Factors influencing the course and the response to treatment in primary focal segmental glomerulosclerosis

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Abstract

Background. The clinical course of primary focal segmental glomerulosclerosis (FSGS) varies and there is considerable controversy as to which factors are of importance in determining prognosis or response to therapy. The aim of this study was to identify clinical, pathological or immunohistochemical features at biopsy that could identify patients with progressive disease who might benefit from treatment, and predict long-term outcome.

Methods. The clinical and pathological findings of 33 adult patients with primary FSGS were retrospectively analysed in order to identify features at biopsy that could be predictive of outcome or response to treatment. For this purpose an immunohistochemical study was also performed, using monoclonal antibodies against intracellular adhesion molecules-1, C5b-9, α3β1 integrin, α-smooth-muscle actin (SMA), and TGF-β1.

Results. At biopsy 17 patients (51%) were nephrotic leading to ESRD. The severity of tubulointerstitial fibrosis is predictive of response to therapy. Of the nephrotic patients, 11 were treated and six received only symptomatic therapy. Initial treatment with prednisolone (Pred) for 6–12 months (average 9 months) resulted in remission in 64% of nephrotic patients. To those with partial or no response, cyclosporin (CsA) or cyclophosphamide was given. At the end of follow-up (mean 57 months) three nephrotic patients (28%) were in complete remission, six (54%) in partial remission, and two (18%) did not respond to the treatment. In the seven treated non-nephrotic patients, Pred induced a complete remission in two (28%), a partial remission in three (44%), while two patients (28%) did not respond. Plasma creatinine remained stable in nephrotic patients who responded and it almost doubled in non-responders. Plasma creatinine also remained unchanged in treated non-nephrotic patients who responded to Pred, while two non-responders reached end-stage renal disease (ESRD). In contrast, 50% of untreated nephrotic patients and 67% of untreated non-nephrotic patients progressed to ESRD. Multivariate analysis showed only age and plasma creatinine at biopsy to have an independent predictive value for renal survival in nephrotic patients. This analysis also demonstrated that only the severity of interstitial fibrosis predicted the response to the treatment. In addition, the tubulointerstitial but not the glomerular expression of C5b-9, α3β1 integrin, α-SMA, and TGF-β1 was significantly more extensive in non-responders and correlated with renal function at biopsy. However, only tubulointerstitial expression of TGF-β1 independently correlated with the degree of renal function impairment at biopsy, but none of the above markers independently predicted renal survival or response to therapy.

Conclusions. Nephrotic patients with FSGS may benefit from a more prolonged course of Pred. Nephrotic patients responding to treatment have a significantly better renal survival than non-responders. Age and plasma creatinine at biopsy are independent risk factors leading to ESRD. The severity of tubulointerstitial fibrosis is predictive of response to therapy.

Keywords: cyclosporin; focal segmental glomerulosclerosis; nephrotic syndrome; prognosis; TGF-β1; treatment

Introduction

Primary focal and segmental glomerulosclerosis (FSGS) is a clinicopathological entity that affects both adults and children and manifests itself clinically by persistent nephrotic syndrome, non-selective proteinuria, microscopic haematuria, hypertension and, commonly, renal insufficiency at presentation [1]. The clinical course of patients with FSGS is one of progressive deterioration of renal function leading to end-stage renal disease (ESRD) over 5–10 years [2,3]. Recent studies have suggested that FSGS in adults is more responsive to corticosteroids than previously believed [4–7] and certain clinical and histological findings have been reported to have prognostic significance [2–4,8,9]. The presence of nephrotic syndrome is an important predictor of poor prognosis. Fifty per cent of patients...
with nephrotic range proteinuria reach ESRD in 6–8 years, in contrast to those with proteinuria alone, who have a much more benign course resulting in a renal survival of >80% at 10 years [2,3,10,11,12]. However, the poor prognosis associated with the nephrotic syndrome is significantly improved for those patients with a complete or even partial remission [1,5–8,12]. Unfortunately, so far there are no reliable clinical or histological features at presentation that allow the nephrologist to predict which patients will respond to therapy [5,12,13].

The aim of this study was to analyse our experience in adult patients with primary FSGS. Furthermore, an attempt was also made to identify clinical, pathological, and immunohistochemical features at presentation that might help to identify patients who will have progressive disease and who might benefit from more aggressive and/or prolonged treatment.

