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journal or publication title	Nephrology Dialysis Transplantation
volume	33
number	1
page range	138-148
year	2018-01-01
URL	http://doi.org/10.24517/00050597

doi: 10.1093/ndt/gfw417

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Running title

Biopsy Study of Diabetic Nephropathy

Word count; Abstract 247, Main text 3587

Number of tables; 6

Number of figures; 2

Key words

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

Source of support

This study was supported in part by a Grant-in-Aid for Diabetic Nephropathy and Nephrosclerosis Research from the Ministry of Health, Labour and Welfare of Japan and Grant-in-Aid for Practical Research Project for Renal Diseases, from the Japan Agency for Medical Research and Development (No. 15ek0310003h0001). This work was also supported in part by Grants-in-Aids from the Ministry of Education, Culture, Sports, Science, and Technology of the Japanese Government.

Financial Disclosure

All authors; none

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ABSTRACT

Background

Clinical and pathological manifestations of diabetic nephropathy in type 2 diabetes are diverse, but large-scale pathological studies with long-term observation have been limited.

Methods

Kidney biopsy and clinical data were collected retrospectively from 13 centers across Japan for 600 patients with Type 2 diabetes. Thirteen pathological 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 Cr, or halving of eGFR), 31 instances of chronic kidney disease (CKD) G5D, 76 cardiovascular events, and 73 deaths. The mean observation period was 72.4 months. The CKD heat map categories for the 600 patients were 103 green or yellow, 149 orange, and 348 red. Diffuse lesions, polar vasculosis, and subendothelial space widening were commonly detected even in the green and yellow category of cases (positive cases: diffuse lesion 81.6%, polar vasculosis 42.6%, and subendothelial space widening 35.1%). Cox proportional hazards analysis revealed that the presence of

nodular lesions, exudative lesions, and mesangiolysis in green and yellow category cases were associated with a significantly greater impact on composite kidney events after adjustment for clinical risk factors (hazard ratio, 95% confidence interval; nodular lesion: 21.1, 5.3–84.6; exudative lesion: 5.1, 1.3–20.3; mesangiolysis: 7.6, 2.0–28.8).

Conclusions

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

INTRODUCTION

The chronic kidney disease (CKD) heat map, issued as part of the Kidney Disease: Improving Global Outcomes (KDIGO) 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease [1], provides a color-coded classification that helps predict the prognosis of CKD. This classification is based on three factors: the cause of the disease, the level of albumin in urine (albuminuria), and the estimated glomerular filtration rate (eGFR). Albuminuria and eGFR are key predictive markers for the prognosis of diabetic nephropathy (DN). In agreement with this, 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]. The CKD heat map classification is simple and clinically relevant. In addition to this classification, other markers of kidney damage and injury can provide further information relevant to CKD prognosis. Pathological findings should be a possible additional marker for CKD.

The clinical and pathological manifestations of DN in type 2 diabetes are diverse [3] [4]. A recent clinical study of DN showed that there were two clinically characterized groups in the progression of kidney dysfunction: rapid decliner and non-decliner groups, classified on the basis of the speed of decrease of glomerular filtration rate (GFRs) [5]. Our previous study indicated that some pathological findings were good predictors for 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 pathological observations of the heterogeneity of diabetic kidney disease motivated us to evaluate how pathological findings and clinical category could assist in predicting renal events in patients with DN.

The pathological classification of diabetic nephropathy (DN) by Tervaert et al. was systematic and organized, predicting renal outcomes in patients with DN [9-11]. However, this classification omitted some important pathological findings, especially in regards to glomerular lesions (Supplementary Table). Accordingly, we evaluated as many pathological 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 reevaluated each DN biopsy specimen. We evaluated the additional predictive value of pathological findings, including details of glomerular lesions, and revealed valuable and specific pathological findings that can predict kidney events in type 2 diabetes patients, particularly those in the low-risk CKD heat map categories.

METHODS

Data for 600 biopsy-confirmed type 2 diabetes patients were collected retrospectively from 13 centers across Japan. The diagnosis of diabetes was based on the criteria of the Japanese Diabetic Society [12]. Patients were classified as having a diabetes if they met one of the following criteria: (i) fasting plasma glucose level ≥126 mg/dl (≥7.0 mmol/l); (ii) 75-g oral glucose tolerance test 2-h value ≥200 mg/dl (≥11.1 mmol/l); or (iii) casual plasma glucose level ≥200 mg/dl (≥11.1 mmol/l). A kidney biopsy was performed for patients for whom it was clinically necessary to obtain a precise diagnosis of kidney lesions. Typical indications included proteinuria without diabetic retinopathy, hematuria, a rapid decline in eGFR, or massive proteinuria with a short duration history of diabetes. Biopsy indications for a 50-case series with normo- or microalbuminuria are presented in Supplementary Table 2. Patients with other glomerular diseases concomitant with DN were excluded from this study. There was no limitation in glomerular number, if the following pathological findings were evaluated. Written informed consent was obtained from each patient, and this study′s protocol was approved by the medical ethics committee of Kanazawa University (Approval No. 1204).

