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ORIGINAL ARTICLE

# Nationwide multicentre kidney biopsy study of Japanese patients with type 2 diabetes

Kengo Furuichi[1], Yukio Yuzawa[2], Miho Shimizu[1], Akinori Hara[1], Tadashi Toyama[1], Hiroshi Kitamura[3], Yoshiki Suzuki[4], Hiroshi Sato[5], Noriko Uesugi[6], Yoshifumi Ubara[7], Satoshi Hisano[8], Yoshihiko Ueda[9], Shinichi Nishi[10], Hitoshi Yokoyama[11], Tomoya Nishino[12], Kentaro Kohagura[13], Daisuke Ogawa[14], Koki Mise[14], Yugo Shibagaki[15], Kenjiro Kimura[16], Masakazu Haneda[17], Hirofumi Makino[14], Seiichi Matsuo[18] and Takashi Wada[19]

[1]Division of Nephrology, Kanazawa University Hospital, Ishikawa, Japan, [2]Department of Nephrology, Fujita Health University Hospital, Aichi, Japan, [3]Department of Pathology, Clinical Research Center, National Hospital Organization Chiba East National Hospital, Chiba, Japan, [4]Health Administration Center, Niigata University, Niigata, Japan, [5]Clinical Pharmacology and Therapeutics, Tohoku University Graduate School of Pharmaceutical Sciences, Sendai, Japan, [6]Department of Pathology, Faculty of Medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan, [7]Nephrology Center, Toranomon Hospital, Tokyo, Japan, [8]Department of Pathology, Faculty of Medicine, Fukuoka University, Fukuoka, Japan, [9]Department of Diagnostic Pathology, Dokkyo Medical University Koshigaya Hospital, Saitama, Japan, [10]Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Hyogo, Japan, [11]Department of Nephrology, Kanazawa Medical University School of Medicine, Ishikawa, Japan, [12]Second Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan, [13]Department of Cardiovascular Medicine, Nephrology and Neurology, University of the Ryukyus School of Medicine, Okinawa, Japan, [14]Department of Diabetic Nephropathy, Okayama University, Okayama, Japan, [15]Division of Nephrology and Hypertension, Department of Internal Medicine, St Marianna University School of Medicine, Kanagawa, Japan, [16]Tokyo Takanawa Hospital, Tokyo, Japan, [17]Division of Metabolism and Biosystemic Science, Department of Medicine, Asahikawa Medical University, Hokkaido, Japan, [18]Division of Nephrology, Department of Internal Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan and [19]Division of Nephrology, Department of Nephrology and Laboratory Medicine, Kanazawa University, Ishikawa, Japan

Correspondence and offprint requests to: Takashi Wada; E-mail: twada@m-kanazawa.jp

## ABSTRACT

**Background.** The clinical and pathologic manifestations of nephropathy due to type 2 diabetes are diverse, but large-scale pathologic studies with long-term observations are limited.

**Methods.** Kidney biopsies and clinical data of 600 patients with type 2 diabetes were collected retrospectively from 13 centres across Japan. Thirteen pathologic findings (nine glomerular lesions, two interstitial lesions and two vascular lesions) were clearly defined and scored.

**Results.** During the observation period, there were 304 composite kidney events [dialysis, doubling of creatinine or reduction of estimated glomerular filtration rate (eGFR) by half], 31 instances of chronic kidney disease (CKD) G5D, 76 cardiovascular events and 73 deaths. The mean observation period was 72.4 months. The distribution of CKD heat map categories for the 600 patients was 103 green or yellow, 149 orange and 348 red. Even in the cases in the green and yellow category, diffuse lesions (81.6%), polar vasculosis (42.6%) and subendothelial space widening (35.1%) were commonly detected. Cox proportional hazard analysis revealed that the presence of nodular lesions [hazard ratio (HR) 21.1, 95% confidence interval (CI) 5.3–84.6], exudative lesions (HR 5.1, 95% CI 1.3–20.3) and mesangiolysis (HR 7.6, 95% CI 2.0–28.8) in cases in the green and yellow category were associated with significantly great impact on composite kidney events after adjustment for clinical risk factors.

**Conclusions.** This nationwide study on kidney biopsy of 600 cases with type 2 diabetes revealed that pathologic findings (presence of nodular lesions, exudative lesions and mesangiolysis) were strong predictors of kidney events in low-risk patients.

**Keywords:** CKD heat map, diabetes mellitus, diabetic nephropathy, kidney biopsy, nodular lesion

## INTRODUCTION

The chronic kidney disease (CKD) heat map, which was issued as part of the Kidney Disease: Improving Global Outcomes (KDIGO) 2012 Clinical Practice Guideline for the Evaluation and Management of CKD [1], provides a simple and clinically relevant colour-coded classification that helps predict the prognosis of CKD. This classification is based on three factors: the cause of the disease, the degree of albuminuria and the estimated glomerular filtration rate (eGFR). Albuminuria and eGFR are key prognostic markers for diabetic nephropathy (DN). In fact, our previous data clearly showed that increased albuminuria and reduced eGFR were strong predictors of kidney events, cardiovascular events and all-cause mortality in Japanese patients with type 2 diabetes [2]. In addition to the CKD heat map classification, other markers of kidney damage and injury can provide further information that is relevant to CKD prognosis. Pathologic findings should be one of these possible additional markers for CKD.

The clinical and pathologic manifestations of DN due to type 2 diabetes are diverse [3, 4]. A recent clinical study on the progression of kidney dysfunction in DN characterized two clinical groups, based on the rate of decline in GFR: the rapid decliner and non-decliner groups [5]. Our previous study indicated that some pathologic findings were good predictors of kidney events, cardiovascular events and all-cause mortality [6, 7]. A recent study revealed that advanced diabetic kidney lesions were incidentally detected even in normoalbuminuric cases [8]. These clinical and pathologic observations of the heterogeneity of diabetic kidney disease motivated us to evaluate how pathologic findings and clinical category could assist in predicting renal events in patients with DN.

The pathologic classification of DN by Tervaert *et al.* was systematic and organized in predicting renal outcomes [9–11]. However, this classification omitted some important pathologic findings, especially with regard to glomerular lesions. Accordingly, we evaluated as many pathologic parameters as possible in the present study, with clear definitions and standardized scores for each. With the support of the Ministry of Health, Labour and Welfare of Japan, we collected kidney biopsy samples on a national scale and re-evaluated each DN biopsy specimen. We evaluated the additional value of specific pathologic findings, including details of glomerular lesions, in predicting kidney events in type 2 diabetes patients, particularly those in the low-risk CKD heat map categories.

