

Steroid-resistant idiopathic nephrotic syndrome in children: long-term follow-up and risk factors for end-stage renal disease

Síndrome nefrótica idiopática córtico-resistente na criança: evolução e fatores de risco para falência renal crônica

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ABSTRACT

Introduction: Steroid resistant idiopathic nephrotic syndrome (SRINS) in children is one of the leading causes of progression to chronic kidney disease stage V (CKD V)/end stage renal disease (ESRD). **Objective:** The aim of this retrospective study is to evaluate the efficacy of immunosuppressive drugs (IS) and to identify risk factors for progression to ESRD in this population. **Methods:** Clinical and biochemical variables at presentation, early or late steroid resistance, histological pattern and response to cyclosporine A (CsA) and cyclophosphamide (CP) were reviewed in 136 children with SRINS. The analyzed outcome was the progression to ESRD. Univariate as well as multivariate Cox-regression analysis were performed. **Results:** Median age at onset was 5.54 years (0.67-17.22) and median follow up time was 6.1 years (0.25-30.83). Early steroid-resistance was observed in 114 patients and late resistance in 22. Resistance to CP and CsA was 62.9% and 35% respectively. At last follow-up 57 patients reached ESRD. The renal survival rate was 71.5%, 58.4%, 55.3%, 35.6% and 28.5% at 5, 10, 15, 20 and 25 years respectively. Univariate analysis demonstrated that older age at onset, early steroid-resistance, hematuria, hypertension, focal segmental glomerulosclerosis (FSGS), and resistance to IS were risk factors for ESRD. The Cox proportional-hazards regression identified CsA-resistance and FSGS as the only predictors for ESRD. **Conclusion:** Our findings showed that CsA-resistance and FSGS were risk factors for ESRD.

Keywords: child; cyclosporine; glomerulosclerosis, focal segmental; kidney failure, chronic; nephrotic syndrome.

RESUMO

Introdução: A síndrome nefrótica idiopática córtico-resistente (SNICR) é uma das principais causas de falência renal crônica (FRC)/doença renal crônica estágio V (DRC V) em crianças. **Objetivo:** Avaliar a resposta aos imunossupressores e identificar fatores de risco para a FRC. **Métodos:** Variáveis clínicas e bioquímicas na apresentação, resistência inicial ou tardia aos esteróides, lesão histológica e resposta à ciclosporina A (CsA) e à ciclofosfamida (CF) foram analisados retrospectivamente em 136 crianças com SNICR. O desfecho analisado foi a progressão para FRC e os métodos utilizados foram a análise univariada e a regressão multivariada de Cox. **Resultados:** A idade mediana do início da doença foi de 5,54 anos (0,67-17,22) e o tempo mediano de seguimento foi de 6,1 anos (0,25-30,83). Resistência inicial aos esteróides ocorreu em 114 pacientes e tardia em 22. Resistência à CF e à CsA ocorreu em 62,9% e 35% dos pacientes, respectivamente. FRC ocorreu em 57 pacientes. A sobrevida renal foi de 71,5%, 58,4%, 55,3%, 35,6% e 28,5% aos 5, 10, 15, 20 e 25 anos, respectivamente. A análise univariada demonstrou que a idade maior ao início da doença, resistência inicial aos esteróides, hematúria, hipertensão, glomeruloesclerose segmentar e focal (GESF) e resistência aos imunossupressores foram fatores de risco para FRC. A regressão de Cox identificou a resistência à CsA e a GESF como os únicos fatores preditores para FRC. **Conclusão:** Nossos achados mostraram que a resistência à ciclosporina e a presença de GESF foram fatores de risco para a progressão para DRC V.

Palavras-chave: ciclosporina; criança; falência renal crônica; glomeruloesclerose segmentar e focal; síndrome nefrótica.

