Low glomerular density is a risk factor for progression in idiopathic membranous nephropathy

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Abstract

Background. The adverse histological features predicting a progressive loss of renal function in idiopathic membranous nephropathy (IMN), before the establishment of impaired renal function with advanced glomerulosclerosis and/or interstitial fibrosis, are still poorly understood. The present study examined the relationship between the glomerular density (GD; non-sclerotic glomerular number/renal cortical area of biopsy) and the renal prognosis in IMN patients, especially in those without any apparent renal dysfunction at the time of diagnosis.

Methods. The predictive value of the factors at biopsy, including the GD, on the renal outcome was retrospectively analyzed in the 65 IMN patients with an estimated glomerular filtration rate (eGFR) of ≥60 mL/min/1.73m² (mean, 80 mL/min/1.73m²) at biopsy.

Results. The individual values for GD ranged from 1.6 to 6.5/mm² with 4-fold variation. A lower GD was associated with progression based on a ≥50% reduction in eGFR or reaching to end-stage renal disease. An association between a lower GD and progression was observed, especially in patients with persistent proteinuria of ≥1 g/day at follow-up. In contrast, any patients who achieved proteinuria of <1 g/day at follow-up did not show progression regardless of their GD levels. In addition, among the various clinicopathological factors observed, the GD was the only factor at biopsy that independently predicted the slope of the renal function during the observation periods.

Conclusion. These results suggest that low GD is a plausible risk factor for progression in IMN patients, especially in those that do not achieve a remission of proteinuria during the follow-up.

Keywords: glomerular density; membranous nephropathy; remission; renal biopsy; renal outcome

Introduction

Idiopathic membranous nephropathy (IMN) is the second most common cause of end-stage renal disease (ESRD) in

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adults with primary glomerulonephritis [1, 2]. Previous studies report that the long-term outcome of IMN is quite variable between individuals [3]. It is often resistant to immunosuppressive drugs, resulting in ESRD, whereas spontaneous complete remission occurs in many patients [4, 5]. Various clinical and histological parameters, such as heavy proteinuria, reduced renal function at diagnosis, development of tubulointerstitial lesions or the presence of segmental glomerular sclerosis in a biopsy, have been proposed as independent risk factors for progression toward renal failure in IMN [6–12]. However, such factors as an impaired renal function and/or severe chronic lesions actually characterize already advanced renal injury rather than reflect the progression rate of renal diseases [13].

Patients with IMN achieving either complete or partial remission show a significantly reduced risk for progression, even though they had nephrotic range proteinuria at onset [14]. On the other hand, a recent study found that a significant number of the patients with IMN showing subnephrotic range proteinuria at onset subsequently develop nephrotic-range proteinuria and then progress more rapidly in comparison to those who continued to have subnephrotic proteinuria [15]. These results suggest that it is difficult to predict the renal outcome in IMN using only the severity of proteinuria at the time of diagnosis. The time-dependent predictors, such as the amount of proteinuria over time or spontaneous remission, are more reliable [4, 5, 14]. However, this means that it takes months or even years before therapeutic decisions can be made, with a risk of the progression of chronic renal injuries. Therefore, reliable early predictors are still needed to ensure that potent immunosuppressive treatment can target patients who are at the highest risk of progression.

Our recent studies have demonstrated the predictive value of glomerular density (GD; non-sclerotic glomerular number per renal cortical area of biopsy) on the renal outcome in patients with IgA nephropathy (IgAN) [16, 17]. Interestingly, the individual value of GD in a biopsy showed approximately a 7-fold variation, even though patients with any apparent renal dysfunction at the time of biopsy were excluded from the analysis. In addition, the results showed that low GD was a plausible independent predictor of progression in those patients. These findings suggest that GD could be used as an important histological predictor of the final outcome of progressive renal diseases. The present study examined the relationship between the GD in a diagnostic biopsy and the renal outcome of the patients with IMN to determine the prognostic value of the GD as a common mediator of progression in chronic renal diseases.