Subjects and methods

Clinical and histological data from 1984 until 1996 of all adult patients (>15 years of age) who had renal biopsies showing ‘classic’ FSGS were reviewed. The diagnosis of FSGS was based on the following criteria: (i) a lesion involving some of the glomeruli in the biopsy with others remaining uninvolved, (ii) the involved glomeruli having a portion that has undergone collapse of capillaries with obliteration of capillary lumina with or without adhesions, and (iii) no clinical or pathological evidence for primary disease that might produce secondary FSGS [13]. Patients with systemic diseases, primary or secondary glomerulopathies, a history of reflex nephropathy, human immunodeficiency virus infection, or intravenous drug abuse were excluded. Only patients with FSGS as their initial glomerular lesion and a well-documented follow-up were included for study.

Based on these criteria, a total of 33 patients were identified. Clinical and laboratory information for all patients was available both at the time of biopsy and throughout their follow-up.

Definitions

The nephrotic syndrome was defined as proteinuria of >3 g/24 h together with a serum albumin of <3 g/dl. Haematuria was defined as >5 red blood cells per high-power field. Normal renal function was defined as a plasma creatinine (Pcr) <1.3 mg/dl. Renal insufficiency was defined as a persistent rise in Pcr >1.3 mg/dl, and ESRD was the point at which dialysis treatment was started or Pcr exceeded 10 mg/dl. Patients with diastolic blood pressures >90 mmHg or systolic blood pressures >140 mmHg were considered hypertensive. The outcome was defined as follows: complete remission was when there was a stable reduction in urinary protein excretion to <0.25 g/24 h and partial remission was when proteinuria ranged between 0.26 and <3 g/24 h in the presence of stable renal function or a Pcr persistently <3.5 mg/dl. Minimal proteinuria, or the presence of contraindications for treatment with corticosteroids or immunosuppressive agents.

Treatment protocol

All treated patients (n=18) initially received Pred at a dose of 1 mg/kg BW for >1 month with progressive tapering. The average duration of treatment was 9 months (range 6–12 months). Patients with partial or no response to Pred were given cyclophosphamide or CsA in combination with low-dose oral Pred. Cyclophosphamide was given orally at a daily dose of 1–2 mg/kg BW for 2 months and CsA at a daily dose of 2–3 mg/kg BW (target whole blood levels 200–300 ng/ml) for an average period of 25 months (range 8–60 months).

Renal biopsy evaluation

All but two biopsies were taken using a standard Trucut needle. In each case, glass slides stained with haematoxylin–eosin, Masson trichrome, periodic acid–Schiff, and metha-mine silver periodic acid–Schiff (Jones) were available for review. Light microscopy findings were analysed and classified by a pathologist (AP) and a nephrologist (EA) without knowledge of patient identity or outcome. In addition to establishing the diagnosis of FSGS, the following features were recorded: the proportion of obsolescent glomeruli and the proportion of glomeruli with segmental scars, the presence of mesangial hypercellularity (>3 cells per mesangial area), the presence of mesangial sclerosis, the presence of epithelial cell proliferation, the presence of synchiae with the Bowman’s capsule, and the presence of diffuse mesangial deposits of IgM and C3. Furthermore, the severity of tubulointerstitial fibrosis, the extent of interstitial infiltrate, and the presence of arteriosclerosis were also evaluated and graded on a scale from 0 to 3.

Immunohistochemical study

Snap-frozen tissue from 13 treated patients (7 responders) was available for immunohistochemistry. The monoclonal antibodies employed were specific for intracellular adhesion molecules (ICAM)-1 (NCL-CD54, Novocastra), Csb-9 (MO 777, Dako), z-smooth-muscle actin (SMA) (M714, Dako), z3/f1 integrin (NCL, CD49c, Novocastra) and transforming growth factor z1 (TGF-f1) (anti-hLAP, MAB 246, R and D Systems). For the purpose of the study an indirect immunoperoxidase technique, as previously described [14,15], was used. Glomerular expression of ICAM-1, Csb-9, z3/f1 integrin and TGF-f1 was graded on a scale of intensity from 0 to 3 for statistical evaluation. The number of glomerular or interstitial SMA-positive cells was finally expressed as the number of cells per glomerular cross-section or per interstitial mm2. Tubulointerstitial expression of Csb-9 and z3/f1 integrin was expressed as the percentage of tubules expressing the antibodies. The tubulointerstitial expression of ICAM-1 and TGF-f1 was assessed and graded on a scale of intensity from 0 to 3.

