Maximal glomerular diameter as a 10-year prognostic indicator for IgA nephropathy

Hiroshi Kataoka¹, Mamiko Ohara², Kazuho Honda³, Takahiro Mochizuki² and Kosaku Nitta¹

¹Department of Medicine, Kidney Center, Tokyo Women’s Medical University, Tokyo, Japan, ²Department of Nephrology, Kameda Medical Center, Chiba, Japan and ³Department of Pathology II, Tokyo Women’s Medical University, Tokyo, Japan

Correspondence and offprint requests to: Kosaku Nitta; E-mail: knitta@kc.twmu.ac.jp

Abstract

Background. Although there have been many reports on clinicopathological studies of immunoglobulin A nephropathy (IgAN), reliable outcome predictors are still lacking. We therefore assessed maximal glomerular diameter (Max GD), an indicator of glomerular size, as a predictor of the long-term evolution of renal histopathology.

Methods. Forty-three adult patients, diagnosed with IgAN, who had estimated glomerular filtration rate (eGFR) ≥50 mL/min/1.73 m², were enrolled in this study. Prognostic variables for renal survival were examined by using the multivariate Cox proportional hazards method. The optimal cut-off value of Max GD was 242.3 μm (AUC = 0.78, sensitivity = 62.5%, specificity = 81.5%) by using receiver operating characteristics analysis. In order to assess the characteristics of glomerular hypertrophy, we divided the cases into two groups according to the Max GD value (Group A, ≥242 μm; Group B, <242 μm). Renal survival was also assessed by Kaplan–Meier curves with the log-rank test.

Results. The Max GD was significantly correlated with age, body mass index and serum triglyceride levels at the time of renal biopsy. During the 10-year follow-up period, the Max GD was significantly correlated with eGFR decline per year, and proteinuria, but not with hematuria. A multiple regression analysis by the Cox method adjusted for age, sex and eGFR showed that the Max GD values were significantly associated with a 1.5-fold increase in serum creatinine (Cr) values (hazard ratio = 1.04, P = 0.03). Renal function in 66.7% of the patients whose Max GD was ≥242 μm had at least a 1.5-fold increase in their serum Cr value at the 10-year follow-up examination (log-rank, P = 0.003).

Conclusions. The results of this study suggest that Max GD is a simple quantitative prognostic indicator of the disease progression in IgAN patients.

Keywords: glomerular hypertrophy; glomerular size; IgA nephropathy; prognosis; renal biopsy

Introduction

Immunoglobulin A nephropathy (IgAN) is the most common form of primary glomerulonephritis, and a major cause of end-stage renal failure worldwide [1]. Recent studies have shown that 30–40% of patients progress to end-stage renal failure within 10–25 years [2, 3]. Identifying factors that accelerate disease progression is extremely important, particularly if they can be modified by treatment. Advanced age, hypertension, proteinuria and impaired renal function at presentation are known to be indicators of a poor prognosis [4, 5]. Several studies have investigated the histological features of IgAN in renal biopsy specimens for possible correlation with the clinical outcome [6–9], and most of them consistently found that advanced glomerulosclerosis and interstitial fibrosis with tubular atrophy are indicators of a poor prognosis in IgAN. Roufosse and Cook [10] have recently reviewed the classifications of individual histological characteristics, each ascribed a semiquantitative score, using routine histological examination or morphometric methods or both. Most of them consistently found that advanced glomerulosclerosis and interstitial fibrosis with tubular atrophy are indicators of a poor prognosis in IgAN. However, parameters that indicate advanced chronic disease cannot be the target of treatment.

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The lack of consensus on the histological prognostic indicators of IgAN has led to an abundance of studies that have used unique combinations of a variable number of individual parameters, with their own definitions and semiquantitative scoring system, and sometimes a unique combination of individual semiquantitative scores to ascribe global compartment scores as well as a global biopsy score [11]. On the other hand, large glomeruli are a common finding in the early stages of renal disease in populations at high risk of renal failure, such as blacks, Pima Indians and Aboriginal Australians [12–14]. These observations have led to the hypothesis that kidneys in which the glomeruli are enlarged are more susceptible to subsequent renal injury and functional decline [15].

