Prediction of corticosteroid responsiveness based on fibroblast-specific protein 1 (FSP1) in patients with IgA nephropathy

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Abstract

Background. Corticosteroids are frequently used to treat patients with active IgA nephropathy (IgAN); however, there have been few reports describing factors that are predictive of the response to corticosteroid treatment. The purpose of this study is to determine the extent to which fibroblast-specific protein 1-positive (FSP1+) cells are predictive of corticosteroid responsiveness in patients with IgAN.

Methods. Fifty biopsy-proven IgAN patients who received corticosteroid therapy were enrolled and followed for 7.1 ± 3.0 years. FSP1+ cells were identified using an anti-FSP1 antibody.

Results. Twelve patients showed progression of renal impairment or no reduction of urinary protein (non-responders) after steroid therapy. In the remaining 38 patients, renal function was stable during follow-up, and their urinary protein declined to <1.0 g/day (responders). Serum creatinine, estimated GFR, severity of mesangial proliferation, percent glomerulosclerosis/total glomeruli, extent of interstitial damage and FSP1+ cell number were all significantly higher in non-responders than in responders. Cox regression analysis using two covariates with every possible combination of factors indicated that FSP1+ cell number was the strongest and most significant predictor of corticosteroid responsiveness. When IgAN patients had >32.6 FSP1+ cells/HPF at diagnosis, they were the more likely to show steroid resistance.

Conclusion. FSP1+ cell number can serve as an excellent predictor of corticosteroid responsiveness in patients with IgAN.

Keywords: corticosteroid; FSP1; IgA nephropathy; responsiveness

Introduction

IgA nephropathy (IgAN) is the most commonly occurring glomerulopathy worldwide [1]. IgAN was once thought to be relatively benign and to have a reasonably good long-term prognosis. However, recent studies indicate that 5–25% of IgAN patients progress to end-stage renal disease within 10 years [1–3] and 25–50% do so within 20 years [2]. Prognostic factors for the future development of renal failure include the presence of persistent and severe proteinuria, elevated serum creatinine (Scr) at diagnosis and the presence of hypertension [4–10]. Clinically, proteinuria is the most powerful predictor of poor renal survival, and reduction of proteinuria correlates with improved renal outcome [1–10]. Histologically, renal damage (e.g., crescent formation or severe tubulointerstitial involvement) is the most reliable predictor of an unfavourable renal prognosis [4,6,8,9,11], and two morphometric studies suggest that the extent of tubulointerstitial fibrosis correlates better with reduced renal function than glomerular histology does [12,13].

Recent studies have shown that corticosteroids are an effective treatment for IgAN patients exhibiting a mild reduction of renal function, moderate proteinuria and active histological findings [14–17]; however, there are few published reports describing factors predictive of the response to steroid treatment. According to the Japanese IgAN Treatment Guideline, corticosteroid therapy is recommended for IgAN patients who meet the following criteria: creatinine clearance (CrCl) ≥70 ml/min, daily urinary protein excretion ≥0.5 g/day and active histological lesions on renal biopsy [18]. Nonetheless, recent studies suggest that steroid therapy for IgAN could be beneficial, even when the disease is in an advanced stage [19–21]. We previously reported that renal biopsy specimens from IgAN patients show elevated numbers of FSP1+ fibroblasts, which correlate significantly with the degree of interstitial fibrosis and the deterioration of renal function [22]. The apparent capacity of FSP1+ cell number to serve as a predictive maker of IgAN may make it a useful indicator of the appropriateness of steroid therapy. In the present study, we investigated the
relationship between FSP1+ cell number and responsiveness to corticosteroid therapy.

Materials and methods

Patients

Fifty patients with IgAN (20 males, 30 females) were enrolled in this study after they gave fully informed consent. At the beginning of the study, percutaneous renal biopsies were performed on all patients, and two renal pathologists histologically confirmed the diagnosis of IgAN. Patients with purpura nephritis, lupus erythematosus, diabetes mellitus, neoplasia, viral hepatitis or other infections were excluded. The patients ranged from 14 to 63 years of age (mean 38.2 ± 13.0 years old), and all presented with persistent proteinuria with a baseline that exceeded 2.4 ± 1.8 (0.5–9.6) g/day. Recent studies have described the benefits of steroid therapy for advanced IgAN. We therefore determined the indication for steroid treatment based on the level of proteinuria (≥0.5 g/day) and the presence of active lesions in renal biopsies (cellular/fibrocellular crescents, moderate to severe mesangial proliferation and/or interstitial cell infiltration). The patients were treated with prednisolone at a dose of 30 mg/day for the first 6 months, after which the dose was gradually tapered until it was discontinued at 2 years. In addition, 31 patients received an angiotensin-converting enzyme inhibitor (ACEI) plus an antiplatelet agent during the follow-up, and 38 patients received an angiotensin receptor blocker (ARB) plus an antiplatelet agent.

