

Nephron Number and Time to Remission in Steroid-Sensitive Minimal Change Disease



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Rationale & Objective: The response to corticosteroid therapy may differ among patients with minimal change disease (MCD). Previous studies have suggested that glomerular hypertrophy or low areal glomerular density in biopsy specimens, which may be related to fewer nephrons, is associated with such a difference. We examined the associations between nephron number and the therapeutic response to corticosteroids in patients with MCD.

Study Design: Retrospective cohort study.

Setting & Participants: 75 adult patients with a histologic diagnosis of MCD.

Exposure: Nephron number per kidney estimated based on the combination of unenhanced computed tomography and nonsclerotic volumetric glomerular density in kidney biopsy specimens.

Outcomes: Complete remission and relapse following corticosteroid therapy.

Analytical Approach: Multivariable Cox proportional hazard analyses of associations between factors, including nephron number, and outcomes.

Results: Mean age of patients was 45.9 years and 60.0% were men. Patients had an estimated glomerular filtration rate of 64.6 mL/min/1.73 m² and proteinuria of 8.7 g/d. The estimated total number of nonsclerotic glomeruli ranged from 1.07 to 18.77 × 10⁵ per kidney among all patients. There were no significant differences in total amounts or selectivity of urinary protein excretion at biopsy among the tertile groups categorized by nephron number. All patients responded to corticosteroid therapy, but those with fewer nephrons had a delayed achievement of complete remission. Multivariable Cox proportional hazard analyses identified nephron number as a significant independent explanatory variable for the achievement of complete remission, with a hazard ratio of 1.10 (95% CI, 1.02-1.19)/100,000 nephrons per kidney. Nephron number in these patients was not associated with achievement of partial remission or relapse following complete remission.

Limitation: Retrospective design and sampling bias of needle biopsy.

Conclusions: A small nephron number in patients with MCD is associated with longer time to complete remission.

Visual Abstract included

Complete author and article information provided before references.

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Minimal change disease (MCD) accounts for >10% of adult-onset primary nephrotic syndrome worldwide and is characterized by an acute-onset clinical course of systemic edema, hypoalbuminemia, and massive urinary protein excretion (UPE) due to increased protein perme-

ability of the kidneys.^{1,2} In Asian populations, including the Japanese, MCD accounts for ~40% of adult patients with nephrotic syndrome.^{3,4} Patients with typical MCD exhibit a high response rate to corticosteroid therapy and rarely progress to end-stage kidney disease. However, some patients with MCD exhibit a frequently relapsing clinical course and become dependent on corticosteroids and/or immunosuppressants.⁵ Several factors, including age, preexisting chronic kidney disease, coexistence of acute kidney injury, amount of proteinuria, and treatment regimen, contribute to the clinical course and prognosis of MCD.^{5,6} In addition, multiple factors such as circulating permeability factor(s) and/or abnormalities in T lymphocytes may be involved in the cause of MCD.⁷⁻¹⁰ However, the pathogenesis of MCD is unclear. Therefore, it would be clinically valuable to identify the kidney

histologic determinants of the clinical course and prognosis of MCD.

Kidney histopathologic findings of MCD are defined as no apparent abnormalities in light microscopic examination findings and a high rate of podocyte foot-process effacement in electron microscopic examinations.³ Morphologic factors may be related to the clinical phenotypes of MCD, including the therapeutic response to corticosteroid therapy. Patients with steroid-dependent and steroid-resistant MCD tend to have larger glomeruli and glomerular hypertrophy, which are linked to greater risk for subsequent progression to focal segmental glomerulosclerosis.¹¹ The time to complete remission is longer in patients with MCD with a low areal glomerular density,¹² which is considered a morphologic surrogate marker for nephron number.¹³⁻¹⁸ These results raise the possibility that a small nephron number contributes to worse clinical outcomes of MCD. However, no previous study has directly examined the relationship between nephron number per kidney and the clinical course in patients with MCD due to technical difficulties in counting nephrons in a clinical setting.

For accurate enumeration of the total number of nephrons, a stereology based detailed analysis of autopsy

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ability of the kidneys.^{1,2} In Asian populations, including the Japanese, MCD accounts for ~40% of adult patients with nephrotic syndrome.^{3,4} Patients with typical MCD exhibit a high response rate to corticosteroid therapy and rarely progress to end-stage kidney disease. However, some patients with MCD exhibit a frequently relapsing clinical course and become dependent on corticosteroids and/or immunosuppressants.⁵ Several factors, including age, preexisting chronic kidney disease, coexistence of acute kidney injury, amount of proteinuria, and treatment regimen, contribute to the clinical course and prognosis of MCD.^{5,6} In addition, multiple factors such as circulating permeability factor(s) and/or abnormalities in T lymphocytes may be involved in the cause of MCD.⁷⁻¹⁰ However, the pathogenesis of MCD is unclear. Therefore, it would be clinically valuable to identify the kidney

PLAIN-LANGUAGE SUMMARY

The kidney is an internal organ made of functional units called nephrons. Studies based on deceased people have shown that nephron counts range widely in humans. However, counting nephrons in living humans was impractical until recently. In this study, a new method was used to count nephrons in individual patients with nephrotic syndrome, a refractory kidney disease that causes severe systemic damage resulting from a large amount of proteins leaking from the kidneys. Our results showed that patients with a small number of nephrons required a longer time to heal from protein leakage after drug therapy. Counting nephrons may improve our understanding of the condition and treatment of patients with nephrotic syndrome.

kidneys is required.¹⁹ In recent years, it has been postulated that the number of nephrons per kidney can be estimated in healthy kidney transplant donors using a combination of kidney cortical volume measured using enhanced computed tomography (CT) and the volumetric glomerular density of kidney biopsy specimens.²⁰⁻²² However, that method is not suitable for patients with kidney disease or kidney insufficiency because enhanced CT requires contrast intermediate, which may be toxic to kidneys.