INTRODUCTION

Steroid-resistant idiopathic nephrotic syndrome (SRINS) occurs in approximately 10-20% of children with idiopathic nephrotic syndrome (INS). The true incidence of SRINS cannot be determined due to the great variability of definitions. According to the International Study of Kidney Disease in Children (ISKDC)¹ 90% of sensitive patients enter into remission within 4 weeks after starting steroids, leading to the definition of steroid resistance as failure to achieve remission after 4 weeks. Another definition which also appeared from ISKDC study² was that the initial non-responders were patients who failed to respond during the first 8 weeks of prednisone therapy (60 mg per m²/day for 4 weeks, followed by 40 mg/m² three times a week for 4 weeks). The French Pediatric Society of Nephrology³ defined steroid resistance as a failure to go into remission after a treatment of four weeks of daily steroid therapy (60 mg per m²/day) followed by three pulses of methylprednisolone (1000 mg/1.73 m²) every other day. Recently, the KDIGO Clinical Practice Guideline for Glomerulonephritis⁴ suggested a minimum of 8 weeks of treatment with steroids to define steroid resistance.

SRINS is associated with increased risk of complications due to persistent proteinuria and therapeutic drug side effects. Bacterial infections, malnutrition, hyperlipidemia, thromboembolic phenomena and progression to ESRD are usually seen during the course of SRINS. The most prevalent histological pattern in SRINS is FSGS,⁵⁻⁷ which is the major glomerular etiology of ESRD in children.⁸ The probability of occurrence of ESRD in 10 years in children with SRINS varies between 34-64%.⁹⁻¹¹ Several risk factors for progression to chronic renal failure or ESRD, as persistent proteinuria, older age at onset, initial renal impairment, and extensive focal sclerosis in biopsy specimens have been reported in the literature.⁹⁻¹¹

The optimal management of SRINS still remains a medical challenge. According to the recent Practical Guidelines from KDIGO,⁴ the recommendations for the treatment of SRINS are: 1) Calcineurin inhibitors (CsA and tacrolimus) associated with steroids must be the first line drugs to treat patients with SRINS. 2) Mycophenolate mofetil (MMF) may be indicated in children who did not respond to CsA (low evidence). 3) Cyclophosphamide (CP) is not suggested for the treatment of SRINS (moderate evidence). 4) Rituximab is still not recommended as a treatment

option for SRINS due to the lack of RCTs and risk of serious adverse events. 5) Angiotensin-converting enzyme inhibitors (ACE-i) and angiotensin receptor blockers (ARB) are recommended for treatment (moderate evidence). The response to therapeutic drugs is a good predictor of a long-term renal survival in children with FSGS.¹²

The objective of this retrospective cohort study is to evaluate the efficacy of immunosuppressive (IS) drugs, namely cyclophosphamide (CP) and cyclosporine A (CsA) and to identify risk factors for ESRD in SRINS.

METHODS

We retrospectively analyzed 136 children with SRINS who were followed at Hospital Federal de Bonsucesso, Rio de Janeiro, Brazil. The analyzed period was between January 1974 and September 2010. Inclusion criteria were children with INS with early or late steroid resistance, age at onset ≥ 3 month and ≤ 18 years and follow-up ≥ 1 year except for patients who developed prematurely ESRD. Exclusion criteria consisted of children with familial history of SRINS, congenital or syndromic forms of nephrotic syndrome (NS), Membranous Glomerulonephritis, Membranoproliferative Glomerulonephritis and secondary forms of NS such as Hepatitis B and C infections, HIV nephropathy, IgA nephropathy and systemic lupus erythematosus.

Clinical and laboratorial data as age of NS onset, gender, blood pressure, estimated glomerular filtration rate (Schwartz's formula) and hematuria were assessed at presentation and during clinical course. Genetic testing was not performed. Immunosuppressor therapy efficacy was evaluated. The patient's race and ethnicity were not evaluated in our study, due to the high rate of miscegenation in Brazil. For statistical purposes, we divided the patients in two groups: Group I, those who developed ESRD (ESRD+) and Group, II those without ESRD (ESRD-).

DEFINITIONS

Idiopathic nephrotic syndrome (INS) was defined by the combination of nephrotic syndrome and non-specific histological abnormalities of the kidney, including minimal changes, focal and segmental glomerular sclerosis, and diffuse mesangial proliferation. Glomeruli show a fusion of epithelial cell foot process on electron microscopy and no significant deposits of immunoglobulins or complement.¹³

Early (primary) steroid resistance was defined as failure to achieve remission during the initial 8 weeks of daily prednisolone therapy of 60 mg/m²/day or 2 mg/kg/day during the first episode of NS. Late steroid resistance was defined as no response to 8 weeks of daily prednisone therapy at a dose of 60 mg/m²/day or 2 mg/kg/day in a child previously known to have a steroid sensitive course.