## MATERIALS AND METHODS

The data from 600 biopsy-confirmed DN cases were collected retrospectively from 13 centres across Japan. The diagnosis of diabetes was based on the criteria of the Japanese Diabetes Society [12]. Patients were classified as having diabetes if they met one of the following criteria: (i) fasting plasma glucose level $\geq$126 mg/dL ($\geq$7.0 mmol/L); (ii) $\geq$200 mg/dL ($\geq$11.1 mmol/L) glucose level at 2 h after a 75-g oral glucose tolerance test; or (iii) random plasma glucose level $\geq$200 mg/dL ($\geq$11.1 mmol/L). Kidney biopsy was performed for patients for whom it was clinically necessary to obtain a precise diagnosis of the kidney lesions. Typical indications for biopsy included proteinuria without diabetic retinopathy, haematuria, rapid decline in eGFR or massive proteinuria in patients with short duration of diabetes (Supplementary Table S1). Patients with other glomerular diseases concomitant with DN were excluded from this study. There was no limitation in glomerular number if the following pathologic findings were evaluated. Written informed consent was obtained from each patient. The study protocol was approved by the medical ethics committee of Kanazawa University (Approval No. 1204).

### Pathologic examinations

Biopsy samples were examined by light microscopy after staining with periodic acid–Schiff, periodic acid methenamine silver, haematoxylin–eosin and Mallory–Azan or Masson's Trichrome stains. Pathologic stages were defined in detail based on typical morphology [13]. Nine glomerular lesions, two interstitial lesions and two vascular lesions were defined. The nine glomerular lesions comprised one each of a diffuse lesion (mesangial expansion), nodular lesion (nodular sclerosis), subendothelial space widening or duplication of the basement membrane, exudative lesion, mesangiolysis/microaneurysm, peri-hilar neovascularization or polar vasculosis, global glomerulosclerosis/collapsing glomerular change and ischaemic glomerular change, segmental glomerulosclerosis and glomerulomegaly. The two interstitial lesions were interstitial fibrosis and tubular atrophy, and interstitial cell infiltration. The two vascular lesions were arteriolar hyalinosis and intimal thickening. Pathologists in each centre evaluated all the pathologic scoring as described below.

**Table 1. Definition of pathologic findings**

| | Pathologic findings | Score | Definition of score |
|---|---|---|---|
| Glomerular lesions | Diffuse lesion (mesangial expansion) | 0–3 | 0 normal or mild mesangial expansion,<br>1 mesangial expansion ≤ capillary lumen,<br>2 mesangial expansion = capillary lumen,<br>3 mesangial expansion ≥ capillary lumen |
| | Nodular lesion (nodular sclerosis) | 0, 1 | 0 (no nodular lesion),<br>1 (one or more lesions detected in all biopsy specimens, whatever the nodular size) |
| | Subendothelial space widening (double contour of basement membrane) | 0–3 | Double contour basement membrane (%) (determined in peripheral capillary of the most severe glomerulus);<br>0 (<10%), 1 (10–25%), 2 (25–50%), 3 (≥50%) |
| | Exudative lesion | 0, 1 | 0 (not detected),<br>1 (detected one or more lesion in all biopsy specimen) |
| | Mesangiolysis/microaneurysm | 0, 1 | 0 (not detected),<br>1 (detected one or more lesion in all biopsy specimen) |
| | Peri-hilar neo-vascularization (polar vasculosis) | 0, 1 | 0 (not detected),<br>1 (detected one or more lesion in all biopsy specimen) |
| | Global glomerulosclerosis/collapsing glomerulopathy ischaemic nephropathy | % | (Number of global glomerulosclerosis and collapsing glomerulopathy ischaemic nephropathy)/number of all glomerulus (%) |
| | Segmental glomerulosclerosis | % | Number of segmental glomerulosclerosis/number of all glomerulus (%) |
| | Glomerulomegaly | 0, 1 | Glomeruli >250 μm in diameter<br>0 (not detected), 1 (detected) |
| Interstitial lesions | Interstitial fibrosis and tubular atorophy (IFTA) | 0–3 | 0 (no IFTA), 1 (<25%), 2 (25–50%), 3 (≥50%) |
| | Interstitial inflammation | 0–3 | 0 (no cell infiltration), 1 (<25%), 2 (25–50%), 3 (≥50%) |
| Vascular lesions | Arteriolar hyalinosis | 0–3 | 0 (no hyalinosis),<br>1 (one or more partial arteriolar hyalinosis),<br>2 (approximately 50% hyalinosis),<br>3 (more than 50% hyalinosis, or penetrating hyalinosis) |
| | Intimal thickening | 0–2 | 0 (no intimal thickening),<br>1 (intimal thickness/media thickness <1),<br>2 (intimal thickening and intimal thickness/media thickness ≥1);<br>Elastica van Gieson staining is helpful for determination |



## Definitions and scoring of the pathologic findings

Agreement on definitions and scoring of all pathologic lesions was reached after reviewing previous studies on DN and several meetings among the authors for more than 2 years. The detailed points on each definition and score were published as a handbook [13]. Supplementary Table S2 presents a simple summary of the definition for each finding, and the scores are shown in Table 1.

IgG deposition in the glomeruli was also evaluated. The score for this was defined as follows: 0, no deposition of IgG; 1, mild deposition of IgG; and 2, obvious deposition of IgG.

## Clinical data

Age, gender, body mass index (BMI), systolic blood pressure (BP), diastolic blood pressure (BP), glycated haemoglobin (HbA1c) and total cholesterol (TC) at the time of the kidney biopsy were used as baseline clinical parameters. eGFR was calculated using the following formula [14]:

$$\text{eGFR (mL/min/1.73 m}^2) = 194 \times \text{serum creatinine (Cr)}^{-1.094} \times \text{age}^{-0.287} (\times 0.739 \text{ for female patients}).$$

HbA1c levels were presented as values based on the national glycohemoglobin standardization program, according to the recommendations of the Japanese Diabetic Society [12] and International Federation of Clinical Chemistry. Based on the CKD classification, albuminuria at baseline was categorized as normoalbuminuria (<30 mg/day or /gCr, category A1), microalbuminuria (30–300 mg/day or /gCr, category A2) or very high and nephrotic albuminuria (≥300 mg/day or /gCr, category A3) [15]. In patients in whom albuminuria was not evaluated, we classified proteinuria as optimal (<0.15 g/day or /gCr, category A1), mild (0.15–0.5 g/day or /gCr, category A2) or severe (≥0.5 g/day or /gCr, category A3). When results were inconsistent, 24-h urinary albumin excretion was performed. The eGFR at baseline was categorized according to CKD category (G1–G5). Based on the CKD heat map category by the KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of CKD, all cases were grouped into four: green (low risk), yellow (moderately increased risk), orange (high risk) and red (very high risk). Due to the limited number of cases, the green and yellow groups were combined in this study.

## Outcomes

The outcomes in this study were composite kidney events (dialysis, reduction of eGFR by half or doubling of serum Cr), CKD G5D (dialysis or kidney transplant), cardiovascular events (cardiovascular death, non-fatal myocardial infarction, coronary intervention or non-fatal stroke) and all-cause mortality. The sources of the outcome data were collected from each centre.

