Total Nephron Number and Single-Nephron Parameters in Patients with IgA Nephropathy

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Key Points

- The first study that estimated total nephron number and related single-nephron parameters in patients with IgA nephropathy.
- Associations of nephron level parameters with CKD stage and clinicopathologic findings were cross-sectionally investigated.
- This study illustrates the feasibility and usefulness of estimating single-nephron dynamics in human glomerulonephritis.

Abstract

Background: Single nephron dynamics in progressive IgA nephropathy (IgAN) have not been studied. We applied novel methodology to explore single nephron parameters in IgAN.

Methods: Non-globally sclerotic glomeruli (NSG) and globally sclerotic glomeruli (GSG) per kidney were estimated using cortical volume assessment via unenhanced computed tomography and biopsy-based stereology. Estimated single-nephron GFR (eSNGFR) and urine protein excretion (SNUPE) were calculated by dividing eGFR and UPE by the number of NSG. Associations with CKD stage and clinicopathologic findings were cross-sectionally investigated.

Results: This study included 245 IgAN patients (mean age 43 y, 62% male, 45% on renin-angiotensin aldosterone system [RAAS] inhibitors pre-biopsy) evaluated at kidney biopsy. CKD stages were 10% CKD1, 43% CKD2, 19% CKD3a, 14% CKD3b and 14% CKD4–5. With advancing CKD stage, NSG decreased from mean 992,000 to 300,000 per kidney whereas GSG increased from median 64,000 to 202,000 per kidney. In multivariable models, advancing CKD stage associated with lower numbers of NSG, higher numbers of GSG, and lower numbers of GSG+NSG, indicating potential resorption of sclerosed glomeruli. In contrast to the higher mean glomerular volume and markedly elevated SNUPE
in advanced CKD, the eSNGFR was largely unaffected by CKD stage. Lower SNGFR associated with Oxford scores for endocapillary hypercellularity and crescents whereas higher SNUPE associated with segmental glomerulosclerosis and tubulointerstitial scarring.

Conclusions: SNUPE emerged as a sensitive biomarker of advancing IgAN. The failure of eSNGFR to increase in response to reduced number of functioning nephrons suggests limited capacity for compensatory hyperfiltration by diseased glomeruli with intrinsic lesions.
Introduction
IgA nephropathy (IgAN) is the most common primary glomerulonephritis worldwide.\textsuperscript{1} Approximately 30–40\% of patients with IgAN will progress to end-stage kidney disease (ESKD) within 20–30 years despite therapeutic interventions.\textsuperscript{2,3} Glomerulonephritis results from the deposition of immunocomplexes containing galactose-deficient IgA1 in the glomerular mesangium, reactive mesangial cell proliferation, variable peripheral capillary wall deposits and endocapillary hypercellularity.\textsuperscript{4} Extracapillary lesions include inflammatory crescents and podocytopathic changes associated with segmental glomerulosclerosis, podocyte injury and depletion, and worsening proteinuria.\textsuperscript{4,5,6} Previous studies have consistently identified impaired kidney function, hypertension, and heavy proteinuria at biopsy as clinical factors predicting worse outcomes in IgAN.\textsuperscript{7,8,9} A proteinuria threshold of more than 1g/day at biopsy has been linked to poor prognosis.\textsuperscript{8,10}

Progressive glomerulosclerosis is a final common pathway to ESKD in chronic nephropathies, in which the abnormalities in single-nephron dynamics are postulated to be critically involved.\textsuperscript{11,12} Early studies used micropuncture to directly assess single nephron filtration dynamics.\textsuperscript{11,13} Animal models of kidney ablation and/or high protein diet demonstrated hyperfunction (hyperfiltration) in individual glomeruli, which was ameliorated by angiotensin-converting inhibitor therapy.\textsuperscript{14,15,16} The renin-angiotensin aldosterone system (RAAS) preferentially vasoconstricts the glomerular efferent arterioles, thereby increasing single-nephron glomerular filtration rate (SNGFR).\textsuperscript{17} Findings from animal studies greatly contributed to the development of RAAS inhibitors to delay progression in patients with CKD, including IgAN.\textsuperscript{18} These studies implicated maladaptive intraglomerular hemodynamics during disease progression, which has not yet been studied in human glomerulonephritis due to the technical difficulty estimating SNGFR in patients.\textsuperscript{19}
Denic et al. recently established a method for estimating the total number of glomeruli in living humans using a combination of contrast-enhanced computed tomography (CT) and biopsy-based stereology, allowing estimation of SNGFR. They showed that total non-sclerotic glomerular number decreases with normal healthy aging, while SNGFR did not vary significantly between different age groups under 70 years of age. To apply this technology to patients with kidney disease, we established a regression equation to estimate kidney cortical volume from measured kidney parenchymal volume (cortex and medulla) in unenhanced CT images. An equation used for estimating cortical volume from parenchymal volume was created based on a mixed cohort of healthy kidney donors and CKD patients including those with CKD stages 4–5, who underwent enhanced and unenhanced CT imaging at the same time. The primary objective of this study was to estimate total glomerular number and single-nephron parameters in different CKD stages at the time of biopsy diagnosis of IgAN. The association of clinical factors at biopsy (CKD stages, hypertension and proteinuria) and histopathological lesions known to predict disease outcomes in IgAN were then studied in relation to these nephron parameters.
Methods

Patient selection

This cross-sectional study included all adult Japanese patients (≥18 years) who underwent native kidney biopsies with diagnosis of primary IgAN at Jikei Hospital, Tokyo, from 2007 to 2017. The sample size was set based on the number of kidney biopsies during the period. Indications for biopsy were kidney functional decline (eGFR <60 mL/min) and/or overt proteinuria (≥0.5 g/day) with or without gross or microscopic hematuria. Diagnosis of IgAN was based on typical histopathological features of mesangial proliferative glomerulonephritis, the presence of dominant or co-dominant glomerular IgA deposition by immunohistochemistry or immunofluorescence, and the presence of electron dense mesangial deposits by electron microscopy. Patients with other systemic diseases associated with glomerular IgA deposition, including IgA vasculitis, liver cirrhosis, and systemic lupus erythematosus were carefully excluded. At our institute, screening evaluation of kidney morphology is routinely performed using unenhanced CT imaging prior to percutaneous kidney biopsy. For this study, exclusion criteria were applied as follows: (i) patients whose CT images were not available within 1 year before kidney biopsy, (ii) patients whose kidney biopsy specimens contained < 5 nonsclerotic glomeruli on light microscopy or a cortical area < 2 mm². Consent was obtained by opting out for individual participants. All participants were provided the opportunity to ask questions and discuss the study.

