

## RESEARCH ARTICLE

# Time series changes in pseudo- $R^2$ values regarding maximum glomerular diameter and the Oxford MEST-C score in patients with IgA nephropathy: A long-term follow-up study

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## Abstract

There is no effectual pathological factor to predict the long-term renal prognosis of IgA nephropathy. Glomerular hypertrophy plays a crucial role in kidney disease outcomes in both experimental models and humans. This study aimed to 1) confirm the long-term prognostic significance of a maximal glomerular diameter (Max GD)  $\geq 242.3 \mu\text{m}$ , 2) test a renal prognosis prediction model adding Max GD  $\geq 242.3 \mu\text{m}$  to the Oxford classification (MEST-C), and 3) examine the time series changes in the long-term renal prognosis of patients with IgA nephropathy. The study included 43 patients diagnosed with IgA nephropathy from 1993 to 1998 at Kameda General Hospital. Renal prognosis with the endpoint of a 50% reduction in estimated glomerular filtration rate (eGFR) or the development of end-stage renal disease requiring dialysis was examined using logistic regression analysis, Cox regression analysis, and the Kaplan-Meier method. Pathological evaluation was performed using MEST-C and Max GD, and the validity of the prediction model was evaluated. Patients with Max GD  $\geq 242.3 \mu\text{m}$  had significantly poor renal prognosis with multivariate Cox analysis ( $P = 0.0293$ ). The results of the Kaplan-Meier analysis showed that kidney survival rates in the high-Max GD group were significantly lower than those in the low-Max GD group (log rank,  $P = 0.0043$ ), which was confirmed in propensity score-matched models (log rank,  $P = 0.0426$ ). Adding Max GD  $\geq 242.3 \mu\text{m}$  to MEST-C improved diagnostic power of the renal prognosis prediction model by renal pathology tissue examination ( $R^2$ : 3.3 to 14.5%, AICc: 71.8 to 68.0, C statistic: 0.657 to 0.772). We confirm that glomerular hypertrophy is useful as a long-term renal prognostic factor.

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## Introduction

Although long-term renal prognoses of kidney diseases are clinically relevant, there is no established histopathological factor to predict the long-term renal prognosis of IgA nephropathy (IgAN). Knoop et al. used data from the Norwegian Kidney Biopsy Registry and found that none of the histopathological variables at the time of biopsy could identify patients with long-term progressive disease [1]. Coppo et al. conducted long-term follow-up analyses of the original Validation Study of the Oxford Classification for IgA Nephropathy cohort, and showed an independent relationship between kidney biopsy findings and the risk of progression towards kidney failure in patients with IgAN. However, in their prognostic analyses, inclusion of the entire set of pathology lesions provided only a slight gain (+1.8%) in discriminatory power over that of the clinical variables alone for the entire follow-up duration [2]. These results suggest that other factors are strongly associated with long-term disease progression of IgAN and that additional markers are clinically required to increase the prognostic efficacy of IgAN [3]. In truth, many studies on renal prognosis include not only histological factors but also clinical factors to predict renal outcome [4, 5]. In 2017, the presence of crescents (C) was added as a fifth parameter to the revised Oxford classification [6]. However, whether the MEST-C score quantitatively improves the long-term prediction of patients' renal prognosis is not yet elucidated. We have previously reported that glomerular hypertrophy (or large renal corpuscles) with a maximum glomerular diameter (Max GD) of 242.3  $\mu\text{m}$  or more as an aggravating factor for renal prognosis of IgAN patients observed for 10 years [7]. The aim of the present study is to examine the prediction efficacy a model in which Max GD  $\geq 242.3 \mu\text{m}$  was added to the Oxford classification (MEST-C) in IgAN cases with long-term data on kidney disease progression.

## Subjects and methods

### Ethics statement

This research was approved by the ethics committee of Kameda Medical Center (No. 17–170), as with our previous study which used the same cohort [7]. After approval by the ethics committee, we used a passive informed consent (opt-out) for subjects. All data were analyzed anonymously.

### Patient selection

Between March 1993 and September 1998, 61 adult patients were diagnosed with IgAN based on their clinical profiles and renal biopsy findings at Kameda Medical Center. Among these, 3 were excluded due to estimated glomerular filtration rate (eGFR)  $< 50 \text{ mL/min/1.73 m}^2$ , 13 were excluded for a follow-up duration  $< 10$  years, and 2 were excluded due to the presence of other renal disease. Finally, 43 patients were enrolled in the present study (S1 Fig). S1 Data include the measurement of covariates, definitions of comorbidities, and the histological assessment of kidney biopsies for all patients in the study.

### Pathological analysis

All kidney tissue specimens were obtained by percutaneous needle biopsy. Each specimen was evaluated for glomerular, interstitial, and vascular changes as previously described [8, 9]. The percentage of glomeruli that exhibited global sclerosis, segmental sclerosis, adhesions, and crescents were estimated. Mesangial cell proliferation, mesangial matrix expansion, interstitial fibrosis, interstitial inflammation, arteriosclerosis, and arteriolar hyalinosis in each patient were semi-quantitatively scored. The Oxford MEST-C criteria [6, 9, 10] was assessed by the

following parameters: mesangial hypercellularity (M0: < 50% of glomeruli showing hypercellularity; M1: > 50% of glomeruli showing hypercellularity), endocapillary hypercellularity (E0: absent; E1: present), segmental glomerulosclerosis (S0: absent; S1: present), tubular atrophy/interstitial fibrosis (T0: < 25%; T1: 25–50%; T2: > 50% of cortical area involved), and cellular/fibrocellular crescents (C0: absent; C1: present in at least 1 but < 25% of glomeruli; C2: present in at least 25% of glomeruli). We also assessed the maximal glomerular area (Max GA) and the Max GD of the maximally-hypertrophied glomerulus (the largest renal corpuscle) identified in serial sections [7]. Max GD was calculated as the mean of two measurements, i.e., the maximal diameter of the maximal profile area in the largest renal corpuscle [11], and the maximal chord perpendicular to the maximal diameter in each specimen [7].

## Study outcome

The primary outcome of the study was kidney disease progression, defined as a  $\geq 50\%$  decline in the eGFR from baseline ( $\geq 50\%$  eGFR decline), or the development of end-stage renal disease (ESRD) requiring dialysis. The patients were followed up until November 2017.

## Statistical analysis

Continuous variables are reported as means and standard deviations, or as medians (minimum–maximum). Categorical variables are reported as percentages, unless otherwise stated. Group differences were evaluated using the unpaired *t*-test, Mann-Whitney U test, Chi-square test, or Fisher's exact test, as appropriate. Logistic regression analyses were used to assess the discriminatory ability and goodness of fit of renal prognostic models. Renal prognostic factors were also evaluated in Cox regression analyses, and the Kaplan-Meier method was used for survival analyses. The prognostic variables for the renal outcomes were assessed using univariate and multivariate Cox proportional hazards models. Variables with *P* values < 0.1 in the univariate model, as well as age, sex, and eGFR, were included in the multivariate model. Based on the previous report [8], we divided the patients into 2 groups consisting of the high-Max GD group (Max GD  $\geq 242.3 \mu\text{m}$ ) and low-Max GD group (Max GD < 242.3  $\mu\text{m}$ ). Survival curves were plotted using the Kaplan-Meier method and evaluated using the log-rank test. To reduce confounding biases, we fitted propensity score-matched models that included potentially modifying variables, namely, age and eGFR; additionally, subgroup analyses were performed. The caliper-matching method was used, with a maximum tolerance level of 0.1.

