Cyclophosphamide versus cyclosporine A therapy in steroid-resistant nephrotic syndrome: a retrospective study with a mean 5-year follow-up

Yanwei Liu1,#, Ruikun Yang1,#, Chen Yang2,#, Shuhong Dong1,#, Ying Zhu3, Mingdong Zhao4, Fenglai Yuan5 and Keke Gui4

Abstract

Objective: To compare the clinical efficacy of cyclophosphamide (CTX) and cyclosporine A (CSA) in initial treatment of children with steroid-resistant nephrotic syndrome (SRNS).

Methods: Prospectively maintained databases were reviewed to retrospectively compare two cohorts with SRNS that received peroral administration of 2 to 2.5 mg/kg/d CTX for 3 to 6 months or 1 to 5 mg/kg/d CSA for 2 years until the primary analysis cut-off date during 2007 to 2011. The time to first on-study relapse of SRNS was the primary endpoint. The effective rate was the second endpoint.

1Department of Pediatrics, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, 510700, China
2Department of Physical Examination, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, 510700, China
3Department of Radiology, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, 510700, China
4Department of Orthopaedics, Jinshan Hospital, Fudan University, Shanghai, 201508, China
5Department of Orthopaedics and Central Laboratory, The Third Hospital Affiliated to Nantong University, Wuxi, Jiangsu, 214041, China

#These authors contributed equally to this work

Corresponding authors:
Mingdong Zhao, Jinshan Hospital, Fudan University, No. 1508 Longhang Road, Shanghai 201508, China.
Email: zhaonissan@163.com
Fenglai Yuan, The Third Hospital Affiliated to Nantong University, Wuxi, Jiangsu 214041, China.
Email: bjqq88@163.com
Keke Gui, Jinshan Hospital, Fudan University, No. 1508 Longhang Road, Shanghai 201508, China.
Email: gilbird@163.com
Results: A total of 127 children with SRNS were included (CTX-treated cohort: n = 62; CSA-treated cohort: n = 65), with a mean 5-year follow-up. CTX-treated children showed a significantly delayed time to first on-study relapse of SRNS compared with CSA-treated children (hazard ratio 0.66, 95% confidence interval 0.32–1.75). The relapse rate (rate/year) in CTX-treated children (1.1 ± 0.1) at the 24-month follow-up was significantly higher than that with CSA (0.4 ± 0.2). This difference persisted until the final follow-up.

Conclusions: CSA is associated with a significantly lower relapse rate and significantly higher effective rate compared with CTX, especially in children with minimal change disease.

Keywords
Cyclophosphamide, cyclosporine A, steroid-resistant nephrotic syndrome, relapse, children, minimal change disease

Date received: 3 March 2018; accepted: 16 May 2018

Introduction

Primary nephrotic syndrome is a condition in which the glomeruli of the kidney leak protein from the blood into the urine. This syndrome is a common disease of the urinary system in children.1–3 Hormone therapy is effective for most children with nephrotic syndrome, but 10% to 30% of children show resistance to corticosteroids.4,5 Hormone resistance continues to pose a therapeutic challenge.6 Combined treatment with steroids and immunosuppressants is recommended as the initial therapy by the Kidney Disease: Improving Global Outcomes guideline7,8 and the Chinese Association of Pediatric Nephrology. Cyclophosphamide (CTX) and cyclosporine A (CSA) are frequently used for steroid-resistant nephrotic syndrome (SRNS). SRNS is associated with a 50% risk for end-stage renal disease within 5 years of diagnosis if patients fail to achieve partial or complete remission. This combination has been reported to induce and maintain remission in previous randomized, controlled studies.9,10 However, the long-term efficacy and prognosis of this therapy remain controversial.11,12 Most of the published literature has reported a success rate of 50% to 60%, but these studies included a small number of cases and evaluated different therapeutic protocols for SRNS. Recent meta-analyses failed to demonstrate any difference in the efficacy of CTX and CSA in inducing remission in children with SRNS.13–15

The present retrospective study compared the clinical efficacy of CTX and CSA in treating children with SRNS using the time to first on-study relapse of SRNS as the primary endpoint.