Definition

Hypertension was defined as a systolic blood pressure of >140 mmHg, a diastolic blood pressure of >90 mmHg, or the use of antihypertensive medications. Patients who were prescribed RAAS inhibitors for renoprotection despite having normal blood pressure were not defined as having hypertension. Body surface area (BSA) was determined by the following equation: BSA (m²) = Weight^{0.425} (kg) × Height^{0.725} (cm) × 71.84 × 10^{-4}. The
estimated glomerular filtration rate (eGFR) was calculated from serum creatinine using a modified equation for GFR based on Japanese individuals: eGFR = 194 × age−0.287 × (serum creatinine)−1.094 (×0.739 if female).

Chronic kidney disease (CKD) stages were defined based on eGFR for Japanese individuals and were classified into 5 categories as follows: CKD1: ≥90, 2: 60 to 89, 3a: 45 to 59, 3b: 30 to 44, and 4–5: <30 mL/min/1.73m², respectively. Creatinine clearance rate (CCr) was measured using serum and urine creatinine concentrations in 24-hour urine collections. The eGFR and CCr values were calculated without adjustment for BSA (units of mL/min) for the estimation of total GFR and eSNGFR. Urinary protein excretion (UPE) was measured using 24-hour urine collection. Severe proteinuria at biopsy was defined as UPE >1g/day.

Pathological analysis

All kidney tissue specimens were obtained by percutaneous needle biopsy. The tissues were embedded in paraffin, cut into 3 µm sections, and stained with hematoxylin-eosin, periodic acid-Schiff, Masson’s trichrome, and periodic acid silver-methenamine. All biopsy samples were stained by immunohistochemistry or immunofluorescence for IgG, IgA, IgM, C3 and C1q. Globally sclerosed glomeruli (GSG) was defined as the entire glomerulus involved by sclerosis. Non-globally sclerotic glomerulus (NSG) was used when there was no sclerosis or sclerosis only involved part of the glomerulus. Glomeruli containing segmental scars or crescents were included among NSG. The cortical area with interstitial fibrosis/tubular atrophy was semi-quantitatively scored to the nearest 10% and average values were estimated across the entirety of each biopsy specimen. Arteriosclerotic lesions and arteriolar hyalinosis were graded as previously described. The Oxford scores for mesangial hypercellularity (M), endocapillary hypercellularity (E), segmental sclerosis or adhesion (S), interstitial fibrosis and tubular atrophy (T) and crescents (C) were determined as described previously.

Morphological measurements
The thickness of the obtained CT images was 5.0 mm. Kidney parenchymal volumes (PV) were measured as previously described using software (ITK-SNAP version 3.6, University of Pennsylvania, Philadelphia, PA, www.itksnap.org) to semi-automatically segment the parenchymal images obtained from unenhanced CT images of both kidneys.\(^{27}\) Estimated kidney cortical volumes were calculated using an equation as follows: estimated cortical volume (cm\(^3\)) = -1.3 (intercept) + 0.71 \times \text{parenchymal volume (cm}\(^3\)).\(^{21}\) An equation used for estimating cortical volume from parenchymal volume is based on a mixed cohort of healthy kidney donors and a variety of stages of CKD patients. Kidney biopsies were semi-automatically analyzed to measure the individual areas of all glomerular capillary tufts and the total area of the obtained renal cortex using image analysis software (Win roof 2017, Mitani Corporation, Tokyo, Japan). Glomerular area was defined as an averaged area described by outer capillary loops of the tuft. Mean glomerular volume was calculated from the measured glomerular area as follows: Mean glomerular volume =

\[
\frac{\beta}{d} \times (\text{mean glomerular area})^\frac{3}{2}, \text{ where } \beta \text{ is a dimensionless shape coefficient (}\beta = 1.382), \text{ and } d \text{ is a size distribution coefficient (}d = 1.01).^{28}\] The volumetric density of non-sclerotic glomeruli (NSG) was determined using the Weibel-Gomez stereological method as follows:

\[
\text{NSG density} = \frac{1}{\beta} \times \sqrt{\frac{\left(\frac{\text{Total number of glomeruli}}{\text{Area of cortex}}\right)^{\frac{3}{2}}}{\frac{\text{Total area of glomeruli}}{\text{Area of cortex}}}}, \text{ where } \beta \text{ is a dimensionless shape coefficient (}\beta = 1.38).^{28,29}\] The volumetric density of GSG was identically calculated: GSG density =

\[
\frac{1}{\beta} \times \sqrt{\frac{\left(\frac{\text{Total number of sclerotic glomeruli}}{\text{Area of cortex}}\right)^{\frac{3}{2}}}{\frac{\text{Total area of sclerotic glomeruli}}{\text{Area of cortex}}}}. \text{ The total number of all glomeruli was estimated based on the sum of non-globally sclerotic and sclerotic glomeruli, as the sum of the non-sclerotic glomerular density and sclerotic glomerular density. The total number of NSG per kidney was calculated by multiplying the estimated cortical volume (mm}\(^3\)) and the volumetric non-sclerotic glomerular density.}^{30}\] The calculated value was divided by 2 for per kidney, by
1.43 for correcting tissue volume shrinkage due to paraffin embedding, and by 1.268 for correcting volume shrinkage due to loss of tissue perfusion pressure. The single-nephron glomerular filtration rate (eSNGFR) and single-nephron UPE (SNUPE) were calculated by dividing total eGFR (mL/min) or total UPE by the total number of NSG in both kidneys.