To evaluate the Oxford classification, components of the MEST-C score with and without large renal corpuscles (Max GD  $\geq 242.3 \mu\text{m}$ ) were considered. The discriminatory ability of the model was evaluated using the concordance (C)-statistic [12, 13]. Goodness of fit was assessed using McFadden's pseudo-*R*-squared (pseudo- $R^2$ ) [14] and the corrected Akaike Information Criterion (AICc) [15]. The statistical tests were 2-tailed, and *P* < 0.05 was considered statistically significant. Statistical analyses were performed using JMP Pro software, version 14.1.0 (SAS Institute, Cary, NC, USA).

## Results

### Patients

The 43 patients included 25 men and 28 women, and their mean age at the time of the renal biopsy was  $39.8 \pm 9.8$  years. The average value of MBP was  $102.6 \pm 16.8$  mmHg, BMI was  $25.4 \pm 4.3$  kg/m<sup>2</sup>, proteinuria was 1.4 (minimum–maximum, 0–7.0) g/day, and eGFR was  $78.6 \pm 17.5$  mL/min/1.73 m<sup>2</sup>. Of the 43 patients, 22 had been treated with a corticosteroid and 25 with either taking an angiotensin-converting enzyme inhibitor or an angiotensin receptor

blocker during the follow-up period. The median duration of follow-up was 14.4 (3.8–24.2) years, with 23 patients reaching the primary outcome (ESRD,  $n = 16$ ;  $\geq 50\%$  eGFR decline,  $n = 21$ ).

### Histopathological findings

The average number of glomeruli examined per patient was  $13.4 \pm 5.5$  and the average number of serial sections examined per patient was  $14.4 \pm 3.2$ . The global glomerulosclerosis rate was 14.3% (0%–57.1%). The average Max GD was  $221.7 \pm 30.8 \mu\text{m}$ . The median rate of global sclerosis, segmental sclerosis, crescent, and interstitial fibrosis were 14.3%, 14.7%, 6.3%, and 5.0%, respectively (Table 1B). The percentages of patients with regards Oxford classification variables were 81.4%, 53.5%, 81.4%, 2.3%, 2.3%, 46.5%, and 9.3% for M1, E1, S1, T1, T2, C1, and C2, respectively (Table 1B).

### High-Max GD relationship to long-term renal outcomes

To examine whether Max GD has relationship with long term renal function decline, we performed univariate and multivariate Cox regression analyses to detect any associations between the baseline clinical and histopathological findings and a  $\geq 50\%$  eGFR decline or ESRD during the follow-up period (Table 2). The multivariate Cox regression analyses revealed a significant association between the primary outcome ( $\geq 50\%$  eGFR decline or ESRD) and a Max GD  $\geq 242.3 \mu\text{m}$  (HR: 3.08, 95% confidence interval [CI]: 1.12–8.69;  $P = 0.0293$ ). Kaplan-Meier analysis showed that the kidney survival rate in the high-Max GD group was significantly lower than that in the low-Max GD group (log rank,  $P = 0.0043$ ) (Fig 1A).

### Comparison of clinical and pathological findings between high- and low-Max GD groups

Comparative analyses revealed that, in the high-Max GD group, the levels of serum creatinine, triglyceride, hemoglobin A1c, and C3c as well as BMI were higher than those in the low-Max GD group (Table 1A). In histological findings, the level of arteriosclerosis and arteriolar hyalinosis were more severe in the high-Max GD group (Table 1B).

Since Max GD was associated with age and eGFR, we fit propensity score-matched models that included potential modifying variables (age and eGFR) and performed subgroup analyses of both groups. Comparative analyses in a propensity score-matched cohort revealed that there were no significant differences between the high-Max GD group and the low-Max GD group in any of the parameters except those associated with Max GD and Max GA levels (high-Max GD group vs. low-Max GD group,  $253.9 \pm 11.9 \mu\text{m}$  vs.  $218.2 \pm 16.2 \mu\text{m}$ ;  $P < 0.0001$ ) (S1 Table). The results of the Kaplan-Meier analysis with a  $\geq 50\%$  eGFR decline or ESRD as the end-point showed that the kidney survival rate of the high-Max GD of IgAN patients was significantly lower than the low-Max GD group, even after adjustment for the age and eGFR (log rank,  $P = 0.0426$ ) (Fig 1B).

### Prognostic value of Max GD over study follow-up times

To examine prognostic values of Max GD, we made two pathological models of IgAN consisting of MEST-C either with or without high-Max GD; we examined 1) the description ability of the model by C-statistic and 2) the goodness of fit of the model by pseudo- $R^2$  and AICc. Addition of Max GD  $\geq 242.3 \mu\text{m}$  to MEST-C scores improved the prediction of the renal outcome compared to using MEST-C scores alone. There was significant improvement in the ability to discriminate between those who did or did not experience the negative renal outcome after