Pathological examinations

Biopsy samples were stained with Periodic Acid–Schiff reagent, periodic acid methenamine silver, hematoxylin–eosin, and Mallory–Azan or Masson's Trichrome stains and examined by light microscopy. Pathological stages were defined in detail from typical pictures [13]. Nine glomerular lesions, two interstitial lesions, and two

vascular lesions were defined. The nine glomerular lesions comprised one diffuse lesion (mesangial expansion), one nodular lesion (nodular sclerosis), one subendothelial space widening (or duplication of the basement membrane), one exudative lesion, one case of mesangiolysis/microaneurysm, one peri-hilar neovascularization (or polar vasculosis), one global glomerulosclerosis/collapsing glomerular change and ischemic glomerular change, one segmental glomerulosclerosis, and one 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 center evaluated all the pathological scoring as described below.

Definitions and scoring for the pathological findings

Agreement on the definitions and scoring for all pathological lesions was reached after reviewing previous studies of diabetic nephropathy and many face-to-face meetings with all the authors over more than two years. The detailed points on each definition and score were published as a handbook [13]. Supplementary Table 1 and its legend present 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, sex, body mass index (BMI), systolic blood pressure (BP), diastolic BP, hemoglobin (Hb) A1c, and total cholesterol (T-Cho) were used as baseline clinical parameters at the time of the kidney biopsy. eGFR was calculated using the following formula [14]:

eGFR (mL/min/1.73 m^2) = 194 × serum creatinine$^{-1.094}$ × age$^{-0.287}$

(For female patients this value was multiplied by 0.739.)

HbA1c levels were presented as national glycohemoglobin standardization program values according to the recommendations of the Japanese Diabetic Society (9) and International Federation of Clinical Chemistry.

Based on the classification of chronic kidney disease, albuminuria at baseline was categorized as normoalbuminuria (<30 mg/day or /gCr in albuminuria, category A1), microalbuminuria (30–300 mg/day or /gCr in albuminuria, category A2), or very high and nephrotic albuminuria (≥300 mg/day or /gCr, category A3) [15]. In the patients for whom albuminuria was not evaluated, we classified proteinuria as optimal proteinuria (<0.15 g/day or /gCr, category A1), mild proteinuria (0.15–0.5 g/day or /gCr, category A2), or severe proteinuria (≥0.5 g/day or /gCr, category A3). When results were inconsistent, 24-h urinary albumin excretion was prioritized. The eGFR at baseline was categorized according to CKD category (categories G1–5). Based on the CKD heat map category according to the KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease, all cases were categorized into four

groups: Green group: low risk, Yellow group: moderately increased risk, Orange group: high risk, and Red group: very high risk.

Outcomes

The outcomes for this study were composite kidney events (dialysis, halving of eGFR, or doubling of serum Cr), CKD G5D, cardiovascular events (cardiovascular death, nonfatal myocardial infarction, coronary intervention, or nonfatal stroke), and all-cause mortality. The sources of the outcome data were collected from each center.

Statistical analysis

Data are expressed as mean ± SD and (median,interquartile range). Continuous variables were compared between groups using the Mann–Whitney U test for nonparametric data, and categorical variables using the chi-square test. Survival curves were obtained using the Kaplan–Meier method and compared by the log-rank test. For the Kaplan–Meier method, the intensities of global glomerulosclerosis/collapsing glomerulopathy and ischemic nephropathy were divided into three (0, lowest tertile; 1, middle tertile; and 2, highest tertile), and 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

Clinical and pathological baseline characteristics for each CKD heat map category

This study evaluated 600 patients with a mean observation period of 72.4 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 (59, 51-66) years, and 67% of the patients were male. The mean systolic and diastolic BP were 144.5 ± 21.6 (142, 130-160) and 78.9 ± 12.5 (80, 70-88) mmHg, respectively, and the mean HbA1c was 7.6% ± 2.0% (7.1, 6.1-8.8) (mean 59 mmol/mol). There were statistically significant differences between the groups in gender, age, systolic and diastolic BP, Hb, HbA1c, and total cholesterol (Table 2A). Age, systolic and diastolic BP, and total cholesterol were found to be higher in the higher-risk CKD heat map categories, with statistically significant differences in these variables between the Green and Yellow group and the Red group. By contrast, Hb 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 rate of renin–angiotensin system inhibitor treatment was significantly lower in Green and Yellow group than in the other groups (Table 2A).

The baseline pathological characteristics of the patients in this study are shown in Table 2B. Diffuse lesions were advanced and the mean severity score was 2.07 ± 0.98 (2, 1-3). Very few nodular lesions and mesangiolysis cases were observed, and the scores for these were 0.42 ± 0.49 (0, 0-1) and 0.38 ± 0.49 (0, 0-1), respectively. Global

sclerosis and segmental sclerosis were observed in 24.29% ± 22.59% (20, 5-38) and 3.76% ± 8.42% (0, 0-5) of glomeruli. Hyalinosis was widely observed in vascular lesions, with a mean severity score of 2.13 ± 1.00 (2, 1-3).

