepted: 15 January 2014

Abstract
Background The importance of the transition from pediatric to adult healthcare has recently been recognized. In patients with steroid-sensitive nephrotic syndrome (SSNS), the shift in steroid dose during the transition period is a big problem. Thus, change in treatment methods during this transition need to be clarified.
Methods Questionnaires were sent to all councilors of the Japanese Society for Pediatric Nephrology who managed SSNS in children. The questionnaires asked about steroid dose, informed consent, and transition programs. Councilors in 50 of 57 (87.7 %) institutes responded within 2 weeks.
Results About one-third of pediatric nephrologists (PNs) did not transfer patients to adult units, and half of PNs followed patients after they reached adulthood (i.e., age >20 years). The dose of steroids after puberty varied between doctors, but 74 % of PNs provided short-term daily therapy. 72 % of PNs informed the patients of the shift in steroid dose, but 26 % of PNs did not. About two-thirds of PNs did not consult with adult nephrologists before the transition from pediatric to adult care. No institute had a transition program for SSNS and 2 institutes had transition coordinators.
Conclusion Transition programs are needed in Japan. But the difference in the steroid regimen between pediatric and adult patients with SSNS is a barrier to transition. This difference needs to be discussed.

Keywords Transition · Nephrotic syndrome · Steroid · Adolescent · Young adult

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Introduction

The "transition from pediatric to adult renal services, a consensus statement" was recently issued by the International Society of Nephrology (ISN) and the International Pediatric Nephrology Association (IPNA) [1].

The position paper of the Society for Adolescent Medicine in 1993 defines the term transition as a process that involves purposeful, planned efforts to prepare the pediatric patient to move from caregiver-directed care to disease self-management in the adult unit [2]. And, many statements and papers have indicated the importance of the transition process in the treatment of chronic pediatric illness including pediatric renal disease [2–9]. But, the problem of transition in patients with steroid-sensitive nephrotic syndrome (SSNS) has not been addressed until now.

Many patients with SSNS have relapses in adult life [10–13], and steroid doses differ between pediatric and adult renal units [14–16], which has been a big problem for patients who transfer to adult renal units. Therefore, we sent questionnaires about steroid dose, informed consent,

Published online: 02 February 2014

Table 1 Items of the questionnaire

1	At what age do you usually transfer patients to the adult health care unit?
	(a) 15–20 years
	(b) After 20 years
	(c) Do not transfer patients
2	What steroid dose do you use after the pubertal period in relapsed patients?
	(a) Use adult regimen after puberty
	(b) Use the maximum dose of 40 mg or lower every day and decrease the dose on alternate days after disappearance of proteinuria for 3 days
	(c) Use dose generally in accordance with the ISKDC regimen[a]
3	Do you discuss steroid doses with doctors in the adult unit before patient transfer?
	(a) Discuss with doctors in the adult unit
	(a-1) Ask to use the dose used in the pediatric unit
	(a-2) Accept the doses used in the pediatric and adult units, though different
	(b) Do not discuss doses
4	Do you usually inform patients about the different doses used in the pediatric and adult units and receive their consent?
	(a) Yes. I receive their consent.
	(b) No. I do not give an explanation.
5	Do you have a transition program?
	(a) Yes
	(b) No
6	Do you have a transition coordinator?
	(a) Yes
	(b) No

[a] ISKDC (International Study group of Kidney Disease in Children) regimen: 60 mg/m^2 (the maximum dose of 80 mg) and decrease the dose 35 mg/m^2 on alternate days after disappearance of proteinuria for 3 days for 4 weeks