Statistical analyses

Patients’ characteristics at baseline are presented as mean ± standard deviation (SD) or median (25th–75th percentile) for continuous variables, and frequencies and proportions for categorical variables. Data were log-transformed as appropriate. The Mann–Whitney U test was used to compare continuous variables between two groups. For three or more groups, trends were tested using linear regression or Jonckheere–Terpstra test. To analyze the correlation with each factor, Spearman’s rank correlation coefficient analysis was used. The associations between clinical factors and glomerular number or single-nephron parameters were analyzed using a linear regression model. To rule out the outliers, sensitivity analyses were performed in subpopulations of the study participants. All reported p values were two-sided. P values of <0.05 were considered to be statistically significant. All statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University), a graphical user interface for R (R Foundation for Statistical Computing, version 3.5.2).
Results

Clinicopathological and morphometric findings

Total 245 IgAN patients were included in this study (Figure 1). Table 1 shows the clinicopathological and morphometric findings of the patients at the time of biopsy diagnosis. The biopsies included 239 initial kidney biopsies and six repeat biopsies. Overall, the mean age was 42.6 years, 152 (62.0%) patients were males, and 52 (19.6%) had systemic hypertension. One hundred-eleven patients (45.3%) had been administered RAAS inhibitors before biopsy diagnosis. The initial indications of RAAS inhibitors were as follows; 81 (72.9%) patients were treated for hypertension and/or renoprotection in the presence of hypertension and 30 (27.1%) patients were treated for renoprotection in the absence of hypertension. The specific RAAS inhibitor therapies prescribed are listed in Supplemental Table S1. eGFR was 61 ± 25 mL/min/1.73m$^2$ and UPE was 835 (433–1536) mg/day. One hundred-one patients (41.2%) had UPE ≥1g/day. Histopathology showed varying active and chronic lesions as indicated by Oxford MESTC scores.

Clinicopathological characteristics of each CKD subgroup are separately shown in Table 1. The distribution of CKD stage was 1 (9.8%), 2 (43.3%), 3a (19.2%), 3b (14.3%), and 4–5 (13.5%). With advancing CKD stages, the characteristics of the patients shifted to older ages, more systemic hypertension, heavier proteinuria and more chronic histopathological lesions.

Comparisons of total glomerular number and single-nephron parameters among different CKD stages

Total glomerular number and single-nephron parameters for the CKD stage subgroups are shown in Figure 2. With advancing CKD stages, trend tests showed the number of NSG to decrease, the number of GSG to increase, and the combined number of NSG and GSG to decrease, respectively. Mean glomerular volume showed a trend to increase with advancing
CKD stages. The eSNGFR showed a slight change with CKD progression, but no statistically significant trends between CKD stages, which was in sharp contrast to the profoundly increased SNUPE levels identified with advancing CKD stages. Similar associations with total glomerular number and single-nephron parameters were obtained in sub-analyses restricted to patients who had not been administered RAAS inhibitors before biopsy (Supplemental Figure S1, S2). Comparisons of total glomerular number and single-nephron parameters among patients treated with or without RAAS inhibitors prior to biopsy diagnosis are shown in Supplemental Figure S3. Patients treated with RAAS inhibitors had fewer numbers of NSG and larger numbers of GSG at biopsy and showed larger glomeruli, higher eSNGFR and higher SNUPE as compared to those without RAAS inhibitors.

Univariable and multivariable linear regression models for clinical factors associated with total glomerular number and single-nephron parameters

Table 2 shows univariable and multivariable linear regression models that analyzed the effects of clinical factors, including CKD stage, hypertension and proteinuria (UPE ≥1g/day) at biopsy on total glomerular number and single-nephron parameters. In univariable models, more advanced CKD was associated with lower numbers of NSG, higher numbers of GSG and lower numbers of GSG + NSG, higher mean glomerular volume and higher SNUPE. All of these variables associating with advancing CKD stages in univariate analyses were significant in multivariable models adjusted for age, sex and BSA. Hypertension was associated with lower numbers of NSG, lower numbers of GSG + NSG, higher eSNGFR and higher SNUPE in univariable models and was associated with lower numbers of GSG + NSG and higher eSNGFR in the multivariable models. Proteinuria was associated with lower numbers of NSG, lower numbers of GSG + NSG, higher mean glomerular volume and higher SNUPE in univariable models and was associated with higher SNUPE in multivariable models. NSG, GGS and GSG + NSG were closely associated with eGFR and age in linear
regression models (Supplemental Table S2). In the sub-analyses restricted to patients not receiving RAAS inhibitors, similar results were obtained (Supplemental Table S3). The simple linear estimates of age-dependent reduction rate in the total numbers of NSG in the present study of IgAN patients was greater than those for healthy donors (Supplemental Figure S4).

Effects of hypertension on total glomerular number and single-nephron parameters in relation to CKD stage at biopsy diagnosis

Figure 3 shows total glomerular number and single-nephron parameters in patients categorized based on CKD stage (stage 1–2 vs. 3–5) and presence or absence of hypertension at biopsy. Hypertension was associated with lower numbers of NSG and GSG + NSG than normotension, higher eSNGFR levels in patients with CKD stage 1–2, and higher SNUPE levels in patients with CKD stage 3–5. In the sub-analyses restricted to patients not receiving RAAS inhibitors, similar results were obtained (Supplemental Figure S5). Comparisons in patients categorized based on CKD stage and presence or absence of UPE ≥1g/day at biopsy did not show any differences in total glomerular number and single-nephron parameters, except that UPE ≥1g/day was associated with higher SNUPE in patients with CKD stage 3–5 (Supplemental Figure S6).