**Table 2. (A) Clinical baseline characteristics of this study and (B) pathological baseline characteristics of this study**

| (A) | G & Y ($n = 103$) Mean ± SD | Orange ($n = 149$) Mean ± SD | Red ($n = 348$) Mean ± SD | All ($n = 600$) Mean ± SD | P |
|---|---|---|---|---|---|
| Gender (% of male) | 53 | 60 | 73[a,b] | 67 | <0.01 |
| Age (years) | 54.2 ± 12.2 | 56.3 ± 11.1 | 59.6 ± 11.3[a,b] | 57.8 ± 11.6 | <0.01 |
| BMI | 23.3 ± 5.5 | 23.2 ± 3.4 | 24.1 ± 4.0 | 23.7 ± 4.2 | 0.10 |
| sysBP (mmHg) | 131.3 ± 20.2 | 141.9 ± 20.1[a] | 149.4 ± 20.8[a,b] | 144.5 ± 21.6 | <0.01 |
| diaBP (mmHg) | 75.9 ± 11.8 | 78.9 ± 11.4 | 79.8 ± 13.1[a] | 78.9 ± 12.5 | <0.05 |
| Haemoglobin (g/dL) | 13.5 ± 1.8 | 12.8 ± 2.0[a] | 11.2 ± 2.2[a,b] | 12.0 ± 2.3 | <0.01 |
| HbA1c (%) | 8.4 ± 2.3 | 8.4 ± 2.2 | 7.1 ± 1.7[a,b] | 7.6 ± 2.0 | <0.01 |
| TC (mg/dL) | 196.1 ± 48.6 | 218.7 ± 57.2[a] | 222.6 ± 81.1[a] | 217.3 ± 71.9 | <0.01 |
| RAS (%) | 11.5 | 56.1[a] | 65.5[a] | 58.3 | <0.01 |

| (B) | | G & Y Mean ± SD (or median, IQR) | Orange Mean ± SD (or median, IQR) | Red Mean ± SD (or median, IQR) | All Mean ± SD (or median, IQR) | P |
|---|---|---|---|---|---|---|
| Glomerular lesions | Diffuse | 1, 1–2 | 2, 1–3[a] | 3, 2–3[a,b] | 2, 1–3 | <0.01 |
| | Nodular | 0, 0–0 | 0, 0–1[a] | 1, 0–1[a,b] | 0, 0–1 | <0.01 |
| | SubendW | 0, 0–1 | 1, 0–1[a] | 1, 1–2[a,b] | 1, 0–2 | <0.01 |
| | Exudative | 0, 0–0 | 0, 0–1[a] | 0, 0–1[a,b] | 0, 0–1 | <0.01 |
| | MesLy | 0, 0–0 | 0, 0–1[a] | 0, 0–1[a,b] | 0, 0–1 | <0.01 |
| | PVas | 0, 0–1 | 1, 0–1[a] | 1, 1–1[a,b] | 1, 0–1 | <0.01 |
| | GScl (%) | 8.21 ± 10.99 | 17.09 ± 19.80 | 32.35 ± 22.72[a,b] | 24.29 ± 22.59 | <0.01 |
| | SScl (%) | 2.16 ± 7.32 | 2.66 ± 8.14 | 4.74 ± 8.74[a,b] | 3.76 ± 8.42 | <0.01 |
| | GMeg | 0, 0–0 | 0, 0–1 | 0, 0–1[a,b] | 0, 0–1 | <0.01 |
| Interstitial lesions | IFTA | 1, 0–1 | 1, 1–2[a] | 2, 2–3[a,b] | 2, 1–3 | <0.01 |
| | ICell | 1, 0–1 | 1, 1–1[a] | 1, 1–2[a,b] | 1, 1–2 | <0.01 |
| Vascular lesions | Hyalin | 1, 1–3 | 2, 1–3[a] | 3, 2–3[a,b] | 2, 1–3 | <0.01 |
| | Arterio | 1, 0–1 | 1, 1–2[a] | 1, 1–2[a,b] | 1, 1–2 | <0.01 |
| IgG deposition | | 2, 1–2 | 2, 1–2 | 1, 0–2 | 2, 1–2 | 0.18 |

P was calculated by one-way ANOVA test. G & Y, green and yellow; sysBP, systolic BP; diaBP, diastolic BP; RAS, rate of renin–angiotensin system inhibitor treatment; Diffuse, diffuse lesion (mesangial expansion); Nodular, nodular lesion (nodular sclerosis); SubendW, subendothelial space widening (double contour of basement membrane); Exudative, exudative lesion; MesLy, mesangiolysis/microaneurysm; PVas, peri-hilar neo-vascularization (polar vasculosis); GScl, global glomerulosclerosis/collapsing glomerular change ischaemic glomerular change; SScl, segmental glomerulosclerosis; GMeg, glomerulomegaly; IFTA, interstitial fibrosis and tubular atrpophy; ICell, interstitial cell infiltration; Hyalin, arteriolar hyalinosis; Arterio, Arteriosclerosis with intimal thickening.

[a]Indicates statistical significance compared with G & Y by paired *t*-test.

[b]Indicates statistical significance compared with orange by paired *t*-test.

**Table 3. The incidence rates of composite kidney end points and CKD G5D were increased accompanied with an increase of proteinuria or decrease of eGFR**

| (A) Actual number of events in each category | | | | | |
|---|---|---|---|---|---|
| | | G & Y | Orange | Red | All |
| Composite kidney event | Event − | 72 | 73 | 120 | 265 |
| | Event + | 20 | 68 | 216 | 304 |
| CKD G5D | Event − | 58 | 103 | 231 | 392 |
| | Event + | 2 | 5 | 24 | 31 |
| CV event | Event − | 62 | 96 | 239 | 397 |
| | Event + | 16 | 20 | 40 | 76 |
| All-cause mortality | Event − | 86 | 129 | 285 | 500 |
| | Event + | 7 | 14 | 52 | 73 |

| (B) Incidence rates of each outcome (/100 person-years) | | | | |
|---|---|---|---|---|
| | G & Y | Orange | Red | All |
| Composite kidney event | 2.31 | 6.59 | 14.52 | 8.97 |
| CKD G5D | 0.36 | 0.75 | 2.20 | 1.34 |
| CV event | 2.05 | 2.15 | 3.21 | 2.57 |
| All-cause mortality | 0.81 | 1.35 | 3.44 | 2.13 |

The composite kidney end point is defined as dialysis, doubling of Cr or halving of eGFR. CKD G5D is defined as dialysis or kidney transplantation. G & Y, green and yellow; CV event, cardiovascular event.