To overcome this, we recently established an equation to accurately estimate kidney cortical volume from kidney parenchymal volume based on 3-dimensionally constructed unenhanced computed tomographic images.²³ In this study, we estimated the total number of nephrons per kidney in patients with MCD using unenhanced CT and biopsy-based stereology to examine the effects of individual differences in nephron number on the clinical phenotype, including the therapeutic response to corticosteroids.

METHODS

Selection of Patients

We recruited patients who exhibited acute-onset nephrotic syndrome histopathologically diagnosed as MCD from January 1, 2007, to March 31, 2017, at Jikei University Hospital and Jikei Kashiwa Hospital. At these institutes, screening evaluation is routinely performed using unenhanced CT before kidney biopsy if there is no contraindication. Exclusion criteria were as follows^{13,20-22}: cases for whom clinical onset could not be estimated, who had been previously diagnosed and/or treated for other kidney diseases; whose kidney biopsy specimens contained fewer than 5 glomeruli (including globally sclerotic glomeruli) in light microscopy images, whose kidney biopsy specimens contained >50% global glomerular sclerosis (GGS) in light microscopy images,

who did not fulfill criteria for nephrotic syndrome and for whom kidney biopsy was performed more than 6 months after the onset of symptoms suggestive of nephrotic syndrome. Nephrotic syndrome was defined as UPE ≥ 3.5 g/d with a serum albumin concentration ≤ 3.0 mg/dL. At our hospitals, typical initial treatment for patients with MCD diagnosed was prednisolone at a dosage of 0.8 to 1.0 mg/kg per day.

During the study period, we identified 80 patients with biopsy-proven MCD. Three of those patients were excluded because images from CT were not available. Two patients were excluded because fewer than 5 glomeruli were obtained. Ultimately, 75 patients were included in the analyses.

This study was approved by the Ethics Review Board of Jikei University School of Medicine (30-385/9406). We followed the tenets of the Declaration of Helsinki. Because this was a retrospective cohort study, information on the research plan was posted, an opportunity to refuse participation was provided, and individual informed consent was not required.

Morphologic Measurements

The thickness of the obtained computed tomographic images was 5.0 mm. Kidney parenchymal volume was measured as previously described using ITK-SNAP, version 1.1, software (University of Pennsylvania) to semi-automatically segment the cortex and medulla from transverse unenhanced computed tomographic images (Fig 1A-C). We recently developed a regression equation to estimate kidney cortical volume using kidney parenchymal volume measured from unenhanced images from CT.²³ The 5 equation models developed were well validated. Adding age, sex, kidney function, and body size did not improve the performance of the equations compared with that using only renal parenchymal volume. Therefore, we used the following equation: estimated cortical volume (cm³) = -1.3 (intercept) + 0.71 \times parenchymal volume (cm³).²³

Pathologic Measurement of Kidney Biopsy Specimens

Kidney tissue specimens were obtained using percutaneous needle biopsy. The tissues were embedded in paraffin and sectioned at an ~ 3 - μ m thickness. The embedded tissues were stained with hematoxylin-eosin, periodic acid-Schiff, Masson trichrome, and periodic acid silver-methenamine. GGS was defined when the entire glomerulus was involved in sclerosis. The degree of interstitial fibrosis/tubular atrophy was semi-quantitatively evaluated as increased extracellular matrix separating tubules and atrophic tubules and expressed as percentage of the affected area over the observed cortical area, with 1% to 5% treated as 5% and the other values rounded to the closest 5%. The degree of podocyte foot-process effacement was semi-quantitatively evaluated using electron microscopy.