We therefore assessed maximal glomerular diameter (Max GD) as a representative index of glomerular size as a marker of a predictor of the long-term evolution of a renal histopathology.

Materials and methods

Patient selection

We reviewed 61 adult cases of biopsy-confirmed IgAN diagnosed between March 1993 and September 1998 at Kameda Medical Center. The inclusion criteria were: (i) estimated glomerular filtration rate (eGFR) ≥50 mL/min/1.73 m$^2$ (which excluded three patients), (ii) duration of follow-up ≥10 years (which excluded 13 patients), (iii) absence of any other renal disease (which excluded 2 patients), (iv) a renal biopsy specimen that contained ≥5 glomeruli (which excluded none of the patients). As a result, 43 cases were ultimately enrolled in the present study. eGFR was calculated by using the formula for Japanese patients as previously described [16].

The clinical parameters assessed at the time of the renal biopsy were age, gender, mean arterial blood pressure (MAP), body mass index (BMI), duration of symptoms, proteinuria (grams per day), hematuria (0–3), eGFR and serum levels of creatinine (Cr), uric acid, triglyceride and total cholesterol and the presence of nephrotic syndrome. We also reviewed treatment regimens after the renal biopsy and the clinical data at the time of the 10-year follow-up examination including the level of proteinuria (0–4) and hematuria (0–3), MAP and BMI. Follow-up examinations were done every 6 months.

Pathological analysis

All kidney tissue specimens were obtained by percutaneous needle biopsy. The tissue was embedded in paraffin, cut into 3-μm sections and stained with hematoxylin–eosin, periodic acid-Schiff stain, Masson’s trichrome stain and periodic acid-methenamine stain. Each specimen was evaluated for glomerular, interstitial and vascular changes as previously described [17]. Two independent observers without any knowledge of the clinical data semiquantitatively scored the pathological findings. Mesangial cell proliferation in each patient was scored according to four degrees of severity: 0, no abnormality; 1, mild; 2, moderate and 3, severe. The specimens were also examined for the presence of endocapillary hypercellularity and extracapillary cellular proliferation.

The percentage of glomeruli that exhibited global sclerosis and the percentage that exhibited segmental sclerosis were estimated. Arterio-arteriolar sclerotic changes were also scored on a similar scale: 0, no abnormality; 1, mild; 2, moderate and 3, severe. The extent of interstitial fibrosis was semiquantitatively scored according to the proportion of fibrotic lesions to the total cortical area: 0, less than 5% of total cortical area; 1, 5–20%; 2, 20–40% and 3, >40%.

We also assessed the maximal glomerular area (Max GA) and the Max GD of the maximally hypertrophied glomerulus in each specimen. Max GA was identified in serial sections, and the Max GD was calculated as the mean of two measurements, i.e. of the maximal diameter of the glomerulus and the maximal chord perpendicular to the maximal diameter (Figure 1). A computer system connected to a camera (DP20; Olympus, Tokyo, Japan) and a microscope (BX51, Olympus) was used to make the measure-ments. Images were analyzed with a computer software program (DP2-BSW; Olympus).

Statistical analysis

The results are expressed as the mean ± SD and compared by means of unpaired t-test or chi-square test, as required. Our principal goal was to investigate if Max GD is a prognostic indicator of IgAN. Simple and multiple linear regression analyses were performed to assess the relationships between continuous variables. Proportional hazard assumptions were confirmed by visual inspection and confirmed that plotting kidney function versus survival time yielded a graph with parallel curves and plotting the log (−log[survival]) versus the log of renal survival time yielded a graph whose lines were parallel. Receiver operating characteristic (ROC) analysis is a method of evaluating the accuracy of a diagnostic test by summarizing the ability of the test to discriminate between the absence and the presence of an abnormal finding. In the context of the present study, the optimal cut-off value refers to the ability of Max GD to discriminate between IgAN patients according to whether their serum Cr value increased ≥1.5-fold. Prognostic variables for renal survival were examined by using the Cox proportional hazards method. P-values <0.05 were considered statistically significant. All statistical analyses were performed by using the StatView 5.0 (SAS Institute Inc., Cary, NC) or the GraphPad Prism4 (GraphPad Software Inc., Tokyo, Japan) software program.