Clinical and histological parameters

Hypertension was defined as a systolic blood pressure (BP) > 140 mmHg and a diastolic pressure > 90 mmHg. Urine and blood samples collected at the time of renal biopsy (baseline) were analysed for Scr and 24-h total protein excretion (Up). To measure estimated glomerular filtration rate (eGFR), we selected the modified MDRD equation for Japanese published during the 51st annual meeting of the Japanese Society of Nephrology. Scr was measured every 2 months. At each visit, basic clinical data, including body weight, BP, Scr and Up, were recorded. Renal biopsy specimens that contained at least 10 glomeruli were considered to be adequate for histological analysis and were analysed semiquantitatively based on the following previously described features [23,24]: (i) percentage of glomeruli showing global or segmental sclerosis (%GS); (ii) severity of mesangial cell proliferation (0 = no proliferation to mild proliferation, with three to four mesangial cells per peripheral lobule; 1 = severe segmental proliferation, with more than four mesangial cells per peripheral lobule; 2 = severe global proliferation. Each glomerulus was scored individually, and the mean scores were calculated for all non-sclerosed glomeruli); (iii) percentage of glomeruli showing crescent formation; (iv) percentage of glomeruli showing adhesions; (v) extent of interstitial damage and chronic inflammation (0 = 0–24%, 1 = 25–50%, 2 = > 50% of biopsy area showing damage or inflammation); (vi) severity of arteriolosclerosis or arteriolar hyalinosis (0 = no hyalinosis to mild hyaline thickening in at least 1 arteriole; 1 = severe hyaline thickening in at least 1 arteriole; 2 = severe hyaline thickening in many arterioles).

Immunohistochemistry

Renal biopsy specimens were fixed in 10% buffered formalin for 12 h, dehydrated, embedded in paraffin and sectioned according to standard procedures. After deparaffinizing the sections, they were incubated with proteinase K (0.4 mg/ml) for 5 min at room temperature, after which endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide. Nonspecific protein binding was blocked with 5% normal goat serum in PBS containing 2% bovine serum albumin (BSA). The sections were then incubated for 60 min at room temperature with a primary polyclonal anti-FSP1 antibody (1:5000 dilution) [22]. The antibody was then detected using a DAKO Envision+ System peroxidase (diaminobenzidine, DAB) kit (DakoCytomation Inc., Carpinteria, CA, USA) and the slides were counterstained with haematoxylin. The specificity of FSP1 staining was confirmed using control rabbit serum and by absorption with anti-FSP1 antibody using an excess of recombinant FSP1 protein. The numbers of FSP1+ fibroblasts within the cortex were counted in each of 10 random and non-overlapped microscope fields (magnification 200×) and then averaged by two examiners.

Outcome definitions

Patients receiving steroid therapy were divided into two groups based on the changes in their renal function and urinary excretion of protein during the follow-up period. Patients who showed an increase in Scr of < 100% from baseline, a reduction in eGFR of < 50% from baseline and a decrease in Up of < 1.0 g/day were deemed responders (Rs). Patients who showed a total increase in Scr of ≥ 100%, a total decline in eGFR of ≥ 50% from baseline or a Up that did not improve to ≤ 1.0 g/day were deemed to be non-responders (NRs).

Statistical analysis

Statistical calculations were made using the Statview 5.0 and JMP 5.1 software packages. Results were expressed as means ± SD, and the statistical analysis was carried out using non-parametric tests. The Mann–Whitney U-test and the Wilcoxon signed-rank test were used for paired and unpaired subjects, respectively. Differences in the parameters between the two groups were analysed using the Friedman and Scheffe’s multiple comparison test. Spearman rank correlation analysis was used to assess the correlation between the number of FSP1+ cells and several parameters. Receiver operating characteristic (ROC) curve analysis was used to explore the predictive cut-off value for FSP1+ cell number and %GS during corticosteroid treatment. To assess the prognostic impact of FSP1+ cell number on renal survival, the relationship between covariates and renal survival were evaluated using the Cox proportional hazards model. Values of P < 0.05 were considered significant in all analyses.
Results