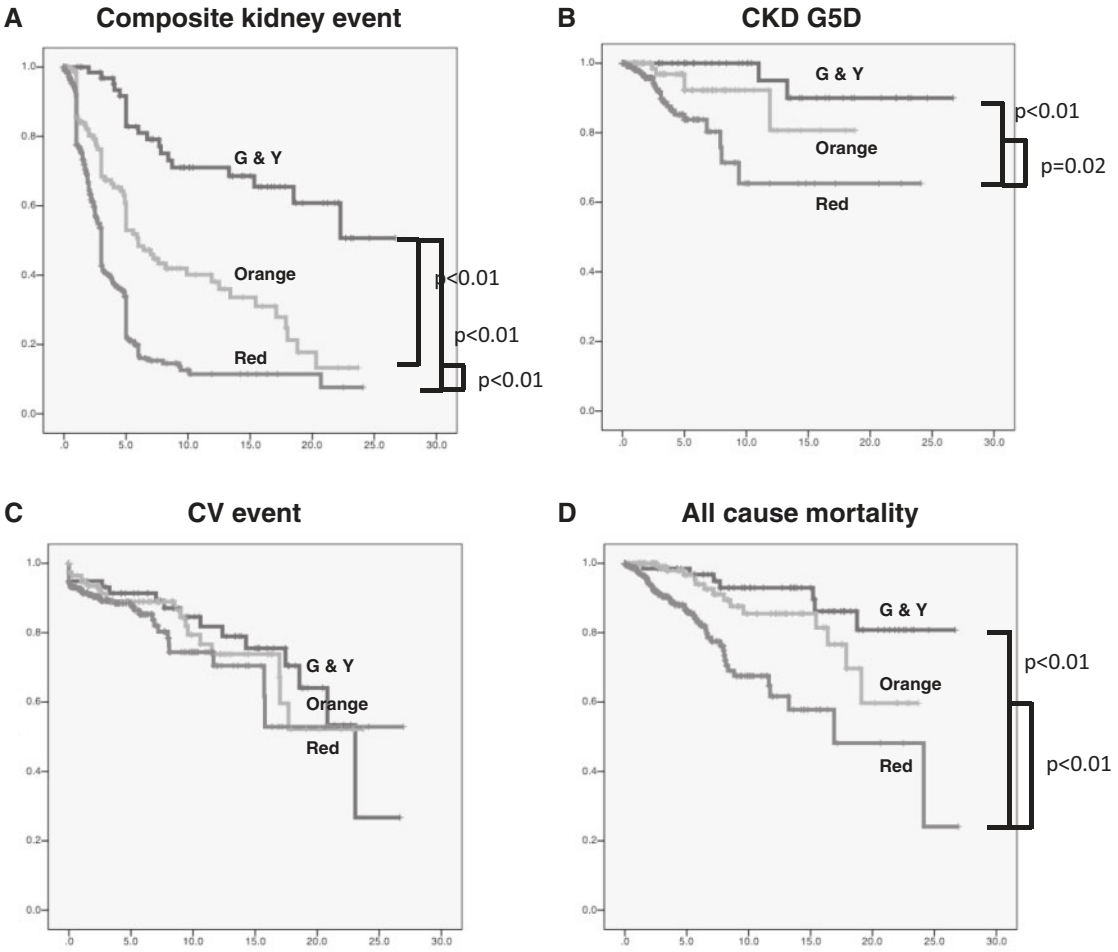


FIGURE 1: Kaplan–Meier survival curves for each outcome. (**A**) Composite kidney end points, (**B**) CKD G5D, (**C**) cardiovascular events and (**D**) all-cause mortality for each CKD heat map category are shown. Differences between the groups were compared using the log-rank test. G & Y, green and yellow.

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### Statistical analysis

Data were expressed as mean ± standard deviation (SD) for continuous variables with symmetric distribution or as median and interquartile range (IQR) for variables with asymmetric distribution. Based on the CKD heat map category, all cases were categorized into four groups: green group, yellow group, orange group and red group. Due to a limited number of cases, the green and yellow groups were combined as one group (green and yellow group). Therefore, data were evaluated among three groups (green and yellow group, orange group and red group). Continuous variables were compared among the three groups of CKD heat map category using the Mann–Whitney *U* test for non-parametric data and the chi-square test for categorical variables. Survival curves were obtained using the Kaplan–Meier method and were compared by the log-rank test. For the Kaplan–Meier method, the intensities of global glomerulosclerosis/collapsing glomerulopathy and ischaemic nephropathy were divided into three: 0, lowest tertile; 1, middle tertile; and 2, highest tertile. Those for segmental glomerulosclerosis were divided into two: 0, 0% and 1, >0%. All analyses were conducted using SPSS, version 19 (SPSS, Tokyo, Japan). A two-sided P-value of $<0.05$ was considered statistically significant.

## RESULTS

### Baseline clinical and pathologic characteristics for each CKD heat map category

This study evaluated 600 patients for a mean observation period of 72.4 ± 70.4 months. The baseline clinical characteristics of the patients at the time of kidney biopsy are shown in Table 2A. The mean age was 57.8 ± 11.6 years and 67% of the patients were men. The mean systolic and diastolic BPs were 144.5 ± 21.6 and 78.9 ± 12.5 mmHg, respectively. The mean HbA1c was 7.6 ± 2.0% (mean, 59 mmol/mol). There were statistically significant differences between the groups in terms of gender, age, systolic and diastolic BP, haemoglobin, HbA1c and TC. Age, systolic and diastolic BP and TC were found to be higher in the higher risk CKD heat map categories, with statistically significant differences between the green and yellow group and the red group. In contrast, haemoglobin and HbA1c were found to be lower in the higher-risk categories; again, there was a statistically significant difference between the green and yellow group and the red group. The use of renin–angiotensin system inhibitor treatment was significantly lower in the green and yellow group than in the other groups.

**Table 4. The percent of each histological score in clinical stage**

**Diffuse**

| Score | G & Y | Orange | Red | Total |
|---|---|---|---|---|
| 0 | 18.4 | 6.0 | 3.9 | 7.0 |
| 1 | 46.6 | 28.2 | 14.3 | 23.5 |
| 2 | 23.3 | 26.8 | 25.0 | 25.2 |
| 3 | 11.7 | 38.9 | 56.8 | 44.4 |
| | 100.0 | 100.0 | 100.0 | 100.0 |

**Nodular**

| Score | G & Y | Orange | Red | Total |
|---|---|---|---|---|
| 0 | 84.2 | 67.6 | 46.4 | 58.3 |
| 1 | 15.8 | 32.4 | 53.6 | 41.7 |
| | 100.0 | 100.0 | 100.0 | 100.0 |

**SubendW**

| Score | G & Y | Orange | Red | Total |
|---|---|---|---|---|
| 0 | 64.9 | 48.6 | 23.8 | 37.2 |
| 1 | 25.8 | 32.6 | 40.4 | 35.9 |
| 2 | 4.1 | 13.2 | 21.0 | 16.1 |
| 3 | 5.2 | 5.6 | 14.8 | 10.8 |
| | 100.0 | 100.0 | 100.0 | 100.0 |

