ORIGINAL ARTICLE

Predictors of long-term outcome of children with idiopathic focal segmental glomerulosclerosis

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Abstract Clinical and histological data of children presenting with steroid-resistant nephrotic syndrome and renal biopsy showing focal and segmental glomerulosclerosis from 1980 with a follow-up of over 10 years were reviewed. There were 66 patients; 38 male and 28 female. Age at onset ranged from 0.4–14.1 years (mean 6.4). Tubular atrophy was present at first biopsy in 50/66, capsular adhesions in 35/66, glomerular tip lesions in 8/66 and mesangial expansion in 31/66 patients. In 51 children, cyclophosphamide was prescribed as the first cytotoxic agent, while 15 received cyclosporine A and complete remission was induced in 43 and 40% of the children, respectively. Complete and stable remission was maintained in 35 children, while 22 had reduction of proteinuria with symptomatic relief. Nine were refractory to cytotoxic therapy. Of the 35 patients who entered complete and stable remission, the renal survival was over 90%, while in the 31 non-responders it was 48% in 10 years. The multivariate analysis using unconditional logistic regression method identified the presence of mesangial expansion ($p=0.011$) and tip lesions ($p=0.005$) as the independent predictors of favourable response to cytotoxic therapy and the presence of renal impairment ($p=0.008$) and extensive focal segmental sclerosis ($p=0.025$) as independent predictors of unfavourable response.

Keywords Renal failure · Nephrotic syndrome · Cytotoxic therapy · Renal histology · Steroid resistance

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Introduction

Focal segmental glomerulosclerosis (FSGS) is a clinico-pathological diagnosis that may be associated with several diseases or syndromes. In its idiopathic form, FSGS accounts for 10% of all children presenting with nephrotic syndrome (NS) [1]. Although FSGS is generally believed to carry a poor prognosis, there appears to be considerable heterogeneity in clinical features, renal histology and natural history, suggesting a diverse pathogenesis [2, 3]. In association with its relative steroid unresponsiveness and unpredictable response to cytotoxic therapy, it has a propensity to progress to end-stage renal failure (ESRF) in refractory nephrotic patients [1, 4]. The favourable long-term renal survival found in children who enter sustained remission has led to the emergence of more aggressive immunosuppressive regimens. Recent therapeutic experience gleaned from several studies of groups with idiopathic FSGS has provided a cause for optimism in the response to treatment of this otherwise progressive glomerular disease [5, 6].

Primary idiopathic FSGS has no demonstrable aetiological factor and currently there are no universally accepted clinical criteria or histological features that predict a poor response to immunosuppressive therapy [7]. The presence of tubulointerstitial lesions and widespread capillary loop collapse on renal histology are generally thought to be associated with poor response to immunosuppressive therapy [8, 9], whereas mesangial hypercellularity may indicate a more favourable response [10].

Evidence regarding the efficacy of cytotoxic therapy has been principally based on retrospective reviews and clinical trials, which remains inconclusive due to the variable beneficial effects reported with different cytotoxic agents [5, 6]. In the light of this, there is no uniform agreement on

the initial and best cytotoxic agent that should be prescribed or how far one should proceed with cytotoxic therapy in order to achieve complete remission [7]. The aim of this study was to analyse our experience and outcome of children with primary idiopathic steroid resistant FSGS referred to Great Ormond Street Children's Hospital (GOSH), London, from 1980 who had completed at least 10 years of follow-up. The study also focused on clinical, pathological, and immunohistochemical features at presentation that might help to identify children who would have a favourable response to immunosuppressive therapy.