Clinical characteristics of the patients

The mean follow-up period was 7.1 ± 3.0 years. During the follow-up, 38 patients maintained stable renal function, and Up declined to < 1.0 g/day. These patients were deemed to be Rs. The remaining 12 patients were classified as NRs. Among the NRs, there were five patients whose renal function was stable, but whose Up continued to be > 1.0 g/day. The clinical characteristics of the Rs and NRs at the time of enrolment are shown in Table 1. There were no differences between the two groups with respect to age, gender, incidence of hypertension, administration of ACEI/ARB and antiplatelet agents, administration of additional immunosuppressives, and total serum protein, albumin and IgA levels. On the other hand, Scr at the time of enrolment was significantly higher in the NRs than in Rs (1.4 ± 0.5 versus 1.0 ± 0.4 mg/ml, P = 0.0109), and eGFR was significantly lower in the NRs than the Rs (43.0 ± 18.0 versus 68.8 ± 31.8 ml/min, P = 0.0142). During follow-up, the Rs showed no significant change in renal function. By contrast, the NRs showed a decline in renal function that was significant by the end of the study (Figure 1). At the time of enrolment, there was no significant difference in Up between the two groups, and Up was significantly lower in both groups after corticosteroid therapy, but the reduction was greater in the Rs (Figure 1). Notably, whereas the Rs showed a significant decline in blood pressure during the follow-up period, the NRs did not (Figure 2). Total serum protein and albumin also significantly increased during the follow-up in the Rs but not in the NRs (Figure 2).

Histological characteristics and steroid responsiveness

The histological features of the two groups at the time of renal biopsy are shown in Table 2. The NRs had significantly more severe mesangial proliferation and interstitial damage than the Rs. Percent GS and FSP1⁺ cell numbers were also significantly higher in NRs. When we then performed ROC curve analyses to determine the predictive cut-off values for %GS and FSP1⁺ cell number, we found that the cut-offs for corticosteroid responsiveness were 53.5% (sensitivity 0.50, specificity 0.95) and 32.6/HPF (sensitivity 0.75, specificity 0.87), respectively (Figure 3). Moreover, when IgAN patients had > 32.6 FSP1⁺ cells/HPF at the time of diagnosis, they were the more likely to show steroid resistance (Figure 3, Figure 4).

Correlation between the number of FSP1⁺ cells and the clinical or histological parameters

To clarify the significance of FSP1⁺ cells during the progression of IgAN, we examined the correlation between various clinical and histological parameters and the numbers of FSP1⁺ cells (Table 3). Among the clinical parameters examined, FSP1⁺ cell number was positively correlated with Scr at the time of enrolment [Spearman rank-correlation coefficient (Rs) = 0.552, P = 0.0003] and was inversely correlated with eGFR at the time of enrolment (Rs = −0.444, P = 0.0019). Among the histological parameters tested, FSP1⁺ cell number was positively correlated with the %GS (Rs = 0.503, P = 0.0004) and the extent of interstitial damage and chronic inflammation (Rs = 0.662, P < 0.0001).

Predictive factors for corticosteroid responsiveness

Univariate Cox regression analysis showed that Up, eGFR, the severity of mesangial proliferation, the extent of interstitial damage and chronic inflammation, %GS and FSP1⁺ cell number all significantly correlated with corticosteroid responsiveness, so their respective risk ratios (RR) were calculated. Estimated GFR [RR 9.63 (95% CI 1.24–74.7, $P = 0.0302$)], the extent of interstitial damage [RR 6.19 (95% CI 1.83–20.9, $P = 0.0033$)], %GS [RR 10.9 (95% CI...
Fig. 1. Changes in Ccr and Up during the follow-up period. During the follow-up period, responders (Rs) showed no significant changes in renal function, whereas non-responders (NRs) showed significantly diminished renal function by the end of the study. At the time of enrolment, there was no significant difference in urinary protein (Up) between the two groups, and Up was significantly reduced in both groups after steroid therapy. At the end of the follow-up, however, the NRs showed significantly higher Up than the Rs.

Fig. 2. Changes in mean blood pressure, total protein and albumin during the follow-up period. The responders (Rs) showed a significant decline in blood pressure during the follow-up, but the non-responders (NRs) did not. The Rs also showed significant increases in total protein and albumin during the follow-up, but the NRs did not.