We define cyclosporine A resistance (CsA-R) as the failure to achieve partial or complete remission after 24 weeks of CsA treatment. Cyclophosphamide resistance was defined as a failure to achieve complete or partial remission after 8-12 weeks of CP treatment at dose of 2-2.5 mg/kg/d. Complete remission was defined as negative or trace proteinuria on the dipstick method or a urinary protein/creatinine ratio ≤ 0.20 on urinalysis or a protein excretion of < 4 mg/m²/hour. Partial remission was defined as absence of edema and a proteinuria between 4 and 40 mg/m²/hour.

Hypertension was defined as exceeding the 95th percentile for systolic or diastolic blood pressure for age, gender, and height.¹⁴ Hematuria was determined by the presence of more than five red blood cells per high power field. Decreased kidney function was defined as the glomerular filtration rate (GFR) below 90 ml/m/1.73 m².¹⁵ The GFR was estimated using the Schwartz formula.¹⁶ End-stage renal disease (ESRD) was defined as the requirement for dialysis or renal transplantation.

IMMUNOSUPPRESSIVE TREATMENT

After the diagnosis of steroid resistance, patients were treated with one or more of the following immunosuppressors: 1) Cyclophosphamide (CP) given orally (PO) at a dose of 2-2.5 mg/kg/day for 8-12 weeks, usually as the first IS agent; 2) Cyclosporine (CsA) PO at a dose of 4-6 mg/kg/day for a minimum of 12 months, associated with Prednisone. Prednisone was given as 2 mg/kg/day for the first 4 weeks followed by alternate days in the same dose for another four weeks. Thereafter it was tapered and withdrawn during the following 6 to 12 months. The CsA dose was adjusted to reach the trough level of 100-150 ng/ml or C2 level at 600-800 ng/ml. 3) Mycophenolate mofetil (MMF) was usually initiated after CsA dependence, CsA resistance or chronic CsA nephrotoxicity (CCsAN) at a dose of 1.200 mg/m² daily in 2 divided doses and associated with Pred in same manner as in CsA treatment.

RENAL BIOPSY

Kidney biopsy was performed in all patients with steroid resistance. All specimens were examined by light as well as immunofluorescence microscopy. Subsequent biopsies were performed to evaluate CsA nephrotoxicity and in patients who presented unexpected clinical deterioration. For analysis purpose, we considered the result of the last biopsy.

STATISTICAL ANALYSIS

MedCalc[®] for Windows, Statistics for Biomedical Research Software, v. 9 was used for the statistical analysis: *Student's t* test to compare differences between means and Mann-Whitney U test for nonparametric comparisons; Fisher's exact test to compare frequencies of qualitative variables; renal survival probability rates (until development of ESRD) were calculated according to Kaplan-Meier, log-rank test to compare survival curves, and Cox proportional hazards analysis to examine the effect of various factors on renal survival. A *p* value < 0.05 was set to indicate a significant difference.

RESULTS

CLINICAL DATA AND DEMOGRAPHIC CHARACTERISTICS

One hundred thirty six children (88 boys and 48 girls) with SRINS met the inclusion criteria in our study. Early SRINS was observed in 114 patients (84%) and late SRINS in 22 (16%). Overall, the median age at presentation was 5.54 years (range 0.67-17.22), and only two patients had less than one year of age at presentation. Median follow time was 6.1 years (range 0.25-30.83) and 6 of 136 patients were followed less than one year due to an early progression to ESRD. At presentation, hypertension was found in 18/120 patients (15%), hematuria in 63/107 patients (59%) and estimated creatinine clearance < 90 ml/1.73 m² in 50/119 patients (42%). Initial renal histology showed MCD in 53 patients (39%), FSGS in 74 (54.4%) and DMP in 9 (6.6%). In 33 patients a 2nd biopsy was performed; in 14 a 3rd and in 3 a 4th biopsy. Thirteen with initial MCD had a transition to FSGS. The histological findings of the last biopsy were: MCD in 41 patients (30%), FSGS in 87 (64%) and DMP in 8 (6%).

Late SRINS developed in 22 out of our 639 (3.4%) children with steroid sensitive nephrotic syndrome. The median duration of NS from onset to appearance of late resistance was 51 months (range 3-296). The

initial renal biopsy in these patients showed MCD in 18, FSGS in 2 and DMP in 2 patients. six had a second renal biopsy and 5 patients a change from MCD to FSGS was observed. The characteristic of patients according to early or late SRINS is shown in Table 1.