Statistical analysis

Cumulative renal survival was established according to the method of Kaplan and Meier [16]. Renal survival was calculated from the time of biopsy. Comparisons of clinical
or histological data between different subgroups were performed using Student’s t-test for paired and unpaired data as well as chi-squared statistics. Relationships between the histological and immunohistological features with clinical parameters were determined using simple linear regression analysis and Spearman’s correlation coefficient. Multivariate analysis was used to detect independent effects on outcome or response to therapy of each clinical, histological or immunohistological parameter assessed.

Statistical analysis was performed using a computer program package (Statview version 4.5, Abacus Concepts Inc., Berkeley CA, USA), and a P value of <0.05 was considered significant.

Results

Incidence of FSGS

From 1984 till 1996, among 751 renal biopsies done on native kidneys, 33 biopsies (4.4%) fulfilled the criteria of primary FSGS. The rate of diagnosis was 1.3% (four of 288 biopsies) in the period 1984–1990 and rose to 6.3% (29 of 463 biopsies) in the period 1990–1996.

Patient characteristics

The clinical data of patients at biopsy are shown in Table 1. All patients had proteinuria and 17 (51%) had nephrotic syndrome. Microscopic haematuria was present in nine patients (27%). Mean Pcr at presentation was 1.9 (±1.5) mg/dl (168 ± 133 μmol/l). Thirteen patients (39%) had a Pcr > 1.5 mg/dl. The mean period of follow-up was 55 months (range 8–142 months).

Nephrotic and non-nephrotic patients

Clinical characteristics of nephrotic and non-nephrotic patients at biopsy are shown in Table 2. Non-nephrotic patients were older, although the difference was of marginal significance (P <0.05). Otherwise both groups were similar with respect to plasma creatinine and hypertension. The histological features of nephrotic and non-nephrotic patients are comparatively illustrated in Table 2. The mean number of glomeruli per biopsy did not differ between nephrotic and non-nephrotic patients. The proportion of glomeruli with segmental sclerosis (± SD) was 33 ± 23% (range 5–83%) in nephrotic patients and 29 ± 19% (range 7–74%) in non-nephrotic patients (P = NS). However, the proportion of obsolescent glomeruli was significantly higher in non-nephrotic patients (26 ± 22%, range 0–71%) compared to nephrotic patients (10 ± 14%, range 0–15%) (P <0.02). When compared with non-nephrotic patients, biopsies from nephrotic patients had significantly less prominent mesangial sclerosis (P <0.02) and interstitial infiltrate (P <0.01), but more often glomerular epithelial cell hyperplasia (P <0.02).

Significant tubulointerstitial fibrosis (>2 +) was present in 41% of biopsies from nephrotic patients and 37% from non-nephrotic patients (P = NS). However, non-nephrotic patients had more frequently intense mononuclear cell infiltrate in their tubulointerstitium (69 vs 41%, P <0.01). The average follow-up for nephrotic patients was 57 months (range 6–140 months) and for non-nephrotic patients 33 months (range 5–80 months).

Response to treatment and outcome

Nephrotic patients

Eleven nephrotic patients were treated and six received only symptomatic treatment. Treated patients received prednisolone as initial therapy. Seven patients (64%) responded to prednisolone (three completely and four partially) (Table 3). The time of remission ranged from 4 to 9 months with most patients responding within 6 months. During follow-up, non-nephrotic proteinuria reappeared in one patient with initial complete remission but no additional treatment was tried again. In those with partial remission or no response, cyclophosphamide (n = 2) or CsA (n = 6) in combination with low-dose oral prednisolone were given. Of four patients with initial partial response, a complete remission was achieved in one patient treated with CsA and a sustained partial remission in the remaining three treated with cyclophosphamide (n = 1) or CsA (n = 2). Of four patients who did not respond to prednisolone, two went to a partial remission (one with cyclophosphamide and one with CsA) and two showed no response at all. After a mean follow-up of 57 months (range 6–140 months) three of 11 nephrotic patients (28%) were in complete remission, six (54%) in partial remission, and two (18%) did not respond and remained nephrotic (Table 3).

There was no significant difference in the overall treatment time or total dose of prednisolone between patients with complete, partial, or no response (Table 4). The total dose of cyclophosphamide in the two treated patients was 6.5 and 7.5 g respectively. The mean dose of CsA was 2.2 ± 0.4 mg/kg/day. The additional complete remission with CsA was achieved after 48 months of the initiation of the drug.