2.69–43.9, \( P = 0.0008 \}) and FSP1\(^+\) cell number [RR 23.5 (95\% CI 4.80–115, \( P < 0.0001 \}) were all significantly predictive of corticosteroid responsiveness (Table 4). Because multiple Cox regression analysis was underpowered in this study, we performed an additional Cox regression analysis using two covariates with every possible combination of factors (FSP1\(^+\) cells versus eGFR, interstitial damage and chronic inflammation or %GS) to identify which factor is the strongest predictor. In all analyses, FSP1\(^+\) cell number was the strongest and most significant predictor of
**Table 2.** Histological findings and steroid responsiveness

<table>
<thead>
<tr>
<th>Histological findings</th>
<th>Responder</th>
<th>Non-responder</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severity of mesangial proliferation</td>
<td>0.55 ± 0.72</td>
<td>1.25 ± 0.75</td>
<td>0.0072</td>
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<tr>
<td>Percentage of glomeruli showing crescent</td>
<td>13.0 ± 13.4</td>
<td>7.84 ± 8.09</td>
<td>ns</td>
</tr>
<tr>
<td>Percentage of glomeruli showing adhesion</td>
<td>9.15 ± 10.9</td>
<td>10.8 ± 10.7</td>
<td>ns</td>
</tr>
<tr>
<td>Percentage of glomeruli showing global or segmental sclerosis</td>
<td>20.3 ± 18.9</td>
<td>34.0 ± 24.8</td>
<td>0.0178</td>
</tr>
<tr>
<td>Extent of interstitial damage and chronic inflammation</td>
<td>0.947 ± 0.613</td>
<td>1.50 ± 0.798</td>
<td>0.0126</td>
</tr>
<tr>
<td>Severity of arteriolosclerosis or arteriolar hyalinosis</td>
<td>0.45 ± 0.60</td>
<td>0.67 ± 0.65</td>
<td>ns</td>
</tr>
<tr>
<td>FSP1+ cell</td>
<td>23.0 ± 9.80</td>
<td>35.9 ± 13.1</td>
<td>0.00250</td>
</tr>
</tbody>
</table>

Fig. 3. ROC curve analysis of the association between responsiveness to corticosteroids and FSP1+ cell number and %GS. We performed ROC curve analysis to determine the predictive cut-off values for percentage of glomeruli showing global or segmental sclerosis (%GS) and FSP1+ cell number with respect to responsiveness to corticosteroids. The values obtained were 53.5% and 32.6/HPF for %GS and FSP1+ cell number, respectively.

Corticosteroid responsiveness and renal outcome in patients with IgAN.

**Side effect of corticosteroid treatment**

One patient developed bacterial pneumonia, one patient developed a cataract that required surgery and two patients experienced upper gastrointestinal upset. None of these side effects necessitated discontinuation of treatment.

**Discussion**

The novel finding of this study is that the FSP1+ cell number at the time of diagnosis is closely associated with responsiveness to corticosteroid therapy and renal outcome in patients with IgAN. Corticosteroids are usually administered to IgAN patients with active histological findings, but an earlier report showed that 30% of those patients do not respond to steroid therapy [16]. Because corticosteroids have many adverse side effects, it would be greatly beneficial if, prior to administration, we were able to identify those patients who will most likely respond to steroid treatment. In addition to conventional clinical and histological parameters, we chose FSP1+ cell number as a possible predictor of steroid responsiveness. FSP1 was first cloned in mice by Strutz et al. [25]. Iwano et al. then showed that FSP1 is a specific maker of activated fibroblasts and that FSP1+ fibroblasts accumulate in areas of interstitial fibrosis [25,26], which suggests that FSP1+ fibroblasts are the principal effector cells in renal fibrosis [27].

We previously found that the renal cortices of patients with IgAN contain large numbers of FSP1+ fibroblasts, which correlate closely with the interstitial fibrosis and renal prognosis [22]. Moreover, patients with ≥20 interstitial FSP1+ fibroblasts per HPF had a poor renal outcome. Because tubulointerstitial fibrosis correlates with renal survival [12,13], we decided to evaluate the correlation between the extent of interstitial damage and the responsiveness of IgAN patients to corticosteroid therapy. In the past, the extent of renal fibrosis was studied using computer-assisted morphometric analysis of stained renal biopsy specimens. With this semi-quantitative assay, it takes at least several weeks before the final results are available. This assay is also limited by the degree of methodological variation among examiners. In the present study, we focused on FSP1+ cells, which appear to be a more sensitive and specific marker of both active fibroblasts and renal fibrosis. This approach is simple and quantitative, and the results are available within a few days after renal biopsy (Figure 4a and b).
FSP1 as a maker for steroid responsiveness