Results

Patients

Between 1993 and 1998, we performed a renal biopsy in 61 patients diagnosed with primary IgAN in order to evaluate the histopathological changes in their kidneys. After excluding the 18 of the 61 patients who did not meet the inclusion criteria described above, 43 of the IgAN patients were eligible for inclusion as subject of this study. The 43 patients consisted of 26 males and 17 females, and their mean age at the time of the renal biopsy was 40.0 ± 10.3 years (range 15–65 years). Average time from onset to biopsy was 80.1 ± 88.9 months. The average value of MAP was 102.6 ± 16.8 mmHg, BMI was 25.4 ± 4.3 kg/m$^2$, proteinuria was 1.8 ± 1.5 g/day and eGFR was 78.3 ± 17.8 ml/min/1.73 m$^2$.

The follow-up period of all the patients was 10 years. Of the 43 patients, 22 had been treated with a corticosteroid and 25 with either an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker during the follow-up period. None of the 43 patients had received any...
medication for the treatment of IgAN before the renal biopsy. The rate of progression as measured by eGFR decline was 2.9 ± 2.4 mL/min/1.73 m²/year. Sixteen patients had reached a 1.5-fold increase in serum Cr value during the 10-year follow-up.

**Correlations between the Max GD and clinical findings at the renal biopsy and at the time of the 10-year follow-up examination**

The average number of glomeruli examined per subject was 13.4 (range 5–34) and the average number of section examined per subject was 14.4 (range 3–22). The rate of global glomerulosclerosis was 17.4 ± 16.1%. The average value of Max GD was 221.7 ± 30.8 μm. The Max GD values were significantly positively correlated with the Max GA values (r = 0.98, P < 0.0001).

The Max GD values were also tested for correlations with clinical parameters at the time of the renal biopsy. As shown in Table 1, simple regression analysis showed that the Max GD was significantly positively correlated with age (β = 0.38, P = 0.02) and BMI (β = 0.60, P = 0.001). We also examined the relationship between the Max GD and the 10-year follow-up parameters. At the 10-year follow-up examination, simple regression analysis showed a significant association between the Max GD and the level of proteinuria (r = 0.46, P = 0.002, Figure 2), BMI (r = 0.46, P = 0.002) and eGFR decline per year (r = 0.33, P = 0.03), but there were no significant correlations with MAP (r = 0.18, P = 0.30) or hematuria (r = 0.17, P = 0.30), as shown in Table 1.

**The Max GD as a 10-year prognostic indicator for IgA nephropathy**

To determine whether Max GD at the time of the renal biopsy was an independent predictor of a decline in renal function, we performed a multivariate regression analysis based on the Cox hazard model for the association of histological findings with a 1.5-fold increase in serum Cr value during the 10-year follow-up (Table 2). When adjusted for age, sex and eGFR, the results showed that Max GD (hazard ratio = 1.04, P = 0.03) and arteriolosclerosis (hazard ratio = 4.30, P = 0.03) were significantly related to an increase in serum Cr value of ≥1.5-fold. The results of the ROC analysis showed that the optimal cut-off value of Max GD was 242.3 μm (AUC = 0.78, sensitivity = 62.5%, specificity = 81.5%, Figure 3).

We also performed a Kaplan–Meier analysis to assess the renal survival. The end point of the renal survival was a 1.5-fold increase in serum Cr value. According to the renal survival curves, the renal survival rate of the IgAN patients whose Max GD was <242 μm was significantly higher than that of patients whose Max GD was ≥242 μm (Figure 4). Renal function in 66.7% of the patients whose Max GD was ≥242 μm had at least a 1.5-fold increase in their serum Cr value at the 10-year follow-up examination (log-rank, P = 0.003).

**Comparison of clinical and pathological findings between groups divided by Max GD**

In order to assess the characteristics of glomerular hypertrophy, we divided the cases into two groups according to the Max GD value (Group A, ≥242 μm and Group B, <242 μm). The comparison of clinical and laboratory findings at the time of renal biopsy in two groups is summarized in Table 3. BMI in Group A (28.5 ± 4.2 kg/m²) was higher than in Group B (23.7 ± 3.4 kg/m², P = 0.0003), and the level of serum triglyceride in Group A (228.7 ± 201.3 mg/dL) was higher than in Group B (129.8 ± 58.9 mg/dL, P = 0.02).