**Table 1. Patient characteristics according to baseline Max GD levels.**

| <b>(Table 1A. Clinical and laboratory findings; Entire cohort, n = 43)</b> |                         |                             |                        |               |                          |
|--|-------------------------|-----------------------------|------------------------|---------------|--------------------------|
| Variables  | Entire cohort           | Max GD $\geq$ 242.3 $\mu$ m | Max GD < 242.3 $\mu$ m | P-value       | Standardized Differences |
|  | n = 43                  | n = 15                      | n = 28                 |               |                          |
| <i>Clinical Findings</i>   |                         |                             |                        |               |                          |
| Age (years)  | 39.8 $\pm$ 9.8 [43]     | 41.4 $\pm$ 9.5              | 39.0 $\pm$ 10.0        | 0.4539        | 0.246                    |
| Sex (Men; n (%))   | 26 (60.5) [43]          | 11 (73.3)                   | 15 (53.6)              | 0.3274        | 0.418                    |
| BMI (kg/m <sup>2</sup> )   | 25.4 $\pm$ 4.3 [43]     | 28.5 $\pm$ 4.2              | 23.7 $\pm$ 3.4         | <b>0.0003</b> | 1.256                    |
| MBP (mmHg)   | 102.6 $\pm$ 16.8 [43]   | 104.8 $\pm$ 15.9            | 101.4 $\pm$ 17.5       | 0.5417        | 0.203                    |
| <i>Laboratory Findings</i>   |                         |                             |                        |               |                          |
| Total Protein (g/dL)   | 6.48 $\pm$ 0.59 [43]    | 6.71 $\pm$ 0.51             | 6.36 $\pm$ 0.59        | 0.0566        | 0.635                    |
| Serum Albumin (g/dL)   | 3.79 $\pm$ 0.39 [43]    | 3.91 $\pm$ 0.40             | 3.73 $\pm$ 0.38        | 0.1487        | 0.461                    |
| Blood Urea Nitrogen (mg/dL)  | 14.5 $\pm$ 3.4 [43]     | 15.6 $\pm$ 2.7              | 13.9 $\pm$ 3.6         | 0.1127        | 0.534                    |
| Serum Creatinine (mg/dL)   | 0.83 $\pm$ 0.20 [43]    | 0.91 $\pm$ 0.22             | 0.79 $\pm$ 0.19        | 0.0719        | 0.584                    |
| eGFR (mL/min/1.73m <sup>2</sup> )  | 78.6 $\pm$ 17.5 [43]    | 72.2 $\pm$ 12.9             | 81.9 $\pm$ 18.9        | 0.0842        | 0.599                    |
| Uric Acid (mg/dL)  | 5.74 $\pm$ 1.58 [43]    | 6.01 $\pm$ 1.56             | 5.60 $\pm$ 1.60        | 0.4210        | 0.259                    |
| Total Cholesterol (mg/dL)  | 195.8 $\pm$ 44.3 [43]   | 197.3 $\pm$ 20.0            | 194.9 $\pm$ 53.3       | 0.8677        | 0.060                    |
| Triglyceride (mg/dL)   | 164.3 $\pm$ 134.2 [43]  | 228.7 $\pm$ 201.3           | 129.8 $\pm$ 58.9       | <b>0.0193</b> | 0.667                    |
| Hemoglobin A1c (NGSP) (%)  | 5.43 $\pm$ 0.94 [30]    | 6.08 $\pm$ 1.32             | 5.11 $\pm$ 0.44        | <b>0.0052</b> | 0.986                    |
| IgG (mg/dL)  | 1141.8 $\pm$ 321.0 [43] | 1173.8 $\pm$ 401.4          | 1124.6 $\pm$ 275.4     | 0.6376        | 0.143                    |
| IgA (mg/dL)  | 337.1 $\pm$ 139.7 [43]  | 362.1 $\pm$ 162.2           | 323.7 $\pm$ 127.2      | 0.3971        | 0.263                    |
| IgM (mg/dL)  | 166.0 $\pm$ 92.0 [43]   | 160.6 $\pm$ 84.3            | 168.9 $\pm$ 97.3       | 0.7832        | 0.091                    |
| CH50 (mg/dL)   | 40.8 $\pm$ 5.9 [34]     | 40.4 $\pm$ 5.5              | 41.0 $\pm$ 6.3         | 0.7791        | 0.101                    |
| C3 (mg/dL)   | 90.4 $\pm$ 19.2 [42]    | 103.6 $\pm$ 21.6            | 83.9 $\pm$ 14.2        | <b>0.0010</b> | 1.078                    |
| C4 (mg/dL)   | 36.9 $\pm$ 10.9 [42]    | 38.7 $\pm$ 11.3             | 36.0 $\pm$ 10.8        | 0.4590        | 0.244                    |
| IgA/C3 ratio   | 3.85 $\pm$ 1.81 [42]    | 3.52 $\pm$ 1.64             | 4.01 $\pm$ 1.90        | 0.4158        | 0.276                    |
| U-Prot (g/day)   | 1.4 (0.0–7.0) [43]      | 1.5 (0.6–5.7)               | 1.1 (0.0–7.0)          | 0.5493        | 0.028                    |
| U-RBC (counts/HPF)   | 10 (0–200) [41]         | 1 (0–100)                   | 10 (0–200)             | 0.0828        | 0.386                    |
| <i>Initial treatments</i>  |                         |                             |                        |               |                          |
| Corticosteroids (n (%))  | 22 (51.2) [43]          | 6 (40.0)                    | 16 (57.1)              | 0.3475        | 0.347                    |
| Tonsillectomy (n (%))  | 2 (4.7) [43]            | 0 (0.0)                     | 2 (7.1)                | 0.5349        | 0.391                    |
| Immunosuppressants (n (%))   | 0 (0.0) [43]            | 0 (0.0)                     | 0 (0.0)                | NA            | NA                       |
| <i>Concomitant drugs</i>   |                         |                             |                        |               |                          |
| Antihypertensive agents (n (%))  | 30 (69.8) [43]          | 11 (73.3)                   | 19 (67.9)              | 1.0000        | 0.119                    |
| ARB and or ACEI (n (%))  | 25 (58.1) [43]          | 9 (60.0)                    | 16 (57.1)              | 1.0000        | 0.059                    |
| Others (n (%))   | 14 (32.6) [43]          | 7 (46.7)                    | 7 (25.0)               | 0.1837        | 0.465                    |
| Anti-platelet agents   | 42 (97.7) [43]          | 14 (93.3)                   | 28 (100.0)             | 0.3488        | 0.379                    |
| Anti-coagulation   | 11 (25.6) [43]          | 3 (20.0)                    | 8 (28.6)               | 0.7190        | 0.202                    |
| Antidyslipidemic agents (n (%))  | 1 (2.3) [43]            | 1 (6.7)                     | 0 (0.0)                | 0.3488        | 0.379                    |
| Antihyperuricemic agents (n (%))   | 5 (11.6) [43]           | 2 (13.3)                    | 3 (10.7)               | 1.0000        | 0.080                    |
| <i>Comorbidities</i>   |                         |                             |                        |               |                          |
| Hypertension (n (%))   | 33 (76.7) [43]          | 12 (80.0)                   | 21 (75.0)              | 1.0000        | 0.120                    |
| Hyperuricemia (n (%))  | 12 (27.9) [43]          | 7 (46.7)                    | 5 (17.9)               | 0.0739        | 0.647                    |
| Hypertriglyceridemia (n (%))   | 16 (37.2) [43]          | 8 (53.3)                    | 8 (28.6)               | 0.1849        | 0.519                    |
| Hypercholesterolemia (n (%))   | 10 (23.3) [43]          | 3 (20.0)                    | 7 (25.0)               | 1.0000        | 0.120                    |
| <b>(Table 1B. Histological findings; Entire cohort, n = 43)</b>            |                         |                             |                        |               |                          |
| Variables  | Entire cohort           | Max GD $\geq$ 242.3 $\mu$ m | Max GD < 242.3 $\mu$ m | P-value       | Standardized Differences |
|  | n = 43                  | n = 15                      | n = 28                 |               |                          |
| Number of glomeruli  | 13.4 $\pm$ 5.5 [43]     | 12.1 $\pm$ 4.5              | 14.1 $\pm$ 5.9         | 0.2767        | 0.381                    |

(Continued)