Patients and methods

GOSH is a tertiary referral centre where a majority of children with NS are referred as a result of steroid resistance or steroid dependence. From a population of 863 patients with NS (age at onset >3 months and <16 years) referred between 1980 and 2000, 254 were due to steroid-resistant disease. Patients with systemic diseases, primary or secondary glomerulopathies, a history of reflux and hepatitis B infection were excluded. Of the remaining 153 children who had FSGS as their initial glomerular lesion, 91 had a well-documented follow-up for 10 years and were included for the histological review. Repeat biopsies following the diagnosis of FSGS were not included in the study. The review of histolopathological features (light microscopy, immunohistochemistry and electron microscopy) was performed by two independent paediatric renal histopathologists who were blinded to the clinical details and outcome of these patients. The diagnosis of FSGS was confirmed based on the following criteria: (i) a lesion involving some of the glomeruli in the biopsy while others remain uninvolved, (ii) the involved glomeruli having a segmental sclerotic lesion with or without discreet capsular adhesions, and (iii) no clinical or pathological evidence for underlying primary disease that might produce secondary FSGS. In addition to confirming the diagnosis of FSGS, the following features were recorded for each biopsy: the proportion of globally sclerosed glomeruli, proportion of glomeruli with segmental sclerosis, the presence of mesangial expansion, capsular adhesions, tip lesions, tubular atrophy/interstitial fibrosis, diffuse mesangial deposits of IgG, IgM, C1q and C3 and electron microscopic changes.

Based on these criteria, a further 25 patients were excluded for suspected other aetiologies and a total of 66 were identified as having 'pure' idiopathic FSGS. Clinical and laboratory information on all patients was available both at the time of biopsy and throughout their follow-up and was obtained from the case notes.

Definitions

Nephrotic syndrome was defined as proteinuria >40 mg/m^2/h or protein/creatinine ratio >200 mg/mmol, hypoalbuminaemia (<25 g/l) and oedema [11]. Normal renal function was defined as a plasma creatinine (Pcr) below the upper limit of the normal for the age. Renal impairment was considered when the estimated glomerular filtration rate (Schwartz formula) or formal measurement was at least 20% below the normal range and ESRF was the point at which dialysis treatment was started or when Pcr exceeded 500 μmol/l. Steroid resistance was defined as a failure to enter remission following 4 weeks of daily prednisolone at 60 mg/m^2/day. Remission was defined as urinary protein excretion <4 mg/m^2/h or reagent strip (Albustix) negative or trace for three consecutive days [11]. Partial remission was defined as a 50% reduction of initial proteinuria.

Treatment strategies

In children who failed to enter remission following 4 weeks of prednisolone therapy prescribed at 60 mg/m^2/day, a renal biopsy was performed. In most patients, daily prednisolone was continued for a further 2 weeks. The decision to introduce a cytotoxic treatment was considered when no response was observed following 6 weeks of daily corticosteroid therapy.

Cyclophosphamide was prescribed in a majority of patients as the first cytotoxic agent in a dose of 3 mg/kg daily for 8 weeks (cumulative dose equivalent to 168 mg/kg/ per course). Steroids were administered on alternate days at 40 mg/m^2 and tapered over 8–16 weeks. A relapse following cyclophosphamide therapy was treated with steroids and/or cytotoxic agents. Initially, when cyclosporine A became available in 1987, it was prescribed mostly for resistant cases, at 3–6 mg/kg in two divided doses to achieve a 12-h trough level between 50–150 μg/l. However, increasingly it was prescribed as the first cytotoxic agent especially for children around puberty. Cyclosporine A levels and the plasma creatinine were monitored every 3 months. The glomerular filtration rate was measured formally if plasma creatinine was elevated. If the glomerular filtration rate was significantly reduced, a renal biopsy was arranged to check for cyclosporine A-induced nephrotoxicity and the dose of cyclosporine A was reduced if evidence of nephrotoxicity was found.

Chlorambucil was used only in children who were resistant to other forms of therapy and administered in a dose 0.2 mg/kg/day for 12 weeks. Vincristine was also prescribed for refractory cases in a dose of 1.5 mg/m^2 weekly for 8 weeks, mostly in combination with cyclophosphamide and prednisolone.