RESPONSE TO IMMUNOSUPPRESSIVE AGENTS

ORAL CYCLOPHOSPHAMIDE (CP)

We did not find an important effect in 105 patients treated with CP. Resistance to CP was found in 66 patients (62.9%). Of the 39 patients CP-sensitive, only 8 reached a long term remission (≥ 2 years). Patients with late resistance were significantly more sensitive to CP than those with early resistance (Table 1), but no difference was found in patients treated with CP with early or late SRINS who reached a long-term remission ≥ 2 years ($p = 0.72$). CP was used in 89% of our patients with early SRINS in decade 1990-1999, versus 54.5% in decade 2000-2009.

CYCLOSPORINE A

Eighty patients were treated with CsA and 52 (65%) of these were sensitive to the drug. Ninety-five percent (95%) of patients with late resistance were sensitive to CsA versus 55% of those with early resistance, $p = 0.002$. In relation to histological pattern, sensitivity to CsA was found in 24/30 (80%) of patients with MCD and in 28/48 (58.3%) with FSGS, $p = 0.08$. Only 2 patients with DMP were treated with CsA. The CsA-S patients were treated for a median of 41 months (range 12-111)

with a mean dose of 4.14 ± 0.74 mg/kg/day. Forty one were treated for more than 24 months. Late resistance to CsA developed in 19 patients. The median follow-up pos CsA treatment for CsA-S and CsA-R was 7.65 years (range 1.5-20) and 2.16 years (range 0.4-16.92) respectively. Seven patients remained in complete remission 52 ± 32.3 months (11-117) after the treatment was withdraw. CsA was administrated to 67 patients who used CP previously; 47 patients were CsA-S (of whom 24 were CP-resistant) and 20 patients were CsA-R (of whom 16 were CP-resistant). To evaluate chronic cyclosporine nephrotoxicity (CCsAN) we performed 52 serial biopsies in 36 patients who were treated with CsA for a median of 37.5 months (range 6.5-73). CCsAN was observed in 8 from 52 biopsies (15.4%). Seven biopsies had a grade II tubulointerstitial lesion (TIL) and one grade III, from Habib's classification.¹⁷

MYCOPHENOLATE MOFETIL

MMF was used in only 13 patients. Four patients were MMF resistant and 9 were treated for a median of 15 months (range 8-57) with a mean of 1.12 relapse/year. Due to a small number of patients treated with MMF and a short period of follow-up, we did not consider the MMF treatment in our review.

OUTCOME AND PREDICTORS TO ESRD

The overall probability of renal survival rate was 71.5%, 58.4%, 55.3%, 35.6% and 28.5% at 5, 10, 15, 20 and 25 years respectively. At last follow-up 57

TABLE 1 CHARACTERISTIC OF PATIENTS WITH EARLY OR SECONDARY SRNIS

	Early (n = 114)	Late (n = 22)	p
Age at onset-years	6.52 (0.67-17.22)	2.96 (1.17-14.1)	0.002
Sex: male/female	74/40	14/8	0.89
First renal biopsy: FSGS/MCD/DMP	72/35/7	2/18/2	< 0.0001
Last renal biopsy: FSGS/MCD/DMP	80/27/7	7/14/1	0.0009
CP: yes/no	86/28	19/3	0.4
CP-resistance: yes/no	62/24	4/15	0.0001
Remission at 2 years in CP - sensitive	20.8%	20%	0.72
CsA: yes/no	60/54	20/2	0.001
CsA resistance: yes/no	27/33	1/19	0.002
Hematuria at presentation: yes/no	58/32	5/12	0.01
Hypertension at presentation: yes/no	17/86	1/16	0.44
eClearance at presentation (ml/min/1.73m ²)	105.5 \pm 47.7 (n = 102)	122.6 \pm 68.6 (n = 17)	0.2
eClearance < 90 ml/m/1.73 m ² : yes/no	43/59	7/10	0.84
Follow-up in years	5.04 (0.25-30.83)	11.05 (1-26.13)	0.002

SRINS: Steroid resistant idiopathic nephrotic syndrome; FSGS: Focal segmental glomerulosclerosis; MCD: Minimal change disease; DMP: Diffuse mesangial proliferation; CP: Cyclophosphamide, CsA: Cyclosporine; eCCI: Estimated creatinine clearance.

of 136 (41.9%) patients progressed to end stage renal disease. Table 2 shows the characteristic of patients with or without ESRD. Univariate analysis demonstrated that an older age at onset, FSGS, early steroid resistance, resistance to immunosuppressive agents, hematuria and hypertension at presentation were risk factors for ESRD.