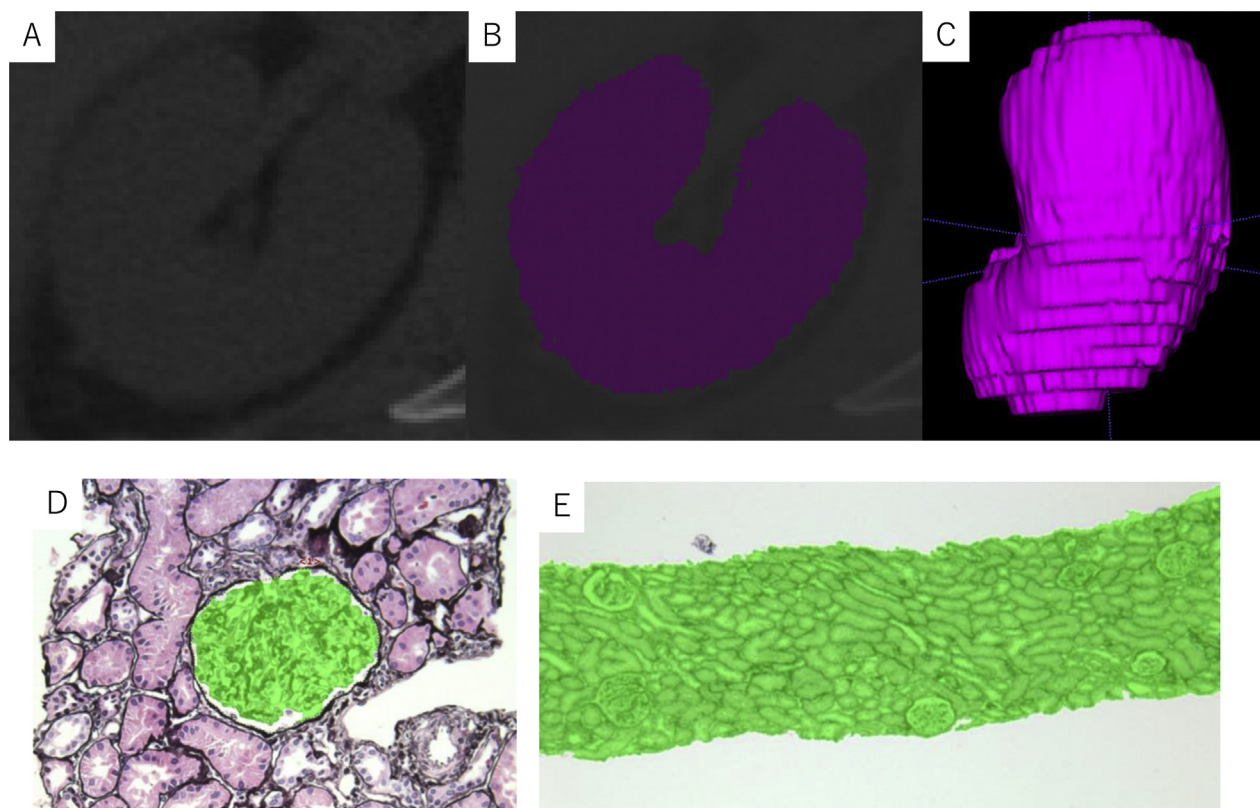


Figure 1. Measurement of kidney parenchymal volume and kidney biopsy morphometry. (A-C) Three-dimensional images were semi-automatically constructed from nonenhanced computed tomographic images of specific density regions of kidneys. The green semi-transparent overlays show (D) the glomerular capillary area and (E) a portion of the cortical area, which are semi-automatically measured.

Kidney biopsy specimens were semi-automatically analyzed to measure the individual area of glomerular capillary tufts and total area of the obtained kidney cortex using Win roof 2017 image-analysis software (Mitani Corp) (Fig 1D and E). Glomerular area was defined as the average area described by the outer capillary loops of the tuft. Mean glomerular volume was calculated from the measured glomerular area as follows: Mean glomerular volume = $\frac{\beta}{d} \times (\text{glomerular area})^{\frac{3}{2}}$, where β is a dimensionless shape coefficient ($\beta = 1.382$) and d is a size distribution coefficient ($d = 1.01$).^{24,25} Volumetric glomerular density excluding globally sclerotic glomeruli (number per mm^3 of the cortex in biopsy specimens) was determined using the Weibel-Gomez stereologic method as follows:

Numerical glomerular density

$$= \frac{1}{\beta} \times \sqrt[2]{\frac{\left(\frac{\text{Total number of glomeruli}}{\text{Area of cortex}}\right)^3}{\frac{\text{Total area of glomerulus}}{\text{Area of cortex}}}},$$

where β is a dimensionless shape coefficient ($\beta = 1.382$).^{24,25} The sclerotic glomerular density (per mm^3

of the cortex in biopsy specimens) was calculated identically:

Sclerotic glomerular density

$$= \frac{1}{\beta} \times \sqrt[2]{\frac{\left(\frac{\text{Total number of sclerotic glomeruli}}{\text{Area of cortex}}\right)^3}{\frac{\text{Total area of sclerotic glomeruli}}{\text{Area of cortex}}}}$$

The total number of glomeruli, including GGS, was estimated based on the glomerular density (per mm^3 of the cortex in biopsy specimens), including globally sclerotic glomeruli, as the sum of nonsclerotic glomerular density and sclerotic glomerular density. Total glomerular volume was calculated by multiplying glomerular volume by total number of nonsclerotic glomeruli.

Estimation of Nephron Number

The nephron number was estimated using the method of Denic et al.^{20,21,26,27} The total number of nonsclerotic glomeruli per kidney was calculated by multiplying estimated cortical volume (mm^3) by volumetric nonsclerotic glomerular density. The calculated value was divided by 2 per kidney, by 1.43 to correct for tissue volume shrinkage

due to paraffin embedding, and by 1.268 to correct for volume shrinkage due to loss of tissue perfusion pressure.