Per remained stable or improved in nephrotic patients who responded to therapy and it almost doubled in the two non-responders (Table 3). However, none of the treated nephrotic patients progressed to ESRD (Table 4). In the untreated nephrotic patients, renal function remained unchanged in three
Table 2. Characteristics of nephrotic and non-nephrotic patients with FSGS at biopsy

<table>
<thead>
<tr>
<th></th>
<th>Nephrotic (n=17)</th>
<th>Non-nephrotic (n=16)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>35</td>
<td>47</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(range)</td>
<td>(14–58)</td>
<td>(19–77)</td>
<td></td>
</tr>
<tr>
<td>Plasma creatinine (mg/dl)</td>
<td>1.8 (±1.8)</td>
<td>1.9 (±1.2)</td>
<td>NS</td>
</tr>
<tr>
<td>(153±153 μmol/l)</td>
<td>(168±106 μmol/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>5.3 (±3.9)</td>
<td>1.6 (±0.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>7 (41%)</td>
<td>9 (56%)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Histological</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glomeruli per biopsy</td>
<td>18 (±7)</td>
<td>18 (±10)</td>
<td>NS</td>
</tr>
<tr>
<td>(range 6–32)</td>
<td>(range 3–31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%Segmental sclerosis</td>
<td>33 (±23)</td>
<td>29 (±19)</td>
<td>NS</td>
</tr>
<tr>
<td>%Obsolescent glomeruli</td>
<td>10 (±14)</td>
<td>26 (±22)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Mesangial hypercellularity</td>
<td>5(53%)</td>
<td>8(50%)</td>
<td>NS</td>
</tr>
<tr>
<td>Mesangial sclerosis</td>
<td>12 (70%)</td>
<td>14 (87%)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>GEC hyperplasia</td>
<td>8 (47%)</td>
<td>4 (25%)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Synechiae</td>
<td>11 (65%)</td>
<td>12 (75%)</td>
<td>NS</td>
</tr>
<tr>
<td>TIN fibrosis (≥2+)</td>
<td>7 (41%)</td>
<td>6 (37%)</td>
<td>NS</td>
</tr>
<tr>
<td>Interstitial infiltrate</td>
<td>7 (41%)</td>
<td>11 (69%)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

GEC, glomerular epithelial cell; TIN, tubulointerstitial.

Table 3. Effect of treatment on proteinuria and renal function in 11 nephrotic patients with FSGS

<table>
<thead>
<tr>
<th>Response</th>
<th>Proteinuria</th>
<th>Plasma creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Corticosteroids</td>
<td>Final</td>
</tr>
<tr>
<td>Complete remission</td>
<td>3 (28%)</td>
<td>3 (28%)</td>
</tr>
<tr>
<td>Partial remission</td>
<td>4 (36%)</td>
<td>6 (54%)</td>
</tr>
<tr>
<td>No response</td>
<td>4 (36%)</td>
<td>2 (18%)</td>
</tr>
</tbody>
</table>

*P = NS.

Table 4. Dosage of prednisolone according to outcome in nephrotic patients

<table>
<thead>
<tr>
<th>Response</th>
<th>Prednisolone (mg/kg/month)*</th>
<th>Total duration*</th>
<th>Time of remission (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>0.6±0.4</td>
<td>8.3±3.9</td>
<td>5.4±3.4</td>
</tr>
<tr>
<td>Partial</td>
<td>0.5±0.3</td>
<td>9.1±4.5</td>
<td>9.7±3.3</td>
</tr>
<tr>
<td>None</td>
<td>0.7±0.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*aP = NS between the groups.

With normal Pcr at biopsy (1.2±0.1 vs 1.4±0.4 mg/dl) but progressed to ESRD in the remaining three patients who had advanced renal insufficiency at the time of biopsy (4.7±3.5 mg/dl) (Table 5). Renal survival at 5 years was significantly better for treated (86%) than for untreated (65%) nephrotic patients (*P*<0.03, Figure 1).

**Non-nephrotic patients**

Of the 16 non-nephrotic patients, seven received prednisolone as initial therapy. Two patients (28%) showed a complete resolution of proteinuria, three...
(44%) a partial one (>50% diminution of pre-existing proteinuria), and two (28%) did not respond at all. The addition of CsA to those with partial or no response did not change the results of the treatment significantly. Pcr remained unchanged in treated non-nephrotic patients with complete response (0.8 ± 0.2 vs 0.9 ± 0.1 mg/dl, P = NS) or partial response (1.4 ± 0.5 vs 1.4 ± 0.7 mg/dl, P = NS). The two non-responders in this group developed ESRD during follow-up (mean 35 months, range 5–80 months) (Table 5).