Comparisons of single-nephron parameters among patients with or without histopathological lesions based on Oxford classification criteria

Figure 4 shows comparisons of single-nephron parameters in patients categorized based on the presence or absence of lesions defined by Oxford MESTC scores. Mean glomerular volumes were not different among patients with or without each MESTC component. The eSNGFR was significantly lower in the presence of E and C lesions. The SNUPE was significantly higher in the presence of S and T lesions. Comparisons of total single-nephron parameters among patients with or without kidney functional decline or histopathological
lesions defined by Oxford MESTC scores are shown in **Supplemental Figure S7**. Univariate linear regression models for the estimations of NSG and GGS per % or grade changes in each renal histopathological variable are shown in **Supplemental Table S4**.

**Sensitivity analyses**

Sensitivity analyses were performed in patients whose number of NSG was within the 5–95th percentile of the overall values (n = 221) or in patients whose biopsy specimens contained a cortical area ≥4 mm² (n = 233). Sensitivity analyses for eSNGFR were performed in patients whose eSNGFR values were within the 5–95th percentile of the overall values (n = 221). The results were similar to those of the original study population (**Supplemental Table S5**, **Supplemental Figure S8**).
Discussion

In this study, we estimated the total number of glomeruli with and without global glomerulosclerosis in patients with biopsy-proven IgAN. To estimate glomerular numbers, we employed a newly established method that combines unenhanced CT scan images and biopsy-based stereology. This approach has enabled us to estimate total glomerular numbers in renal disease patients who are often not suitable candidates for contrast media.\textsuperscript{32,33} Our results clearly show that the total numbers of NSG decreases and the total numbers of GSG increases with advancing CKD stages in IgAN patients, as expected. Unexpectedly, the total numbers of all glomeruli (the sum of GSG + NSG) showed a clear trend to decrease with advancing CKD stages indicating that many glomeruli had disappeared without trace, presumably through a process of resorption. This study further analyzed single-nephron functional parameters in IgAN patients, providing important insights into the pathophysiology of IgAN progression.

Kidney functional decline, hypertension and heavy proteinuria at biopsy diagnosis are established predictors of worse outcomes in IgAN.\textsuperscript{34,35,36} Among these factors, advanced CKD stage and hypertension were both associated with lower numbers of NSG and NSG + GSG. In addition, the numbers of NSG in patients with preserved kidney function (CKD stage 1–2) were fewer in hypertensive than normotensive subjects, whereas there were no differences in the numbers of GSG in these subjects. Given the evidence from epidemiological and experimental studies showing a link between low nephron (glomerular) endowment and essential hypertension, these results suggest that the presence of fewer glomeruli in hypertensive IgAN patients may reflect lower nephron endowment.\textsuperscript{37} As compared to kidney function or hypertension, proteinuria levels showed weaker association with glomerular number. The preferential effects of CKD stage on total glomerular number
parameters are likely due to the fact that the number of glomeruli directly represents filtration capacity.

Total glomerular number is known to decrease with normal aging. In this study population of IgAN patients, the univariable linear estimate of decrease in NSG per kidney was 11,504 per year (Supplemental Table S2), which is nearly double the rate of nephron loss reported in aging subjects without kidney disease (6,200–6,700 per kidney per year). In agreement with these observations, the simple linear estimates of age-dependent reduction rate in the total numbers of NSG in the present study of IgAN patients was 1.8-fold greater than those for healthy donors in our previous study using the same methodology (Supplemental Figure S4). Of note, the total number of glomeruli in patients with CKD stage 3b or more advanced was approximately 45% less than for patients in CKD stage 1.

In this study population, reliable surrogates for total glomerular number at birth, such as birth weight, were not available. The requirement for biopsy diagnosis in IgAN may produce a lead-time bias that could mask any substantial disease progression and loss of glomeruli. Thus, we could not determine to what extent the low glomerular numbers identified in advanced CKD stages in the present study were the cause or consequence of disease progression of IgAN. The substantial decrease in the total glomerular number (GSG + NSG) during aging as demonstrated in healthy living kidney donors suggests that some globally sclerosed glomeruli are resorbed and disappear without trace. The same process of decrease in total glomerular number suggesting resorption was observed in our IgAN patients with advancing CKD stages, although to a more accelerated degree, as expected for a progressive glomerulonephritis. Accordingly, chronic histopathological indices for glomerular, vascular and tubulointerstitial injury were preferentially associated with lower numbers of NSG (Supplemental Table S4).
A conceptual schematic that summarizes the factors involved in IgAN progression is shown in Figure 5. An advantage of estimating total glomerular number in the clinical setting is the capacity to estimate single-nephron parameters based on the corresponding clinical data. Although values for eSNGFR did not differ between CKD stages, our results showed that hypertension had an independent effect on higher SNGFR. In a study that examined glomerular filtration in a rat model of nephrotoxic serum nephritis (NSN), SNGFR was kept constant by increasing transcapillary hydraulic pressure difference (ΔP) while compensating for a decrease in glomerular capillary ultrafiltration coefficient (Kf). These findings in NSN rats are consistent with the findings in our present study of IgAN patients, where eSNGFR is largely unaffected with advancing the CKD stages. On the other hand, the wide variation in eSNGFR within each CKD stage suggests that additional factors other than systemic hypertension such as aging and arteriosclerotic lesions of the kidney may affect intra-renal plasma flow rate and thereby SNGFR. Diversity in SNGFR among patients with the same CKD stages may represent "biphasic" vulnerability of SNGFR (hyperfiltration or hypofiltration). Thus, evaluating SNGFR in a clinical setting would allow us to detect dynamic changes in filtration function at the single-nephron level rather than to simply count the number of nephrons that appear to be functioning.