Materials and Methods

Study population and endpoints

This study was approved by the Medical Ethics Committee (First Affiliated Hospital of Sun Yat-sen University) and exemption from informed consent was obtained from our responsible Investigational Ethics Review Board. A retrospective study was performed of the medical records of 127 children with SRNS who were admitted to two medical centres between April 2007 and
November 2011. The following inclusion criteria were used: age range of 3 to 15 years; patients with SRNS who had previously not been treated with immunosuppressive medications; patients who were initially enrolled and were receiving peroral administration of 2 to 2.5 mg/kg/d CTX for 3 to 6 months or 1 to 5 mg/kg/d CSA for 2 years as a second-line immunosuppressive drug due to their SRNS; renal biopsy was performed in all patients at inclusion and the end of therapy; and renal pathological type was based on a previous classification standard\textsuperscript{16} as minimal change disease (MCD), focal segmental glomerulosclerosis (FSG), or mesangial proliferative glomerulonephritis (MPG). The following exclusion criteria were used: clear secondary causes for nephrotic syndrome (e.g., systemic lupus erythematosus); previous CTX or CSA exposure or the use of any immunosuppressant or antineoplastic drugs; membranoproliferative glomerulonephritis; immunoglobulin (Ig) A nephropathy or IgM nephropathy; tumours; discontinuation or interruption of CTX or CSA treatment; abnormal endogenous creatinine clearance, liver function, or blood count; idiopathic membranous nephropathy or C3 glomerulonephritis; organ failure; or severe infectious diseases (e.g., systemic inflammatory response syndrome). The relapse rate was the primary endpoint. The effective rate was the secondary endpoint.

**Definitions of descriptive variables**

Definitions of descriptive variables were based on previous descriptions.\textsuperscript{8} Nephrotic syndrome was defined as oedema, a urine protein/creatinine ratio (uPCR) \(\geq 2000\ \text{mg/g (20–200 mg/mmol)}\), \(\geq 300\ \text{mg/dL protein, or 3+ protein on a urine dipstick, and hypoalbuminemia} \leq 2.5\ \text{mg/L (\leq 25 g/L). SRNS was defined as failure to achieve complete remission after 2 months of prednisolone therapy of 1.5 to 2 mg/kg/d. Complete remission was defined as a uPCR < 200 mg/g (<20 mg/mmol) or < 1+ protein on a urine dipstick for 3 consecutive days. Partial remission was defined as a reduction in proteinuria of 50% or greater from the presenting value and an absolute uPCR between 200 and 2000 mg/g (20–200 mg/mmol). No remission was defined as failure to reduce urine protein excretion by 50% from baseline or persistent excretion of a uPCR \(> 2000\ \text{mg/g (}> 200 \text{mg/mmol)}\). The definition of relapse was based on evidence of a uPCR \(\geq 2000\ \text{mg/g (}\geq 200 \text{mg/mmol)}, \geq 300\ \text{mg/dL protein, or 3+ protein on a urine dipstick.**

**Study design and treatment**

A retrospective, multicentre study was performed, in which eligible patients received peroral administration of 2 to 2.5 mg/kg/d CTX for 3 to 6 months or 1 to 5 mg/kg/d CSA for 2 years. The dose of these two drugs was adjusted. Follow-up was performed at 1, 3, 6, and 12 months postoperatively and yearly thereafter. Clinical assessments (oedema, urine volume, gastrointestinal reaction, gingival hyperplasia, etc.) and the following measurements were assessed at each follow-up: 24-h urinary protein quantification, endogenous creatinine (Cl creat), estimated glomerular filtration rate (eGFR) calculated using the Schwartz formula\textsuperscript{5}: 
\[
\text{GFR} = \frac{k \times \text{height (cm)}}{\text{serum creatinine (mg/dL)}} \quad (k = 0.55 \text{ or } k = 0.7 \text{ [if boys \geq 12 years old]})
\]
routine blood examination, routine urine examination, N-acetyl-\(\beta\)-d-glucosaminidase levels monitored every 3 months, liver function, biochemical analysis of blood, and drug serum concentrations.

CTX or CSA was administered in combination with prednisone (1 mg/kg/d). The prednisone dose was gradually reduced after 2 weeks of negative urine protein. Prednisone was maintained at small doses (0.25–0.5 mg/kg) or stopped after 6 months. CTX was introduced at 2 to 2.5 mg/kg/d orally for 3 to 6 months. The mean duration
of CTX treatment was 4.4 ± 1.6 months. The initial dose of CSA was introduced at 3 to 5 mg/kg/d orally, two times per day. The total blood valley concentration of initial CSA was measured after 4 to 7 days, and the CSA dosage was adjusted according to the serum concentration. The maximum dose of CSA was no greater than 6 mg/kg/d. The dose of CSA decreased when serum concentrations attained trough levels of 100 to 200 l g/L 6 to 9 months after onset. A small dose of 1 to 3 mg/kg/d was maintained after 12 months to maintain serum concentrations of 40 to 70 l g/L for a total duration of 2 years. CSA treatments that were ineffective or produced severe side effects were discontinued after 6 months.