**Exudative**

| Score | G & Y | Orange | Red | Total |
|---|---|---|---|---|
| 0 | 84.5 | 64.4 | 40.4 | 54.2 |
| 1 | 15.5 | 35.6 | 59.6 | 45.8 |
| | 100.0 | 100.0 | 100.0 | 100.0 |

**MesLy**

| Score | G & Y | Orange | Red | Total |
|---|---|---|---|---|
| 0 | 87.4 | 68.0 | 52.3 | 62.5 |
| 1 | 12.6 | 32.0 | 47.7 | 37.5 |
| | 100.0 | 100.0 | 100.0 | 100.0 |

**PVas**

| Score | G & Y | Orange | Red | Total |
|---|---|---|---|---|
| 0 | 57.4 | 33.3 | 23.4 | 31.8 |
| 1 | 42.6 | 66.7 | 76.6 | 68.2 |
| | 100.0 | 100.0 | 100.0 | 100.0 |

**GMeg**

| Score | G & Y | Orange | Red | Total |
|---|---|---|---|---|
| 0 | 78.0 | 69.4 | 59.7 | 65.3 |
| 1 | 22.0 | 30.6 | 40.3 | 34.7 |
| | 100.0 | 100.0 | 100.0 | 100.0 |

**IFTA**

| Score | G & Y | Orange | Red | Total |
|---|---|---|---|---|
| 0 | 35.9 | 13.4 | 2.3 | 10.9 |
| 1 | 42.7 | 44.3 | 20.5 | 30.3 |
| 2 | 18.4 | 28.9 | 35.4 | 30.8 |
| 3 | 2.9 | 13.4 | 41.8 | 27.9 |
| | 100.0 | 100.0 | 100.0 | 100.0 |

**ICell**

| Score | G & Y | Orange | Red | Total |
|---|---|---|---|---|
| 0 | 40.8 | 20.8 | 5.9 | 15.7 |
| 1 | 44.7 | 59.7 | 51.3 | 52.3 |
| 2 | 13.6 | 12.8 | 27.0 | 21.1 |
| 3 | 1.0 | 6.7 | 15.8 | 11.0 |
| | 100.0 | 100.0 | 100.0 | 100.0 |

**Hyalin**

| Score | G & Y | Orange | Red | Total |
|---|---|---|---|---|
| 0 | 24.3 | 6.8 | 3.3 | 7.8 |
| 1 | 30.1 | 27.7 | 15.4 | 21.1 |
| 2 | 17.5 | 19.6 | 23.1 | 21.3 |
| 3 | 28.2 | 45.9 | 58.2 | 49.8 |
| | 100.0 | 100.0 | 100.0 | 100.0 |

**Arterio**

| Score | G & Y | Orange | Red | Total |
|---|---|---|---|---|
| 0 | 36.8 | 23.4 | 8.3 | 16.9 |
| 1 | 38.9 | 46.8 | 46.9 | 45.6 |
| 2 | 24.2 | 27.7 | 42.9 | 35.9 |
| 3 | 0.0 | 2.1 | 1.8 | 1.6 |
| | 100.0 | 100.0 | 100.0 | 100.0 |

100-80% 80-60% 60-40% 40-20%

G & Y, green and yellow; Diffuse, diffuse lesion (mesangial expansion); Nodular, nodular lesion (nodular sclerosis); SubendW, subendothelial space widening (double contour of basement membrane); Exudative, exudative lesion; MesLy, mesangiolysis/microaneurysm; PVas, peri-hilar neo-vascularization (polar vasculosis); GMeg, glomerulomegaly; IFTA, interstitial fibrosis and tubular atrpophy; ICell, interstitial cell infiltration; Hyalin, arteriolar hyalinosis; Arterio, arteriosclerosis with intimal thickening.

The baseline pathologic characteristics of the patients are shown in Table 2B. Diffuse lesions were advanced and the mean severity score was 2 (IQR 1–3). Very few nodular lesions and mesangiolysis cases were observed and the scores for both of these were 0 (IQR 0–1). Global sclerosis and segmental sclerosis were observed in 24.29 ± 22.59% and 3.76 ± 8.42% of glomeruli, respectively. Hyalinosis was widely observed in vascular lesions, with a mean severity score of 2 (IQR 1–3).

All pathologic findings differed significantly among the three groups (Table 2B). The pathologic scores for glomerular, interstitial and vascular lesions were found to be higher in the higher-risk categories and were highest for the red group. Scores differed significantly between the green and yellow group and the red group. Each pathologic finding correlated strongly with the scores for the other pathologic findings. However, there was no significant difference in the IgG deposition score among the groups (green and yellow: 2, IQR 1–2; orange: 2, IQR 1–2; and red: 1, IQR 0–2). The incidences of composite kidney end points, dialysis and all-cause mortality were significantly higher in the higher-risk categories.

The composite kidney end points, CKD G5D, cardiovascular events and all-cause mortality were observed in 304, 31, 76 and 73 patients, respectively (Table 3A). The incidence of each outcome was presented as 100 person-years in Table 3B. The rates of composite kidney end points, dialysis, cardiovascular events and all-cause mortality were higher in the higher-risk categories. The incidence rates per 100 person-years for the green and yellow, orange and red groups were as follows: 2.31, 6.59 and 14.52, respectively, for the composite kidney end points; 0.36, 0.75 and 2.20, respectively, for CKD G5D; 2.05, 2.15 and 3.21, respectively, for cardiovascular events; and 0.81, 1.35 and 3.44, respectively, for all-cause mortality (Table 3B).

The above-mentioned findings were confirmed by the Kaplan–Meier survival curves and log-rank test for each outcome (Figure 1). For the composite kidney end points, the survival curve for the green and yellow group was significantly better than those for the orange and red groups. For CKD G5D and all-cause mortality, the survival curves for the red group were significantly poorer than those of the other two groups. However, there was almost no difference in the survival curves for cardiovascular events among the three groups.