Materials and methods

The selection of the patients

This study included patients with IMN that underwent renal biopsies at theJikei Hospital, Tokyo, during the period from 1972 to 2004, and were followed for >5 years. Patients with secondary membranous nephropathies, such as lupus nephritis or membranous nephropathies related to hepatitis B virus or malignancy, were excluded. Any patients with a moderately impaired renal function [estimated glomerular filtration rate (eGFR) <60 mL/min/1.73m²] at the time of the biopsy and those whose renal tissue specimens contained <10 glomeruli (including globally sclerotic glomeruli) were also excluded.

Ninety-two IMN patients who were followed for >5 years from the time of diagnosis or who had reached ESRD due to the progression of IMN were recruited from the outpatient population at this hospital. Twenty-three of these 92 patients showed impaired renal function at the time of diagnosis (eGFR <60 mL/min/1.73m²) and thus were excluded. Four of remaining 69 renal biopsy samples contained <10 glomeruli and thus were excluded. Finally, 65 biopsies from 65 patients were recruited for this study. All the 65 patients were Japanese. In this study population, some patients had a moderate percentage of global sclerosis (13 of 65 patients had 10–20% global sclerosis) and one patient had a significant percentage (30%) of global sclerosis.

Definitions

The eGFR was calculated by applying a modified three-variable equation for estimating the GFR for Japanese [15]: eGFR = 194 × Age−0.287 × sCr−1.094 × (0.739 if female), where sCr is the serum creatinine. Hypertension was defined as systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or usage of antihypertensive medication. Progression was defined as the patients who had achieved a ≥50% reduction in the eGFR from the baseline or the patients who had to undergo a renal replacement therapy. In addition, the percent change in the eGFR from the baseline per year (ΔeGFR) was used to express the progression as the slope of the renal function since the duration of follow-up varied in the patients. The eGFR was calculated by applying the formula: ΔeGFR (%/years) = [eGFR at the last observation] – [eGFR at biopsy]) × 100(eGFR at biopsy) × (the time of observation in years). Improvement of clinical status was defined as a urinary-protein excretion (UPE) of ≤1 g/day according to the criterion of good long-term renal outcome in the Japanese cooperative study that analyzed the largest cohort of the patients with IMN [4]. The cutoff value of GD for the comparison of the findings between the patients with lower GD and those with higher GD was the median of GD in this study population, i.e. 3.1/mm².

Pathological analysis

All kidney tissue specimens were obtained by performing a percutaneous needle biopsy. The tissues were embedded in paraffin, cut into 3- to 4-μm sections and stained with hematoxylin–eosin, periodic acid-Schiff, Masson’s trichrome and periodic acid-methenamine silver stain. The百分ages of the glomeruli affected by global or segmental sclerosis were assessed. Sclerosis was defined as the obliteration of the capillary lumen by increased extracellular matrices, with or without hyalinosis or foam cells. Global glomerular sclerosis was defined when the entire glomerulus was involved in the sclerosis, and segmental sclerosis was defined when any amount of the glomerulus was involved in the sclerosis, but the entire glomerulus was not affected. The area of interstitial fibrosis/tubular atrophy (IF/TA) was quantitatively evaluated using a computed imaging analyzer (Scion Image; Scion Corporation, Frederick, MD) according to the percentage of cortical area involvement. Masson’s trichrome staining was used for this analysis, and the area of interstitial fibrosis with atrophic tubules was measured. In the measurement of the IF/TA, glomerular area was included, but the immediate subcapsular area and the larger vessels at the cortico-medullary junction were not included. We also performed a semiquantitative measurement of the IF/TA that considered the area percentage of the cortex occupied by fibrous tissue and atrophic tubules. A semiquantitative evaluation of the IF/TA was done to the nearest 5% by NT and TK independently without prior knowledge of the clinical and patient information. The inter-observer variability was good (r = 0.793), and the data shown are the averaged scores. The results of IF/TA using this semiquantitative method were similar to those of the quantitative method (Supplementary figure S1). The GD was determined by calculating the number of glomeruli that were not globally sclerotic per total renal cortical area, which was measured using a computed imaging analyzer (Scion Image). The segmentally sclerotic glomeruli were included in the calculation of the GD. We also simply measured the length and width of the biopsy on the glass slide and determined the GD. The results using such an alternative method were similar to those obtained by an image analysis (Supplementary figure S2).