Of the nine untreated non-nephrotic patients, renal function remained stable in three with normal Pcr at presentation (0.9 ± 0.3 vs 0.9 ± 0.2 mg/dl, P = NS) but progressed to ESRD in six who already had advanced renal insufficiency at the time of biopsy (3.0 ± 1.1 mg/dl) (Table 5).

Recorded side-effects, presumably related to the use of corticosteroids, were cushingoid in six patients (33%), proximal myopathy in three (16%), hypertension in one (5%), and diabetes mellitus in one (5%). Of the 11 patients who received CsA, two (18%) showed an aggravation of pre-existing hypertension and two (18%) developed mild hypertrichosis. Side-effects were usually mild and reversed after appropriate treatment or adjustment of the dosage of the drugs.

### Prognostic significance of presenting features

Pcr at presentation was correlated with the proportion of obsolescent glomeruli in both, nephrotic (r = 0.7, P < 0.005) and non-nephrotic (r = 0.5, P < 0.04) patients.

A multivariate analysis of clinical characteristics showed that only age and Pcr at the time of biopsy were predictive of renal survival in nephrotic patients (P < 0.003 and P < 0.01 respectively). In non-nephrotic patients, however, only the age at biopsy was found to be predictive of renal survival (P < 0.01). No other clinical parameters studied, including gender, proteinuria, and hypertension were predictive of renal survival.

When this analysis was performed to evaluate pathological findings in nephrotic patients, no histological feature that we measured was found to be significant as a risk factor for outcome (ESRD). In non-nephrotic patients, however, this analysis showed that mesangial sclerosis and the severity of interstitial infiltrates were predictive of renal survival (P < 0.005 and P < 0.002 respectively).

Treated patients, either nephrotic or non-nephrotic patients with complete or partial response to the treatment, were younger in comparison to those who did not respond at all (Table 6). Otherwise the groups were similar with respect to Pcr and severity of proteinuria.

In comparison with non-responders, respondents had more often mesangial hypercellularity (50 vs 25%, P < 0.005) and glomerular epithelial cell hyperplasia (43 vs 0%) but less often focal adhesions with the Bowman’s capsule (43 vs 75%, P < 0.001), mesangial sclerosis (25 vs 50%, P < 0.005) or severe tubulointerstitial fibrosis (Table 6). In addition the proportion of obsolescent glomeruli was higher in non-responders (14 ± 14%) than in responders (6 ± 9%) although this difference did not reach the level of significance. A multivariate analysis was also performed to determine if any of the same clinical or histological parameters had an influence on the response to the treatment. From this analysis only the severity of interstitial fibrosis (P < 0.001) was predictive of a response to therapy.

Of the untreated patients, those who progressed were significantly older and had significantly higher Pcr at presentation when compared to patients with stable renal function (Table 7). In addition, this subgroup also showed mesangial sclerosis (P < 0.001) and tubulointerstitial fibrosis (P < 0.001) more frequently. The degree of proteinuria did not differ significantly between the two subgroups. A multivariate analysis of presenting clinical and histological features showed that only Pcr at the time of biopsy was predictive of outcome (ESRD) (P < 0.0005).

The mean blood pressure during treatment did not differ significantly between responders (completely or partially) and non-responders (105 ± 10 vs 107 ± 8 mmHg, P = NS). However, it tended to be higher in the untreated group of patients with deteriorating renal function (110 ± 12 mmHg) when compared with those with stable renal function (103 ± 9 mmHg) although this difference did not reach the statistical significance. Angiotensin-converting enzyme inhibitors had an in

### Table 6. Clinical and histological characteristics at biopsy of treated patients according to the final response to therapy

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Responders (n = 14)</th>
<th>Non-responders (n = 4)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>29</td>
<td>47</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Plasma creatinine (mg/dl)</td>
<td>1.5 (± 0.5)</td>
<td>1.7 (± 0.3)</td>
<td>NS</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>4.7 (± 4.6)</td>
<td>4.2 (± 2.1)</td>
<td>NS</td>
</tr>
<tr>
<td>% Obsolescent glomeruli</td>
<td>6 (± 9)</td>
<td>14 (± 14)</td>
<td>NS</td>
</tr>
<tr>
<td>Mesangial hypercellularity</td>
<td>7 (50%)</td>
<td>1 (25%)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Mesangial sclerosis</td>
<td>4 (25%)</td>
<td>2 (50%)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>GEC hyperplasia</td>
<td>6 (43%)</td>
<td>—</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Synechiae</td>
<td>6 (43%)</td>
<td>3 (75%)</td>
<td></td>
</tr>
<tr>
<td>TIN fibrosis (&gt; 2 +)</td>
<td>—</td>
<td>1 (25%)</td>
<td></td>
</tr>
</tbody>
</table>

GEC, glomerular epithelial cell; TIN, tubulointerstitial.