Despite an increase in mean glomerular volume, the SNGFR did not increase with advancing CKD stage in response to reduced number of NSG. A discrepancy between SNGFR and mean glomerular volume suggests a failure of compensatory glomerular hyperfiltration. Abnormalities in single-nephron dynamics may be the effects of histopathological lesions specific to IgAN, including segmental glomerular scarring, compromise of Bowman’s space by crescents, and reduced glomerular capillary luminal diameter caused by variable mesangial and/or endocapillary hypercellularity, increased matrix and immune deposits. To address these possibilities, we compared total glomerular
number and single-nephron parameters among patients with and without Oxford M, E, S, T and C lesions. We found eSNGFR was significantly lower in patients with E or C lesions, supporting their contribution to reduced glomerular filtration at the single-nephron level in IgAN (Figure 4, Supplemental Figure S7). Among the Oxford histopathological lesions, T lesion is known to be closely correlated to global glomerulosclerosis and may influence the eSNGFR values. However, we could not find any significant difference in eSNGFR values among patients with or without T lesions. These results are consistent with the results that single-nephron GFR is fairly unchanged with advancing CKD stages, which may be explained by diversity in GFR values possibly due to the biphasic vulnerability (hyperfiltration or hypofiltration) of individual glomeruli in patients with various degrees of T lesions.

A novel biomarker used in this study, the SNUPE, may help elucidate the pathophysiology of proteinuric glomerulopathies. Our results showed that the increase in SNUPE was disproportionately much greater than for total UPE with advancing CKD stages. Compared to the CKD stage 1 as reference, the stage 4–5 exhibited a 5-fold increase in total UPE and a striking 19-fold increase in SNUPE. Interestingly, the SNUPE were significantly higher in patients with S or T lesions, suggesting a pathogenetic link. Given the potential for heavy proteinuria to reflect podocyte injury and loss of filtration barrier integrity, as well as to cause tubulointerstitial injury via excessive protein trafficking, SNUPE may provide a highly sensitive marker of and risk factor for disease progression.48

In this study, 111 (45.3%) of patients were treated with RAAS inhibitors prior to biopsy diagnosis. According to renoprotective mechanisms, RAAS inhibition is expected to attenuate glomerular hyperfiltration and thereby eSNGFR and SNUPE. However, patients treated with RAAS inhibitors had fewer numbers of NSG and larger numbers of GSG at biopsy as compared to those without RAAS inhibitors (Supplemental Figure S2), indicating
that these patients already had more advanced kidney injury, which probably influenced their selection for treatment. Due to this treatment bias, it is difficult to determine whether RAAS inhibitors effectively attenuated hyperfiltration and the associated proteinuria at the single-nephron level in these patients.

This study has notable limitations. First, the method of estimating glomerular number was based on needle biopsy specimens. The sensitivity analyses demonstrated that our estimates were robust (Supplemental Table S5); however, this does not rule out the possibility that differences in glomerular density between biopsy sites and SNGFR within a kidney could cause sampling bias. Second, the single-nephron parameters are determined as averaged values and do not always represent true ‘single’ values. Glomerular lesions in IgAN are heterogeneous and not uniformly distributed throughout the kidney. The focality of some glomerular lesions may cause divergence in eSNGFR and SNUPE within a kidney. Third, like creatinine-based eGFR, creatinine-based estimation of SNGFR also reflects the tubular secretion of creatinine. In this study cohort, we did not have measured GFR available as it is not routinely obtained in clinical care of IgAN patients. Fourth, to determine cortical volume (needed to estimate nephron number and SNGFR), total kidney volume was measured on CT scans and the fraction of cortical volume was estimated from the total kidney volume. Intravenous contrast is needed to separately measure cortical volume on CT scans but is avoided in patients with CKD due to concerns for contrast nephropathy. Finally, all patients included in this study are Japanese. Given the potential difference in total glomerular number among races, validation studies among different races are required.

In conclusion, this study estimated total glomerular number and related single-nephron parameters in 245 patients with IgAN. The findings support progressive reduction in NSG and total glomeruli (GSG + NSG) with advancing CKD stage, indicating that sclerosed glomeruli can be resorbed over time. The finding of lower numbers of NSG in
hypertensive than normotensive patients with preserved kidney function (CKD stage 1–2) implicates a predisposing role for low nephron endowment. SNUPE emerged as a stronger biomarker than SNGFR in advanced stage CKD, likely reflecting the limited ability of diseased hypercellular glomeruli to respond by compensatory hyperfiltration. These findings illustrate the feasibility and usefulness of estimating single-nephron dynamics in human glomerulonephritis. Future studies are needed to determine the generalizability of these findings to other forms of glomerulonephritis.
Disclosures

J. Bertram reports Scientific Advisor or Membership: Kidney International. A. Rule reports Scientific Advisor or Membership: NIDDK - CKD Biomarker Consortium External Expert Panel, JASN – Associate Editor, Mayo Clinic Proceedings - Section Editor; Other Interests/Relationships: UpToDate. T. Yokoo reports Scientific Advisor or Membership: Editorial Board Member of "HUMAN CELL". All remaining authors have nothing to disclose.

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This study was approved by the ethics review board of the Jikei University School of Medicine [30-385 (9406)] and conducted according to the Declaration of Helsinki. Parts of this study were presented at the 62th Annual Meeting of The American Society of Nephrology, Nov 5–10, 2019, Washington D.C., USA.

Authors’ Contributions

H Marumoto: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Validation; Visualization; Writing - original draft; Writing - review and editing

N Tsuboi: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Supervision; Validation; Writing - original draft; Writing - review and editing

V D'Agati: Writing - review and editing

T Sasaki: Writing - review and editing

Y Okabayashi: Writing - review and editing

K Haruhara: Writing - review and editing
Supplemental Material

Supplemental Table S1. Specific RAAS inhibitor therapies prescribed prior to biopsy diagnosis

Supplemental Table S2. Unadjusted estimates of total numbers of glomeruli depending on clinical variables in linear regression models

Supplemental Table S3. The association of clinical characteristics with total glomerular number per kidney and single-nephron parameters among IgAN patients who had not been treated with RAAS inhibitors before biopsy

Supplemental Table S4. Renal histopathological variables associated with non-globally sclerotic glomeruli and globally sclerotic glomeruli per kidney in univariate linear regression models

Supplemental Table S5. Sensitivity analyses for clinical characteristics as predictors of non-globally and globally sclerotic glomerular numbers in univariate linear regression models
Supplemental Figure S1. Comparisons of total glomerular number and single-nephron parameters among IgAN patients who had not been treated with RAAS inhibitors before biopsy.