Statistical analysis

The continuous variables are expressed as the mean ± SD. The variables were assessed for normality both visually (normal probability plot) and by inferential statistics (Shapiro–Wilk W and Kolmogorov–Smirnov tests). For continuous variables, the Wilcoxon rank-sum test was utilized to assess the data for significant differences among groups. Categorical variables were
expressed as a percentage and compared using the chi-square test. A univariate or the multivariate regression analysis was applied to determine the relationship between the continuous variables and the GFR. All clinically relevant parameters were included in the analysis. Because the distribution of the UPE and the global/segmental sclerosis were skewed, these variables were log transformed before performing both univariate and multivariate regression analyses. Values of $P < 0.05$ were considered to be statistically significant. All statistical analyses were performed using the SPSS software package.

**Results**

**The baseline characteristics**

The baseline characteristics of the patients are summarized in Table 1. The amount of proteinuria at biopsy varied between the individuals. The majority of the patients showed only mild degrees of segmental/global glomerular sclerosis and IF/TA. The variation in the GD between the individuals was >4-fold. The median GD was 3.1/mm$^2$ in this study population, which was almost the same level with that of patients with IgAN, i.e. 3.0/mm$^2$ [17].

**The clinical findings during the follow-up**

The clinical findings during the follow-up are summarized in Table 2. Forty-two percent of the patients were treated with corticosteroids and/or immunosuppressants during the observation periods, and 86% of the patients were treated with either angiotensin-converting enzyme inhibitor (ACE-I) or angiotensin receptor blocker (ARB) or both. Forty percent of the patients showed UPE of $\geq 1$ g/day at the time of the last observation. The mean eGFR was $-3.8\%$ per year, but it was highly variable between the individuals. Fifteen and 8% of the patients achieved a $\geq 50\%$ reduction in the eGFR and ESRD, respectively, at the time of last observation.

**Comparison of the clinicopathological findings and renal outcome between the patients with lower GD and those with higher GD**

The association between the GD and the clinicopathological findings at biopsy and renal prognosis was examined by comparing them in the patients with lower GD ($<3.1/mm^2$) and those with higher GD ($\geq 3.1/mm^2$) (Table 3). The age, renal function, amount of UPE, frequencies of the patients with nephrotic-range proteinuria (UPE $\geq 3.5$ g/day), hypertension, degree of global/segmental glomerular sclerosis and degree of IF/TA at the time of the biopsy in the patients with lower GD were not significantly different than in those with higher GD. Furthermore, the duration of follow-up and frequencies of the patients treated with immunosuppressants and ACE-I/ARB during follow-up showed no difference between the groups. However, the frequencies of the patients with UPE of $\geq 1$ g/day, those that achieved a $\geq 50\%$ reduction in eGFR and those with ESRD at the last observation, were significantly higher in the patients with lower GD than those with higher GD. Similar results were obtained in the analysis of the patients who were treated with immunosuppressants during the follow-up (Supplementary table S1). Similar results were also obtained using the GD with other definitions that included global sclerosis or without the area of IF/TA (data not shown).

**The effects of persistent proteinuria on the progression in relation to the GD levels**

Both of the patient groups with lower GD and higher GD were further divided into two groups, i.e. patients with UPE $<1$ g/day at follow-up and those with UPE $\geq 1$ g/day at follow-up, respectively, to further examine whether the incidence of progression could be influenced by the presence or absence of an improvement of proteinuria. Figure 1 demonstrates that the incidence of progression was significantly higher in those with lower GD than those with higher GD in patients with UPE $\geq 1$ g/day (56 versus 13%, $P < 0.05$). In contrast, any patients who achieved proteinuria $<1$ g/day did not show progression regardless of their GD levels.