Table 2 Results from the questionnaires

Items	Number	NT group (n = 18)	T group (n = 32)
Timing of transition[a]			
(a) 15–20 years	12 (24 %)		
(b) >20 years	26 (52 %)		
(c) Do not transfer	18 (36 %)		
(d) Others	1 (2 %)		
Steroid dose[b]			
(a) Adult regimen*	15 (30 %)	2 (11.1 %)	13 (40.6 %)
(b) ≤40 mg short-term every day therapy	23 (46 %)	8 (44.4 %)	15 (46.9 %)
(c) ISKDC methods	14 (28 %)	7 (38.9 %)	7 (21.9 %)
(d) Others	2 (4 %)	1 (5.6 %)	1 (3.1 %)
Discussion with adult doctor[c]			
(a-1) Use of the pediatric dose	3 (6 %)	2 (11.1 %)	1 (3.1 %)
(a-2) Use of a different dose	18 (36 %)	6 (33.3 %)	12 (37.5 %)
(b) No	33 (66 %)	12 (66.7 %)	21 (65.6 %)
(c) Others	1 (2 %)	1 (5.6 %)	0 (0.0 %)
Patients informed about different doses[d]			
(a) Yes**	36 (72 %)	8 (44.4 %)	28 (87.5 %)
(b) No**	13 (26 %)	9 (50.0 %)	4 (12.5 %)
(c) Others	2 (4 %)	2 (11.1 %)	0 (0.0 %)
Transition program			
(a) Yes	0 (0 %)	0 (0.0 %)	0 (0.0 %)
(b) No	50 (100 %)	18 (100.0 %)	32 (100.0 %)
Transition coordinator			
(a) Yes	2 (4 %)	1 (5.6 %)	1 (3.1 %)
(b) No	48 (96 %)	17 (94.4 %)	31 (96.9 %)

NT not transferred, *T* transferred

* $p < 0.05$: NT versus T, ** $p < 0.01$: NT versus T

[a] 7 institutes answered 2 or 3 (a + b:1, b + c:5, a + b + c:1)

[b] 4 institutes answered 2 (a + b:2, b + c:2)

[c] 5 institutes answered 2 (a-1 + c:1, a-2 + b:4)

[d] 1 institute answered 2 (a + b:1)

and transition programs to expert pediatric nephrologists (PNs) who serve as councilors of the Japanese Society for Pediatric Nephrology (JSPN) to clarify the issue of transition in patients with SSNS.

Methods

Questionnaires asking about usage of steroid and transition programs were sent by E-mail to all JSPN councilors who managed patients with SSNS. These questionnaires contained 6 questions soliciting information about the timing of transfer, steroid doses, informed consent, consultation with doctors providing adult services, transition programs, and transition coordinators (Table 1).

E-mails were sent on May 16, 2010 to 57 institutes which managed pediatric SSNS patients and 50 (87.7 %) institutes responded within 2 weeks.

We divided PNs into two groups: those who did not transfer patients (NT) and those who did transfer patients (T) to adult units. PNs who selected multiple conflicting answers to question about transition were included in NT group, since they did transition only if the patient desired it or lived far away. The association between NT and T was assessed with Pearson's χ^2 test. Two-sided p value < 0.05 was considered statistically significant. All analyses were done using JMP® statistical software version 7.0.2 (SAS Institute, Inc., Cary, North Carolina).

Results

About one-third of PNs did not transfer patients to adult units, and half of PNs followed patients after they reached adulthoods (age >20 years) (Table 2).

The steroid dose after puberty varied and no standard dosing protocol could be ascertained from the questionnaires. But more than 70 % of PNs prescribed steroids as short-term daily therapy and alternate day therapy after the absence of proteinuria for 3 days. The percentage of PNs using adult doses of steroids was lower in the NT group (2 out of 18, 11.1 %) than in the T group (13 out of 32, 40.6 %, $p < 0.05$) (Table 2).

Most doctors (87.5 %) who transferred patients to adult units informed their patients about the differences between adult and pediatric steroid usage, but two-thirds of doctors did not discuss these differences with the doctors providing adult care.

In regard to the transition program, no institute had a transition program for SSNS and only 2 institutes had transition coordinators (a nurse and a child life specialist).

Discussion

The need for transition programs in Japan has been recently acknowledged [17, 18]. Analysis of data from 23 Japanese Association of Children's Hospitals and related institutions showed that three-fourths of pediatricians and all nurses believed that transition programs are necessary. However, only 22 % of pediatricians and 37.5 % of nurses had established processes in place [17]. The first manual in Japan was produced by nurses as recently as 2010 [18].

In the nephrology area, we intend to set up a transition program. A consensus statement by the International Society of Nephrology (ISN) and the International Pediatric Nephrology Association (IPNA) published in 2011 indicated that transfer should occur from pediatric to adult nephrology services only after the adolescent/young adult has been assessed and prepared, and after the receiving adult service has been sent the necessary patient care information [1].