Outcomes

The main outcomes were complete remission and first relapse of nephrotic syndrome. Complete remission was defined as UPE < 0.3 g/d or g/g of creatinine (g/gCr). Partial remission was defined as UPE of 0.3 to <3.5 g/d or g/gCr.³ Time to complete remission was defined as the period from administration of corticosteroids to achievement of complete remission. Time to partial remission was defined in the same way as complete remission. Relapse was defined as the reappearance of UPE > 1.0 g/d or g/gCr, followed by an increase in corticosteroid dose, administration of additional immunosuppressive agents, or both after achieving complete remission.⁶ Time to first relapse was defined as the period from time of complete remission to that of first relapse. During hospitalization, blood and urinary tests were performed at least once a week to determine remission. In principle, patients were discharged after achieving complete or partial remission. Because patients do not need weekly urinalysis after achieving complete remission, UPE was treated as 0 g/d for plotting changes over time when the patient had no apparent relapse and did not undergo urinalysis after complete remission. Blood and urinary tests were performed at approximately 1- to 3-month intervals after discharge from the hospital.

Definitions of Other Risk Factors and Single-Nephron Parameters

Hypertension was defined as systolic blood pressure > 140 mm Hg, diastolic blood pressure > 90 mm Hg, or both, or use of antihypertensive medications. Body weight at admission was used as the weight. Body surface area (BSA) was determined by the equation: $BSA (m^2) = \text{weight}^{0.425} (\text{kg}) \times \text{height}^{0.725} (\text{cm}) \times 71.84 \times 10^{-4}$.²⁸ Estimated glomerular filtration rate (eGFR) was calculated from serum creatinine (Scr) level using a modified equation for the GFR of Japanese individuals: $eGFR = 194 \times \text{age}^{-0.287} \times \text{Scr}^{-1.094} (\times 0.739 \text{ if female})$.²⁹ Creatinine clearance rate (CL_{cr}) was calculated from Scr and urine creatinine concentrations. CL_{cr} values were calculated before and after adjustment for BSA. Single-nephron GFR (SNGFR) and single-nephron UPE (SNUPE) values were calculated by dividing total kidney GFR (eGFR and CL_{cr}) or UPE by total number of nonsclerotic glomeruli.

Statistical Analyses

Imputation was not performed because there were no missing values for baseline covariates. We were able to track all cases until complete remission was achieved. Participants who did not experience relapse until the last consultation were treated as censored. Continuous variables are expressed as mean \pm standard deviation or median with interquartile range. Variables were assessed for

normality using a normal probability plot, histogram, and Shapiro-Wilk test. We used the Jonckheere-Terpstra test or Cochran-Armitage test to detect trends in baseline characteristics and morphologic measurements according to tertile of nephron number. Log-rank test was performed to analyze survival curves. Cox proportional hazard analyses were conducted to calculate the hazard ratio (HR) for complete remission and relapse. Stratified analyses were performed based on age and sex. Interactions were analyzed using the interaction term for age or sex and nephron number as a continuous value. Sensitivity analysis was performed for patients within the 5th to 95th percentiles of nephron number. $P < 0.05$ was considered indicative of statistical significance. Statistical analyses were performed with EZR (Saitama Medical Center, Jichi University) based on the R software package (The R Foundation for Statistical Computing, version 3.2.2).³⁰

RESULTS

Baseline Characteristics and Treatments of Patients With MCD

Clinical and laboratory findings at the time of biopsy diagnosis are shown in Table 1. A total of 75 patients with biopsy-diagnosed MCD were recruited, and their mean age at diagnosis was 45.9 years. These patients were characterized by typical clinical findings of MCD, such as very low serum albumin level and massive amount and high selectivity of UPE. At the time of biopsy diagnosis, 26 (35%) patients showed kidney function impairment, which was defined as $eGFR < 60 \text{ mL/min/1.73 m}^2$. Seventeen (23%) patients showed kidney function impairment at discharge. Patients in the low-nephron categories were older and had lower height, higher serum albumin level, and higher incidence of hypertension, GGS, and interstitial fibrosis/tubular atrophy. Sex, weight, body mass index, blood pressure, and kidney function parameters were not different among the groups. Regarding therapeutic regimens, including initial corticosteroid dose, no significant differences were found among the 3 groups.

Morphologic Measurements of Patients With MCD

In morphologic findings, the degree of chronic kidney injury, such as GGS or interstitial fibrosis/tubular atrophy, was low (Table 2). The estimated nonsclerotic nephron number in this cohort ranged from 1.07 to 18.77×10^5 per kidney, a maximal 17-fold difference, with a mean value of 8.78×10^5 per kidney. Measured parenchymal volume and estimated cortical volume were smaller in the low-nephron group compared with the intermediate- and high-nephron groups. Mean numbers of nonsclerotic glomeruli per kidney differed by almost 3-fold between the low- and high-nephron groups. Patients in the low-nephron group had the lowest total glomerular volume and highest SNGFR and SNUPE values among the groups.