Refractory oedema was treated with regular albumin infusions combined with frusemide in haemodynamically unstable patients while in haemodynamically stable patients it was controlled with frusemide alone or with spironolactone and/or metolazone. Nifedepine, atenolol, frusemide and captopril was prescribed either alone or in combination to control hypertension. Lipid-lowering drugs were not routinely prescribed for this cohort of patients. Angiotensin-converting enzyme inhibitors such as captopril were not routinely prescribed to control proteinuria in this cohort of patients.

Statistical analysis

Univariate analysis to detect the relationship between each clinical, histological or immunohistological parameters and response to therapy and progression to ESRF was performed by using Fisher's exact test and chi-square test as the standard statistical tests. The odds ratios (OR) are presented with 95% confidence interval's (CI) and p values. Variables with significant associations were further analyzed to identify their independent associations. Unconditional logistic regression method was used to detect independent effects on outcome or response to therapy. Statistical analysis was performed using a computer program package SSPS (version 11.0, 2001).

Results

Patient and histological characteristics

There were 66 patients: 38 males and 28 females. Age at onset ranged from 0.4–14.1 years (mean 6.4). All patients had nephrotic-range proteinuria. Hypertension was noted in 19/66 (29%) of patients while microscopic haematuria was detected in 35/66 (53%) of patients at presentation. None of these patients had macroscopic haematuria at presentation. Six children had evidence of renal impairment at diagnosis. Complete remission was induced in 11/66 (16.6%) with an

extended course of daily prednisolone (6 weeks) but later developed steroid dependency with toxicity or secondary steroid resistance needing cytotoxic therapy. The number of glomeruli per biopsy ranged from 7–68 (mean 37). Tubular atrophy was present in 47/66 (71.2%), capsular adhesions in 36/66 (54.5%), glomerular tip lesions in 8/66 (12.5%) and mesangial expansion in 31/66 (47%) patients. Twenty-seven patients also demonstrated globally sclerosed glomeruli ranging from 12–44%. The number of glomeruli with segmental changes ranged from 6–56%.

Response to treatment and outcome

Cyclophosphamide was used as the first cytotoxic agent in 51 patients and induced complete remission in 22 (43.1%). Duration to enter complete remission ranged from 8–78 days (mean 46). The patients who relapsed following cyclophosphamide and patients who failed to respond were treated with further cytotoxic therapy such as chlorambucil, vincristine, cyclosporine A or a second course of cyclophosphamide. In 15 patients, cyclosporine A was used as the first cytotoxic agent and induced remission in six patients (40%). The choice of prescription of different cytotoxic therapies is given in Table 1. The initial responses to individual cytotoxic therapies are shown in Table 2. Overall, with the prescription of different combination of cytotoxic therapy, complete and stable remission was induced in 35 patients while 22 patients had partial remission with symptomatic relief. Of the 35 patients who entered complete remission, 13 remained in sustained remission while 22 suffered relapse of the disease which responded to further immunosuppressive therapy. Nine children were refractory to cytotoxic therapy. The renal survival of all patients and specifically of responders and the non-responders are shown in Fig. 1. At 10 years the renal survival was 94% for the responders while it was 48% for the non-responders. The relationship between different histological and clinical features with the response to immunosuppressive therapy and the progression to ESRF is shown in Tables 3 and 4, respectively. The univariate

Table 1 The choice of cytotoxic therapy

Number	First	Second	Third	Fourth
21	Cyclophosphamide	–	–	–
9	Cyclophosphamide	Cyclosporin A	–	–
7	Cyclophosphamide	Cyclophosphamide -		–
6	Cyclophosphamide	Cyclophosphamide + vincristine		–
5	Cyclophosphamide	Vincristine	Cyclosporin A	–
3	Cyclophosphamide	Cyclophosphamide cyclosporin A		Chlorambucil
6	Cyclosporin A	–	–	–
5	Cyclosporin A	Cyclophosphamide	–	–
4	Cyclosporin A	Cyclophosphamide chlorambucil		–