Statistical analysis
Categorical variables are expressed as counts and percentages and were analysed using the χ² test or Mann–Whitney U-test. Continuous numerical variables are expressed as the mean and standard deviation and were analysed using the Student’s t-test. IBM SPSS Statistics, version 24.0 (IBM Corp., Chicago, IL, USA) was used for statistical analyses. A P value less than 0.05 was considered a statistically significant difference for all statistical tests.

Results
Comparison of patients and treatment characteristics
A total of 363 patients were assessed for study eligibility, and 127 patients (CTX-treated cohort: n = 62, mean age: 9.6 ± 5.2 years; CSA-treated cohort: n = 65, 9.5 ± 6.5 years) met the inclusion criteria (Figure 1). Table 1 shows comparison of patients’ demographics between the groups. The mean duration of the study at the primary analysis cut-off date was 65 months (interquartile range: 56.7–74.5) for children on CTX and 61 months (interquartile range: 57.3–66.5) for children on CSA. There was no correlation between the efficacy of CTX or CSA and onset age and clinical classification (simple or nephritic type) of the children (odds ratio [OR] 1.32, 95% confidence interval [CI] 0.21–2.47, P = 0.65). Serum creatinine levels did not significantly change before or after treatment in 20 children, but two showed acute kidney injury within 1 week after treatment, both of which were reversible. Three patients who were treated with CSA for 2 years showed no CSA-related tubulointerstitial fibrosis after repeated renal biopsy. Two patients had end-stage renal lesions, and the pathological type was focal segmental glomerulosclerosis. One patient with tubulointerstitial lesions before treatment was invalid for CSA treatment. One patient with partial remission following 2-year CSA treatment underwent repeated renal biopsy, and we found no change in CSA-related renal interstitial fibrosis. Two patients entered end-stage renal failure at 4 and 5 years of disease duration.

Comparison of the relapse rate between the groups
The relapse rate per year of the CTX and CSA groups was significantly decreased at the end of treatment (P < 0.05) and at the final follow-up (P < 0.05) compared with before therapy (Table 2). The 5-year total relapse rates of the CTX and CSA groups were 11.2% and 6.2%, respectively. The 5-year relapse rates of the CTX and CSA groups were 10.5% (4/38) and 5.7% (2/35), respectively, in children with MCD. The CSA group showed a significantly delayed time to first on-study relapse of SRNS compared with the CTX group (hazard ratio
0.82, 95% CI 0.47–1.93; \( P < 0.0001 \). Six patients showed complete remission following initial CSA treatment, relapse during small doses of maintenance therapy, and the presence of CSA resistance, in which conversion to tacrolimus was effective. Two children with FSG showed a rise in serum creatinine levels at the end of 2 years of treatment, the eGFR decreased to 60 mL/(min × 1.73 m²), and the disease entered the stage of renal failure in 5 years. Eight children were no longer treated with CSA after 6 months because of CSA resistance. Five of these patients showed normal renal function, and three with FSG entered end-stage renal failure after 1 year and were required to undergo peritoneal dialysis treatment.
Comparison of the effective rate of treatment between the groups

The mean time to first on-study relapse was 6 months with CTX compared with 10 months with CSA (hazard ratio 0.63, 95% confidence interval 0.12–3.71; \( P = 0.0001 \)). Twenty-four patients in the CTX group showed complete remission at the final follow-up, eight showed partial remission, and 30 showed no effect. The total effective rate of CTX was 51.6%. Thirty-five patients in the CSA group showed complete remission at the final follow-up, 11 showed partial remission, and 19 showed no effect. The total effective rate of CSA was 70.8%. The total effective rate of the CTX group was significantly lower than that in the CSA group \( (P = 0.027) \) (Table 3). The effective rate of CSA treatment (47.5%, 19/40) was significantly higher than that for CTX treatment (21.1%, 8/38) in children with MCD \( (P < 0.001) \). The effective rate of CTX treatment (31.3%, 5/16) was significantly different from that of CSA treatment (33.3%, 5/15) in children with FSG. No significant differences were detected in the distributions of the sex ratio, pathological type, and clinical type between the groups.