**A univariate and the multivariate analysis of the factors associated with the slope of renal function**

The continuous variables at biopsy were tested for the association with the slope of renal function ($\Delta eGFR$). Table 4 shows that only the GD was a factor that significantly associated with the slope in the univariate analyses. The GD remained a factor that was significantly associated with the slope according to a multivariate analysis. Figure 2 shows the relationship between the GD and the $\Delta eGFR$. The patients with a low GD were frequently associated with a steeper slope of the renal function, whereas most of the

### Table 1. Clinicopathological findings at biopsy

<table>
<thead>
<tr>
<th>Clinical</th>
<th>Number of patients</th>
<th>Age (years)</th>
<th>Gender (%male)</th>
<th>eGFR (mL/min/1.73m$^2$)</th>
<th>UPE (g/day)</th>
<th>Serum albumin (g/dL)</th>
<th>Hypertension (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>65</td>
<td>56 ± 14</td>
<td>68</td>
<td>80 ± 19 (60–141)</td>
<td>3.1 ± 2.4 (0.3–13.4)</td>
<td>3.2 ± 0.7 (1.7–4.6)</td>
<td>38</td>
</tr>
<tr>
<td>Histopathological</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total cortical area</td>
<td></td>
<td>5.9 ± 2.7 (1.9–14.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total glomerular number</td>
<td></td>
<td>25 ± 11 (10–58)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Global glomerular sclerosis (%)</td>
<td></td>
<td>5.1 ± 6.3 (0–30)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Segmental glomerular sclerosis (%)</td>
<td></td>
<td>0.7 ± 2.6 (0–17)</td>
<td></td>
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</tr>
<tr>
<td>IF/TA; semiquantitative (%)</td>
<td></td>
<td>9.0 ± 6.0 (0–27.7)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>IF/TA; quantitative (%)</td>
<td></td>
<td>11.2 ± 8.1 (0–37.1)</td>
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<tr>
<td>GD (mm$^2$)</td>
<td></td>
<td>3.4 ± 1.1 (1.6–6.5)</td>
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</tr>
</tbody>
</table>

### Table 2. Clinical findings during the follow-up

| Duration of follow-up (years) | 8.1 ± 3.8 (5–24) |
| Therapies during the follow-up | 42 |
| Immunosuppressants (%) | 86 |
| ACE-I/ARB (%) | 40 |
| Last observation | 61 ± 28 (3–129) |
| UPE (g/day) | $-3.8 ± 5.6 (-32.8–2.8$) |
| UPE $>1$ g/day (%) | 15 |
| ESRD (%) | 8 |
patients with a high GD showed a lower gradient of renal function. Similar results were obtained using the GD with other definitions that included global sclerosis or without the area of IF/TA (data not shown).

**Discussion**

Although a number of clinical and histological markers are associated with an increased risk of progression to renal failure in IMN, it is still difficult to recognize the long-term outcome for a patient that presents with IMN. This study asked whether the GD observed in a diagnostic renal biopsy could predict the renal outcome in the patients with IMN. The results suggest that low GD is a plausible predictor of progression in IMN patients, especially in those without any apparent renal dysfunction at the time of a diagnostic renal biopsy. A similar result of the relationship was found between the GD and the renal outcome in the analysis of patients with IgAN [16, 17]. These results, therefore, suggest the prognostic value of the GD as a common mediator of progression in chronic renal diseases.

The results from recent observational studies suggest that proteinuria during the follow-up, rather than that at onset, has a significant impact on the renal outcome in IMN [4, 5, 14]. Indeed, the severity of proteinuria at the time of diagnosis was not associated with GFR in the current study. In
GD and progression of IMN

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>P</td>
</tr>
<tr>
<td>Age (years)</td>
<td>-0.200</td>
<td>0.110</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>0.145</td>
<td>0.249</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>-0.134</td>
<td>0.288</td>
</tr>
<tr>
<td>UPE (g/day)</td>
<td>-0.112</td>
<td>0.375</td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>0.130</td>
<td>0.303</td>
</tr>
<tr>
<td>Global glomerular sclerosis (%)a</td>
<td>-0.134</td>
<td>0.286</td>
</tr>
<tr>
<td>Segmental glomerular sclerosis (%)</td>
<td>-0.119</td>
<td>0.345</td>
</tr>
<tr>
<td>IF/TA; quantitative (%)</td>
<td>0.127</td>
<td>0.313</td>
</tr>
<tr>
<td>GD (/mm²)</td>
<td>0.319</td>
<td>0.010</td>
</tr>
</tbody>
</table>

*aVariables were log transformed before analysis.