Table 1. (Continued)

|                                    |                            |                      |                      |         |       |
|------------------------------------|----------------------------|----------------------|----------------------|---------|-------|
| Global sclerosis (%)               | 14.3 (0.0–57.1) [43]       | 12.5 (0.0–55.6)      | 14.5 (0.0–57.1)      | 0.9693  | 0.037 |
| Segmental sclerosis (%)            | 14.7 (0.0–69.2) [43]       | 11.1 (0.0–55.6)      | 17.7 (0.0–69.2)      | 0.2460  | 0.277 |
| Crescent (%)                       | 6.3 (0.0–83.3) [43]        | 6.3 (0.0–33.3)       | 7.4 (0.0–83.3)       | 0.6312  | 0.232 |
| Cellular or Fibro-Cellular (%)     | 6.3 (0.0–83.3) [43]        | 6.3 (0.0–22.2)       | 6.1 (0.0–83.3)       | 0.5938  | 0.297 |
| Fibrous (%)                        | 0.0 (0.0–12.5) [43]        | 0.0 (0.0–12.5)       | 0.0 (0.0–8.3)        | 0.3101  | 0.410 |
| Mesangial cell proliferation (0–3) | 2 (1–3) [43]               | 3 (1–3)              | 2 (1–3)              | 0.1908  | 0.428 |
| Interstitial fibrosis (%)          | 5.0 (0.0–60.0) [43]        | 5.0 (0.0–60.0)       | 5.0 (0.0–30.0)       | 0.7766  | 0.174 |
| Interstitial fibrosis (0–3)        | 1 (0–2) [43]               | 1 (0–2)              | 1 (0–2)              | 0.8161  | 0.098 |
| Interstitial inflammation (%)      | 5.0 (0.0–30.0) [43]        | 5.0 (0.0–25.0)       | 5.0 (0.0–30.0)       | 0.5248  | 0.277 |
| Arteriosclerosis (0–3)             | 1 (0–2) [43]               | 1 (0–2)              | 0 (0–2)              | 0.0082  | 0.878 |
| Arteriolar hyalinosis (0–3)        | 1 (0–3) [43]               | 1 (0–3)              | 1 (0–2)              | 0.0017  | 1.132 |
| Max GD ( $\mu\text{m}$ )           | 221.7 $\pm$ 30.8 [43]      | 253.1 $\pm$ 10.9     | 204.9 $\pm$ 24.0     | <0.0001 | 2.586 |
| Max GA ( $\mu\text{m}$ )           | 37333.8 $\pm$ 10230.7 [43] | 48024.0 $\pm$ 5034.0 | 31606.9 $\pm$ 7223.2 | <0.0001 | 2.637 |
| <i>Oxford Classification</i>       |                            |                      |                      |         |       |
| M1                                 | 35 (81.4) [43]             | 12 (80.0)            | 23 (82.1)            | 1.0000  | 0.054 |
| E1                                 | 23 (53.5) [43]             | 9 (60.0)             | 14 (50.0)            | 0.7492  | 0.202 |
| S1                                 | 35 (81.4) [43]             | 11 (73.3)            | 24 (85.7)            | 0.4188  | 0.311 |
| T1                                 | 1 (2.3) [43]               | 0 (0.0)              | 1 (3.6)              | 1.0000  | 0.273 |
| T2                                 | 1 (2.3) [43]               | 1 (6.7)              | 0 (0.0)              | 0.3488  | 0.379 |
| C1                                 | 20 (46.5) [43]             | 8 (53.3)             | 12 (42.9)            | 0.5401  | 0.209 |
| C2                                 | 4 (9.3) [43]               | 0 (0.0)              | 4 (14.3)             | 0.2802  | 0.578 |

Continuous variables are expressed as means  $\pm$  standard deviation or median (minimum–maximum). Count data are expressed as n (%). For each variable, the number of patients with non-missing data is shown in [].

Abbreviations: n, number; %, percentages; BMI, body mass index; MBP, mean blood pressure; GFR, estimated glomerular filtration rate; NGSP, national glycohemoglobin standardization program; IgG, immunoglobulin G; IgA, immunoglobulin A; IgM, immunoglobulin M; CH50, 50% hemolytic complement activity; C3, complement component 3; C4, complement component 4; U-Prot, Urinary protein excretion; U-RBC, urinary red blood cells; HPF, high-power field; ARB, angiotensin receptor blocker; ACEI, angiotensin-converting enzyme inhibitor; Max GD, maximal glomerular diameter; Max GA, maximal glomerular area; M, mesangial hypercellularity; E, endocapillary hypercellularity; S, segmental glomerulosclerosis; T, tubular atrophy/interstitial fibrosis; C, cellular/fibrocellular crescents

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biopsy as measured by the change in C-statistic (0.657 up to 0.772) (Fig 2). There was also an increase in pseudo- $R^2$  by 0.112 (0.033 vs. 0.145) and a reduction in AICc by 3.8 (71.8 down to 68.0), demonstrating better model fit.

### Time series changes in pseudo- $R^2$ regarding prognostic efficacy

Furthermore, to elucidate time series changes regarding renal prognostic ability of kidney pathological factors, we made further examination regarding  $R^2$  of Max GD, MEST-C score, and the sum of the MEST-C score with or without Max GD (Table 3, Fig 3). As shown in Fig 3A and Table 3, the values of the pseudo- $R^2$  of the Oxford MEST-C were highest 4 years after kidney biopsy (0.4410), gradually fell until 8 years after kidney biopsy (0.0548), and then remained almost the same through the end of the follow-up period. The values of the pseudo- $R^2$  of the model of 'Max GD  $\geq$  242.3  $\mu\text{m}$  with Oxford MEST-C' showed the highest value of 0.6658 at the 4-years-post-kidney biopsy timepoint, fell to 0.0616 at the 8-years-post-kidney biopsy timepoint, and then rose to the value of 0.1449 at the end of the follow-up period. The values of the pseudo- $R^2$  of the model of 'Max GD  $\geq$  242.3  $\mu\text{m}$  with Oxford MEST-C' was higher than those of 'Oxford MEST-C' at all the follow-up timepoints, demonstrating better model fit even in time series follow-up. As shown in Fig 3B and Table 3, the values of the pseudo- $R^2$  of



**Table 2. Univariate and multivariate analysis of risk factors associated with a  $\geq 50\%$  eGFR decline or ESRD (Entire cohort, n = 43).**