Fig. 4. Representative photomicrograph illustrating FSP1+ expression in the renal interstitium of responders and non-responders. Higher numbers of FSP1+ cells accumulate in the interstitium of non-responder (b, d) than responder (a, c). FSP1 was also localized in tubular epithelial cells (arrow). The original magnification in a and b is 100×; c and d are the high power images of the boxed areas in a and b.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Spearman rank correlation coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>0.160</td>
<td>0.2627</td>
</tr>
<tr>
<td>Urinary excretion of protein for 24 h</td>
<td>0.291</td>
<td>0.0419</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>0.552</td>
<td>0.0003</td>
</tr>
<tr>
<td>Estimated GFR</td>
<td>−0.444</td>
<td>0.0019</td>
</tr>
<tr>
<td>Histological parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severity of mesangial proliferation</td>
<td>0.141</td>
<td>0.3243</td>
</tr>
<tr>
<td>Percent of crescent/total glomeruli</td>
<td>0.267</td>
<td>0.3760</td>
</tr>
<tr>
<td>Percent of adhesion/total glomeruli</td>
<td>−0.171</td>
<td>0.5710</td>
</tr>
<tr>
<td>Percentage of glomeruli showing global or segmental sclerosis</td>
<td>0.503</td>
<td>0.0004</td>
</tr>
<tr>
<td>Extent of interstitial damage and chronic inflammation</td>
<td>0.662</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Severity of arteriolosclerosis or arteriolar hyalinosis</td>
<td>0.329</td>
<td>0.0212</td>
</tr>
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</table>

We also showed that Scr, eGFR, severity of mesangial proliferation, extent of interstitial damage and chronic inflammation, %GS and FSP1+ cell number were significant predictors of responsiveness to corticosteroids. Interestingly, Cox regression analysis revealed that the number of interstitial FSP1+ fibroblasts was the strongest predictor of corticosteroid responsiveness and renal outcome in patients with IgAN. Moreover, ROC curve analysis showed that the cut-off value is 32.6 FSP1+ cells per HPF. In other words, when IgAN patients have >32.6 FSP1+ cells/HPF, they are much more likely to show steroid resistance.

It is noteworthy that some of the tubular epithelial cells surrounding areas of fibrosis also expressed FSP1, which is consistent with earlier studies describing epithelial–mesenchymal transition (EMT) in regions around areas...
of fibrosis [27,28]. Using FSP1 staining as a predictor of steroid responsiveness, we were able to assess not only tissue fibroblasts, but also the emergence of fibroblasts via EMT, which could be indicative of future fibrotic processes. This would make counting FSP1+ cells a more sensitive and specific method than traditional morphometric analysis for evaluating current and future renal fibrosis, and could explain why the statistical power of counting FSP1+ cells is much greater than traditional clinical and histologic parameters for predicting responsiveness to corticosteroids.

In the present study, 24.0% of patients with IgAN did not respond to corticosteroid treatment. That level of responsiveness is compatible with the level reported earlier by Pozzi [16], who found that ~30% of IgAN patients did not respond to steroids. We suggest that FSP1+ cells, including fibroblasts emerging via EMT, represent an ongoing fibrotic process, and the presence of large numbers of FSP1+ cells indicates a need for more intensive therapy, using additional immunosuppressive drugs, from the outset.

Conclusion

Our findings indicate that the renal FSP1+ cell number is a novel marker to guide steroid treatment in IgAN patients. However, further prospective studies will be needed to fully define the role of FSP1+ cells in the treatment strategy for patients with IgAN.

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Conflict of interest statement. None declared. The results were presented in 2006 Renal Week held in San Diego, CA, USA.

References


Table 4. Univariate Cox regression analysis of predictive factors affecting steroid resistance

<table>
<thead>
<tr>
<th>Variables</th>
<th>Risk ratio (95%-CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary excretion of protein for 24 h (≥ 3.0 g/day)</td>
<td>1.72 (0.52–5.72)</td>
<td>0.3791</td>
</tr>
<tr>
<td>Estimated GFR (&lt;60 ml/min/1.73 m²)</td>
<td>9.63 (1.24–74.7)</td>
<td>0.0302</td>
</tr>
<tr>
<td>Histological parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severity of mesangial proliferation (severity score ≥1)</td>
<td>2.97 (0.64–13.8)</td>
<td>0.1654</td>
</tr>
<tr>
<td>Extent of interstitial damage and chronic inflammation (score ≥2)</td>
<td>6.19 (1.83–20.9)</td>
<td>0.0033</td>
</tr>
<tr>
<td>%Global or segmental sclerosis (≥50.0%)</td>
<td>10.9 (2.69–43.9)</td>
<td>0.0008</td>
</tr>
<tr>
<td>FSP1+ cell (≥32.6/HPF)</td>
<td>23.5 (4.80–115)</td>
<td>&lt;0.0001</td>
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