Fig. 2. Relationship between the GD and the slope of the renal function (ΔeGFR). The patients with a lower GD frequently had a steeper slope of the renal function during the long-term follow-up.

contrast, persistent proteinuria of ≥1 g/day during follow-up was apparently associated with a poor renal outcome in the same patient population. Notably, a low GD was frequently associated with progression and this association was synergistically enhanced by a UPE ≥1 g/day at follow-up. These results suggest that both a low GD and persistent proteinuria during the follow-up independently contribute to the progression of IMN, at least in part, by different mechanisms. The rate of the patients with persistent proteinuria (≥1 g/day) was significantly higher in the patients with lower GD than those with higher GD. Such a difference may be partially explained by the fact that a significantly larger number of patients with lower GD already have advanced renal failure at the last observation.

Although not statistically significant, the patients in the low GD group seemed to have relatively worse renal injuries than the high GD group, especially in their degree of global glomerular sclerosis. This result was likely obtained because the patients with moderately advanced glomerulosclerosis, due to a progressive loss of functioning nephrons, tended to be included in the low GD group. To exclude the influence of such already advanced renal injuries, more precisely determined inclusion criteria, such as only patients with global glomerulosclerosis of <10%, would thus be required. However, we did not exclude the patients with moderately advanced glomerulosclerosis or IF/TA from the present study because there is no formal definition of what percentage of global sclerosis represents compensated renal impairment. In addition, such compensation would be differently determined for each individual with a different GD.

Previous studies have suggested the development of tubulointerstitial lesions [7, 8] or segmental glomerular sclerosis [9, 10] as significant predictors of progression to renal failure in IMN. However, these two factors at the time of biopsy were not associated with the progression in the current series (Table 4 and Supplementary table S2). This was probably because the patients with moderately impaired renal function were excluded from the analysis, and none of the patients showed severely advanced IF/TA or segmental glomerular sclerosis that were simply associated with renal dysfunction. Nevertheless, our present results do not refute previous reports showing that the degree of IF/TA or segmental glomerular sclerosis predicts the renal outcome in IMN patients.

A detailed study using serial renal biopsies of progressive IMN patients suggested that hyperfiltration induced by loss of functioning nephrons as well as impaired hydraulic permeability plays a crucial role in the progressive loss of renal function [19]. On the other hand, a low nephron number is associated with low birth weight, which has also been implicated in the poor outcome of glomerular diseases including IMN [20–24]. These observations suggest that a reduction in nephron number, either acquired or later in life, may have a significant impact on the renal outcome in IMN. The present results showing a relationship between the lower GD in a biopsy specimen and the higher incidence of progression in IMN may indirectly support this possibility. Finally, there were no significant differences in the usage of either immunosuppressants or ACE-I/ARB between the patients with lower GD and those with higher GD. However, since this study did not include therapeutic intervention, it is difficult to interpret the relationship between the GD and the effects of the therapies in each individual.

Supplementary data

Supplementary data are available online at http://ndt.oxfordjournals.org.

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Conflict of interest statement. None declared.

(See related article by Fogo. Relativity and the kidney: observations regarding glomerular density. Nephrol Dial Transplant 2011; 26: 3425–3426.)

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The impact of self-management support on the progression of chronic kidney disease—a prospective randomized controlled trial

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Abstract
Background. Chronic kidney disease (CKD) is a public health problem worldwide. Multidisciplinary intervention helps improve outcomes for CKD patients. We conducted an open-label, randomized controlled trial to examine the impact of self-management support (SMS) in the outcome of late-stage CKD patients.

Methods. Incidental CKD (Stages III–V) patients were randomized into self-management support (SMS) and non-SMS groups and followed up for 12 months. SMS comprised health information, patient education, telephone-based support and the aid of a support group. The primary end points were absolute estimated glomerular filtration rate (eGFR) alteration and number of hospitalization...