| Variables                                    | Univariate analysis   |         | Multivariate analysis |         |
|--|-----------------------|---------|-----------------------|---------|
|  | Hazard Ratio (95% CI) | P-value | Hazard Ratio (95% CI) | P-Value |
| <i>Clinical and Laboratory Findings</i>      |                       |         |                       |         |
| Age (1 year increase)                        | 1.02 (0.98–1.07)      | 0.3017  | 0.97 (0.91–1.05)      | 0.5115  |
| Men (vs. women)                              | 1.95 (0.83–5.07)      | 0.1283  | 1.62 (0.45–6.05)      | 0.4513  |
| BMI (1 kg/m <sup>2</sup> increase)           | 1.08 (0.97–1.18)      | 0.1449  | -                     | -       |
| MBP (1 mmHg increase)                        | 1.00 (0.98–1.03)      | 0.8917  | -                     | -       |
| eGFR (1 mL/min/1.73 m <sup>2</sup> increase) | 0.93 (0.89–0.96)      | <0.0001 | 0.94 (0.89–0.98)      | 0.0082  |
| Hemoglobin (1 g/dL increase)                 | 0.96 (0.78–1.17)      | 0.6879  | -                     | -       |
| Serum albumin (1 g/dL increase)              | 1.02 (0.637–3.08)     | 0.9653  | -                     | -       |
| U-Prot (g/day)                               | 1.00 (0.74–1.27)      | 0.9797  | -                     | -       |
| Hypercholesterolemia (vs. no)                | 0.58 (0.17–1.55)      | 0.2950  | -                     | -       |
| Hypertriglyceridemia (vs. no)                | 1.07 (0.44–2.46)      | 0.8699  | -                     | -       |
| Hyperuricemia (vs. no)                       | 3.41 (1.44–7.84)      | 0.0062  | 0.76 (0.21–2.76)      | 0.6661  |
| <i>Initial treatments</i>                    |                       |         |                       |         |
| Corticosteroids (vs. no)                     | 0.47 (0.20–1.09)      | 0.0789  | 0.65 (0.22–1.82)      | 0.4136  |
| Tonsillectomy (vs. no)                       | 2.24 (0.36–7.72)      | 0.3289  | -                     | -       |
| <i>Histological findings</i>                 |                       |         |                       |         |
| Global sclerosis (%)                         | 1.01 (0.99–1.04)      | 0.3190  | -                     | -       |
| Segmental sclerosis (%)                      | 0.99 (0.96–1.02)      | 0.4142  | -                     | -       |
| Crescent (%)                                 | 1.00 (0.97–1.03)      | 0.7976  | -                     | -       |
| Mesangial cell proliferation (0–3)           | 1.26 (0.54–2.86)      | 0.5855  | -                     | -       |
| Interstitial fibrosis (0–3)                  | 3.77 (1.53–8.55)      | 0.0049  | 2.26 (0.79–6.58)      | 0.1240  |
| Interstitial inflammation (0–3)              | 1.64 (0.77–3.52)      | 0.2021  | -                     | -       |
| Arteriosclerosis (0–3)                       | 1.10 (0.63–1.87)      | 0.7247  | -                     | -       |
| Arteriolar hyalinosis (0–3)                  | 2.22 (1.26–3.82)      | 0.0062  | 1.02 (0.47–2.25)      | 0.9551  |
| Max GD (1 $\mu$ m increase)                  | 1.02 (1.01–1.04)      | 0.0061  | NA                    | -       |
| Max GD $\geq 242.3 \mu$ m (vs. not)          | 3.16 (1.37–7.38)      | 0.0076  | 3.08 (1.12–8.69)      | 0.0293  |
| <i>Oxford Classification</i>                 |                       |         |                       |         |
| M0/M1  | 1.64 (0.56–6.95)      | 0.3977  | NA                    | -       |
| E0/E1  | 1.28 (0.56–2.99)      | 0.5618  | NA                    | -       |
| S0/S1  | 1.22 (0.46–4.23)      | 0.7077  | NA                    | -       |
| T0/T1/T2                                     | 2.07 (0.36–6.28)      | 0.3475  | NA                    | -       |
| C0/C1/C2                                     | 0.96 (0.50–1.75)      | 0.8960  | NA                    | -       |

Variables with  $P$ -values of less than 0.1 in the univariate model, age, sex, and eGFR were included in the multivariate model.

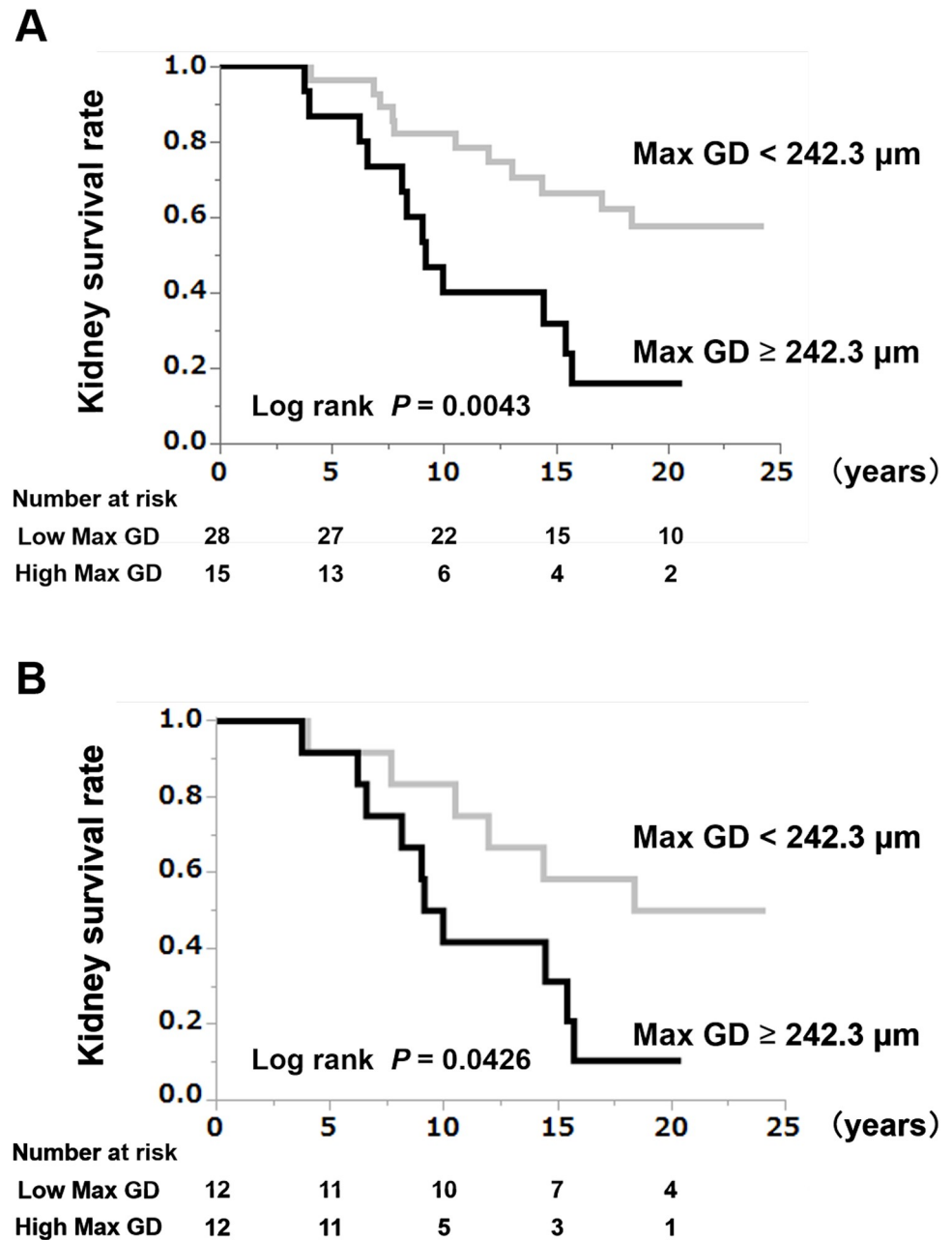
Abbreviation: eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; n, number; %, percentages; CI = confidence interval; vs, versus; BMI, body mass index; MBP, mean blood pressure; U-Prot, Urinary protein excretion; Max GD, maximal glomerular diameter; NA, not applicable; M, mesangial hypercellularity; E, endocapillary hypercellularity; S, segmental glomerulosclerosis; T, tubular atrophy/interstitial fibrosis; C, cellular/fibrocellular crescents

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‘Max GD  $\geq 242.3 \mu$ m’ showed a highest value of 0.2718 at the 4-years-post-kidney biopsy, fell to 0.0101 at the 8-years-post-kidney biopsy, and then rose to the value of 0.1156 at the end of the follow-up period.