Table 4 shows the pathological findings in the renal biopsy specimens. Comparison between the renal biopsy findings in Groups A and B revealed that the scores of arteriolosclerosis (1.53 versus 0.71, P = 0.0006), arterio-sclerosis (1.07 versus 0.46, P = 0.01), Max GD (253.1 μm versus 204.9 μm, P < 0.0001) and Max GA (48024 μm² versus 31607 μm², P < 0.0001) were significantly higher in Group A than in Group B. No significant difference was detected in mesangial cell proliferation, endocapillary hypercellularity, extracapillary cellular proliferation, global glomerulosclerosis and segmental glomerulosclerosis.

**Table 1. Correlation between Max GD and clinical findings at the renal biopsy and at the time of the 10-year follow-up examination**

<table>
<thead>
<tr>
<th></th>
<th>Renal biopsy</th>
<th>10-year follow-up</th>
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<tbody>
<tr>
<td></td>
<td>r</td>
<td>P-value</td>
</tr>
<tr>
<td>Proteinuria (0–4)</td>
<td>0.11</td>
<td>0.5</td>
</tr>
<tr>
<td>Hematuria (0–3)</td>
<td>−0.35</td>
<td>0.02</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.42</td>
<td>0.006</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>0.51</td>
<td>0.0005</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>0.21</td>
<td>0.2</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>0.42</td>
<td>0.005</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>−0.34</td>
<td>0.03</td>
</tr>
<tr>
<td>eGFR decline per year (mL/min/1.73 m²/year)</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NA, not applicable.
between the groups. During the 10-year follow-up examination, the Max GD was significantly related to an increase in serum Cr value of \( \geq 1.5 \)-fold \((P = 0.005)\), but not Max GA.

**Discussion**

The aim of this study was to establish a simple, reproducible and quantitative histological prognostic indicator in renal biopsy specimens. The Cox multivariate analysis adjusted for age, sex and eGFR in this study showed that Max GD and arteriolosclerosis were significantly related to a \( \geq 1.5 \)-fold increase in serum Cr value. Considering that the assessment of arteriolosclerosis had a poor interobserver reproducibility \([18]\), Max GD can be a useful indicator of disease progression in IgAN patients. On the other hand, endocapillary hypercellularity and extracapillary cellular proliferation failed to predict disease progression at the time of the 10-year follow-up examination. These findings suggest that acute disease activity at the time of the renal biopsy is not a major determinant of disease progression in IgAN, if patients were treated with adequate therapy.

Histopathological risk factors identified thus far, such as global glomerulosclerosis and interstitial fibrosis, have been reported to explain \(<30\%\) of the decline in GFR in IgAN patients \([10]\). In the Oxford classification of IgAN, four histological markers—mesangial hypercellularity, segmental glomerulosclerosis, endocapillary hypercellularity and tubular atrophy/interstitial fibrosis—were chosen to predict renal outcome \([18, 19]\). Even though all four markers are useful histological markers, and conventional markers of disease progression in IgAN are clinically desired.

On the other hand, it has been well established by a great number of studies that glomerular hypertrophy plays crucial roles in the outcomes of kidney diseases in experimental models \([20–23]\) and in humans \([24–26]\). For example, the presence of glomerular hypertrophy in minimal-change disease is an indicator of progression to focal segmental glomerular sclerosis \([20]\). Glomerular hypertrophy and renal hypertrophy are also seen soon after the onset of type 1 diabetic nephropathy \([21]\). Persistent renal hypertrophy precedes the development of microalbuminuria and a decline in GFR \([22]\). Thus, the presence of glomerular hypertrophy is of great significance in regard to susceptibility to glomerular sclerosis and renal insufficiency. Measurements of glomerular size have been to be valuable to predict the progression of kidney diseases \([27]\). However, many problems exist in regard to the measurement of glomerular size as a means of clinical diagnosis:

**Table 2.** Multivariate Cox hazard model for the association of histological findings with a 1.5-fold increase in serum Cr value during the 10-year follow-up