All pathological findings differed significantly between the three groups (Table 2B). The pathological 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 pathological finding correlated strongly with the scores for the other pathological findings. However, there was no difference in IgG deposition score between the groups (Green and Yellow, 1.5 ± 0.8; Orange, 1.4 ± 0.8; and Red, 1.2 ± 0.8). The incidences of composite kidney end points, dialysis, and all-cause mortality were significantly higher in the higher-risk categories.

The composite kidney end point (dialysis, doubling of Cr, or halving of eGFR), CKD G5D (dialysis or kidney transplantation), cardiovascular events, and death were observed in 304, 31, 76, and 73 patients, respectively (Table 3A). The incidence of each outcome is presented as 100 person-years in Table 3B. The rates of composite kidney end point, dialysis, cardiovascular events, and all-cause mortality in the three groups were found to be 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 for the composite kidney end point; 0.36, 0.75, and 2.20 for CKD G5D; 2.05, 2.15, and 3.21 for cardiovascular events; and 0.81, 1.35, and 3.44 for death (Table 3B).

These findings were confirmed by the survival curves for each outcome derived using the Kaplan–Meier method and analyzed with the log-rank test (Figure 1). For the composite kidney end point, the survival curve for the Green and Yellow group was significantly better than that for the Orange and Red groups (Figure 1A). For CKD G5D and all-cause mortality, the survival curves of the Red group were significantly poorer than those of the other two groups (Figure 1B, D). However, there was little or no difference between the three groups in the curves for cardiovascular events (Figure 1C).

Pathological findings were detected even in the green or yellow CKD heat map category cases

Pathological scores increased in accordance with increasing CKD heat map category (Table 4). However, diffuse lesions, polar vasculosis, and subendothelial space widening were commonly detected even in the Green and Yellow group (positive cases: diffuse lesions 81.6%, polar vasculosis 42.6%, and subendothelial space widening 35.1%).

In contrast, around half of the Red group patients were negative for nodular lesions, exudative lesions, mesangiolysis, and glomerulomegaly (negative cases: nodular lesions 46.4%, exudative lesions 40.4%, mesangiolysis 52.3%, and glomerulomegaly 59.7%).

Pathological scores could clearly predict the occurrence of composite kidney events in each CKD heat map category, particularly in the low-risk green and yellow categories.

In addition to the CKD heat map category, all thirteen pathological measures could clearly predict the occurrence of composite kidney events (Supplementary Figure 2). Among the thirteen pathological measures, nodular lesions, exudative lesions, and mesangiolysis showed special effects on prediction of composite kidney events, especially in the low-risk green and yellow categories (Figure 2).

The adjusted hazard ratios (HRs) of nodular lesions, exudative lesions, and mesangiolysis for the composite kidney event are shown in Table 5. Cox proportional hazards analysis revealed that these three pathological factors were positively associated with the composite kidney event in the Green and Yellow, Orange, and Red groups after adjustment for BMI, systolic BP, HbA1c, and T-cho. However, the HRs of the Green and Yellow group compared with the Orange and Red groups for the outcomes of nodular lesion, exudative lesion, and mesangiolysis were significantly high (HR, 95% confidence interval: nodular lesion 21.1, 5.3–84.6; exudative lesion 5.1, 1.3–20.3; mesangiolysis 7.6, 2.0–28.3).

Although the HR for the composite kidney events of nodular lesion, exudative lesion, and mesangiolysis were significantly high in the Green and Yellow group, the baseline data for the clinical factors showed almost no difference between positive and negative cases of nodular lesion, exudative lesion, and mesangiolysis (Table 6).

IgG deposition and renin–angiotensin system inhibitor treatment at kidney biopsy did not show any significant differences for each outcome (data not shown).

DISCUSSION

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

Our results demonstrated that pathological findings of nodular lesions, exudative lesions, and mesangiolysis add predictive information for composite kidney events, particularly for patients in the low-risk green and yellow CKD heat map categories. It has been well established that raised albuminuria is a risk for the progression of kidney dysfunction [7]. Our data also indicated that in cases with advanced albuminuria and decreased eGFR, albuminuria and eGFR were good predictors for kidney dysfunction, cardiovascular events, and all-cause mortality. Although all our pathological scores were statistically significant for the prediction of kidney events in all cases, these pathological findings added negligible predictive information for the outcomes in the

higher-risk orange and red categories. In contrast, the green and yellow category patients with nodular lesions, exudative lesions, and mesangiolysis showed remarkably poorer outcomes with regard to composite kidney events than did the cases with the particular pathological findings. Nodular lesions and mesangiolysis were characteristic pathological findings for DN and have been reported as predictive markers for improper renal function [7, 8]. Exudative lesion is also a typical pathological change in DN. A previous study indicated that patients without exudative lesions had a significantly better renal survival rate than those with such lesions [10]. It is important to perform a renal biopsy for patients with type 2 diabetes and to limit clinical changes for two reasons: to exclude non-diabetic renal diseases, which may benefit from specific treatment [16]; and second, to define the risk worse renal outcomes (end-stage kidney disease) developing and guide therapeutic management [17]. In this study, we indicated that the effects were more significant in the low-risk CKD heat map categories, and emphasize the importance of these pathological 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 most cases of diabetes with normoalbuminuric and microalbuminuric remaining stable for a long period, some cases with microalbuminuria showed a rapid decline in glomerular filtration rate [18]. However, to date no good biomarker or clinical finding exists for predicting the progression of diabetic kidney disease in patients with low-grade albuminuria and preserved eGFR. Our study has

identified pathological 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 pathological changes may provide clear indication 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 categories with normal kidney function and normo- to microalbuminuria, particular pathological findings (diffuse lesions, polar vasculosis, and subendothelial space widening) were commonly detected. The report by Fioretto et al. as well as other recent histological studies indicates that heterogeneity is present at an early stage in diabetic nephropathy [4, 19, 20]. Furthermore, some of these pathological 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 pathological findings for DN.