## Discussion

To grasp the risk for ESRD over extended follow-up periods, lifetime risk is important knowledge for chronic kidney disease patients. IgAN is considered to have a variable progression rate due to differences in pathophysiological pathways. As mentioned in the previous report

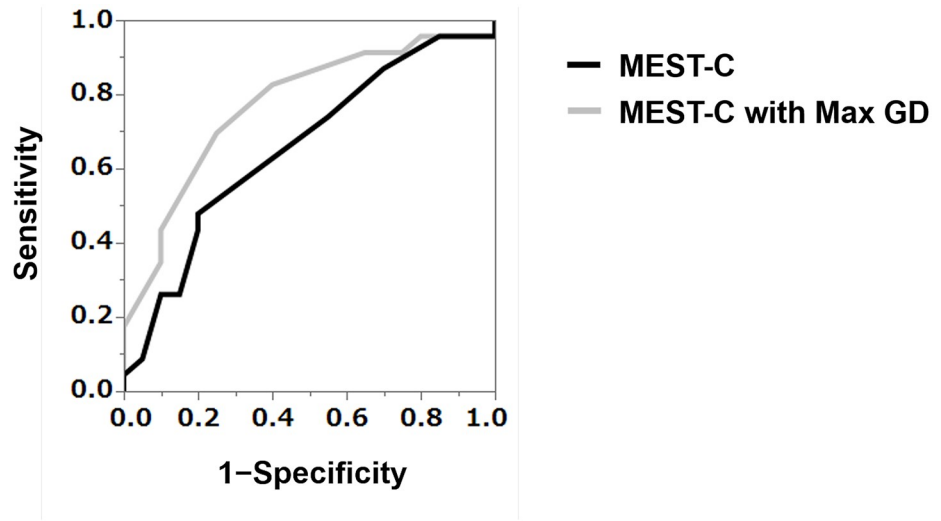


**Fig 1.** (A) Kidney survival rates in the high-Max GD group (Max GD  $\geq 242.3 \mu\text{m}$ ) and low-Max GD group (Max GD  $< 242.3 \mu\text{m}$ ) within the entire cohort. The renal prognosis for patients with glomerular hypertrophy with a Max GD  $\geq 242.3 \mu\text{m}$  was poor. Max GD: maximal glomerular diameter. (B) Kidney survival rate in the high-Max GD group (Max GD  $\geq 242.3 \mu\text{m}$ ) and low-Max GD group (Max GD  $< 242.3 \mu\text{m}$ ) in the propensity score-matched cohort. The renal prognosis for patients with glomerular hypertrophy and a Max GD  $\geq 242.3 \mu\text{m}$  was poor after matching the groups for age and eGFR. Max GD: maximal glomerular diameter; eGFR, estimated glomerular filtration rate.

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[2], some patients may not develop ESRD during the first year of follow-up, but they still have a substantial risk for ESRD over their lifetimes. Investigating the relationships of MEST-C scores or Max GD with long-term renal outcomes is clinically significant. In the present study, we found that long-term prediction was confirmed in the time series change of pseudo- $R^2$





**Fig 2. Receiver operating characteristic curves and the C-statistic (area under the curve) for the models predicting the risk of a  $\geq 50\%$  eGFR or ESRD using the Oxford (MEST-C) score with or without a maximal glomerular diameter (Max GD)  $\geq 242.3 \mu\text{m}$ .** Adding a Max GD  $\geq 242.3 \mu\text{m}$  to the MEST-C score significantly improved discrimination regarding the renal outcome, as measured by the change in the C-statistic from 0.657 to 0.772. eGFR: estimated glomerular filtration rate; ESRD: end-stage renal disease; Max GD: maximal glomerular diameter.

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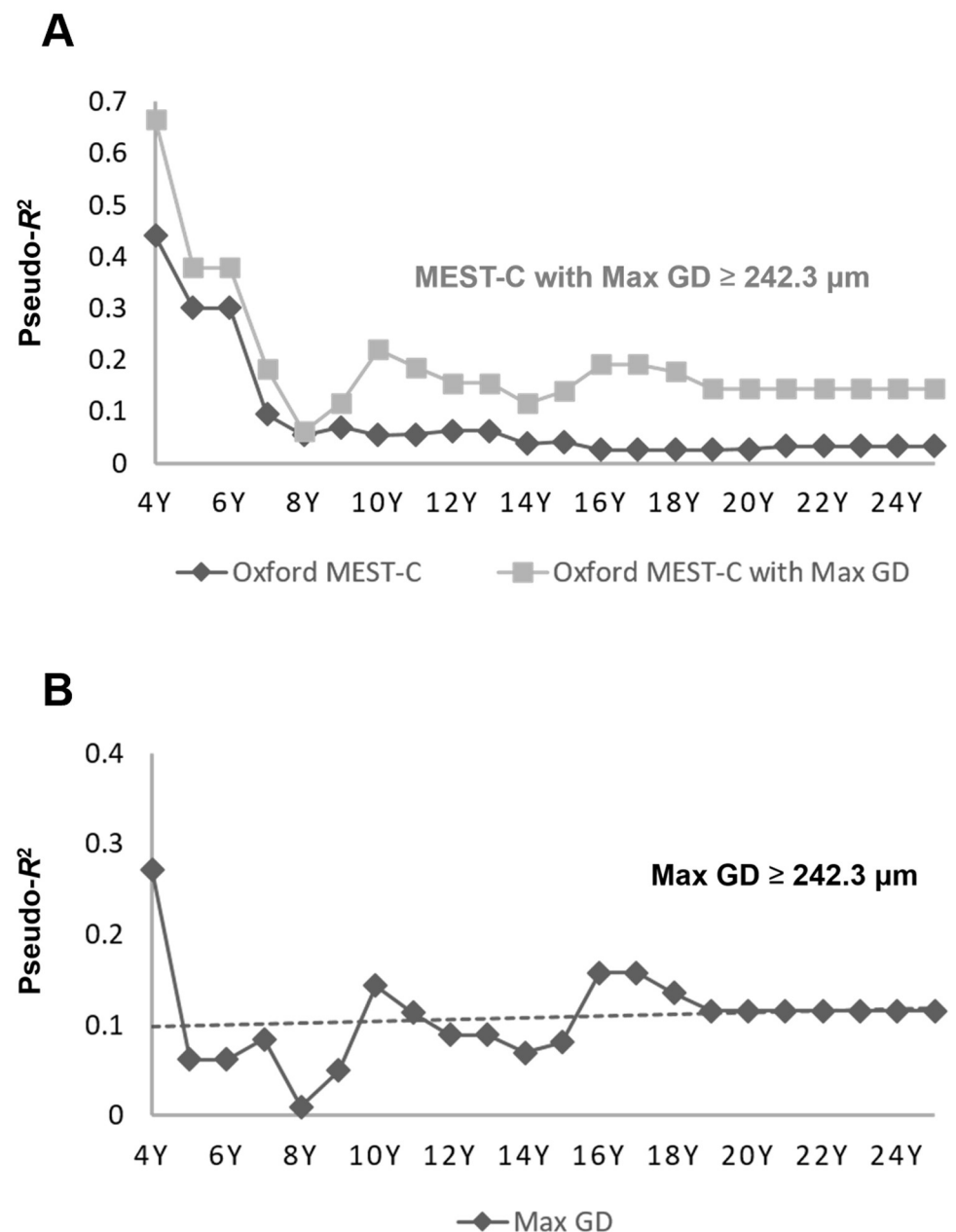
**Table 3. Time series changes in pseudo- $R^2$  regarding prognostic efficacy for renal outcome: Max GD and Oxford MEST-C score.**