Table 1. Baseline Characteristics and Treatments During Follow-up Among All Participants and According to Nephron Number Tertile

Factor	Overall (n = 75)	Nephron No., ×10 ⁵ /Kidney			P for Trend
		Tertile 1 (1.07-7.08) (n = 25)	Tertile 2 (7.09-9.50) (n = 25)	Tertile 3 (9.51-18.77) (n = 25)	
Baseline Characteristics					
Age, y	45.9 ± 18.2	59.6 ± 15.8	43.1 ± 17.7	35.2 ± 11.7	<0.001
Male sex	45 (60.0%)	13 (52.0%)	15 (60.0%)	17 (68.0%)	0.2
Height, cm	164 ± 9	160 ± 10	167 ± 9	166 ± 7	0.01
Weight, kg	63.6 ± 11.8	60.9 ± 13.4	63.7 ± 10.3	66.1 ± 11.5	0.1
BMI, kg/m ²	23.5 ± 3.8	23.8 ± 4.6	22.9 ± 3.0	23.8 ± 3.8	0.8
Hypertension	23 (30.7%)	14 (56.0%)	3 (12.0%)	6 (24.0%)	0.006
SBP, mm Hg	127 ± 19	133 ± 21	124 ± 15	124 ± 19	0.1
DBP, mm Hg	76 ± 11	77 ± 14	77 ± 10	73 ± 10	0.1
Serum albumin, mg/dL	1.6 ± 0.6	1.9 ± 0.6	1.6 ± 0.6	1.4 ± 0.5	0.002
Serum total cholesterol, mg/dL	404 ± 114	364 ± 100	420 ± 106	428 ± 128	0.07
Scr, mg/dL	1.18 ± 0.89	1.26 ± 0.97	1.25 ± 1.10	1.04 ± 0.51	0.9
eGFR, mL/min/1.73 m ²	64.6 ± 26.0	57.4 ± 29.1	65.1 ± 25.4	71.4 ± 22.2	0.003
CL _{cr} , mL/min	89.4 ± 37.5	84.6 ± 32.1	87.7 ± 41.5	95.7 ± 39.0	0.08
CL _{cr} , mL/min/1.73 m ²	86.0 ± 34.8	77.9 ± 28.8	85.4 ± 38.7	94.8 ± 35.5	0.08
UPE, g/d	8.7 ± 4.4	8.6 ± 3.6	8.1 ± 4.3	9.4 ± 5.4	0.9
Selectivity index of UPE	0.11 ± 0.12	0.14 ± 0.17	0.09 ± 0.09	0.09 ± 0.08	0.3
Treatments					
Initial PSL dose, mg	42.3 ± 6.2	40.6 ± 6.5	43.6 ± 6.5	42.8 ± 5.2	0.3
Initial PSL dose/weight, mg/kg	0.69 ± 0.17	0.70 ± 0.23	0.69 ± 0.11	0.67 ± 0.16	0.7
Use of mPSL pulse therapy	7 (9.3%)	3 (12.0%)	3 (12.0%)	1 (4.0%)	0.3
Use of statin	37 (49.3%)	14 (56.0%)	11 (44.0%)	12 (48.0%)	0.6
Use of intravenous 25% albumin	6 (8.0%)	4 (16.0%)	2 (8.0%)	0 (0.0%)	0.04
Use of diuretics	47 (62.7%)	16 (64.0%)	14 (56.0%)	17 (68.0%)	0.8

Note: Values are presented as mean ± standard deviations or number (percent). Conversion factors for units: serum total cholesterol in mg/dL to mmol/L, ×0.02586; Scr in mg/dL to μmol/L, ×88.4.

Abbreviations: BMI, body mass index; CL_{cr}, creatinine clearance; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; mPSL, methylprednisolone; PSL, prednisolone; SBP, systolic blood pressure; Scr, creatinine; UPE, urinary protein excretion.

Comparison of Outcomes According to Nephron Number

Figure 2 shows time series changes in mean UPE values according to tertiles of nephron number. Figure S1 shows serial plots of all measured UPE values for each patient. UPE in the high-nephron group tended to decrease earlier compared with the other groups, and the slowest decrease was in the low-nephron group. Time to complete remission was significantly different, being longest in the low-nephron group and shortest in the high-nephron group, whereas time to partial remission was not significantly different among the groups (Table 3). Similarly, the number of patients achieving complete remission, but not those achieving partial remission, was significantly different among groups at week 4 (Table S1). There were no significant differences among groups in the frequency of patients with relapse or time to relapse (Table 3). A log-rank test revealed that time to complete remission was significantly different among the 3 groups (Fig 3). There were no significant differences in relapse rates according to age or sex, both of which are reportedly associated with time to relapse (Table S2).

Uni- and Multivariable Cox Proportional Hazard Analyses for Complete Remission and Relapse

Univariate and multivariable Cox proportional hazard analyses were performed to examine the influence of factors, including nephron number, on achievement of complete remission and development of relapse. Nephron number was significantly associated with achievement of complete remission, and the relationship remained after multivariable adjustment (Table 4). Conversely, nephron number was not significantly associated with relapse. Similar trends for complete remission and relapse were obtained if the nephron number including GGS was used instead of that excluding GGS (Table S3).

Sensitivity Analysis

To rule out the influence of outliers, we performed sensitivity analysis on patients within the 5th to 95th percentiles of nephron number (n = 67). Nephron number ranged from 2.67 to 16.16 ×10⁵ per kidney (6-fold difference). Log-rank analysis based on tertiles of nephron number revealed that the trend was significant (Fig S2). The observed point estimates of HR were similar to estimates from the main analysis (HR, 1.10 [95% confidence interval, 0.99–1.21]; P = 0.07).