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

This study had several limitations. First, we only evaluated pathological findings by light microscopy. Further evaluation with electron microscopy should be performed in future studies. Furthermore, the reproducibility of scoring for each pathological finding is an important point, and future studies will be required to establish this. Second, this was a retrospective study. The collected laboratory data and outcomes depended on clinical records. Third, biopsy procedures and biopsy sample handling processes were not uniform across the 13 centers. Fourth, subjects were limited to patients who had undergone a kidney biopsy. As a kidney biopsy was only performed when clinically relevant, such as in patients with hematuria or acute kidney injury, these factors may have affected our results. Before generalizing this result to all diabetic cases, the possibility of selection bias from biopsy policy or indication should be considered, and the findings of this study should be confirmed in other cohorts. Additionally, an indication bias for kidney biopsy could potentially explain the limited differences in cardiovascular events between the groups. Fifth, we did not evaluate the therapeutic interventions during follow-up; these may have impacted kidney prognosis. Although the rate of renin–angiotensin system inhibitor treatment was lowest in the Green and Yellow group, the incidence of the composite kidney end point in this group was lower than in the other groups. Furthermore, renin–angiotensin system inhibitor treatment did not prevent survival curve at kidney biopsy for each outcome. Finally, the data for proteinuria were used when data for albuminuria were not available. A comparison between our classification system and that of Tervaert et al. will be our next project. Although these limitations may have affected the interpretation of our results to some

extent, this multicenter study involving 600 kidney biopsy samples with long-term follow-up clinical data is important for understanding the clinicopathological features of diabetic kidney lesions and clinical outcomes.

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

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FIGURE LEGENDS

Figure 1. Kaplan–Meier survival curves for each outcome.

Survival curves for: (A) composite kidney end points, (B) CKD G5D, (C) cardiovascular events, and (D) all-cause mortality. Classification into CKD heat map categories (Green and Yellow [G & Y], Orange, and Red) is shown for all end points. Differences between the groups were compared using the log-rank test.

Figure 2. Kaplan–Meier survival curves for composite kidney end points stratified by each pathological 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 pathological findings (nodular lesion, exudative lesion, and mesangiolysis [MesLy]), and each line indicates the score. The continuous black line indicates score 0, and the blue line indicates score 1. Differences between scores were compared using the log-rank test.

ACKNOWLEDGMENTS

The authors thank Dr. Hiroshi Kida (National Hospital Organization Kanazawa Medical Center) for his helpful comments on this study.

We also thank Dr. Richard J. Glassock (Department of Medicine, Geffen School of Medicine at UCLA) for his conscientious critical review of the manuscript and help in the preparation of this manuscript.

We would like to thank Dr. Kenichi Yoshimura (Kanazawa University Hospital), for invaluable suggestion of statistical analysis and logistical support.

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1

Definition of pathological findings

Diffuse lesion (mesangial expansion)

A diffuse lesion is defined as mesangial matrix expansion that is double the width of the nucleus of a mesangial cell. The lesion should be detected in at least two peripheral lobules in one glomerulus.

Nodular lesion (nodular sclerosis)

A nodular lesion is defined as a rounded mesangial matrix expansion where no normal capillary is present.

Supportive findings: A typical nodular lesion exhibits low PAM and Periodic Acid–Schiff (PAS) staining. Uptake with PAM and PAS should be standardized by arterial media. A low PAM and PAS staining area is usually blue with Masson's trichrome staining. The difference between diffuse and nodular lesions is the presence of normal capillaries around a diffuse lesion and the absence of normal capillaries around a nodular lesion.

Subendothelial space widening

Edematous widening of the subendothelial space indicated subendothelial space widening.

Exudative lesion

An exudative lesion is defined by serum amorphous protein deposits in the subendothelial space (fibrin cap) or Bowman's capsule wall (capsular drop).

Mesangiolysis/microaneurysm

Mesangiolysis is defined as dissolution or attenuation of the mesangial matrix and degeneration of mesangial cells.

Peri-hilar neovascularization

There is neovascularization in the glomerular hilar region around the afferent and efferent arterioles, and the vascular wall often has hyalinosis.

Global glomerulosclerosis, collapsing glomerulopathy, and ischemic nephropathy

All the glomerulocapillaries are sclerosed, the capillary lumen is not detected, and the

glomerulocapillaries are collapsing.