However, the start up of a transition program for patients with SSNS is faced with a difficult steroid dose problem. Recent papers show 30–40 % of pediatric patients relapse after reaching puberty and adulthood [10–13]. In Japan, data indicate frequent relapse in patients with nephrotic syndrome treated with cyclosporine and remission 15 years after treatment in only 27 % of these patients. Therefore, many patients with SSNS require long-term steroid and immunosuppressant treatment after childhood [19].

In the Kidney Disease Improving Global Outcomes (KDIGO) guidelines of 2012 for adults and children [14], Japanese Society for Nephology (JSN) guidelines for treating nephrotic syndrome in adults [15], and JSPN guidelines for treating nephrotic syndrome in children [16], the recommended regimens for patients with SSNS differ. In children, the treatment is a daily dose of prednisone 60 mg/m^2 or 2 mg/kg (maximum of 60 mg)/day until the child has been in complete remission for at least 3 days. In most children, 10–14 daily doses are required because they achieve early complete remission [20]. On the other hand, the treatment for adults is a daily dose of 1 mg/kg (maximum 80 mg) in KDIGO guidelines and a daily dose of 20–40 mg in the JSN guidelines. Reduction regimens after complete remission are also different. Children are given single decreasing doses of prednisolone on alternate days over 4–6 weeks. The dose of prednisolone is tapered slowly over 6 months in the KDIGO guidelines for adults and the daily dose of prednisolone is generally tapered slowly over 1–2 years in the corresponding JSN guidelines. Furthermore, the definitions of steroid dependence and frequent relapse regarding the use of immunosuppressant for steroid sparing effect have remained absent in guidelines for adults (Table 3).

We asked experts of the JSPN about the regimens they administered. One-third of PNs did not transfer patients to the adult unit and about 90 % of them continued to administer pediatric regimens. And, more than 70 % of the doctors used short-term daily steroids and alternate day steroids after the absence of proteinuria for 3 days even after puberty including T group. In the statements of the IPNA and INS, a recurring point raised by young people who have transferred to adult units is the lack of continuity of care and build-up of trust that they experienced in the pediatric unit [1]. Because dosages, especially, and the duration of treatment with daily steroids differ between the pediatric and adult units, patients need to be sufficiently informed to give consent and discussions need to take place between the pediatric and adult units regarding the patient's treatment prior to the transfer. However, we found that the discussion between the units was insufficient.

The present study found that no institute had a transition program for SSNS and 2 institutes have transition coordinators. Many papers have concluded that well-designed transition programs are needed [1–9, 17, 18]. A well-timed transition from child-oriented to adult-oriented healthcare helps young people transition to adult roles and functioning [3]. The most effective time to transfer an adolescent/young adult from a pediatric to an adult renal service occurs after a transition process. Hence, young people should be introduced to the concept of transition in early adolescence (12–14 years). The concepts of identified lead clinicians (transition champions) and transition coordinators deserve further delineation [1]. Although there is no paper regarding transition program for SSNS, those for renal failure were published [8, 9]. In the US, Canada, and Europe in 2004, 33 % of dialysis centers had a transition program with transition coordinators such as nurses and social workers, and 74 % of centers without a transition program believed there was a need for one. They concluded that without

Table 3 Prednisolone treatment in patients with SSNS based on various guidelines

Guideline	KDIGO [14] Adult	KDIGO [14] Children	JSN [15] Adult	JSPN [16] Children
At relapse	1 mg/kg/day (maximum of 80 mg) or alternate day single dose of 2 mg/kg (maximum of 120 mg) for 4–16 weeks	60 mg/m^2 or 2 mg/kg (maximum of 60 mg) until complete remission for at least 3 days.	20–30 mg/day according to patient condition	60 mg/m^2 or 2 mg/kg (maximum of 60 mg) until complete remission for at least 3 days (maximum of 4 weeks).
After remission	Tapering slowly over 6 months	A single dose on alternate days (40 mg/m^2 per dose or 1.5 mg/kg per dose: maximum of 40 mg on alternate days) for at least 4 weeks	Gradually decreased to 5–10 mg for 1 or 2 years	A single dose on alternate days for 6 weeks (60 mg/m^2 per dose or 2.0 mg/kg per dose for 2 weeks, then, 30 mg/m^2 or 1.0 mg/kg per dose for 2 weeks, and 15 mg/m^2 or 0.5 mg/kg for 2 weeks). Long-term use is permitted according to patient condition
Frequent relapse[a]	No clearly defined	Two or more relapses within 6 months of initial response, or four or more relapses in any 12-month period	No clearly defined	Two or more relapses within 6 months of initial response, or four or more relapses in any 12-month period
Steroid dependence[a]	No clearly defined	Two consecutive relapses during corticosteroid therapy, or within 14 days of ceasing therapy	No clearly defined	Two consecutive relapses during corticosteroid therapy, or within 14 days of ceasing therapy