Table 2. Morphologic and Single-Nephron Parameters at Baseline Among All Participants and According to Nephron Number Tertile

Parameter	Overall (n = 75)	Nephron No., ×10 ⁵ /Kidney			P for Trend
		Tertile 1 (1.07-7.08) (n = 25)	Tertile 2 (7.09-9.50) (n = 25)	Tertile 3 (9.51-18.77) (n = 25)	
Morphologic Parameters					
Measured parenchymal volume, cm ³ /kidney	150.5 ± 30.7	135.7 ± 26.0	155.7 ± 36.5	160.1 ± 23.4	<0.001
Estimated cortical volume, cm ³ /kidney	105.6 ± 21.8	95.1 ± 18.5	109.3 ± 25.9	112.4 ± 16.6	<0.001
Cortical area in biopsy, mm ²	7.60 ± 3.11	7.83 ± 3.61	7.24 ± 2.66	7.71 ± 3.07	0.8
IF/TA, %	4.6 ± 4.8	7.0 ± 6.0	3.8 ± 4.1	3.0 ± 3.2	0.02
No. of NSG in biopsy	18.7 ± 8.8	13.6 ± 6.7	17.2 ± 6.6	25.2 ± 8.9	<0.001
No. of GGS in biopsy	0.9 ± 1.4	1.7 ± 1.7	0.6 ± 0.9	0.4 ± 0.9	0.003
Numerical nonsclerotic glomerular density, /mm ³	15.1 ± 6.6	9.2 ± 4.6	14.5 ± 3.3	21.7 ± 4.5	<0.001
Numerical total glomerular density including GGS, /mm ³	16.1 ± 6.6	10.9 ± 5.2	15.1 ± 3.5	22.3 ± 4.5	<0.001
Glomerular volume, ×10 ⁶ μm ³	2.33 ± 0.87	2.57 ± 1.18	2.29 ± 0.71	2.13 ± 0.59	0.1
Total nonsclerotic nephron number, ×10 ⁵ /kidney	8.78 ± 0.41	4.71 ± 2.02	8.33 ± 0.70	13.30 ± 2.83	<0.001
Total nephron number including GGS, ×10 ⁵ /kidney	9.31 ± 0.40	5.59 ± 2.41	8.69 ± 0.94	13.65 ± 2.90	<0.001
Total glomerular volume, cm ³	2.02 ± 1.05	1.17 ± 0.59	1.95 ± 0.64	2.94 ± 1.00	<0.001
Podocyte foot-process effacement, %	79.0 ± 24.0	89.0 ± 17.0	75.2 ± 23.1	72.5 ± 28.3	0.01
Single-Nephron Parameters					
Single nephron eGFR, mL/min/1.73 m ²	47 ± 35	73 ± 47	40 ± 17	29 ± 12	<0.001
Single nephron CL _{cr} , mL/min	69 ± 59	113 ± 78	53 ± 26	39 ± 19	<0.001
Single nephron CL _{cr} , mL/min/1.73 m ²	65 ± 50	104 ± 66	52 ± 25	38 ± 18	<0.001
Single nephron UPE, g/d	6.8 ± 6.5	11.8 ± 8.9	5.0 ± 2.8	3.5 ± 1.6	<0.001

Note: Values are presented as mean ± standard deviation.

Abbreviations: CL_{cr}, clearance of creatinine; eGFR, estimated glomerular filtration rate; GGS, global glomerular sclerosis; IF/TA, interstitial fibrosis/tubular atrophy; UPE, urinary protein excretion.

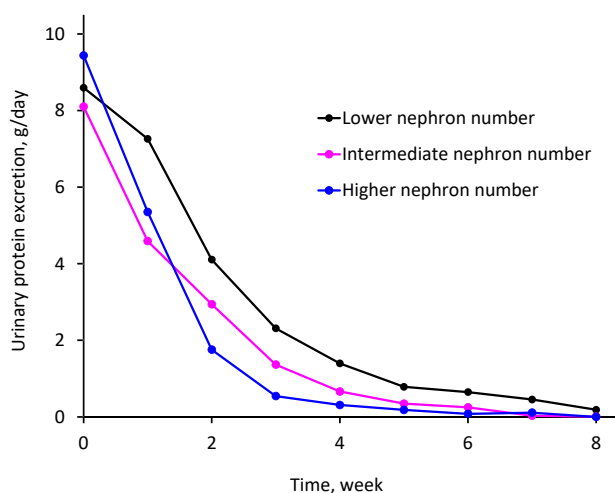


Figure 2. Time course of the average level of urinary protein excretion. The mean value of urinary protein excretion according to nephron number tertile tended to decrease more slowly as nephron number decreased ($P = 0.03$).