### Table 7. Clinical and histological characteristics at biopsy of untreated patients based on outcome of renal function

<table>
<thead>
<tr>
<th>n</th>
<th>Stable</th>
<th>Deteriorating</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>6 (40%)</td>
<td>9 (60%)</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td>Plasma creatinine (mg/dl)</td>
<td>1.1 (± 0.2)</td>
<td>3.4 (± 2)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Proteinuria (g/24 h)</td>
<td>2.4 (± 1.1)</td>
<td>2.8 (± 1.4)</td>
<td>NS</td>
</tr>
<tr>
<td>Mesangial sclerosis</td>
<td>1 (17%)</td>
<td>6 (67%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mesangial hypercellularity</td>
<td>5 (83%)</td>
<td>4 (44%)</td>
<td>NS</td>
</tr>
<tr>
<td>TIN* fibrosis (&gt; 2 +)</td>
<td>3 (50%)</td>
<td>7 (78%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are means ± standard deviation; *TIN, tubulointerstitial.
were used for the treatment of hypertension in four responders (28%) and two non-responders (50%). In addition, a low-protein diet was advised in all patients with renal insufficiency.

Prognostic factors in FSGS

The numbers of glomerular SMA-positive cells did not differ significantly between responders and non-responders (12.8 ± 4.5 vs 10.1 ± 3.7, P = NS). Also the intensity of glomerular ICAM-1, C5b-9, α3β1-integrin and TGF-β1 expression was similar in both groups. Within the tubulointerstitium, the extent of ICAM-1 expression by the tubules was similar in the two groups (1.7 ± 0.3 vs 2.0 ± 0.3, P = NS). However, in the responders, a significantly lower number of tubules expressed C5b-9 (28.2 ± 10 vs 65.2 ± 13%, P < 0.01) and α3β1 integrin (34.3 ± 13 vs 45.7 ± 19%, P < 0.05) as compared with the non-responders (Figure 2). In this group also, the number of interstitial SMA-positive cells was significantly smaller than in non-responders (184 ± 62 vs 364 ± 57, P < 0.005) and the intensity of TGF-β1 expression weaker (1.5 ± 0.2 vs 2.4 ± 0.4, P < 0.05) (Figure 2).

Plasma creatinine at the time of biopsy was highly correlated with the tubulointerstitial, but not glomerular, expression of α3β1 integrin (r = 0.64, P < 0.02), SMA (r = 0.62, P < 0.02), C5b-9 (r = 0.72, P < 0.03), ICAM-1 (P < 0.03), and TGF-β1 (P < 0.005). In addition, tubular expression of α3β1 integrin was significantly correlated with the degree of proteinuria (P < 0.04) and with the interstitial expression of TGF-β1 (P < 0.03). Interestingly, the extent of interstitial expression of TGF-β1 was significantly correlated with the ICAM-1 expression within the tubulointerstitium (P < 0.04). The multivariate analysis showed, however, that only the tubulointerstitial expression of TGF-β1 had independent predictive value for Pcr at the time of biopsy (P < 0.001). None of the above immunohistochemical features was independently predictive of a response to therapy or renal survival.

Discussion

The present study demonstrated a sixfold increase in the incidence of histological diagnosis of FSGS in recent years. This is more than an incidental finding, since our indications for renal biopsy in proteinuric patients have not changed and none of the patients had evidence of HIV infection during the period of study. For still unknown reasons, a similarly marked increase in the incidence of this lesion has been recognized in several recent reports, where the yearly incidence of primary FSGS has risen from 4–10% in 1974 to 12–25% in 1993 [17,18].