Supplemental Figure S2. Comparisons of total glomerular number and single-nephron parameters among IgAN patients who treated with RAAS inhibitors at biopsy.

Supplemental Figure S3. Comparisons of total glomerular number and single-nephron parameters among patients treated with or without RAAS inhibitors prior to biopsy diagnosis.

Supplemental Figure S4. Correlations between age and non-sclerotic glomerular number or total glomerular number.

Supplemental Figure S5. Comparisons of total glomerular number and single-nephron parameters among IgAN patients with and without kidney functional decline or hypertension: Sub-group analyses of patients who had not been treated with RAAS inhibitors before biopsy.

Supplemental Figure S6. Comparisons of total glomerular number and single-nephron parameters among IgAN patients with and without kidney functional decline or presence and absence of UPE ≥1g/day.

Supplemental Figure S7. Comparisons of total single-nephron parameters among patients with or without kidney functional decline or histopathological lesions defined by Oxford MESTC scores.

Supplemental Figure S8. Comparison of single-nephron GFR within the 5–95th percentile of the overall values.
References


## Table 1. Baseline characteristics of patients in the different CKD stages

<table>
<thead>
<tr>
<th>Clinical findings</th>
<th>Overall (n=245)</th>
<th>1 (n=24)</th>
<th>2 (n=106)</th>
<th>CKD stage</th>
<th>3a (n=47)</th>
<th>3b (n=35)</th>
<th>4–5 (n=33)</th>
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<td>Age (years)</td>
<td>42.6 ± 14.1</td>
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<td>38.0 ± 11.5</td>
<td>46.0 ± 10.8</td>
<td>53.9 ± 15.2</td>
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<td>Male (%)</td>
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<td>54.2</td>
<td>55.7</td>
<td>74.5</td>
<td>62.9</td>
<td>69.7</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.0 ± 3.7</td>
<td>21.1 ± 4.3</td>
<td>22.8 ± 3.8</td>
<td>23.7 ± 3.1</td>
<td>23.9 ± 3.8</td>
<td>22.6 ± 3.4</td>
<td></td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.71 ± 0.20</td>
<td>1.63 ± 0.18</td>
<td>1.70 ± 0.20</td>
<td>1.76 ± 0.19</td>
<td>1.70 ± 0.22</td>
<td>1.71 ± 0.22</td>
<td></td>
</tr>
<tr>
<td>Glomeruli with segmental sclerosis (%)</td>
<td>16.9 ± 1.7</td>
<td>11.3 ± 7.6</td>
<td>136 ± 97</td>
<td>165 ± 94</td>
<td>152 ± 51</td>
<td>152 ± 53</td>
<td></td>
</tr>
<tr>
<td>Patients with hypertension (%)</td>
<td>19.6</td>
<td>8.3</td>
<td>18.3</td>
<td>13.6</td>
<td>25.7</td>
<td>33.3</td>
<td></td>
</tr>
<tr>
<td>RAAS inhibitor use (%)</td>
<td>45.3</td>
<td>25</td>
<td>28.3</td>
<td>55</td>
<td>80</td>
<td>63.6</td>
<td></td>
</tr>
<tr>
<td>Laboratory findings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>3.8 ± 0.5</td>
<td>4.1 ± 0.3</td>
<td>3.9 ± 0.5</td>
<td>3.9 ± 0.4</td>
<td>3.6 ± 0.7</td>
<td>3.4 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>1.19 ± 0.63</td>
<td>0.67 ± 0.12</td>
<td>0.84 ± 0.15</td>
<td>1.13 ± 0.14</td>
<td>1.50 ± 0.25</td>
<td>2.45 ± 0.73</td>
<td></td>
</tr>
<tr>
<td>eGFR (mL/min)</td>
<td>60.0 ± 25.1</td>
<td>98.6 ± 27.3</td>
<td>73.0 ± 10.3</td>
<td>54.5 ± 7.2</td>
<td>36.1 ± 6.3</td>
<td>23.1 ± 6.2</td>
<td></td>
</tr>
<tr>
<td>24-hour Ccr (mL/min)</td>
<td>83 ± 34</td>
<td>119 ± 35</td>
<td>99 ± 24</td>
<td>85 ± 18</td>
<td>51 ± 14</td>
<td>37 ± 13</td>
<td></td>
</tr>
<tr>
<td>Serum uric acid (mg/dL)</td>
<td>6.5 ± 1.7</td>
<td>5.1 ± 1.3</td>
<td>5.9 ± 1.4</td>
<td>7.0 ± 1.1</td>
<td>7.7 ± 1.4</td>
<td>7.9 ± 1.8</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>144 ± 83</td>
<td>113 ± 76</td>
<td>136 ± 97</td>
<td>165 ± 94</td>
<td>152 ± 51</td>
<td>152 ± 53</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>62 ± 18</td>
<td>67 ± 17</td>
<td>64 ± 19</td>
<td>58 ± 17</td>
<td>62 ± 18</td>
<td>60 ± 19</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>121 ± 32</td>
<td>110 ± 34</td>
<td>121 ± 32</td>
<td>121 ± 30</td>
<td>122 ± 34</td>
<td>130 ± 35</td>
<td></td>
</tr>
<tr>
<td>Lp(a) (mg/dL)</td>
<td>5.5 ± 0.5</td>
<td>5.4 ± 0.7</td>
<td>5.4 ± 0.4</td>
<td>5.5 ± 0.4</td>
<td>5.6 ± 0.6</td>
<td>5.5 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>IgA (mg/dL)</td>
<td>324 ± 106</td>
<td>315 ± 107</td>
<td>307 ± 91</td>