DISCUSSION

The present study demonstrated that a small nephron number in adult patients with steroid-sensitive MCD is

significantly associated with longer time to complete remission. In comparisons among low-, intermediate-, and high-nephron groups, significant differences in times to complete remission were identified, whereas no such significant difference was found in time to partial remission or relapse rate following complete remission among the groups. The time to complete remission of the low-nephron group (5 weeks on average) was 2 weeks longer than that of the high-nephron group (3 weeks on average), a difference potentially with significant implications for daily clinical practice. The amount and selectivity of UPE at baseline showed no significant difference, suggesting that initial disease status of MCD was not substantially different among the groups. Multivariable analyses using the Cox proportional hazard model identified nephron number per kidney as being independently associated with complete remission. Taken together, results suggest that a difference in nephron number per kidney does not influence the initial response to corticosteroid therapy or sensitivity to relapse, but affects time to complete remission.

The number of nephrons may affect the remission status of proteinuria in patients with MCD. In a previous study of MCD that analyzed areal glomerular density as a surrogate for nephron number, lower glomerular density was

Table 3. Remission and Relapse During Follow-up

	Overall (n = 75)	Nephron No., ×10 ⁵ /Kidney			P for Trend
		Tertile 1 (1.07-7.08) (n = 25)	Tertile 2 (7.09-9.50) (n = 25)	Tertile 3 (9.51-18.77) (n = 25)	
Patients with complete remission at final observation	75 (100.0%)	25 (100.0%)	25 (100.0%)	25 (100.0%)	NA
Time to complete remission, d	26 [20-36]	35 [25-42]	25 [18-34]	21 [20-28]	0.003
Patients with partial remission at final observation	75 (100.0%)	25 (100.0%)	25 (100.0%)	25 (100.0%)	NA
Time to partial remission, d	9 [6-16]	11 [6-24]	9 [6-13]	7 [4-12]	0.051
Patients with relapse	31 (41.3%)	8 (32.0%)	10 (40.0%)	13 (52.0%)	0.2
Time to relapse, mo	20 [9-40]	26 [16-44]	12 [7-27]	17 [9-44]	0.2

Note: Data for categorical variables expressed as number (percent); data for continuous variables expressed as median [interquartile range].
Abbreviation: NA, not applicable.

associated with delayed complete remission.¹² As in other diseases, weight at birth, which is associated with total number of nephrons, may influence the clinical phenotype of MCD.³¹⁻³⁴ In the current study, mean SNGFR and SNUPE values in the low-nephron group were approximately 3.0- and 3.4-fold those in the high-nephron group, respectively, although mean eGFR, CL_{cr}, and UPE per body values of the low-nephron group were comparable to those of the high-nephron group. By contrast, there were no significant differences in body mass index or blood pressure between the subgroups, which could influence SNGFR and SNUPE.^{35,36} These findings indicate that the differences in time to complete remission between subgroups were associated with changes in local glomerular hemodynamics, mainly attributed to the different number of nephrons. As indicated by the difference in total glomerular volume among subgroups in this study,

patients with a small nephron number may have a limited whole filtration surface area per kidney than those with a larger nephron number. The lack of significant trends in mean glomerular volume among the subgroups suggests a maladaptive response in single glomerular size against hemodynamic load due to acute-onset nephrotic syndrome.

The primary cause of the massive proteinuria in MCD is loss of the charge barrier function of glomerular filtration, but nonimmunologic factors including SNGFR can influence the appearance of proteinuria.³⁷⁻⁴⁰ In general, SNGFR is determined by multiple factors such as body size and kidney plasma flow and can be represented at the single-nephron level by the glomerular filtration pressure gradient, glomerular oncotic pressure gradient, and glomerular filtration coefficient.⁴¹ A previous study reported a 69% decrease in glomerular filtration coefficient in patients with MCD during nephrotic syndrome.⁴² However, these are reversible changes and the influence on them of acute-onset nephrotic syndrome is similarly attenuated at least in the near transition phase to complete remission irrespective of nephron number. A recent interventional study demonstrated that the remaining kidney after heminephrectomy in living kidney donors shows a sustained increase in SNGFR compared with that before donation.⁴³ Thus, we assume that patients with a small nephron number exhibit a substantial increase in SNGFR and so may be engaged to an additional load for the appearance of residual proteinuria during remission.

The association between nephron number and clinical phenotype of MCD may be explained by immune-mediated mechanisms. Previous studies indicated that low birth weight, an important explanatory factor for low nephron number, is associated with an increased incidence and relapse of steroid-sensitive MCD in children.^{33,34} Considering that MCD is an immunologic disease frequently triggered by infections, changes in post-infectious response associated with low birth weight may influence kidney disease outcomes, as suggested in a previous cohort study of Australian Aborigines.⁴⁴

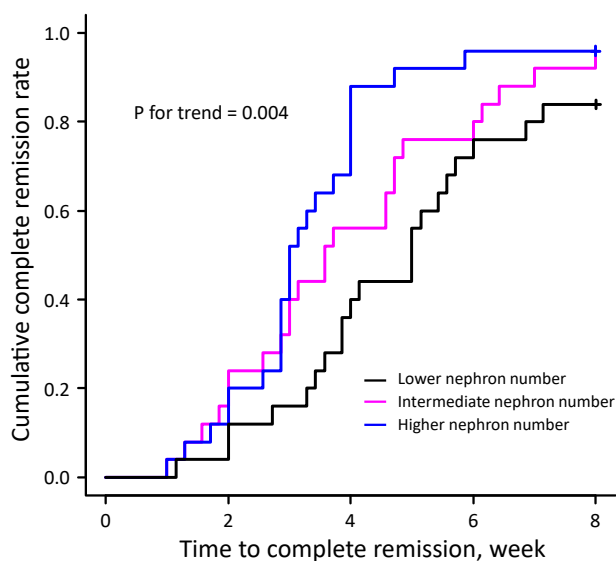


Figure 3. Time to complete remission within 8 weeks according to nephron number group. In log-rank tests, time to complete remission significantly differed among the groups.