<table>
<thead>
<tr>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>1.02</td>
<td>0.95–1.10</td>
</tr>
<tr>
<td>Gender (male; 0, 1)</td>
<td>0.14</td>
<td>0.01–1.61</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m²)</td>
<td>0.94</td>
<td>0.89–0.99</td>
</tr>
<tr>
<td>Mesangial cell proliferation (0–3)</td>
<td>0.73</td>
<td>0.27–2.01</td>
</tr>
<tr>
<td>Endocapillary hypercellularity (0, 1)</td>
<td>0.40</td>
<td>0.05–3.61</td>
</tr>
<tr>
<td>Extracapillary cellular proliferation</td>
<td>1.60</td>
<td>0.20–12.89</td>
</tr>
<tr>
<td>Global glomerulosclerosis (%)</td>
<td>0.97</td>
<td>0.91–1.03</td>
</tr>
<tr>
<td>Segmental glomerulosclerosis (%)</td>
<td>1.01</td>
<td>0.93–1.08</td>
</tr>
<tr>
<td>Arteriosclerosis (0–3)</td>
<td>0.36</td>
<td>0.12–1.10</td>
</tr>
<tr>
<td>Arteriolosclerosis (0–3)</td>
<td>4.30</td>
<td>1.17–15.80</td>
</tr>
<tr>
<td>Interstitial fibrosis (0–3)</td>
<td>10.53</td>
<td>0.75–149.01</td>
</tr>
<tr>
<td>MaxGD (µm)</td>
<td>1.04</td>
<td>1.004–1.068</td>
</tr>
</tbody>
</table>

*Model adjusted for age, sex, and eGFR. Max GD, maximum glomerular diameter.

*CI, confidence interval.*
(i) the size of each glomerulus varies over time, (ii) glomeruli of various size (including hypertrophied glomeruli and collapsing glomeruli) are seen within the same kidney, (iii) different techniques are used to measure glomerular volume, (iv) there is an area limitation in needle biopsy and (v) measuring glomerular size is laborious. Because of these problems, it is very difficult to evaluate and measure glomerular size in needle biopsy specimens. Among these, the most important problem in evaluating and measuring glomerular size in biopsy specimens is how to deal with sclerosing and collapsing glomeruli because it includes the problem of selection bias. Since some injured glomeruli increased in size before sclerosing and collapsing, glomerular hypertrophy precedes glomerulosclerosis [28]. That is, injured glomeruli change their sizes to the opposite direction. Non-immunological mechanisms of glomerular injury are thought to be responsible for the pathological process [29].

Besides, not all the glomeruli are injured simultaneously and thus glomerular sizes vary within the same kidney. Our histogram analysis of glomerular diameter revealed the presence of glomeruli in the process of sclerosing and collapsing in the 100–140 μm diameter range before global glomerulosclerosis had occurred (Figure 5). On the other hand, slightly sclerotic and collapsing glomeruli are often difficult to discriminate from normal glomeruli. Sclerosing and collapsing glomeruli and normal glomeruli both seemed to be present in the 160–180 μm range (Figure 5). Thus, it is difficult to identify sclerosing and collapsing glomeruli in biopsy specimens.

Although previous studies have assessed mean glomerular volume, the values of mean glomerular volume hinder the glomerular heterogeneity and include the volume of sclerosing and collapsing glomeruli [30]. In our study, when the cases were divided into two groups according to the 1.5-fold increase in serum Cr value, our subgroup analysis failed to demonstrate a statistical difference in mean glomerular volume between the groups, whereas Max GD in the group with >1.5-fold increase in serum Cr value was statistically larger than in the group without 1.5-fold increase in serum Cr values. We hypothesize that extremely hypertrophied glomeruli imply the presence of glomerular stress and that sclerosed glomeruli indicate only past glomerular stress. On this hypothesis, it is not necessary to look for collapsing and sclerosing glomeruli but to focus on finding hypertrophied glomeruli in pathological prognostic prediction. We therefore measured the diameter of maximally hypertrophied glomeruli in all needle biopsy specimens.

Among the several techniques that are available to measure glomerular volume, we chose the maximal profile area
Max GD values were significantly correlated with the degree of proteinuria at the time of the 10-year follow-up examination, despite the lack of association with proteinuria at the time of the renal biopsy.

We conclude that Max GD, which is a simple quantitative histological parameter in needle biopsy, can be used as a prognostic indicator for disease progression in IgAN patients.

Conflict of interest statement: None declared.

References


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