<td>340 ± 106</td>
<td>370 ± 140</td>
<td>303 ± 95</td>
<td></td>
</tr>
<tr>
<td>C3 (mg/dL)</td>
<td>100 ± 19</td>
<td>93 ± 17</td>
<td>101 ± 16</td>
<td>103 ± 20</td>
<td>102 ± 25</td>
<td>96 ± 16</td>
<td></td>
</tr>
<tr>
<td>Uric RBC count, grade 1–5 (%)</td>
<td>77.0</td>
<td>66.7</td>
<td>81.0</td>
<td>74.3</td>
<td>60.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall (n=245)</td>
<td>835 (433–1,536)</td>
<td>419 (250–608)</td>
<td>633 (423–1,129)</td>
<td>1,066 (432–1,392)</td>
<td>1,037 (610–2,333)</td>
<td>1,983 (1,263–3,362)</td>
<td></td>
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<tr>
<td>Patients with UPE &gt; 1g/day (%)</td>
<td>41.2</td>
<td>4.2</td>
<td>28.3</td>
<td>53.2</td>
<td>51.4</td>
<td>81.8</td>
<td></td>
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<tr>
<td>Histopathological findings</td>
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<td></td>
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<tr>
<td>Total glomeruli identified in biopsy specimen</td>
<td>23.0 ± 11.0</td>
<td>25.0 ± 12.4</td>
<td>26.0 ± 11.4</td>
<td>21.3 ± 10.4</td>
<td>19.0 ± 8.5</td>
<td>18.8 ± 8.7</td>
<td></td>
</tr>
<tr>
<td>Glomeruli with global sclerosis (%)</td>
<td>16.9 ± 16.1</td>
<td>5.4 ± 7.4</td>
<td>10.7 ± 12.5</td>
<td>19.2 ± 14.6</td>
<td>24.1 ± 14.2</td>
<td>33.8 ± 17.8</td>
<td></td>
</tr>
<tr>
<td>Glomeruli with segmental sclerosis (%)</td>
<td>2.8 ± 4.8</td>
<td>1.1 ± 3.3</td>
<td>2.2 ± 3.2</td>
<td>2.2 ± 4.5</td>
<td>5.2 ± 6.1</td>
<td>4.0 ± 7.3</td>
<td></td>
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<tr>
<td>Glomeruli with cellular/fibrocellular crescents (%)</td>
<td>10.7 ± 27.6</td>
<td>7.1 ± 26.2</td>
<td>11.8 ± 32.7</td>
<td>7.8 ± 15.3</td>
<td>9.9 ± 29.4</td>
<td>14.7 ± 22.1</td>
<td></td>
</tr>
<tr>
<td>Glomeruli with fibrous crescents (%)</td>
<td>2.0 ± 6.8</td>
<td>2.0 ± 6.1</td>
<td>2.6 ± 7.4</td>
<td>0.0 ± 0.3</td>
<td>2.0 ± 4.8</td>
<td>2.7 ± 10.7</td>
<td></td>
</tr>
<tr>
<td>Interstitial fibrosis/tubular atrophy (%)</td>
<td>17.8 ± 17.0</td>
<td>8.1 ± 6.9</td>
<td>9.6 ± 7.2</td>
<td>16.2 ± 12.5</td>
<td>27.5 ± 17.1</td>
<td>43.0 ± 20.2</td>
<td></td>
</tr>
<tr>
<td>Arteriosclerotic lesion†, grade 1–2 (%)</td>
<td>59.0</td>
<td>16.7</td>
<td>55.2</td>
<td>59.6</td>
<td>77.1</td>
<td>81.8</td>
<td></td>
</tr>
<tr>
<td>Arteriolar hyaline, grade† 1–3 (%)</td>
<td>34.7</td>
<td>16.7</td>
<td>30.2</td>
<td>36.2</td>
<td>48.6</td>
<td>45.5</td>
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<tr>
<td>Oxford Classification</td>
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<td></td>
</tr>
<tr>
<td>Patients with M1 (%)</td>
<td>46.5</td>
<td>45.8</td>
<td>46.2</td>
<td>36.2</td>
<td>48.6</td>
<td>60.6</td>
<td></td>
</tr>
<tr>
<td>Patients with E1 (%)</td>
<td>12.2</td>
<td>8.3</td>
<td>13.2</td>
<td>8.5</td>
<td>5.7</td>
<td>24.2</td>
<td></td>
</tr>
<tr>
<td>Patients with S1 (%)</td>
<td>89.4</td>
<td>75.0</td>
<td>85.8</td>
<td>95.7</td>
<td>97.1</td>
<td>93.9</td>
<td></td>
</tr>
<tr>
<td>Patients with T1+2 (%)</td>
<td>24.1</td>
<td>4.2</td>
<td>3.8</td>
<td>19.1</td>
<td>54.3</td>
<td>78.8</td>
<td></td>
</tr>
<tr>
<td>Patients with Cl+2 (%)</td>
<td>38.0</td>
<td>25.0</td>
<td>41.5</td>
<td>38.3</td>
<td>28.6</td>
<td>45.5</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as the means ± standard deviations or median (25th–75th percentile).  
† The urinary RBC count was graded as follows: grade 0, <5/high power field (HPF); grade 1, 5–9/HPF; grade 2, 10–19/HPF; grade 3, 20–49/HPF; grade 4, 50–99/HPF; and grade 5, >99/HPF.  
* Arteriosclerotic lesions were defined as normal (grade 0), and less than 50% (grade 1) or more than 50% of the thickness of media (grade 2). Arteriolar hyaline was graded as the proportion of arterioles affected (grade 0, <1%; grade 1, 1–25%; grade 2, 26–50%; grade 3, >50%). BMI, body mass index; BSA, body surface area; Ccr, Creatinine clearance rate; CKD, chronic kidney disease; GFR, glomerular filtration rate; HDL, high density lipoprotein; IgA, immunoglobulin A; LDL, low density lipoprotein; UPE, urinary protein excretion; RAAS, renin-angiotensin aldosterone system; RBC, red blood cell.
Table 2. The association of clinical characteristics with total glomerular number per kidney and single-nephron parameters