### Table 1. Patients’ demographics between the two groups.

| Variable                      | CTX (n = 62)       | CSA (n = 65)       | \( P \) value |
|-------------------------------|-------------------|-------------------|--------------|
| Age at onset (years)          | 9.6 ± 5.23        | 9.5 ± 6.53        | 0.304\(^a\)  |
| Sex (M:F)                     | 28:34             | 30:35             | 0.910\(^b\)  |
| Pathological type             |                   |                   | 0.903\(^c\)  |
| MCD                           | 38                | 40                |              |
| FSG                           | 16                | 15                |              |
| MPG                           | 8                 | 10                |              |
| Duration of treatment (months)| 68.2 ± 10.13      | 67.7 ± 14.49      | 0.151\(^a\)  |
| Creatinine ratio              |                   |                   | 0.482\(^c\)  |
| <0.2 mg/mg                    | 9                 | 12                |              |
| 0.2–2.0 mg/mg                 | 31                | 33                |              |
| >2.0 mg/mg                    | 22                | 20                |              |
| Urinary protein (g/24 h)      | 2.4 ± 0.93        | 2.5 ± 0.29        | 0.102\(^a\)  |
| SP (mmHg)                     | 93 ± 10.25        | 94 ± 6.69         | 0.213\(^a\)  |
| DP (mmHg)                     | 65 ± 5.47         | 64 ± 8.43         | 0.305\(^a\)  |
| eGFR (mL/min/1.73 m\(^2\))    |                   |                   | 0.782\(^c\)  |
| >90 (stage I)                 | 18                | 20                |              |
| 60–89 (stage II)              | 13                | 15                |              |
| 30–59 (stage III)             | 14                | 13                |              |
| 15–29 (stage IV)              | 10                | 9                 |              |
| <15 (stage V)                 | 7                 | 8                 |              |
| Scr (\(\mu\)mol/L)           | 26.09 ± 6.54      | 26.72 ± 6.22      | 0.475\(^a\)  |
| Cl creat (mL/min/1.73 m\(^2\))| 97.13 ± 42.61     | 96.78 ± 48.24     | 0.197\(^a\)  |

Values are mean ± standard deviation or as specified. \(^a\)Analysed using the independent-samples t-test; \(^b\)analysed using the chi-square test; \(^c\)analysed using the Mann–Whitney U-test. CTX: cyclophosphamide; CSA: cyclosporine A; MCD: minimal change disease; FSG: focal segmental glomerulosclerosis; MPG: mesangial proliferative glomerulonephritis; SRNS: steroid-resistant nephrotic syndrome, eGFR: estimated glomerular filtration rate (according to the Schwartz formula); Scr: serum creatinine; NKF K/DOQI CKD stages: National Kidney Foundation Kidney Disease Outcomes Quality Initiative stages for chronic kidney disease; SP: systolic pressure; DP: diastolic pressure; Cl creat: endogenous creatinine.
Table 2. Comparison of the relapse rate and renal pathological type between the two groups at four-time points.

|                  | CTX (n = 62) | CSA (n = 65) | P value |
|------------------|-------------|-------------|---------|
|                  | UP | Cl creat | Rr/y | UP | Cl creat | Rr/y |
| Before therapy   |    |         |      |    |         |      |
| MCD              | 3.5±2.4 | 98.5±44.4 | 5.4±1.4 | 3.6±1.3 | 97.9±50.3 | 5.4±2.8 |
| FSG              | 6.3±2.7 | 85.2±23.6 | 3.5±2.2 | 6.5±1.3 | 88.6±22.4 | 3.6±1.4 |
| MPG              | 5.8±2.3 | 96.7±27.1 | 2.9±1.9 | 5.6±2.9 | 95.5±32.3 | 2.8±1.2 |
| Rr/y             | 4.2±1.3# |         |        | 4.2±3.7# |         |
| At the end of therapy |    |         |      |    |         |      |
| MCD              | 0.7±0.7 | 57.1±22.7 | 1.7±0.7 | 0.7±0.6 | 50.5±12.1 | 1.2±0.4 |
| FSG              | 1.4±0.3 | 66.3±17.6 | 1.8±0.6 | 1.5±0.4 | 62.2±11.5 | 1.1±0.5 |
| MPG              | 0.5±0.4 | 62.5±28.4 | 0.9±0.4 | 0.3±0.2 | 58.3±17.2 | 0.5±0.7 |
| Rr/y             | 1.7±0.4# |         |        | 1.2±0.6# |         |
| Two years after treatment |    |         |      |    |         |      |
| MCD              | 0.9±0.1 | 45.2±11.3 | 1.1±0.7 | 0.8±0.1 | 39.5±10.3 | 0.5±0.3 |
| FSG              | 1.7±0.6 | 54.5±13.6 | 1.2±0.8 | 1.5±0.4 | 42.2±9.44 | 1.3±0.4 |
| MPG              | 0.6±0.2 | 42.5±18.2 | 0.5±0.2 | 0.5±0.3 | 34.5±11.2 | 0.7±0.3 |
| Rr/y             | 1.1±0.1# |         |        | 0.4±0.2# |         |
| Five years after treatment |    |         |      |    |         |      |
| MCD              | 2.5±1.2 | 65.3±13.8 | 1.6±0.8 | 1.1±0.5 | 43.2±11.4 | 0.6±0.2 |
| FSG              | 3.2±1.6 | 32.5±9.2  | 2.3±1.4 | 2.5±1.1 | 36.7±10.1 | 1.5±0.5 |
| MPG              | 1.7±0.4 | 68.8±11.5 | 1.0±0.5 | 1.2±0.6 | 38.9±11.9 | 1.0±0.4 |
| Rr/y             | 1.8±0.5# |         |        | 0.7±0.3#2 |         |