The median age at NS onset was higher in the ESRD + than the ESRD- group: 7.79 years (range 1.87-14.88) *versus* 4.06 years (range 0.67-17.22) $p = 0.003$. Children with FSGS had significant higher age at onset than patients with MCD; 6.78 years (range 0.67-17.22) *versus* 3.58 years (range 1-14.31) respectively, $p = 0.001$. The age at onset in patients with DMP was 3.9 years (range 1.58-8.17). No statistical differences were found between ages at onset in MCD *versus* DMP. Considering only those patients with FSGS, the median age at onset was not different between the groups ESRD+ and ESRD-; 7.92 (range 1.87-14.88) *vs.* 6.21 (range 0.67-17.22) respectively, $p = 0.46$.

ESRD occurred in 51/87 (58.6%) patients with FSGS, 4/8 (50%) with DMP and 2/41 (4.9%) with MCD. Interestingly was the recurrence of NS soon after a renal transplant in a patient with MCD who progressed to ESRD and in whom 3 subsequent kidney biopsies showed the same pattern. The renal survival rate was significant better in MCD than in FSGS, Figure 1, $p < 0.0001$.

Patients with late SRINS had a better kidney survival rate than those with early SRINS, $p = 0.0007$,

Figure 2. At last follow-up 2 of 22 (9%) children with late resistance *versus* 55 of 114 (48.2%) with early resistance progressed to ESRD and 13 children (12 with early resistance) presented with CKD (stage II-8, stage III-4 and stage IV-1). The two patients with late resistance that progressed to ESRD had a histopathological transition from MCD to FSGS.

Hematuria at presentation was found in 79.5% in ESRD + *versus* 44.4% in the ESRD-, $p = 0.0006$ and was seen in 67.6%, 87.5%, and 28.6% in patients with FSGS, DMP, and MCD respectively. Hypertension at presentation was found in 26.5% in ESRD+ group *versus* 7% in ESRD-, $p = 0.007$. The incidence of hypertension was 20.2%, 25% and 0% in patients with FSGS, DMP and MCD respectively.

The resistance to immunosuppressive agents was significantly associated with ESRD (Table 2).

ESRD occurred in 53% of patients with CP-resistance *versus* in 15.4% of patients with CP-sensitivity ($p = 0.0003$), as well in 60,7% of patients with CsA-R *versus* 17.3% of patients with CsA-S ($p \leq 0.0002$). Figure 3 depicts the renal survival according to CsA response.

The Cox proportional-hazards regression analysis demonstrated that cyclosporine-resistance and FSGS were the only predictors of ESRD (Table 3). Patients with FSGS are 9.25 times more likely to develop ESRD than patients with MCD, as well as patients with cyclosporine-resistance are 4.3 times more likely to develop ESRD than CsA-sensitive patients.

TABLE 2 COMPARISON OF DEMOGRAPHIC, CLINICAL CHARACTERISTIC AND RESPONSE TO IMMUNOSUPPRESSIVE AGENTS IN CHILDREN WITH SRINS WITH OR WITHOUT ESRD

	Group I ESRD+ (n = 57)	Group II ESRD- (n = 79)	<i>p</i>
Age at onset-years	7.79 (1.87-14.88)	4.06 (0.67-17.22)	0.003
Sex: male/female	39/18	49/30	0.55
Early/late SRINS	55/2	59/20	0.001
Last renal biopsy:FSGS/MCD/DMP	51/2/4	36/39/4	< 0.0001
CP: yes/no	41/16	64/15	0.29
CP-resistance: yes/no	35/6	31/33	0.0003
CsA: yes/no	26/31	54/25	0.01
CsA-resistance: yes/no	17/9	11/43	0.0002
Hematuria at presentation: yes/no	35/9	28/35	0.0006
Hypertension at presentation: yes/no	13/36	5/66	0.007
eClearance at presentation (ml/min/1.73m ²)	99.7 ± 44.9 (n = 47)	113.6 ± 54.6 (n = 72)	0.15
eClearance < 90 ml/m/1.73 m ² : yes/no	23/24	27/45	0.31

SRINS: Steroid resistant idiopathic nephrotic syndrome; FSGS: Focal segmental glomerulosclerosis; MCD: Minimal change disease; DMP: Diffuse mesangial proliferation; CP: Cyclophosphamide; CsA: Cyclosporine; eCCI: Estimated creatinine clearance.