<table>
<thead>
<tr>
<th>Clinical variables</th>
<th>Non-globally sclerotic glomeruli (number per kidney)</th>
<th>Globally-sclerotic glomeruli (number per kidney)</th>
<th>Total glomeruli (number per kidney)</th>
<th>Mean glomerular volume ($\times 10^6 \mu m^3$)</th>
<th>Single-nephron GFR (nL/min)</th>
<th>Single-nephron UPE (µg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>$P$ value</td>
<td>Estimate</td>
<td>$P$ value</td>
<td>Estimate</td>
<td>$P$ value</td>
</tr>
<tr>
<td>Univariable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CKD category†</td>
<td>-196,284</td>
<td>&lt;0.001</td>
<td>50,918</td>
<td>&lt;0.001</td>
<td>-145,366</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-154,544</td>
<td>0.02</td>
<td>-5,651</td>
<td>0.88</td>
<td>-160,195</td>
<td>0.02</td>
</tr>
<tr>
<td>UPE ≥ 1 g/day</td>
<td>-190,543</td>
<td>&lt;0.001</td>
<td>56,199</td>
<td>0.053</td>
<td>-134,345</td>
<td>0.02</td>
</tr>
<tr>
<td>Multivariable*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CKD category†</td>
<td>-172,720</td>
<td>&lt;0.001</td>
<td>49,712</td>
<td>&lt;0.001</td>
<td>-123,007</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>-93,575</td>
<td>0.08</td>
<td>-35,891</td>
<td>0.33</td>
<td>-129,466</td>
<td>0.046</td>
</tr>
<tr>
<td>UPE ≥ 1 g/day</td>
<td>20,944</td>
<td>0.66</td>
<td>-12,605</td>
<td>0.70</td>
<td>8,338</td>
<td>0.88</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, and BSA in addition to CKD stage, hypertension, and UPE.
†Five categories of CKD stage were defined: eGFR levels ≥90, 60–89, 45–59, 30–44 and <30 mL/min/1.73m², respectively. BSA, body surface area; CKD, chronic kidney disease; GFR, glomerular filtration rate; UPE, urinary protein excretion.
Figures and legends

Figure 1. Patients selection
During the study period, we identified 324 Japanese patients with primary IgAN. Seventy-nine patients were excluded from this study due to lack of CT data or inadequate glomerular number or cortical area in biopsy specimens. CT, computed tomography; IgAN, IgA nephropathy.

Figure 2. Comparisons of total glomerular number and single-nephron parameters among IgAN patients at different CKD stages
Total number of non-globally sclerotic glomeruli (A), globally sclerotic glomeruli (B), total glomeruli (C), mean glomerular volume (D), single-nephron GFR (E), single-nephron UPE (F) were compared among patients with IgAN at different CKD stages at biopsy diagnosis. Bold horizontal line and scale bar indicates each mean value and the 5–95 percentile, respectively. Percent difference indicates change in mean values compared to mean value in CKD stage 1 as a reference. CKD, chronic kidney disease; IgAN, IgA nephropathy.

Figure 3. Comparisons of total glomerular number and single-nephron parameters among IgAN patients with and without kidney functional decline or hypertension
Patients were categorized based on CKD stage (stage 1–2 vs. stage 3–5) or presence or absence of hypertension at biopsy diagnosis. The numbers of non-globally sclerotic glomeruli (A), globally sclerotic glomeruli (B), total glomeruli (C), and values for mean glomerular volume (D), single-nephron GFR (E), single-nephron UPE (F) were compared among the groups. CKD, chronic kidney disease; GFR, glomerular filtration rate; HT, hypertension; IgAN, IgA nephropathy; UPE, urinary protein excretion.
Figure 4. Comparisons of single-nephron parameters among patients with or without histopathological lesions defined by Oxford MESTC scores

The mean glomerular volume (A, D, G, J, M), single-nephron GFR (B, E, H, K, N) and single-nephron UPE (C, F, I, L, O) were compared between the groups categorized based on the presence or absence of lesions defined by Oxford MESTC scores. Bold horizontal line and scale bar indicates each mean value and the 5–95 percentile, respectively. GFR, glomerular filtration rate; UPE, urinary protein excretion; M, mesangial hypercellularity; E, endocapillary hypercellularity; S, segmental sclerosis or adhesion; T, interstitial fibrosis and tubular atrophy; C, crescents.

Figure 5. Factors involved in IgAN progression to ESKD

Individual nephron endowment may determine the sensitivity to progressive nephron loss when it encounters acquired kidney diseases such as chronic glomerulopathies including IgAN. In 245 IgAN patients analyzed in this study, single-nephron GFR level was largely unchanged among the different CKD stage groups, but varied significantly between individuals in the same CKD stages. Increased UPE levels were more pronounced at the single nephron level in advanced CKD stages. This study therefore indicates "biphasic" vulnerability of single-nephron GFR (hyper- or hypo-glomerular filtration) is involved in the progression of IgAN to ESKD. Some of histopathological findings specific to IgAN may interfere with the ability to achieve compensatory hyperfiltration during progressive nephron loss. CKD, chronic kidney disease; ESKD, end-stage kidney disease; GFR, glomerular filtration rate; IgAN, immunoglobulin A nephropathy; Kf, glomerular capillary ultrafiltration coefficient; ΔP, transcapillary hydraulic pressure difference; UPE, urinary protein excretion.
Native-biopsy cases diagnosed as primary IgAN from 2007 to 2017

n=324

Excluded:
- Unenhanced CT scan data not available (n=72)
- Biopsy specimens containing <5 non-globally sclerotic glomeruli or cortical area <2 mm$^2$ (n=7)

Patients included in final analysis

n=245
Figure 2

A. Non-globally sclerotic glomeruli

B. Globally sclerotic glomeruli

C. Total glomeruli

D. Mean glomerular volume

E. Single-nephron GFR

F. Single-nephron UPE
Figure 4