Table 2 Response to cytotoxic therapy

Drug	Cyclosporin A	Cyclophosphamide	Cyclophosphamide + vincristine
Number	15	51	6
Complete remission	40%	43%	50%

analysis identified the presence of tip lesions (*p*=0.005) and mesangial expansion (*p*=0.012) to be associated with a favourable response to cytotoxic therapy while the presence of renal impairment at presentation (*p*=0.008), tubular atrophy (*p*=0.007), extensive (>20%) focal and segmental sclerosis (*p*=0.015) and capsular adhesions (*p*=0.043) were associated with an unfavourable response to therapy. The multivariate analysis using unconditional logistic regression method identified the presence of mesangial expansion (*p*=0.011) and tip lesions (*p*=0.005) as the independent predictors of favourable response cytotoxic therapy and the presence of renal impairment (*p*=0.008) and extensive segmental sclerosis (*p*=0.025) as independent predictors of unfavourable response. However, tubular atrophy was significantly associated and did not individually predict poor response to therapy (*p*=0.27). Patients with mesangial expansion had 4.6 (CI 1.39–15.24) times higher response rate than those without mesangial expansion. Neither hypertension nor the presence of haematuria at presentation was individually significant as predictors of progression to ESRF (*p*=0.22 and *p*=0.35, respectively). None of the biochemical or histological parameters evaluated were individually significant as predictors of progression to ESRF except renal failure at presentation (*p*<0.001). All of the patients who had evidence of renal impairment at presentation progressed to ESRF by 3 years in spite of immunosuppressive therapy.

Two patients died during follow-up due to complications relating to heavy immunosuppression. Other major complications encountered were peritonitis and septicaemia in eight patients, renal vein thrombosis in one, persistent

hypertension in 11 and convulsions in three. Fifteen patients had transplants and three developed recurrence of FSGS in the transplanted kidney.

Discussion

The initial response to conventional doses of corticosteroid therapy for idiopathic FSGS is poor in contrast to that of minimal change glomerulopathy [1, 4]. In the majority of studies published, the response rate has been less than 30% [5]. Pei et al. reported that by using a more prolonged course of prednisolone therapy, 44% of children with idiopathic FSGS entered complete remission [12]. The results of this review demonstrate that 16.6% of patients entered remission with an extended course of daily prednisolone for 6 weeks followed by alternate day prednisolone for a further 8–16 weeks, thus suggesting that the definition of steroid resistance and the timing for renal biopsy should exceed the recommended duration of 4 weeks [11]. Previous studies addressing the efficacy of cyclophosphamide in inducing remission showed conflicting results [5, 6]. A controlled trial looking at the efficacy of oral cyclophosphamide therapy in inducing remission in FSGS failed to demonstrate a benefit over the placebo therapy [13]. However, many case cohorts studied have reported variable beneficial effects [5, 14, 15]. The results of this review demonstrate that a single course of oral cyclophosphamide-induced remission in 43.1% of patients with FSGS when used as the first cytotoxic agent and thus supports the use of cyclophosphamide in steroid-resistant FSGS. The higher rate of success with induction of stable remission with cyclophosphamide in this cohort of patients is likely to be due to the meticulous review of renal histology thus excluding the FSGS due to systemic disorders and syndromes.

However, there is currently a debate regarding the optimal therapy for patients with steroid-resistant NS and the best available evidence supports the use of cyclosporin A [5, 6, 16]. In this study, of the 15 patients who received cyclosporine A as the first cytotoxic therapy, remission was induced in 40% of patients. However, long-term cyclosporin A therapy is associated with potential nephrotoxicity, while the histological changes are indistinguishable from the progression to FSGS and thereby makes it difficult to quantify the risk of nephrotoxicity [16, 17]. It is therefore still unclear whether cyclosporin A therapy improves long-
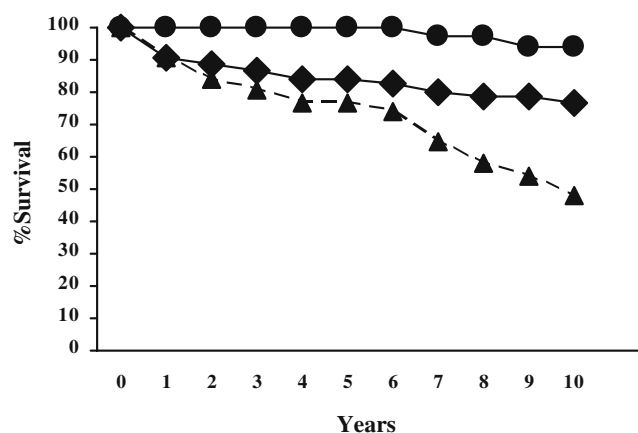