Segmental glomerulosclerosis

In segmental glomerulosclerosis, part of the glomerulocapillaries is sclerosed.

Glomerulomegaly

In glomerulomegaly, one or more glomerular diameter is greater than 250 μm in all biopsy specimens. In 400× visual field microscopy, the glomerular diameter is usually around 500 μm.

Interstitial fibrosis, IFTA

Interstitial fibrosis is defined as the accumulation of collagen and related molecules in the interstitium. Tubular atrophy is defined as a decrease in tubular diameter and number.

Arteriolar hyalinosis

This is the ratio of hyaline thickness to whole arteriolar wall thickness expressed as a percentage. A totally occluded artery and an artery connecting to a globally sclerotic glomerulus are shown for comparison.

Intimal thickening

Fibrous intimal thickening of an arteriole or larger artery (interlobular artery arcuate artery) should be evaluated together with the symmetrical wall. Advanced intimal thickening is dominant in some patients and the media is hard to detect. The branching artery is not evaluated.

REFERENCE for SUPPLEMENTARY METHODS

Wada T, Yuzawa Y (Eds.) (2014). Manual for pathological diagnosis of diabetic nephropathy and hypertensive nephrosclerosis. Tokyo Igakusha

Supplementary Figure 2

Kaplan–Meier survival curves for composite kidney end points stratified by each pathological score.

Event-free curves for composite kidney end points are shown. Each column shows the event-free curves for all cases, the Green and Yellow group (G & Y), the Orange group, and the Red group. Each row shows all the pathological findings (including

nodular lesion, exudative lesion, and mesangiolysis, as shown in Figure 2), and each line indicates the score. The continuous black line indicates score 0, the blue line indicates score 1, the red line indicates score 2, and the purple line indicates score 3. Differences between scores were compared using the log-rank test.

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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, 1 mesangial expansion≦capillary lumen, 2 mesangial expansion=capillary lumen, 3 mesangial expansion≧capillary lumen
	Nodular lesion （nodular sclerosis）	0, 1	0 (no nodular lesion）， 1 (one or more lesions detected in all biopsy specimens , not care of 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 ）； 0(＜10％), 1(10-25％), 2(25-50％), 3（≧50％）
	Exudative lesion	0, 1	0 (not detected）， 1 (detected one or more lesion in all biopsy specimen)
	Mesangiolysis/microaneurysm	0, 1	0 (not detected）， 1 (detected one or more lesion in all biopsy specimen)
	peri-hilar neo-vascularization (polar vasculosis)	0, 1	0 (not detected）， 1 (detected one or more lesion in all biopsy specimen)

	Global glomerulosclerosis/collapsing glomerulopathy · ischemic nephropathy	%	(number of global glomerulosclerosis and collapsing glomerulopathy · ischemic nephropathy)/number of all glomerulus (%)
	Segmental glomerulosclerosis	%	number of segmental glomerulosclerosis/number of all glomerulus (%)
	Glomerulomegaly	0, 1	glomeruli >250 μm in diameter 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), 1 (one or more partial arteriolar hyalinosis), 2 (approximately 50% hyalinosis), 3 (more than 50% hyalinosis, or penetrating hyalinosis)
	Intimal thickening	0-2	0 (no intimal thickening), 1 (intimal thickness/media thickness＜1), 2 (intimal thickening and intimal thickness/media thickness≧1) EVG staining is helpful for determination

Table 2

A ***Clinical baseline characteristics of this study***

	G & Y (n=103)	Orange (n=149)	Red (n=348)	All (n=600)	ANOVA
	Mean±SD （Median, IQR）	Mean±SD （Median, IQR）	Mean±SD （Median, IQR）	Mean±SD （Median, IQR）	p
Gender (% of male)	53	60	73 #, §	67	<0.01
Age (yeas old)	54.2±12.2 (54, 47-64)	56.3±11.1 (58, 49.15-64)	59.6±11.3 #, § (61, 52-68)	57.8±11.6 (59, 51-66)	<0.01
BMI	23.3±5.5 (22.5, 20.3-25.4)	23.2±3.4 (22.7, 21.2-24.9)	24.1±4.0 (23.5, 21.5-26.1)	23.7±4.2 (23.1, 21.3-25.8)	0.10
sysBP (mmHg)	131.3±20.2 (130, 118-140)	141.9±20.1 # (140, 128-154)	149.4±20.8 #, § (147, 136-163)	144.5±21.6 (142, 130-160)	<0.01
diaBP (mmHg)	75.9±11.8 (78, 70-84)	78.9±11.4 (80, 70-88)	79.8±13.1 # (80, 70-90)	78.9±12.5 (80, 70-88)	<0.05
Hb (g/dL)	13.5±1.8 (13.5, 12.4-14.7)	12.8±2.0 # (12.9, 11.7-14.2)	11.2±2.2 #, § (11.1, 9.7-12.55)	12.0±2.3 (12, 10.3-13.7)	<0.01
HbA1c (%)	8.4±2.3 (8.1, 6.7-9.7)	8.4±2.2 (8.1, 6.55-9.8)	7.1±1.7 #, § (6.7, 5.9-8.0)	7.6±2.0 (7.1, 6.1-8.8)	<0.01
Tcho	196.1±48.6	218.7±57.2 #	222.6±81.1 #	217.3±71.9	<0.01