Values are mean ± standard deviation. #No significant difference (P = 0.214) before therapy; #P = 0.031 at the end of therapy; #P = 0.012 at 2 years after treatment; #P = 0.001 at 5 years after treatment. CTX: cyclophosphamide; CSA: cyclosporine A; UP: urinary protein; Cl creat: endogenous creatinine; MCD: minimal change disease; FSG: focal segmental glomerulosclerosis; MPG: mesangial proliferative glomerulonephritis; Rr/y: relapse rate/year.

Table 3. Comparison of the result of treatment of SRNS children undergoing combined treatment between groups at the final follow-up.

| Variable                  | CTX (n = 62) | CSA (n = 65) | P value |
|---------------------------|-------------|-------------|---------|
| Relapse-free period (months) | 32.5±8.47 | 38.3±7.52 | <0.001a |
| Assessment                |             |             | 0.038b  |
| complete remission        | 24          | 35          |         |
| partial remission         | 8           | 11          |         |
| no effect                 | 30          | 19          |         |
| Effective rate            | 51.6% (32/62) | 70.8% (46/65) | 0.027c  |

Values are mean ± standard deviation, n, or % (n). a Analysed using the independent-samples t-test; b Analysed using the Mann–Whitney U-test; c Analysed using the chi-square test. CTX: cyclophosphamide; CSA: cyclosporine A.

Remission analysis in the CTX group

The dose of prednisolone in the CTX group gradually decreased during the treatment period. The time for urinary protein to turn negative in nine children with complete remission ranged from 10 to 240 days, with
an average of 116 days. Twenty-four patients were followed up for 5 years and showed complete remission with no relapse. Six patients with relapse showed remission after treatment combined with azathioprine and small doses of prednisone. Eight cases of partial remission in children showed persistent proteinuria (+ to ++) and normal renal function, and two of these patients successfully converted from CTX to CSA. Seventeen of the 30 children who failed to respond to this CTX course were converted from CTX to CSA. Nine of these patients showed a successful effect, and eight of these patients failed to respond to the CSA course.

**Remission analysis in the CSA group**

The dose of prednisone was significantly reduced from 40 to 10 mg during treatment in the CSA group. The time for urine protein to turn negative in 35 children with complete remission was 5 to 240 days, with an average of 49 days. Thirty patients who showed complete remission were followed up for 5 years and had no relapse. Five (5/35) patients had relapses; three of these patients received additional CSA and two showed reappearance of hormone effects. The other 11 patients showed partial remission following introduction of CSA, and five of these showed sustained proteinuria (+) throughout the follow-up period.

**Adverse effects**

With CTX, nausea was observed in 3/62 cases, fungal infection in 4/62 cases, reversible hair loss in 2/62 cases, leukopenia in 5/62 cases, and alopecia in 2/62 cases. With CSA, nausea was observed in 4/65 cases, fungal infection 3/65 cases, reversible hair loss in 3/65 cases, tremors in 3/65 cases, leukopenia in 3/65 cases, hirsutism in 4/65 cases, and alopecia in 3/65 cases. There was no significant difference in distribution of complications between the groups.

**Discussion**

The present study followed children with SRNS for a mean of 5 years. The most important finding was that hormone treatment in combination with CSA was associated with a significantly lower relapse rate and significantly longer relapse-free period compared with hormone treatment in combination with CTX in children with SRNS and MCD.

These findings are consistent with those by Drube et al. Our 5-year relapse rate (6.2%) indicated that hormone treatment in combination with CSA is reliable. Other studies that compared the clinical efficacy of CTX and CSA for treating children with SRNS reported similar results. Wu et al. detected a lower rate of relapse in children with SRNS receiving hormone treatment in combination with CSA compared with hormone treatment in combination with CTX at a mean follow-up of 23 months. The present study showed a significant difference in relapse rate between the groups, but this is inconsistent with several previous studies that demonstrated an absence of a significant difference in the relapse rate. A prospective study by van Husen et al. included 35 children with SRNS who underwent CTX or CSA treatment and showed no significant difference in relapse rates.