|     | Max GD $\geq 242.3 \mu\text{m}$ | Oxford MEST-C | Oxford MEST-C with Max GD $\geq 242.3 \mu\text{m}$ |
|-----|---------------------------------|---------------|--|
| 4Y  | 0.2718                          | 0.4410        | 0.6658   |
| 5Y  | 0.0622                          | 0.3015        | 0.3795   |
| 6Y  | 0.0622                          | 0.3015        | 0.3795   |
| 7Y  | 0.0848                          | 0.0945        | 0.1835   |
| 8Y  | 0.0101                          | 0.0548        | 0.0616   |
| 9Y  | 0.0498                          | 0.0697        | 0.1174   |
| 10Y | 0.1437                          | 0.0541        | 0.2209   |
| 11Y | 0.1138                          | 0.0561        | 0.1859   |
| 12Y | 0.0896                          | 0.0628        | 0.1552   |
| 13Y | 0.0896                          | 0.0628        | 0.1552   |
| 14Y | 0.0696                          | 0.0375        | 0.1166   |
| 15Y | 0.0808                          | 0.0411        | 0.1400   |
| 16Y | 0.1579                          | 0.0259        | 0.1916   |
| 17Y | 0.1579                          | 0.0259        | 0.1916   |
| 18Y | 0.1355                          | 0.0259        | 0.1775   |
| 19Y | 0.1156                          | 0.0259        | 0.1449   |
| 20Y | 0.1156                          | 0.0276        | 0.1449   |
| 21Y | 0.1156                          | 0.0333        | 0.1449   |
| 22Y | 0.1156                          | 0.0333        | 0.1449   |
| 23Y | 0.1156                          | 0.0333        | 0.1449   |
| 24Y | 0.1156                          | 0.0333        | 0.1449   |
| End | 0.1156                          | 0.0333        | 0.1449   |

Abbreviations: Max GD, maximal glomerular diameter; Y, year; End, end of the study

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## Time-series changes in pseudo- $R^2$



**Fig 3.** (A) Time-series changes in the pseudo- $R^2$  values of the prognostic efficacy in relation to the renal outcome. The lower line represents the time-series changes in the pseudo- $R^2$  values of the Oxford mesangial (M) and endocapillary (E) hypercellularity, segmental sclerosis (S), interstitial fibrosis/tubular atrophy (T), and crescents (C) (MEST-C) score and the upper line shows the time-series changes in the pseudo- $R^2$  values of the Oxford MEST-C score with a maximal glomerular diameter (Max GD)  $\geq 242.3 \mu\text{m}$ . Adding the Max GD improved the model's short-term renal prognostic ability to about 1.5-fold for patients with IgAN 4 years after kidney biopsy, and the model's long-term renal prognostic ability to more than 3-fold for patients with IgAN at the end of the study. Max GD: maximal glomerular diameter; eGFR: estimated glomerular filtration rate; ESRD: end-stage renal disease. (B) Time-series changes in the pseudo- $R^2$  values for prognostic efficacy in relation to the renal outcome: Max GD. Although the prognostic potential of the Max GD tended to decrease after 4 years, it rose the predictive power with respect to renal prognosis after 8 years and sustained to the end of the study. Dotted line marks least-squares regression line. Max GD: maximal glomerular diameter.

<https://doi.org/10.1371/journal.pone.0232885.g003>

regarding renal prognosis. This finding could provide IgAN patients with important information about their disease progression.

### Significance of large renal corpuscles (glomerular hypertrophy)

Although it has been well-established that large renal corpuscles (glomerular hypertrophy) play a crucial role in the outcomes of kidney diseases in experimental models [16–19] and in humans [20–22], large glomerular size and renal corpuscle size as an indicator of renal prognosis have yet to be fully used in clinical settings. We propose that there is a pathological threshold of glomerular size which differentiates morbid glomerular hypertrophy from physiological glomerular hypertrophy [23], and we previously demonstrated poor renal prognosis of the high-Max GD group in patients with IgAN at the 10-year follow-up [7]. The present study demonstrated the poor renal prognosis of the high-Max GD group in patients with IgAN at the long-term follow-up. This result is important because histological risk factors are generally recognized to have weaker predictive power for long-term outcomes than clinical risk factors, such as persistent proteinuria or hypertension [24, 25]. The multivariate Cox regression analysis of the cohort indicated that large renal corpuscles (Max GD  $\geq$  242.3  $\mu\text{m}$ ) were associated with a  $\geq$  50% eGFR decrease or ESRD, confirmed by Kaplan-Meier analysis in age- and eGFR-matched models.

### Evaluation study of the Oxford classification of IgAN with Max GD

The C-statistic originated in diagnostic studies [12] and was generalized for survival data [13]. When comparing two models, an increase in C-statistic suggests improvement in discrimination. In the present study, significant improvement in discrimination regarding the renal outcome in C-statistic (0.657 up to 0.772) was achieved by adding Max GD  $\geq$  242.3  $\mu\text{m}$  to MEST-C scores. Akaike Information Criterion is an estimator of the relative quality of statistical models, and the AICc was generalized to allow for small sample sizes [15], with a reduction in AICc suggesting better model fit. When Max GD was added to Oxford MEST-C scores, there was a reduction in AICc by 3.8 (71.8 down to 68.0), demonstrating better model fit. Pseudo- $R^2$  is a measure of the explained variance that is valid in categorical regression models. Pseudo- $R^2$  ranges from 0 to 1 (or 0% to 100%) with higher values indicating better model fit in logistic regressions [14]. In the present study, there was an increase in pseudo- $R^2$  by 0.112 (0.033 vs. 0.145) when Max GD was added to Oxford MEST-C scores, suggesting better model fit. This result suggests that the score of the Oxford MEST-C combined with Max GD  $\geq$  242.3  $\mu\text{m}$  explains 14.5% of renal outcome at the end of the present study, with long-term follow-up data. From the results of these three indices, we confirmed that the inclusion of the Max GD improves the renal long-term predictive prognostic ability in patients with IgAN.