Table 4. Predictors of Complete Remission and First Relapse

	Crude			Age- and Sex-Adjusted Model			Multivariable Model		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Complete Remission									
Estimated total nephron number, per 100,000 nephron/kidney	1.11	1.05-1.17	<0.001	1.09	1.02-1.17	0.01	1.10	1.02-1.19	0.01
First Relapse									
Estimated total nephron number, per 100,000 nephron/kidney	1.09	0.99-1.19	0.08	1.07	0.95-1.19	0.3	1.07	0.94-1.20	0.3

Note: The multivariable model was adjusted for age, sex, height, estimated glomerular filtration rate, urinary protein excretion, dosage of prednisolone, and methyl-prednisolone pulse.

Abbreviations: CI, confidence interval; HR, hazard ratio.

This study had several important limitations. First, it was a retrospective observational study that included a relatively small number of samples. Second, kidney tissue specimens obtained by needle biopsy may have considerable sampling errors because glomeruli are not distributed equally within a kidney. Third, the relationship between nephron number and long-term prognosis is unknown due to the lack of long-term follow-up in a large number of cases. Long-term observation of patients with steroid-sensitive MCD would enable analyses of, for example, whether frequently relapsing nephrotic syndrome and/or long-term steroid dependency in relation to a small nephron number affects the incidence of or progression to chronic kidney insufficiency. Fourth, all patients included in this study are Japanese. Given the potential racial differences in nephron number, validation studies with participants of other races are needed. Finally, despite consistent results from sensitivity analysis, it is difficult to determine whether the wide variability in nephron number in this study (17-fold) is a result of factors related to patients with MCD or to measurement errors. Progress is being made in the precise measurement of nephron number using magnetic resonance imaging.^{4,5} The clinical application of such a technology may allow validation of our results.

In conclusion, our results suggest that a small nephron number in patients with MCD is associated with longer time to complete remission. The results provide insight into the kidney response to MCD and will assist determination of the optimum therapeutic strategy during follow-up of patients with MCD diagnosed.

SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Figure S1: Time course of the level of urinary protein excretion

Figure S2: Time to complete remission based on number of nephrons in patients within the 5th to 95th percentiles of nephron number (n = 67)

Table S1: Sensitivity to corticosteroids at 4 weeks after initiation of treatment

Table S2: Stratified analyses based on age and sex category

Table S3: Predictors of time to complete remission and first relapse based on total nephron number, including globally sclerotic glomeruli

ARTICLE INFORMATION

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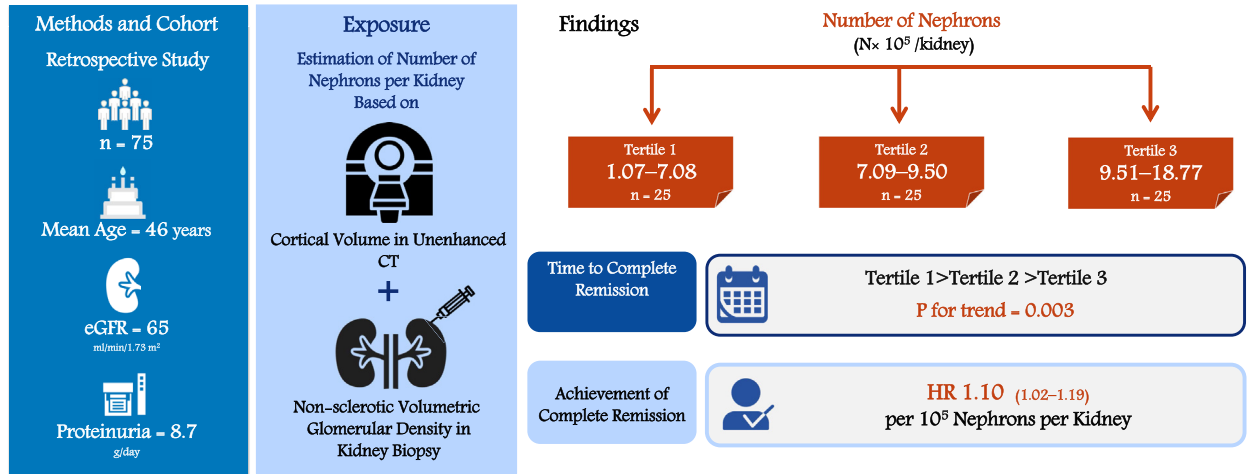
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Is the number of nephrons per kidney associated with response to steroids in adults with minimal change disease?



Conclusion: Having a fewer number of nephrons per kidney in adults with MCD is associated with a longer time to complete remission.

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