The incidence of SRNS in children is reported to be 16/100,000, with a new incidence of $2 \times 10^{-5}$ to $7 \times 10^{-5}$ per year, and one in approximately 6000 children show nephrotic syndrome. SRNS does not respond to hormone therapy, and proper treatment needs to be initiated as soon as possible to avoid the side effects caused by the long-term use of hormones. In children with SRNS, hormone treatment combined with immunosuppressive agents is a
common consensus. Currently, the most common immunosuppressive agents are CTX and CSA, but there are different reports of the efficacy of these agents in the published literature. In the present study, the 5-year total relapse rates of the CTX and CSA groups were 11.2% and 6.2%, respectively. The relapse rates of the CTX and CSA groups were 10.5% and 5.7%, respectively, in children with MCD. A prospective study by Westhoff et al. investigated 112 children with SRNS who were treated with prednisone in combination with CTX or CSA. In their study, the relapse rates of MCD, MPG, and FSG in children were 8.8%, 12.5%, and 6.5%, respectively. The relapse rate of children with SRNS who were treated with CSA and followed for more than 1 year could reach 12% to 27%. However, a meta-analysis by Fu et al. showed no significant difference in the relapse rates of CTX or CSA in children with MCD or FSG. In previous studies on the prognosis of SRNS, multivariate analysis showed that the relapse rate of children with MCD was significantly lower than that of children without MCD. In the present study, no significant difference was detected in the relapse rate of children between MCD and FSG, regardless of CTX or CSA treatment. A recent retrospective study of 67 children with SRNS who received combination CSA and hormone therapy showed that the relapse rate in MCD cases was 0% (0/65) and that in the family hereditary FSG was 88% (46/52), but a significant difference between groups was not detected. The present study did not investigate the genetic background of FSG. Further study is required on the effects of familial and non-familial genetic FSG on immunosuppressive agents. We found that the relapse rate of CSA treatment was lower than that with CTX treatment in children with MCD, but there were no significant advantages for CSA treatment in children with FSG. Evidence-based medicine analysis has shown that the relapse rates of CSA-treated children with MCD and FSG are not superior to placebo. However, the effectiveness of CSA in children with FSG is not significantly different from the placebo group. Therefore, a prospective, randomized, controlled study with a larger sample is required to clarify whether CSA is superior to CTX for treating children with FSG. The present study compared the onset age, clinical classification, and efficacy of CTX or CSA in children. Onset age and clinical classification (simple or nephritic type) of the children were not significantly correlated with the efficacy of CTX or CSA. Multivariate analysis of SRNS has suggested that age is one of the factors influencing prognosis, but previous studies lacked further analyses of confounding factors. This study eliminated the effects of the different treatment regimens and showed that onset age did not affect the prognosis of SRNS. The safety of long-term application of inhibitors, such as CTX or CSA, is a major concern. Infection is a common problem with application of immunosuppressive agents, especially in chickenpox, herpes simplex, carinii, and other special pathogens, with a high fatality rate. Only one case of varicella occurred without herpes simplex encephalitis in the present study. A preliminary study of respiratory and urinary tract infections showed that approximately 20% to 30% of children in the two groups were prone to recurrent respiratory or urinary tract infections. No severe adverse effects of haemorrhagic cystitis were observed in the CTX group because we ensured that patients received adequate hydration and alkalization during the course of clinical treatment. CSA was first used for treating SRNS in 1993, and it is currently widely used, but limited to short-term treatments.
important reason for the short course of treatment is that CSA has obvious side effects of renal interstitial fibrosis.\textsuperscript{12,14,18} Recurrence of renal disease is common after cessation of CSA treatment, with a recurrence rate of 44\% at 12 months of treatment.\textsuperscript{2,4,15,17} This rate has led to clinical extension of CSA treatment. The present study showed that that long-term CSA treatment reduced relapse rates. The renal toxicity of CSA has become the focus of attention in recent years. An open, nonrandomized, retrospective study by Sumegi et al.\textsuperscript{12} examined repeated renal biopsies in children with SRNS who were treated with small doses of CSA for longer than 2 years. These authors found no change in renal fibrosis or drug toxicity. The present study showed that renal failure in children may be associated with persistent proteinuria and the natural course of the disease itself. A small dose of CSA (1–5 mg/kg) maintained at 40 to 70 g/L serum concentrations and long-term maintenance therapy was safe in our study. Notably, four patients developed elevated N-acetyl-\(\beta\)-d-glucosaminidase levels, which indicated renal tubular injury. Further studies and monitoring are required to understand renal injury because of the small number of repeated renal biopsies in this study.