(mg/dL)	(195, 163-229)	(212, 182-241)	(207, 172-249)	(207, 174.5-243)	
RAS (%)	11.5	56.1 #	65.5 #	58.3	<0.01

B. ***Pathological baseline characteristics of this study***

		G & D	Orange	Red	All	ANOVA
		Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	p
Glomerular lesions	**Diffuse**	1.28±0.90 (1, 1-2)	1.99±0.96 # (2, 1-3)	2.35±0.87 #, § (3, 2-3)	2.07±0.98 (2, 1-3)	<0.01
	Nodular	0.16±0.37 (0, 0-0)	0.32±0.47 # (0, 0-1)	0.54±0.50 #, § (1, 0-1)	0.42±0.49 (0, 0-1)	<0.01
	SubendW	0.49±0.81 (0, 0-1)	0.76±0.89 # (1, 0-1)	1.27±0.99 #, § (1, 1-2)	1.01±0.98 (1, 0-2)	<0.01
	Exudative	0.16±0.36 (0, 0-0)	0.36±0.48 # (0, 0-1)	0.60±0.49 #, § (1, 0-1)	0.46±0.50 (0, 0-1)	<0.01
	MesLy	0.13±0.33 (0, 0-0)	0.32±0.47 # (0, 0-1)	0.48±0.50 #, § (0, 0-1)	0.38±0.49 (0, 0-1)	<0.01

	PVas	0.43±0.50 (0, 0-1)	0.67±0.47 # (1, 0-1)	0.77±0.42 #, § (1, 1-1)	0.68±0.47 (1, 0-1)	<0.01
	GScl (%)	8.21±10.99 (5, 0-11)	17.09±19.80 (10, 0-25)	32.35±22.72 #, § (30, 16-47)	24.29±22.59 (20, 5-38)	<0.01
	SScl (%)	2.16±7.32 (0, 0-0)	2.66±8.14 (0, 0-0)	4.74±8.74 #, § (0, 0-8)	3.76±8.42 (0, 0-5)	<0.01
	GMeg	0.22±0.42 (0, 0-0)	0.31±0.46 (0, 0-1)	0.40±0.49 #, § (0, 0-1)	0.35±0.48 (0, 0-1)	<0.01
Interstitial lesions	IFTA	0.88±0.81 (1, 0-1)	1.42±0.89 # (1, 1-2)	2.16±0.83 #, § (2, 2-3)	1.76±0.98 (2, 1-3)	<0.01
	ICell	0.75±0.72 (1, 0-1)	1.05±0.78 # (1, 1-1)	1.53±0.83 #, § (1, 1-2)	1.27±0.86 (1, 1-2)	<0.01
Vascular lesions	Hyalin	1.50±1.15 (1, 1-3)	2.05±1.01 # (2, 1-3)	2.36±0.86 #, § (3, 2-3)	2.13±1.00 (2, 1-3)	<0.01
	Arterio	0.87±0.78 (1, 0-1)	1.09±0.77 # (1, 1-2)	1.38±0.66 #, § (1, 1-2)	1.22±0.73 (1, 1-2)	<0.01

BMI; body mass index, sysBP; systolic blood pressure, diaBP; diastolic blood pressure, Hb; hemoglobin, Tcho; total cholesterol, RAS; rate of renin angiotensin system inhibitor treatment, IQR; interquartile range

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 · ischemic 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

P was calculated by one way ANOVA test. # indicates statistical significance compared with G & Y, and § with Orange by paired t test.

Table 3 The incidence rates of composite kidney end points and kidney death were increased accompanied with 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

		G & Y	Orange	Red	All
Kidney death	Event -	58	103	231	392
	Event +	2	5	24	31

		G & Y	Orange	Red	All
	Event -	62	96	239	397
CV event	Event +	16	20	40	76

		G & Y	Orange	Red	All
All cause	Event -	86	129	285	500

mortality	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
Kidney death	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

CV event; cardiovascular event

The composite kidney end point is defined as dialysis, doubling of Cr or halving of eGFR. Kidney death is defined as dialysis or kidney transplantation.

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		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

MesLy

Score	G & Y	Orange	Red	Total
0		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

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

	100.0	100.0	100.0	100.0

Exudative

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

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

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%

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

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

Univariate ***Model 1*** ***Model 2***

Nodular

		HR 95% CI	p
All	Score 0	Reference	
	Score 1	2.4 (1.9 - 3.1)	<0.01
G & Y	Score 0	Reference	
	Score 1	*10.2 (3.6 - 28.6)*	*<0.01*
Orange	Score 0	Reference	
	Score 1	1.7 (1 - 2.8)	<0.05
Red	Score 0	Reference	
	Score 1	1.7 (1.3 - 2.3)	<0.01

		HR 95% CI	p
All	Score 0	Reference	
	Score 1	2.4 (1.9 - 3.1)	<0.01
G & Y	Score 0	Reference	
	Score 1	*12.9 (3.9 - 43.1)*	*<0.01*
Orange	Score 0	Reference	
	Score 1	1.7 (1.0 - 2.8)	<0.05
Red	Score 0	Reference	
	Score 1	1.7 (1.3 - 2.3)	<0.01