Fig. 1 Renal survival at 10 years; responders (*asterisk*), non-responders (*triangle*) and total (*diamond*)

Table 3 Correlation between clinical and histological features and response to cytotoxic therapy

	Responders (35 patients)	Non-responders (31 patients)	P value	OR (CI)
Age at onset of NS in years	6.8 (1.2–12)	6.1 (0.4–10.1)	–	–
Renal impairment at presentation	0	6	0.008*	Fisher's exact test
Tubular atrophy	20	27	0.007*	0.2 (0.06–0.68)
IgM-C3 deposits	8	6	0.38	1.7 (0.53–5.3)
Mesangial expansion	22	9	0.012	3.67 (1.3–10.3)
Segmental sclerosis >20%	11	19	0.015*	0.29 (0.11–0.79)
Tip lesions	8	0	0.005	Fisher's exact test
Capsular adhesions	15	21	0.043*	0.36 (0.13–0.98)
Globally sclerosed glomeruli >10%	15	12	0.73	1.19 (0.44–3.2)

*Unfavourable response

term renal survival despite recent reports of encouraging short-term success in induction of remission in steroid-resistant FSGS. Interestingly, combination therapy with vincristine and cyclophosphamide used in six patients induced remission in 50%, supporting the recent reports of beneficial effects with vincristine therapy [18, 19]. However, vincristine was prescribed in a majority of patients as second-line agents and therefore their efficacy was difficult to analyse due to the diverse immunosuppressive therapies used previously and therefore need further evaluation. Evidence supporting the concept that patients with FSGS may be undertreated has surfaced in recent studies. In an uncontrolled trial, Tune et al. reported a remission rate of over 50% with an intensive course of corticosteroid therapy for children with idiopathic FSGS resistant to a conventional dose of steroid therapy [14]. In a regimen that could be regarded as an extremely aggressive course of treatment, methylprednisolone infusions along with oral steroids were administered for over 12 months. In spite of the encouraging results, such a treatment protocol has not been widely accepted in view of the associated corticosteroid-related side-effects, namely cataracts (22%), hypertension (17%), retardation of growth (17%) and infectious complications (17%) [14].

There are no firm clinical or laboratory data that predict the response to cytotoxic therapy in idiopathic steroid-resistant FSGS. However, the black African-American race [20] and tubulo-interstitial changes in renal histology [8] have been implicated as predictors for refractory NS. The identification of the underlying gene defect in some cases of congenital NS has recently led to a critical breakthrough in the understanding of the pathogenesis of nephrotic syndromes. The causative gene, NPHS1, encodes a novel protein, nephrin, which is a transmembrane protein belonging to the immunoglobulin superfamily specifically expressed in the glomerular podocyte [21, 22]. In addition, within certain populations, a proportion of individuals with sporadic idiopathic FSGS will have mutations in the gene NPHS2, which encodes for a novel membrane protein named podocin localized at the cytoplasmic part of the slit diaphragm [22, 23]. In such individuals, the disease is characterized by early onset, complete steroid-resistance and rapid progression to ESRF with no recurrence after renal transplantation [24, 25]. Other genes involved in the slit-diaphragm or the nephrotic syndrome are CD2-associated protein (CD2AP), FAT1, WT1, LMX1B and SMAR CAL1 [24]. Altogether, these data demonstrate the pivotal role of the podocyte in the development and the maintenance of the glomerular filtration barrier and the crucial role