Figure 1. Renal survival in children with steroid resistant idiopathic nephrotic syndrome according renal histopathology. MCD: Minimal change disease; FSGS: Focal Segmental Glomerulosclerosis; MCD: Solid line; FSGS: Dotted line.

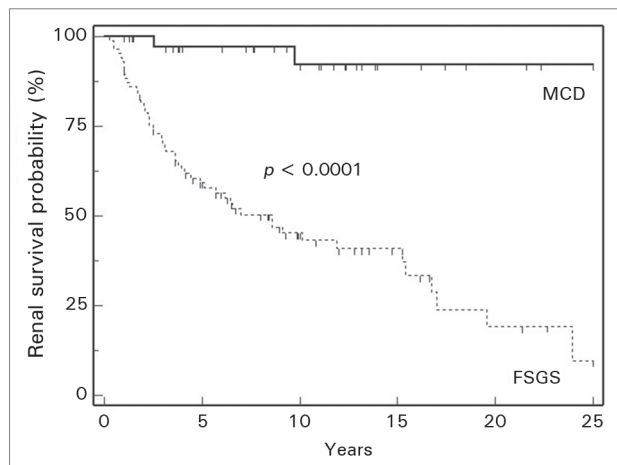


Figure 2. Renal survival in children with steroid resistant idiopathic nephrotic syndrome according early (primary) versus late steroid resistance. Solid line: LSR: Late Steroid Resistance; Dotted line: ESR: early steroid resistance.

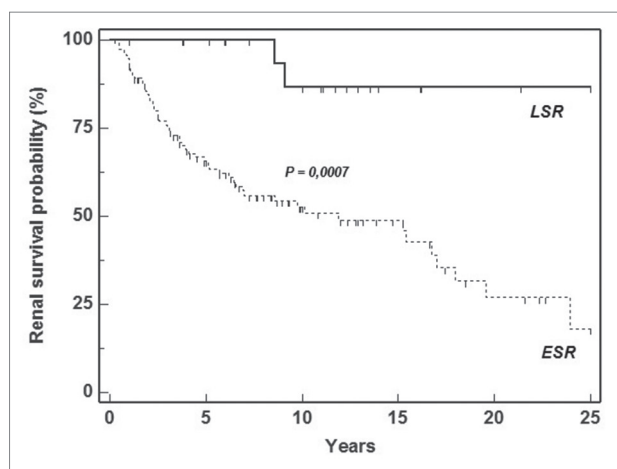


Figure 3. Renal survival in children with steroid resistant idiopathic nephrotic syndrome according to Cyclosporine A response. CsA-S: Cyclosporine A sensitive; CsA-R: Cyclosporine A resistant; CsA-S: Solid line; CsA-R: Dotted line.

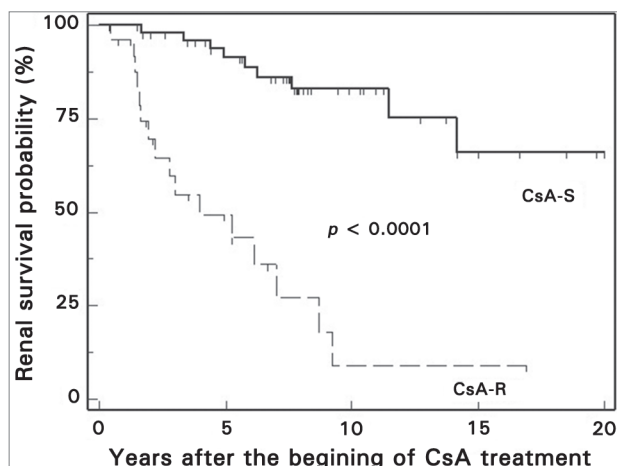


TABLE 3 COX PROPORTIONAL-HAZARD- REGRESSION ANALYSIS TO EXAMINE THE EFFECT OF VARIOUS FACTORS ON RENAL SURVIVAL (STEPWISE METHOD)

Covariate	p	RR	95% CI
FSGS	0.032	9.25	1.21 to 70.23
CsA-R	0.0048	4.30	1.56 to 11.81

Overall Model Fit: $p = 0.00001$; RR: Relative risk of an event; CI: Confidence interval; FSGS: Focal segmental glomerulosclerosis; CsA-R: Cyclosporine resistance.