		HR 95% CI	p
All	Score 0	Reference	
	Score 1	2.2 (1.7 - 2.9)	<0.01
G & Y	Score 0	Reference	
	Score 1	*21.1 (5.3 - 84.6)*	*<0.01*
Orange	Score 0	Reference	
	Score 1	1.2 (0.6 - 2.2)	0.64
Red	Score 0	Reference	
	Score 1	1.7 (1.2 - 2.3)	<0.01

Exudative

		HR 95% CI	p
All	Score 0	Reference	
	Score 1	2.8 (2.2 - 3.6)	<0.01
G & Y	Score 0	Reference	
	Score 1	*4.3 (1.5 - 12.1)*	*<0.01*
Orange	Score 0	Reference	
	Score 1	2.1 (1.3 - 3.5)	<0.01
Red	Score 0	Reference	
	Score 1	1.9 (1.4 - 2.5)	<0.01

		HR 95% CI	p
All	Score 0	Reference	
	Score 1	2.8 (2.2 - 3.6)	<0.01
G & Y	Score 0	Reference	
	Score 1	*4.2 (1.4 - 12.9)*	*<0.01*
Orange	Score 0	Reference	
	Score 1	2.1 (1.3 - 3.5)	<0.01
Red	Score 0	Reference	
	Score 1	1.9 (1.4 - 2.5)	<0.01

		HR 95% CI	p
All	Score 0	Reference	
	Score 1	2.6 (2.0 - 3.5)	<0.01
G & Y	Score 0	Reference	
	Score 1	*5.1 (1.3 - 20.3)*	*<0.05*
Orange	Score 0	Reference	
	Score 1	1.7 (0.9 - 3.3)	0.10
Red	Score 0	Reference	
	Score 1	1.8 (1.2 - 2.5)	<0.01

MesLy

		HR 95% CI	p
All	Score 0	Reference	

		HR 95% CI	p
All	Score 0	Reference	

		HR 95% CI	p
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

Nodular; nodular lesion (nodular sclerosis), Exudative; exudative lesion, MesLy; mesangiolysis/microaneurysm

Model 1: Adjusted for age, gender. Model 2: Adjusted for the covariates in model 1, body mass index, systolic blood pressure, HbA1c, total cholesterol.

Table 6　Clinical background of G&Y group is almost similar between positive and negative cases of each particular pathological findings.

Nodular	Age	Gender	BMI	SysBP	DiaBP	Hb	HbA1c	Tcho	eGFR	UAlb
	Mean±SD (Median, IQR)	%	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)
+	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	Age	Gender	BMI	SysBP	DiaBP	Hb	HbA1c	Tcho	eGFR	UAlb
+	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 #	0.76	0.33	0.21	0.83	0.93	<0.05 #	0.25

MesLysis	Age	Gender	BMI	SysBP	DiaBP	Hb	HbA1c	Tcho	eGFR	UAlb
+	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

BMI; body mass index, sysBP; systolic blood pressure, diaBP; diastolic blood pressure, Hb; hemoglobin, Tcho; total choresterol,

RAS; rate of renin angiotensin system inhibitor treatment, IQR; interquartile range

 # indicates statistical significance by paired t test.

Supplementary Table 1

Comparison of classification system in this study and the classification system of Renal Pathological Society

	This study		RPS 2010
Glomerular lesions	Diffuse	0 normal or mild mesangiumu expansion, 1 mesangiumu expansion≦capimally lumen, 2 mesangiumu expansion=capimally lume, 3 mesangiumu expansion≧capimally lumen	4 grades GScl>50%(Class IV) ↓ Nodular (Class III) ↓ Mesangial expansion>50% (Diffuse) (Class IIB, IIA) ↓ Glomerular basement membrane thickening by electron microscopy (Class I)
	Nodular	0 (no nodular lesion), 1(one or more nodular lesion in all biopsy specimen, not care of nodular size)	
	SubendW	double contour of basement membrane (%); 0(＜10%), 1(10-25%), 2(25-50%), 3(≧50%)	
	Exudative	0 (not detected), 1 (detected one or more lesion in all biopsy specimen)	
	MesLy	0 (not detected), 1 (detected one or more lesion in all biopsy specimen)	
	PVas	0 (not detected), 1 (detected one or more lesion in all biopsy specimen)	
	GScl	(number of global glomerulosclerosis and collapsing glomerulopathy・ischemic nephropathy)/number of all glomerulus (%)	
	SScl	number of segmental glomerulosclerosis/number of all glomerulus (%)	
	GMeg	more than 250μm in glomerular diameter 0 (not detected), 1 (detected)	