Table 4 Correlation between clinical and histological features and ESRF

	Total (n=66)	ESRF at 10 years (n=18)	P value	OR (CI)
Renal impairment at presentation	6	6	<0.001	Fisher's exact test
Tubular atrophy	47	14	0.6	0.7 (0.18–2.69)
IgM-C3 deposits	14	3	0.54	0.38 (0.13–2.17)
Mesangial expansion	31	4	0.08	0.35 (0.1–1.15)
Segmental sclerosis >20%	30	11	0.12	2.4 (0.79–7.3)
Tip lesions	8	0	0.95	Fisher's exact test
Capsular adhesions	36	13	0.08	2.83 (0.87–9.1)
Globally sclerosed glomeruli >10%	27	7	0.72	1.22 (0.41–3.6)

of the genetic factors in the development of SRNS. It has been suggested that all cases of sporadic SRNS be tested for *NPHS2* mutations in an attempt to avoid unnecessary immunosuppression in some children. Whilst in the near future this will be possible, genetic mutational analysis for *NPHS2* is not yet widely available. Thus, currently, this approach is not widely utilized in routine clinical practice.

In this study, more emphasis was given for the histological diagnosis in order to minimise the effects of the observer error. This was achieved by reviewing the histology by two independent paediatric renal pathologists who were blinded to the clinical details and the outcome of these patients. Of the histological features that we evaluated in this group, mesangial expansion and glomerular tip lesions were individually predictive of a favourable response to cytotoxic therapy. A correlation between poor outcome and mesangial expansion has been described [26], but other studies [10] and the results of these studies suggest that it can predict a favourable response. Moreover, another study group recently described glomerular tip lesions as an independent predictor of good renal outcome [27] and therefore the presence of these factors could be useful when deciding on cytotoxic therapy for induction of remission. The presence of renal impairment at presentation and extensive segmental sclerosis were independently predictive of a poor response therapy. Tubular atrophy and capsular adhesions were more prominent and significantly associated with an unfavourable response to cytotoxic therapy but were not individually predictive of unfavourable response.

In primary idiopathic FSGS with nephrotic range, proteinuria patients who achieve a remission have a significantly improved clinical course, in that, the progression to ESRF is less than 15% [1]. In contrast, over 60% of those fail to achieve remission progress to ESRF [1]. In light of this, more intensive immunosuppressive regimens have emerged on the basis that the non-responding patient would finally have to undergo a renal transplantation resulting in life-long immunosuppressive therapy. The results of this study indicate that the renal survival was 94% for the 35 patients who entered complete remission while it was 48% for the non-responders, thus supporting the argument for using aggressive cytotoxic therapy for induction of remission especially when gene defects have been excluded. Arguments against such a practice would be that repeated courses of cytotoxic therapy would increase the risk of future malignancies and gonadal toxicity and possibly increase the risk of post-transplant lymphoproliferative disease [7, 28]. Therefore the intensity of immunosuppressive therapy in NS should be meticulously balanced against its potential side-effects [29]. The results also confirm that patients who have evidence of renal impairment at presentation have a poor renal outcome despite aggressive immunosuppressive therapy [30] and therefore should be considered for conservative management and subsequent renal replacement therapy.

This study is a retrospective review of patients and therefore cannot make firm recommendations. However, the findings suggest that prolonged treatment with corticosteroids increases the chances of a remission and preserves renal function in patients with idiopathic FSGS. The chance of remission may be increased with the addition of cyclophosphamide, vincristine and with the prolonged use of low-dose cyclosporine A. Persistent proteinuria (Fig. 1) and renal impairment at presentation are associated with poor prognosis. The presence of mesangial expansion and tip lesions independently predicts a favourable response to immunosuppressive therapy while renal insufficiency at presentation and extensive focal segmental sclerosis are individual predictors of poor response to therapy, which can help the clinician to decide on the intensity and duration of cytotoxic therapy on an individual basis.

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