CSA-induced nervous system side effects are rare,\textsuperscript{1,7,12,22,26} but headache, insomnia, tremors, and anxiety may occur.\textsuperscript{3,11,12,27} Some symptoms, such as aphasia, hallucinations, convulsions, and coma, may be encountered in severe cases.\textsuperscript{7,14,16} Nervous system side effects are independent of the CSA dose and may occur at small doses.\textsuperscript{6,7,11,17} A total of 30\% of patients with SRNS show a low density of white matter in the brain.\textsuperscript{1,12,13} Rosti et al.\textsuperscript{28} showed that children receiving CSA treatment without persistent abnormalities on electroencephalogram have a better prognosis. One patient suffered from headache 1 day after drug treatment in the present study. One patient suffered convulsions once after a 2-year treatment, but showed no lesions of brain white matter in computed tomography, with mild brain atrophy and a normal electroencephalogram. The neurological symptoms of 2 children were relieved, and their prognosis improved. Our data showed that the toxic and side effects of CSA may be controlled or relieved by treatment. None of our patients discontinued treatment because of side effects.

There are some limitations in this study. First, the retrospective nature of the study limits the level of confidence of our conclusion. Second, there may have been selection bias, which may have reduced the strength of our findings. However, this effect may have been limited because a high participation rate of 76\% of patients was achieved in the present study. Third, our study lacked genetic investigation. Finally, every attempt was made to adjust for all potential confounders, but other unmeasured factors may also be relevant.

In conclusion, the combination of hormone and immunosuppressant (CTX or CSA) treatment achieves good results in children with SRNS. The total effective rate of the CSA-treated cohort was better than that of the CTX-treated cohort, especially in children with MCD. Evaluation of the efficacy of CTX or CSA in children with SRNS should be further investigated using randomized, controlled trials, and the long-term effects and side effects should be noted.

**Declaration of conflicting interest**

The authors declare that there is no conflict of interest.

**Funding**

Funding for this research was received from the Shanghai Municipal Health and Family Planning Commission Fund Project (201640057), the Natural Science Foundation...
of China (81770876, 81270011, 81472125), the Natural Science Foundation of Jiangsu Province (Grant BK20151114), the Foundation of Traditional Chinese Medicine of Jiangsu Province (YB201578), and Fundamental Research Funds for Medical Innovation Center of Health and Family Planning Commission of Wuxi (CXTD006).

References

1. Sinha A, Gupta A, Kalaivani M, et al. Mycophenolate mofetil is inferior to tacrolimus in sustaining remission in children with idiopathic steroid-resistant nephrotic syndrome. Kidney Int 2017; 92: 248–257.

2. Wu B, Mao J, Shen H, et al. Triple immunosuppressive therapy in steroid-resistant nephrotic syndrome children with tacrolimus resistance or tacrolimus sensitivity but frequently relapsing. Nephrology 2015; 20: 18–24.

3. Trautmann A, Schnaitd S, Lipska-Zietkichewicz BS, et al. Long-term outcome of steroid-resistant nephrotic syndrome in children. J Am Soc Nephrol 2017; 28: 3055–3065.

4. Plank C, Kalb V, Hinkes B, et al. Cyclosporin A is superior to cyclophosphamide in children with steroid-resistant nephrotic syndrome - a randomized controlled multicentre trial by the Arbeitsgemeinschaft fur Padiatrische Nephrologie. Pediatr Nephrol 2008; 23: 1483–1493.

5. van Husen M and Kemper MJ. New therapies in steroid-sensitive and steroid-resistant idiopathic nephrotic syndrome. Pediatr Nephrol 2011; 26: 881–892.

6. Ulinski T and Aoun B. Pediatric idiopathic nephrotic syndrome: treatment strategies in steroid dependent and steroid resistant forms. Curr Med Chem 2010; 17: 847–853.

7. Mantan M, Sriram CS, Hari P, et al. Efficacy of intravenous pulse cyclophosphamide treatment versus combination of intravenous dexamethasone and oral cyclophosphamide treatment in steroid-resistant nephrotic syndrome. Pediatr Nephrol 2008; 23: 1495–1502.

8. Lombel RM, Gipson DS and Hodson EM. Treatment of steroid-sensitive nephrotic syndrome: new guidelines from KDIGO. Pediatr Nephrol 2013; 28: 415–426.