### Pathologic findings were detected even in the green or yellow CKD heat map category

Pathologic scores increased in accordance with increasing CKD heat map category (Table 4). Even in the green and yellow

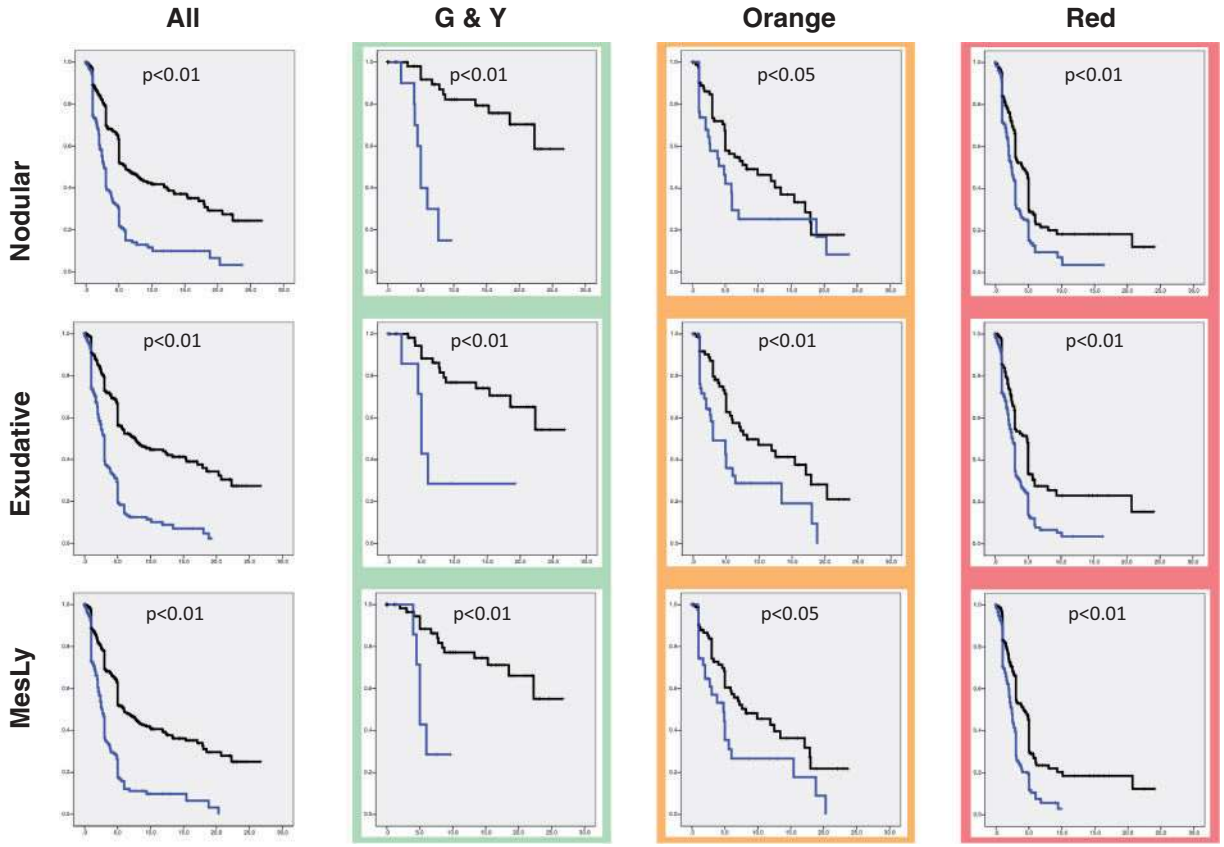


FIGURE 2: Kaplan–Meier survival curves for composite kidney end points stratified by pathologic score. Event-free curves for the composite kidney end points are shown. Each column shows the event-free curves for all cases and for the green and yellow (G & Y), orange and red groups. Each row shows the pathologic findings [nodular lesion, exudative lesion and mesangiolysis (MesLy)] and the corresponding score: score 0 (continuous black line) and score 1 (blue line). Differences between scores were compared by the log-rank test.

group, cases with diffuse lesions (81.6%), polar vasculosis (42.6%) and subendothelial space widening (35.1%) were common. In contrast, around half of the patients in the red group were negative for nodular lesions (46.4%), exudative lesions (40.4%), mesangiolysis (52.3%) and glomerulomegaly (59.7%). Pathologic scores could clearly predict the occurrence of composite kidney events in each CKD heat map category, particularly in the low-risk green and yellow category.

Although all 13 pathologic measures could clearly predict the occurrence of composite kidney events (Supplementary Figure S2), exudative lesions and mesangiolysis showed some special features for prediction of composite kidney events, especially in the low-risk green and yellow category (Figure 2). The adjusted hazard ratios (HRs) of nodular lesions, exudative lesions and mesangiolysis for the composite kidney events are shown in Table 5. Cox proportional hazard analysis revealed that these three pathologic factors were positively associated with composite kidney events in all groups after adjustment for BMI, systolic BP, HbA1c and TC. However, compared with the orange and red groups, the green and yellow group had significantly higher HRs for the outcomes of nodular lesions [HR 21.1, 95% confidence interval (CI) 5.3–84.6], exudative lesions (HR 5.1, 95% CI 1.3–20.3) and mesangiolysis (HR 7.6, 95% CI 2.0–28.3) (Table 5, Supplementary Tables S3–S5).

Although the HR for the composite kidney events of nodular lesions, exudative lesions and mesangiolysis were significantly higher in the green and yellow group, the baseline clinical data showed almost no differences between positive and negative cases of nodular lesions, exudative lesions and mesangiolysis (Table 6). IgG deposition and renin–angiotensin system inhibitor treatment at the time of kidney biopsy did not show any significant difference for each outcome (data not shown).

## DISCUSSION

In this cohort study of biopsy-confirmed DN cases, we found that the CKD heat map category was a good predictor of composite kidney events, CKD G5D and all-cause mortality. We also demonstrated the value of pathologic evaluation for predicting the prognosis of diabetic kidney disease. Certain pathologic findings, such as nodular lesions, exudative lesions and mesangiolysis, were useful predictive factors for composite kidney events, particularly in patients in the low-risk CKD heat map categories. Furthermore, our study demonstrated that even in low-risk categories, diffuse lesions, polar vasculosis and subendothelial space widening were common. These findings indicated that pathologic characteristics provide useful information