Current experience suggests the poor response to therapy previously experienced in adults with FSGS [2,3,19] may have been a result of undertreatment [20]. Since 1980, complete remission rates of over 30% have been associated with more prolonged durations of therapy ranging from 5 to 8 months, as compared with the less than 2 months of therapy used in earlier studies [4–7,12]. In this study, remission (complete and partial) of nephrotic syndrome with corticosteroids was achieved in 64% of our patients with a mean course of treatment of 9 months, and the average time to remission was slightly longer of that of previous reports at 5.4 months. It is of interest that a higher rate of partial remissions (36%) was achieved as compared with other studies (0–26%) [5–7,12,21] and this may be related to the more prolonged duration of corticosteroid therapy [22].

There is controversy about the role of CsA or cytotoxic agents in steroid-resistant FSGS. Treatment with cyclophosphamide resulted in complete remission rates of less than 25% in patients with steroid-resistant FSGS [1]. Also, despite discrepancies between different studies, some 20–25% of adults with steroid-resistant FSGS may have complete remission and another 20–30% a partial remission under CsA [1,23]. In this study, the addition of low-dose CsA or cyclophosphamide was associated with a complete remission in 25% of patients with partial response to prednisolone and a partial remission in 50% of those who did not respond at all. It is worth mentioning that in three patients, CsA-induced remissions were achieved after prolonged treatment (48, 55 and 60 months respectively) without concomitant changes in renal function. These results indicate that cyclophosphamide, and particularly CsA, in low-doses may enhance the likelihood of remission of proteinuria in patients who do not respond completely to corticosteroid therapy [1,24,25].

The value of treatment in non-nephrotic patients with FSGS has not been determined and most authors do not recommend steroids or immunosuppressive drugs [26]. However, since the amount of proteinuria by no means indicates the severity or the development of glomerular sclerosis [27], we decided to treat seven non-nephrotic patients with corticosteroids. Proteinuria remitted completely in two patients (28%), partially in three (44%), and remained unchanged in two (28%). It is interesting that CsA administration to those with partial or no response to corticosteroids did not offer an additional benefit. The question of whether the diminution of proteinuria offers a real advantage to the long-term prognosis of non-nephrotic patients with FSGS remains to be answered. This may be true for patients with normal or near-normal renal function, since two patients in this category with renal insufficiency at biopsy (Pcr 2.8 and 3.3 mg/dl respectively) progressed to ESRD. Certainly, more studies are needed to clarify this important point.

Nephrotic patients with FSGS have a poorer prognosis when compared with non-nephrotic patients [2,3,20,28]. However, several authors were unable to demonstrate that proteinuria at presentation or at biopsy is an independent predictor of ESRD [5,12]. The number of nephrotic patients eventually entering remission (complete or partial) in the current study...
was high (82%) and none progressed to ESRD. In contrast, plasma creatinine almost doubled in nephrotic patients with no response to treatment, while 50% of the untreated nephrotic patients progressed to ESRD. Renal survival at 5 years was significantly better for treated nephrotic than for non-treated nephrotic patients, and these figures are similar to those reported by others [12]. Thus, achieving a remission significantly altered the course of our nephrotic patients with FSGS.

In agreement with others, of the clinical parameters tested only the age of the patients and the level of renal insufficiency at biopsy were significant risk factors for prognosis [12,29,30]. Regarding the prognostic significance of renal histology, the multivariate analysis demonstrated that none of the histological features were independently significant as predictors of ESRD, although the extent of interstitial fibrosis approached significance ($P<0.06$). This is in contrast to the report of Rydel et al. [12], who found that interstitial fibrosis has independent predictive value for outcome. However, their results may not be inconsistent with our findings, since their patients, especially the nephrotic patients, had more advanced renal insuffi-


Prognostic factors in FSGS

The degree of renal function impairment at biopsy. The expression independently predicts the degree of renal
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erular, expression of certain molecules such as SMA, None of the histological features that we evaluated
and nephron loss [20,31]. However, the almost identical levels of proteinuria indicate that treatment should be initiated as early as authors reported no link between prognosis and the function during follow-up and those who progressed
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ciency at biopsy nor the degree of proteinuria were predictive studies are needed to con
predict which patients will enter a remission. In agree-

so far there are no reliable clinical or histological features at presentation that allow the nephrologist to predict which patients will enter a remission. In agreement with others [12] we found that neither Pcr at biopsy nor the degree of proteinuria were predictive of response to treatment. In addition, this study showed that ‘active’ glomerular lesions such as mesangial and glomerular epithelial cell hyperplasia were more often observed in responders, while ‘chronic’ lesions such as mesangial sclerosis and advanced tubulointerstitial fibrosis were more commonly seen in non-responders. The multivariate analysis demonstrated that only the extent of interstitial fibrosis had independent predictive value for response to treatment. Others, however, found that none of the pathological features assessed including the severity of interstitial fibrosis were predictivc of response to treatment [13,31]. Given the frequent concurrence of active lesions and segmental and global scars, and a temporal relationship that suggests that these lesions evolve into scars, our data indicate that treatment should be initiated as early as possible, particularly in patients with more pronounced cellular hyperplasia and less extensive tubulointerstitial fibrosis, to forestall irreversible glomerular scarring and nephron loss [20,31].