9. Hodson EM and Craig JC. Therapies for steroid-resistant nephrotic syndrome. Pediatr Nephrol 2008; 23: 1391–1394.

10. Saito T, Iwano M, Matsumoto K, et al. Significance of combined cyclosporine-prednisolone therapy and cyclosporine blood concentration monitoring for idiopathic membranous nephropathy with steroid-resistant nephrotic syndrome: a randomized controlled multicenter trial. Clin Exp Nephrol 2014; 18: 784–794.

11. Indian Society of Pediatric Nephrology; Gulati A, Bagga A, Gulati A, et al. Management of Steroid Resistant Nephrotic Syndrome. Indian Pediatr 2009; 46: 35–47.

12. Sumegi V, Haszon I, Bereczki C, et al. Long-term follow-up after cyclophosphamide and cyclosporine-A therapy in steroid-dependent and -resistant nephrotic syndrome. Pediatr Nephrol 2008; 23: 1085–1092.

13. Inaba A, Hamaasaki Y, Ishikura K, et al. Long-term outcome of idiopathic steroid-resistant nephrotic syndrome in children. Pediatr Nephrol 2016; 31: 425–434.

14. Fu HD, Qian GL and Jiang ZY. Comparison of second-line immunosuppressants for childhood refractory nephrotic syndrome: a systematic review and network meta-analysis. J Invest Med 2017; 65: 65–71.

15. Mulic B, Milosevski-Lomic G, Paripovic D, et al. Congenital nephrotic syndrome may respond to cyclosporine A - A case report and review of literature. Srp Arh Celok Lek 2017; 145: 407–410.

16. Churg J, Bernstein J and Glassock RJ. Classification of glomerular disease. In: J Churg, J Bernstein, RJ Glassock, editors. Renal disease classification and atlas of glomerular diseases. 2nd ed. Tokyo: Igaku-Shoin; 1995. p. 151–156.

17. Drube J, Geerlings C, Taylor R, et al. Fifteen-year remission of a steroid-resistant nephrotic syndrome sustained by cyclosporine A. Pediatr Nephrol 2007; 22: 600–602.

18. Pena A, Bravo J, Melgosa M, et al. Steroid-resistant nephrotic syndrome: long-term
evolution after sequential therapy. Pediatr Nephrol 2007; 22: 1875–1880.
19. Matveeva M, Leonova L, Kucherenko A, et al. Control of cyclosporine a treatment in children with steroid-resistant and steroid-dependent nephrotic syndrome. Pediatr Nephrol 2011; 26: 1653–1654.
20. Moudgil A, Bagga A and Jordan SC. Mycophenolate mofetil therapy in frequently relapsing steroid-dependent and steroid-resistant nephrotic syndrome of childhood: current status and future directions. Pediatr Nephrol 2005; 20: 1376–1381.
21. Eckardt KU and Kasiske BL. KDIGO clinical practice guideline for glomerulonephritis. Foreword. Kidney Int Suppl 2012; 2: 140–140.
22. Cattran DC, Alexopoulos E, Heering P, et al. Cyclosporin in idiopathic glomerular disease associated with the nephrotic syndrome: Workshop recommendations. Kidney Int 2007; 72: 1429–1447.
23. Samuel S, Bitzan M, Zappitelli M, et al. Canadian Society of Nephrology commentary on the 2012 KDIGO clinical practice guideline for glomerulonephritis: management of nephrotic syndrome in children. Am J Kidney Dis 2014; 63: 354–362.
24. Robinson RF, Nahata MC, Mahan JD, et al. Management of nephrotic syndrome in children. Pharmacotherapy 2003; 23: 1021–1036.
25. Hodson EM, Wong SC, Willis NS, et al. Interventions for idiopathic steroid-resistant nephrotic syndrome in children. Cochrane Database Syst Rev 2016; 10: CD003594.
26. El-Reshaid K, El-Reshaid W and Madda J. Combination of immunosuppressive agents in treatment of steroid-resistant minimal change disease and primary focal segmental glomerulosclerosis. Ren Fail 2005; 27: 523–530.
27. Mekahli D, Liutkus A, Ranchin B, et al. Long-term outcome of idiopathic steroid-resistant nephrotic syndrome: a multicenter study. Pediatr Nephrol 2009; 24: 1525–1532.
28. Rosti RO, Sotak BN, Bielas SL, et al. Homozygous mutation in NUP107 leads to microcephaly with steroid-resistant nephrotic condition similar to Galloway-Mowat syndrome. J Med Genet 2017; 54: 399–403.