**Table 5. HRs of nodular lesion, exudative lesion and mesangiolysis for the composite kidney events**

| Univariate | | | | Model 1 | | | | Model 2 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | HR 95% CI | *P* | | | HR 95% CI | *P* | | | HR 95% CI | *P* |
| Nodular | | | | | | | | | | | |
| All | Score 0 | Reference | | All | Score 0 | Reference | | All | Score 0 | Reference | |
| | Score 1 | 2.4 (1.9–3.1) | <0.01 | | Score 1 | 2.4 (1.9–3.1) | <0.01 | | Score 1 | 2.2 (1.7–2.9) | <0.01 |
| G & Y | Score 0 | Reference | | G & Y | Score 0 | Reference | | G & Y | Score 0 | Reference | |
| | Score 1 | *10.2 (3.6–28.6)* | *≤0.01* | | Score 1 | *12.9 (3.9–43.1)* | *≤0.01* | | Score 1 | *21.1 (5.3–84.6)* | *≤0.01* |
| Orange | Score 0 | Reference | | Orange | Score 0 | Reference | | Orange | Score 0 | Reference | |
| | Score 1 | 1.7 (1–2.8) | <0.05 | | Score 1 | 1.7 (1.0–2.8) | <0.05 | | Score 1 | 1.2 (0.6–2.2) | 0.64 |
| Red | Score 0 | Reference | | Red | Score 0 | Reference | | Red | Score 0 | Reference | |
| | Score 1 | 1.7 (1.3–2.3) | <0.01 | | Score 1 | 1.7 (1.3–2.3) | <0.01 | | Score 1 | 1.7 (1.2–2.3) | <0.01 |
| Exudative | | | | | | | | | | | |
| All | Score 0 | Reference | | All | Score 0 | Reference | | All | Score 0 | Reference | |
| | Score 1 | 2.8 (2.2–3.6) | <0.01 | | Score 1 | 2.8 (2.2–3.6) | <0.01 | | Score 1 | 2.6 (2.0–3.5) | <0.01 |
| G & Y | Score 0 | Reference | | G & Y | Score 0 | Reference | | G & Y | Score 0 | Reference | |
| | Score 1 | *4.3 (1.5–12.1)* | *≤0.01* | | Score 1 | *4.2 (1.4–12.9)* | *≤0.01* | | Score 1 | *5.1 (1.3–20.3)* | *≤0.05* |
| Orange | Score 0 | Reference | | Orange | Score 0 | Reference | | Orange | Score 0 | Reference | |
| | Score 1 | 2.1 (1.3–3.5) | <0.01 | | Score 1 | 2.1 (1.3–3.5) | <0.01 | | Score 1 | 1.7 (0.9–3.3) | 0.10 |
| Red | Score 0 | Reference | | Red | Score 0 | Reference | | Red | Score 0 | Reference | |
| | Score 1 | 1.9 (1.4–2.5) | <0.01 | | Score 1 | 1.9 (1.4–2.5) | <0.01 | | Score 1 | 1.8 (1.2–2.5) | <0.01 |
| MesLy | | | | | | | | | | | |
| All | Score 0 | Reference | | All | Score 0 | Reference | | All | Score 0 | Reference | |
| | Score 1 | 2.7 (2.1–3.4) | <0.01 | | Score 1 | 2.7 (2.1–3.4) | <0.01 | | Score 1 | 2.4 (1.8–3.2) | <0.01 |
| G & Y | Score 0 | Reference | | G & Y | Score 0 | Reference | | G & Y | Score 0 | Reference | |
| | Score 1 | *5.6 (1.9–16.8)* | *≤0.01* | | Score 1 | *5.4 (1.7–17.4)* | *≤0.01* | | Score 1 | *7.6 (2.0–28.8)* | *≤0.01* |
| Orange | Score 0 | Reference | | Orange | Score 0 | Reference | | Orange | Score 0 | Reference | |
| | Score 1 | 1.9 (1.1– 3.1) | <0.05 | | Score 1 | 1.8 (1.1–3.1) | <0.05 | | Score 1 | 1.4 (0.7–2.8) | 0.31 |
| Red | Score 0 | Reference | | Red | Score 0 | Reference | | Red | Score 0 | Reference | |
| | Score 1 | 1.9 (1.5–2.6) | <0.01 | | Score 1 | 1.9 (1.5–2.6) | <0.01 | | Score 1 | 1.8 (1.3–2.6) | <0.01 |

Model 1: adjusted for age, gender. Model 2: adjusted for the covariates in model 1, BMI, systolic BP, HbA1c, total cholesterol. G & Y, green and yellow; Nodular, nodular lesion (nodular sclerosis); Exudative, exudative lesion; MesLy, mesangiolysis/microaneurysm.

for predicting the prognosis of diabetic kidney disease, especially in cases in the low-risk CKD heat map categories.

Our results demonstrated that pathologic findings of nodular lesions, exudative lesions and mesangiolysis added predictive information for composite kidney events, particularly for patients in the low-risk green and yellow CKD heat map categories. Albuminuria has been well established as a risk factor for the progression of kidney dysfunction [7]. Our data indicated that in some advanced cases, albuminuria and eGFR were good predictors of kidney dysfunction, cardiovascular events and all-cause mortality. Although all the pathologic scores in this study significantly predicted kidney events in all cases, these pathologic findings added negligible predictive information on the outcomes of patients in the higher-risk categories. On the other hand, the green and yellow category patients with nodular lesions, exudative lesions and mesangiolysis showed remarkably poorer outcomes of composite kidney events than the cases with the particular pathologic findings.

Nodular lesions and mesangiolysis were characteristic pathologic findings for DN and have been reported as predictive markers of impaired renal function [7, 8]. Exudative lesion is also a typical pathologic change in DN. A previous study indicated that patients without exudative lesions had a significantly better rate of renal survival than those with such lesions [10]. Therefore, it is important to perform renal biopsy of patients with type 2 diabetes and to limit the clinical changes for two reasons: to exclude non-diabetic renal diseases that may benefit from specific treatment [16] and to evaluate the risk of developing end-stage kidney disease and guide therapeutic management [17]. In this study, the prognostic significance was more pronounced in patients in the low-risk CKD heat map categories. Therefore, we emphasize the importance of these pathologic findings for predicting kidney dysfunction, particularly in cases with normoalbuminuria to microalbuminuria and preserved kidney function. The progression of diabetic kidney disease is heterogeneous [5]. A previous study showed that despite being stable for a long period, some cases with microalbuminuria showed rapid decline in GFR [18]. However, to date, no good biomarker or clinical finding can predict the progression of diabetic kidney disease in patients with low-grade albuminuria and preserved eGFR. Our study has identified pathologic findings that add important predictive information about the progression of kidney disease, particularly in patients in the low-risk CKD heat map categories. Urinary or serum biomarkers that correlate well with pathologic changes may provide clear indications of when kidney biopsy is required for diabetic patients. Such biomarkers should be considered in future studies.

The present study demonstrated that even in low-risk patients with normal kidney function or normo- to microalbuminuria, particular pathologic findings, including diffuse lesions, polar vasculosis and subendothelial space widening were commonly detected. The report by Fioretto *et al.*, as well as other recent histologic studies, indicated that heterogeneity was present