Time series changes in pseudo- $R^2$  regarding prognostic efficacy are rarely examined. In this study, we investigated the time series change of pseudo- $R^2$ , because long-term renal prognosis [2, 26] and time series changes regarding prognostic efficacy of risk factors are clinically relevant issues. The present study is the first to show the long-term follow-up time series change in pseudo- $R^2$  values in relation to the prognostic abilities of renal pathological factors in IgAN patients. Though time series changes of prognostic abilities for renal outcome in IgAN patients were unknown, the values of the pseudo- $R^2$ s of MEST-C gradually fell over time, as we expected. The pseudo- $R^2$  regarding the MEST-C fell from 0.4410 to 0.0548 between the 4- and 8-years-post-kidney biopsy timepoints, validating previous studies that found histological risk factors are worse predictors for the long-term outcome than clinical risk factors [24, 25]. To the contrary, although the values of the pseudo- $R^2$  of 'Max GD  $\geq$  242.3  $\mu\text{m}$ ' also fell to the minimum of 0.0101 at the 8-years-post-kidney biopsy, it increased again to the value of 0.1156

at the end of the follow-up period. Assisted by the prognostic characteristics of 'Max GD  $\geq 242.3 \mu\text{m}$ ', the 'MEST-C score combined with a Max GD  $\geq 242.3 \mu\text{m}$ ' model explained 66.6% of renal outcomes 4 years after kidney biopsy, and 14.5% of renal outcomes at the end of the study. Adding Max GD improved the model's short-term renal prognostic ability to 150.9% for patients with IgAN at the 4-year follow-up and improved the model's long-term renal prognostic ability to 435.1% for patients with IgAN at the end of the study (calculations based on [Table 3](#)).

We propose that the reason for the rise of the pseudo- $R^2$  value of 'Max GD  $\geq 242.3 \mu\text{m}$ ' is based on the multifactorial characteristics of glomerular hypertrophy. Glomerular hypertrophy has been well-reported by many studies to occur in various pathophysiological conditions and to play crucial roles in the outcomes in animal models of kidney disease, such as high-fructose diet-fed rat [27], high-fat diet-fed rhesus monkey [28], Otsuka Long-Evans Tokushima Fatty rat [18, 29], high protein diet-fed mouse [30], high cholesterol-fed rat [31], Dahl salt-sensitive rat [32], streptozotocin diabetic rat [33, 34], obese Zucker rat [35], human immunodeficiency virus type-1 transgenic mouse [36], SV40 transgenic mouse [37], growth hormone transgenic mouse [38], uninephrectomized rat received angiotensin II [17], and others [16, 19, 39–56]. In humans, glomerular hypertrophy is thought to reflect the activity of the original disease and the occurrence of renal damage caused by the presence of various metabolic risk factors such as obesity and diabetic mellitus [23]. In the present study, Max GD was affected by BMI and serum triglyceride and C3 levels, which were found to be higher in the high-Max GD group (Max GD  $\geq 242.3 \mu\text{m}$ ) ([Table 1A](#)). Although no patients were found to have diabetic nephropathy at the beginning of the study, it is important to consider diabetes or metabolic disorders when glomerular hypertrophy is detected on renal biopsy.

Generally, chronic kidney disease including IgAN is affected by multiple risk factors for disease progression [57, 58]. Therefore, it is extremely important to identify not only acute risk factors but also chronic risk factors for the acceleration of the kidney disease progression. Short-term renal prognoses are influenced by inflammation and lifestyle-related diseases, resulting in time-series decreases of the pseudo- $R^2$  when patients were treated successfully. Long-term renal prognoses are influenced by irreversible lesions and lifestyle-related diseases, including obesity and atherosclerosis, generally resulting in time-series increases of the pseudo- $R^2$  when patients were not treated successfully. The relationships between the Max GD pseudo- $R^2$  value and short- and long-term renal prognoses suggest that the Max GD may represent a variety of pathological conditions, including immunological inflammation, lifestyle-related diseases, and irreversible damage.

In the present study, proteinuria and some Oxford lesions (mesangial hypercellularity, crescents, and segmental sclerosis) were not associated with the primary outcome ([Table 2](#)). At first glance, this seems discrepant with numerous published clinical and pathologic studies. However, the time series changes in pseudo- $R^2$  values regarding prognostic efficacy provides elucidating information. As shown in [Fig 3A](#) and [Table 3](#), although the value of the pseudo- $R^2$  of the Oxford MEST-C was only 0.055 at 8 years after kidney biopsy, it remained greater than 0.3 until 6 years after kidney biopsy. Considering that most patients had active disease (with Oxford scores of M1 in 81.4%, E1 in 53.5%, S1 in 81.4%, T0 in 95.3%, C1 in 46.5%, and C2 in 9.3% of patients), and that 22 (51.2%) of the patients received steroid immunosuppression, the long-term renal prognoses might have been improved by successful treatment in the present study. Although the Oxford classification of IgAN [9, 10] was originally introduced to improve the individual risk prediction of IgAN progression, two major issues required resolution: the low renal prognostic ability of the Oxford classification [4, 26] and inconsistency in the renal prognostic power of each marker in the Oxford classification [59–62]. Differences regarding treatment, outcome measures, and patient selection criteria are thought to cause

inconsistencies regarding the predictive values of the Oxford components. The assessment of the time series changes in pseudo- $R^2$  values regarding renal prognostic efficacy might help resolve these inconsistencies.

### Limitations

The current results may have broader implications for patients with a variety of diseases due to our assessment of time series changes in pseudo- $R^2$  of disease risk factors. However, our patient characteristics were determined only at baseline, and data during the follow-up period were not considered. Additionally, the sample size was relatively small, and further studies with a larger cohort are needed to confirm our findings. This study was observational in nature; thus, any observed associations do not prove causality.

### Conclusion

In conclusion, Max GD can be used as a long-term prognostic indicator for disease progression in IgAN patients. By adding Max GD  $\geq 242.3 \mu\text{m}$  to Oxford MEST-C scores, there was significant improvement in the prediction of renal outcome in patients with IgAN at the long-term follow-up. Time series changes in pseudo- $R^2$  regarding the Max GD provided highly suggestive information on renal progression of IgAN, which has multifactorial risk factors. We hope that the Max GD may be used as an early prognostic factor for the long-term outcomes of IgAN patients.

### Supporting information

**S1 Fig. Patient selection flow chart.**

(DOCX)

**S1 Data. Measurements of the covariates and definitions of comorbidities.**

(DOCX)

**S1 Table. Patient characteristics according to baseline Max GD levels (propensity score-matched cohort).**

(DOCX)

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**Writing – original draft:** Hiroshi Kataoka.

**Writing – review & editing:** Toshio Mochizuki.

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