The analysis of the immunohistological findings in a group of treated patients showed that responders exhibited less extensive tubulointersttial, but not glomerular, expression of certain molecules such as SMA, α3β1 integrin, C5b-9, and TGF-β1 compared to non-responders. Expression of SMA characterizes myofibroblasts, which play a key role in fibrogenesis [32]. In addition, TGF-β1 induces the expression of β1 integrins by renal cells [33] and this is further supported by the highly significant correlation between the two molecules within the tubulointerstitium. Both TGF-β1 and β1 integrins critically participate in matrix assembly and affect tissue remodelling [34,35]. In addition, C5b-9 has numerous toxic effects to the cells and may trigger collagen synthesis by the tubular cells [36,37]. The relationship between proteinuria and the extent of α3β1 integrin expression by the tubular cells is an interesting finding. Since proteinuria induces integrin expression by the tubules [38], we may hypothesize that proteinuria in FSGS is one, but not the unique, factor responsible for the tubulointerstitial damage.

These differences may simply reflect differences in the base pathology between the two groups; however, they confirm previous observations that it is the degree of interstitial disease that determines the overall prognosis and the response to treatment in glomerular diseases [14,15]. This is further supported by the significant relationship between the tubulointerstitial (but not glomerular) expression of these markers with the degree of renal function impairment at biopsy. The multivariate analysis demonstrated that the extent of tubulointerstitial expression of TGF-β1 was the only independent factor that predicted the degree of renal insufficiency at biopsy. However, none of these markers were found to be of independent value in predicting the response to treatment or the subsequent outcome of the disease. Our findings may be explained by the relatively small number of cases studied, but also on the basis of the high rate of remissions, all of whom had excellent long-term renal survival. Certainly, larger studies are needed to confirm our preliminary findings and to identify patients more likely to respond to the treatment.

In the untreated group of patients, the multivariate analysis demonstrated that only Pcr at biopsy had an independent prognostic value of ESRD, and this is compatible with some [27,39,40] but not all [6,29] studies. A Pcr >1.5 mg/dl was associated with a significantly poorer renal survival than those <1.5 mg/dl, regardless of the level of proteinuria. The severity of proteinuria did not differ significantly between untreated patients who retained stable renal function during follow-up and those who progressed to ESRD. Although the presence of nephrotic-range proteinuria has consistently been associated with a poor outcome in primary FSGS [2–4,28,39], several authors reported no link between prognosis and the degree of proteinuria [6,40–42]. In our study, 50% of untreated nephrotic patients and 67% of untreated non-nephrotic patients progressed finally to ESRD. However, the almost identical levels of proteinuria between untreated patients with stable or deteriorating renal function may be attributed to the already advanced renal insufficiency in the latter subgroup.

None of the histological features that we evaluated in this group of untreated patients were independently significant as predictors of ESRD. However, glomerular sclerotic changes and the severity of tubulointerstitial fibrosis were more prominent in patients with progressive renal disease. A correlation between outcome and the extent of tubulointerstitial lesions has been confirmed in several [6,13,28,39] but not all [41] studies, although in some reports the prognosis has been associated with the extent of mesangial hypercellularity [43,44]. Moreover, in one series it was concluded that renal biopsy, while indispensable for adequate diagnosis, is of little help for determining prognosis [2].

In conclusion, this study showed that prolonged treatment with corticosteroids increases the chances of a remission and preserves renal function in nephrotic patients with FSGS. The chance of remission may be increased with the addition of cyclophosphamide and, particularly, with the prolonged use of low-dose CsA. Persistent nephrotic syndrome and advanced renal insufficiency at presentation are associated with poor prognosis. Treatment should be initiated as early as possible since ‘active’ lesions on renal biopsy are more likely to be associated with a favourable response to treatment. The extent of tubulointerstitial TGF-β1 expression independently predicts the degree of renal insufficiency at biopsy, while the severity of interstitial fibrosis is the only predictor of response to the therapy.

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