DISCUSSION

SRINS is responsible for an increased risk of ESRD, leading to a 34-64% of probability of developing ESRD in 10 years. Various factors have been reported to influence the outcome in SRINS. Age, hematuria, hypertension, decreased creatinine clearance at initial clinical presentation, histopathological pattern as well as early versus late steroid resistance have been described as risk factors for ESRD.

FSGS is the most prevalent histological pattern in SRINS^{5,18} and also the major cause of ESRD.⁸ Instead; few reports in literature have shown that the initial histological lesion has no influence on the development of ESRD. In a European multicenter study¹⁰ involving children with SRINS, the initial histopathological pattern was not a significant predictor for ESRD. Niaudet *et al.*¹³ also found that in patients with SRNS the progression to ESRD was similar in patients with MCD or FSGS on initial biopsy; however, patients with MCD who progressed to ESRD and had a subsequent renal biopsy always developed FSGS (personal communication; August 30, 2011). In this present study, FSGS was the most prevalent lesion in early SRINS and also the most frequent cause of ESRD, allowing the association of early steroid-unresponsiveness and a higher probability of progression to ESRD in the analyzed population.

Patients with late resistance seem to have a better outcome than those with early resistance. Otukesh *et al.*¹⁹ showed a better kidney survival rate in patients with late SRINS, versus in those with early resistance. Schwaderer *et al.*²⁰ demonstrated no case of decreased renal function in 14 patients with late SRNS, but in his paper the review of the literature concerning late SRINS showed that the overall incidence of decreased renal function was 23% in 126 patients. In our study we also demonstrate a better kidney survival in patients with late SRIN.

Renal impairment at presentation^{11,21-23} and older age^{10,23} were considered risk factors for chronic kidney disease and ESRD. Our results showed that the age at NS onset was significantly higher in the ESRD+ group than in ESRD- group. This fact could be explained by the higher incidence of FSGS than MCD and DMP in this group. The age at onset in children with FSGS was higher than in children with MCD or DMP. When only patients with FSGS are analyzed, the age at onset was not different between both groups (ESRD+ and ESRD-). In relation to initial creatinine clearance and the initial renal impairment we did not find any difference between the 2 groups. The presence of hematuria and hypertension at onset were risk factors for ESRD by univariate analysis, and this fact could be explained by the higher incidence of hematuria and hypertension in patients with FSGS than those with MCD. Others studies^{10,22} showed that hematuria at presentation was not a predictive factor for ESRD.

The achievement of a complete or partial remission is one of most important factors related to a better outcome on SRINS.^{5,12,22} Cyclophosphamide has yet been used in SRINS despite conflicting results. In a recent meta-analysis²⁴ no significant difference in the number of patients who achieved complete remission between oral cyclophosphamide with prednisone *versus* prednisone alone, intravenous (IV) *versus* oral cyclophosphamide or IV cyclophosphamide *versus* oral cyclophosphamide with IV dexamethasone was observed. Nammalwar²⁵ in a prospective study involving children with SRINS treated with a IV methyl prednisolone plus oral prednisolone for one year with 6 pulses monthly IV CP demonstrated a better remission rate in children with MCD and DMP than in those with FSGS (81.8%, 66.7% and 16.7%, respectively) at end of three years of study. Bajpai²⁶ showed that therapy with intravenous cyclophosphamide has limited efficacy in inducing sustained remission in patients with initial corticosteroid resistance. We also showed a low efficiency of CP in patients with SRINS, as described in the results section.