**Table 6. Clinical background of green and yellow group is almost similar between positive and negative cases of each particular pathological findings**

| | Age Mean ± SD (Median, IQR) | Gender % | BMI Mean ± SD (Median, IQR) | SysBP Mean ± SD (Median, IQR) | DiaBP Mean ± SD (Median, IQR) | Haemoglobin Mean ± SD (Median, IQR) | HbA1c Mean ± SD (Median, IQR) | TC Mean ± SD (Median, IQR) | eGFR Mean ± SD (Median, IQR) | UAlb Mean ± SD (Median, IQR) |
|---|---|---|---|---|---|---|---|---|---|---|
| Nodular | | | | | | | | | | |
| + | 58.5 ± 11.0 (55, 52–66) | 62 | 20.9 ± 6.6 (21.8, 19.8–24.5) | 134.9 ± 21.5 (140, 120–148) | 75.4 ± 9.4 (78, 70–80) | 13.0 ± 2.0 (13.0, 12.0–14.7) | 8.3 ± 1.9 (8.1, 7.4–8.9) | 201.4 ± 64.0 (190, 155–241) | 74.0 ± 13.9 (66.8, 64.4–85.5) | 0.12 ± 0.11 (0.13, 0–0.22) |
| − | 53.3 ± 12.4 (54, 45–63) | 52 | 23.6 ± 5.1 (22.7, 20.8–25.4) | 130.5 ± 20.3 (129, 118–140) | 75.7 ± 12.4 (78, 70–84) | 13.6 ± 1.7 (13.6, 12.6–14.9) | 8.4 ± 2.4 (8, 6.5.0–10.4) | 195.4 ± 45.7 (196, 165–229) | 83.7 ± 24.5 (79.7, 65.4–94.1) | 0.06 ± 0.08 (0.02, 0–0.11) |
| *P* | 0.12 | 0.44 | 0.08 | 0.48 | 0.92 | 0.22 | 0.95 | 0.67 | 0.13 | 0.06 |
| Exudative | | | | | | | | | | |
| + | 57.9 ± 9.7 (57, 52–65) | 62 | 20.4 ± 6.7 (21.2, 19.5–23.4) | 129.7 ± 20.0 (132, 112–140) | 72.9 ± 7.8 (72, 70–80) | 13.0 ± 1.9 (12.8.0, 12.0–14.0) | 8.5 ± 2.1 (8.0, 7.2–9.0) | 197.1 ± 61.0 (188, 155–220) | 68.5 ± 14.1 (66.1, 60.8–74.4) | 0.10 ± 0.12 (0.02, 0–0.22) |
| − | 53.5 ± 12.6 (54, 45–63) | 52 | 23.9 ± 5.1 (22.8, 21.0–25.4) | 131.6 ± 20.4 (130, 118–142) | 76.4 ± 12.4 (78, 70–84) | 13.6 ± 1.7 (13.7, 12.7–15.0) | 8.3 ± 2.3 (8.1, 6.5–9.9) | 195.9 ± 46.2 (196, 164–231) | 84.5 ± 23.8 (79.8, 67.8–94.1) | 0.06 ± 0.08 (0.03, 0–0.12) |
| *P* | 0.19 | 0.44 | <0.05[a] | 0.76 | 0.33 | 0.21 | 0.83 | 0.93 | <0.05[a] | 0.25 |
| MesLy | | | | | | | | | | |
| + | 59.7 ± 8.6 (57, 52–65) | 69 | 23.0 ± 4.1 (22.0, 19.7–25.8) | 136.9 ± 19.7 (140, 132–148) | 76.2 ± 7.8 (80, 70–80) | 12.9 ± 1.9 (12.9, 11.8–13.8) | 8.1 ± 0.9 (8, 7.5–8.8) | 197.8 ± 64.7 (189, 159–211.5) | 71.2 ± 13.6 (66.5, 61.2–79.7) | 0.11 ± 0.12 (0.03, 0–0.21) |
| − | 53.4 ± 12.5 (54, 45–63) | 51 | 23.3 ± 5.8 (22.7, 20.8–25.4) | 130.4 ± 20.3 (128, 118–140) | 75.8 ± 12.4 (78, 70–84) | 13.6 ± 1.7 (13.7, 12.4–15.0) | 8.4 ± 2.4 (8.1, 6.5–10.3) | 195.8 ± 46.1 (195, 164–232) | 83.6 ± 23.9 (78.9, 66.6–94.1) | 0.06 ± 0.08 (0.02, 0–0.12) |
| *P* | 0.08 | 0.22 | 0.88 | 0.32 | 0.92 | 0.17 | 0.47 | 0.89 | 0.07 | 0.23 |

Nodular, nodular lesion (nodular sclerosis); Exudative, exudative lesion; MesLy, mesangiolysis/microaneurysm; sysBP, systolic BP; diaBP, diastolic BP; UAlb, urinary albumin.
[a]Indicates statistical significance by paired *t*-test.

at an early stage in DN [4, 19, 20]. Furthermore, some of these pathologic changes were not specific to DN. Recent basic studies have indicated the importance of glomerular endothelial injury in various kidney diseases, including DN [9, 21–23]. Widening of the subendothelial space could be a phenotype of endothelial injury. Further studies are needed to evaluate the sensitivity and specificity of these pathologic findings for DN.

Tubulo-interstitial lesions, vascular lesions and global glomerulosclerosis were likewise observed in cases of hypertension, smoking or obesity, and in patients with advanced age [22]. These additional clinical factors could affect the pathologic findings in diabetic patients. Therefore, additional studies on larger number of patients with early-stage diabetic kidney disease are required. We should also try to detect by biopsy or new biomarkers the pathologic changes of diabetic kidney disease before albuminuria or decline in eGFR sets in.

This study had several limitations. First, we evaluated pathologic findings only by light microscopy. Further evaluation by electron microscopy should be performed in future studies. Furthermore, the reproducibility of the scoring for each pathologic finding is an important point that needs to be established by future studies. Second, this was a retrospective study; therefore, the collected laboratory data and outcomes depended on clinical records. Third, the biopsy procedures and handling of biopsy samples were not uniform across the 13 centres. Fourth, the subjects were limited to patients who had undergone kidney biopsy for clinically relevant reasons, such as the presence of haematuria or acute kidney injury; these factors may have affected our results. Before generalizing our results to all diabetic cases, the possibility of selection bias from the biopsy protocol or indications should be considered. In addition, the findings of this study should be confirmed in other cohorts. Moreover, an indication bias for kidney biopsy could potentially explain the limited differences in cardiovascular events among the groups. Fifth, during follow-up, we did not evaluate the therapeutic interventions, which may have created an impact on kidney prognosis. Although the rate of renin–angiotensin system inhibitor treatment was lowest in the green and yellow group, the incidence of composite kidney end points in this group was lower than that in the other groups. Furthermore, treatment with renin–angiotensin system inhibitors at the time of kidney biopsy did not affect the survival curve for each outcome. Finally, data on proteinuria were used when data for albuminuria were not available. A comparison between our classification system and that of Tervaert *et al.* [9] will be our next project. Although these limitations may affect the interpretation of our results to some extent, this multicentre study involving 600 kidney biopsy cases with long-term follow-up could be important in understanding the clinicopathologic features and clinical outcomes of diabetic kidney disease.

In conclusion, among the various clinical and pathologic parameters, the presence of subendothelial space widening, advanced interstitial cell infiltration, segmental sclerosis and glomerulomegaly were predictors of renal and cardiovascular events in type 2 diabetic patients with preserved eGFR and normoalbuminuria to microalbuminuria. These results clearly suggested that pathologic characteristics may provide additional useful prognostic information for diabetic kidney disease.

## SUPPLEMENTARY DATA

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

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## CONFLICT OF INTEREST STATEMENT

None declared.

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