[a] Indication of immunosuppressants

adequate preparation for the transition and without flexibility in the time of transfer, tragic outcomes will continue [8]. In 2005, the Renal Unit at the Royal Children's Hospital, Melbourne Australia, established a well-designed transition program which includes transition coordinators and a transition clinic with adult nephrologists. A survey of the transplant patients concluded that transition planning needs to start earlier and should involve young people more actively, and that the focus should be broadened beyond achieving transfer to more fully reflect what is embodied by the term transition [9].

The above papers mainly indicated that a good transition program should help establish self-management routines that enable individuals to function effectively as adults. Watson [7] pointed out that though the culture of support may often be very different, clinical care within pediatric and adult renal units can be the same. No reports could be found about the difference in SSNS treatment methods between pediatric and adult units. Discussion is needed about the differences in steroid regimens used by pediatric nephrologists and adult nephrologists. We think that this is one of the barriers to implementation of a transition program. Patients with SSNS should be informed about the differences in steroid treatment between the units.

Acknowledgments We gratefully acknowledge the participation of all councilor members of the JSPN who responded to our questionnaires.

Conflict of interest All the authors have declared no competing interest.

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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: 271–278, 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

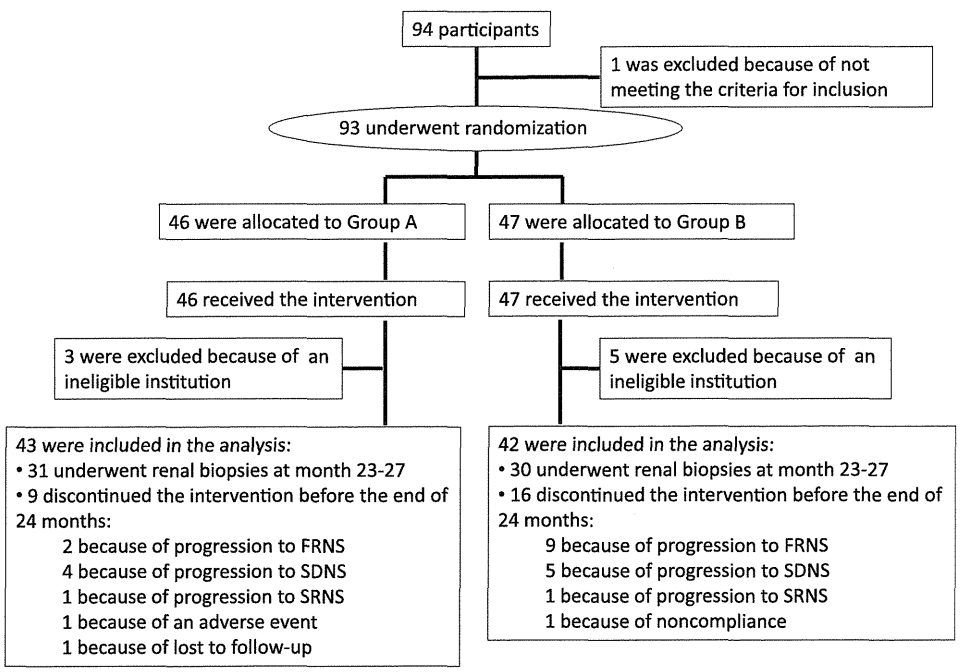

Figure 1. | Flow diagram of the patients. FRNS, frequently relapsing nephrotic syndrome; SDNS, steroid-dependent nephrotic syndrome; SRNS, steroid-resistant nephrotic syndrome.