CsA have been used in SRINS since 1986 and currently is the most common drug used to treat SRINS. Ehrich *et al.*²⁷ reported a 77% rate of remission in 52 patients with non-genetic SRINS FSGS treated with combined Prednisolone + CsA therapy including intravenous-methylprednisolone (IV-MPred) pulses. Patients receiving IV-MPred + oral PRED+CSA had

a significantly better outcome than patients treated with oral PRED+CSA (84 *vs.* 64% of cumulative proportion of sustained complete remission at 60 months). All patients with SRINS MCD achieved remission with oral Pred +CsA or IV-MPred+oral pred+oral CsA. Hamasaki *et al.*²⁸ in an prospective multicentre trial in Japan involving 35 SRINS children, demonstrated that a high remission rates was achieved in 23 of 28 (82.1%) patients in the MC/DMP group treated for 12 months with CsA plus prednisolone and in six of the seven (85.7%) patients in the FSGS group treated for 12 months with CsA and prednisolone plus methyl prednisolone pulse therapy.

In a multicenter, randomized, controlled study CsA therapy was superior in inducing at least partial remission in children with early SRINS when compared to cyclophosphamide IV pulse therapy.²⁹ Actually, CsA therapy must be considered as the first line in children with SRINS.^{4,29,30} A major problem concerning the use of CsA is the high rate of relapses when CsA is withdrawn or tapered and a more prolonged course is necessary to obtain a prolonged remission. The long-term use of CsA can result in the development of a high rate of CsA-induced nephrotoxicity.^{31,32} In the other side, a low incidence of CCsAN has been reported with a low dose of CsA, even in a long-term treatment.³³⁻³⁵ The occurrence of CCsAN in our study was low (15.4%) despite long-term treatment and moderate dose of CsA.

CsA may also have a protective effect in renal function by reducing proteinuria. In addition to the immunological mechanism involved in proteinuria reduction, Faul *et al.*³⁶ showed that the antiproteinuric effect of CsA results from the stabilization of the actin cytoskeleton in kidney podocytes. Ingulli *et al.*³⁷ demonstrated that long-term CsA therapy successfully reduces the proteinuria in black and Hispanic children with steroid-resistant FSGS, and the incidence of ESRD was 24% in treated children *versus* 78% in historical controls. Catran *et al.*³⁸ in a randomized controlled trial in 49 cases of steroid-resistant FSGS comparing 26 weeks of CsA plus low-dose prednisone to placebo plus prednisone, observed a long-term decrease in proteinuria and preservation of renal function in the CsA-treated patients. Ghiggeri *et al.*³³ in a retrospective multicentre study involving 139 patients (children and adults) with FSGS SRINS without genetic mutation in four Italian centers, showed that progression to ESRF occurred in 10% of CsA-responsive patients *versus* 60% of CsA-resistant patients and

62% of non-treated patients ($p = 0.002$). Fifty five patients were treated with CsA: 20 were CsA-responsive and 35 were CsA-resistant.

Our study revealed that resistance to CsA was associated with a high rate of progression to ESRD. Figure 3 shows a significant difference in survival rates between CsA-S *versus* CsA-R patients. ESRD occurred in 17.3% of CsA-S, 60.7% of CsA-R, and 55.4% in non CsA-treated patients ($p < 0.0001$).

The present study has some limitations, especially regarding the historical design as well as the lack of genetic study of our patients, which in turn was difficult to perform in developing countries in the past (although we excluded patients with a familial history and syndromic forms of NS). NPHS2 mutations have been identified in 20.4% in patients with sporadic form of steroid-resistant FSGS,³⁹ meaning that about 20% of our patients with FSGS might have a genetic form. Children with SRNS with NPHS2 homozygous mutations show poor response to CsA.

CONCLUSION

Our study contributes to the understanding of the particularities associated with steroid-resistant idiopathic nephrotic syndrome in children, the long-term outcome and risk factors for ESRD, emphasizing the importance of the renal histology and the importance of early *versus* late steroid resistance on outcome. We showed also that cyclosporine-resistance and FSGS were predictors for ESRD; patients with FSGS are 9.25 times more likely to develop ESRD than patients with MCD, as well as patients with cyclosporine-resistance are 4.3 times more likely to develop ESRD than CsA-sensitive patients. Furthermore, the anti-proteinuric effect of cyclosporine with preservation of renal function over time and also minimal change disease are predictors of better renal survival in children with steroid resistant idiopathic nephrotic syndrome. The use cyclophosphamide should not be encouraged in patients with SRINS due a low rate of response and potential side effects.

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