Interstitial lesions	IFTA	0 (no IFTA), 1 (<25%), 2 (25-50%), 3 (≧50%)	0 (no IFTA), 1 (<25%), 2 (25-50%), 3 (≧50%)
	ICell	0 (no cell infiltration), 1 (<25%), 2 (25-50%), 3 (≧50%)	0 (no infiltration), 1(infiltration only in relation to IFTA), 2 (infiltration in areas without IFTA)
Vascular lesions	Hyalin	0 (no hyalinosis), 1 (one or more partial arterioral hyalinosis), 2 (around 50% hyalinosis), 3 (more than 50 % hyalinosis , or penetrating hyalinosis)	0 (no hyalinosis), 1 (at least one area of arteriolar hyalinosis), 2 (more than one area of arteriolar hyalinosis)
	Arterio	0 (no intimal thickning), 1 (intimal thickness/media thickness＜1), 2 (intimal thickning and intimal thickness/media thickness≧ 1) EVG staining is helpful for determination	Intimal thickening greater than thickness of media

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 · ischemic 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

RPS2010; the classification system of Renal Pathological Society (Ref. 6)

Supplementary Table 2

Biopsy indication (single center survey of 50 case series with normo- or micro-albuminuria**)**

Cause of biopsy		Number of cases
Normo-albuminuria		**31 cases (62%)**
	Decreased eGFR	**19 cases (38%)**
Micro-albuminuria		**17 cases (34%)**
	Decreased eGFR	**10 cases (20%)**
	No diabetic retinopathy	**8 cases (16%)**
	Short duration of diabetic history	**6 cases (12%)**
	Highly speculated other diesases	**2 cases (4%)**

58% was cases of rapid decline in eGFR, 16% was cases with proteinuria without diabetic retinopathy, and 12% was cases of proteinuria with short duration of diabetic history

Supplementary Table 3

HRs of nodular lesion, exudative lesion and mesangiolysis for the cardiovascular events

		Univariate			***Model 1***			***Model 2***	
Nodular									
		HR 95% CI	**p**			**HR 95% CI**	**p**		**HR 95% CI**	**p**
All	**Score 0**	**Reference**		**All**	**Score 0**	**Reference**		**All**	**Score 0**	**Reference**	
	Score 1	**1.6(0.9-2.8)**	**0.102**		**Score 1**	**1.4(0.8-2.6)**	**0.228**		**Score 1**	**1.4(0.7-2.6)**	**0.310**
G & Y	**Score 0**	**Reference**		**G & Y**	**Score 0**	**Reference**		**G & Y**	**Score 0**	**Reference**	
	Score 1	**2.6(0.5-12.6)**	**0.242**		**Score 1**	**2.2(0.4-12.6)**	**0.382**		**Score 1**	**3.2(0.3-30.2)**	**0.319**
Orange	**Score 0**	**Reference**		**Orange**	**Score 0**	**Reference**		**Orange**	**Score 0**	**Reference**	
	Score 1	**1.4(0.5-3.8)**	**0.515**		**Score 1**	**1.2(0.4-3.2)**	**0.773**		**Score 1**	**1.3(0.4-3.8)**	**0.617**
Red	**Score 0**	**Reference**		**Red**	**Score 0**	**Reference**		**Red**	**Score 0**	**Reference**	
	Score 1	**1.5(0.6-3.4)**	**0.372**		**Score 1**	**1.4(0.6-3.3)**	**0.404**		**Score 1**	**1.2(0.5-3.3)**	**0.683**
Exudative									
		HR 95% CI	**p**			**HR 95% CI**	**p**		**HR 95% CI**	**p**
All	**Score 0**	**Reference**		**All**	**Score 0**	**Reference**		**All**	**Score 0**	**Reference**	
	Score 1	**1.6(0.9-2.8)**	**0.120**		**Score 1**	**1.5(0.9-2.7)**	**0.147**		**Score 1**	**1.4(0.8-2.7)**	**0.277**
G & Y	**Score 0**	**Reference**		**G & Y**	**Score 0**	**Reference**		**G & Y**	**Score 0**	**Reference**	
	Score 1	**NA(**			**Score 1**	**NA**			**Score 1**	**NA**	

Orange	Score 0	Reference		Orange	Score 0	Reference		Orange	Score 0	Reference	
	Score 1	1.2(0.4-3.3)	0.709		Score 1	1.2(0.5-3.4)	0.667		Score 1	1.1(0.4-3.6)	0.839
Red	Score 0	Reference		Red	Score 0	Reference		Red	Score 0	Reference	
	Score 1	1.9(0.8-4.5)	0.141		Score 1	2.0(0.8-4.7)	0.124		Score 1	2.6(0.9-7.5)	0.079

MesLy

		HR 95% CI	p			HR 95% CI	p			HR 95% CI	p
All	Score 0	Reference		All	Score 0	Reference		All	Score 0	Reference	
	Score 1	1.4(0.8-2.7)	0.246		Score 1	1.5(0.8-2.8)	0.219		Score 1	1.3(0.7-2.7)	0.427
G & Y	Score 0	Reference		G & Y	Score 0	Reference		G & Y	Score 0	Reference	
	Score 1	2.2(0.3-18.0)	0.470		Score 1	2.0(0.2-17.1)	0.543		Score 1	3.9(0.4-42.7)	0.263
Orange	Score 0	Reference		Orange	Score 0	Reference		Orange	Score 0	Reference	
	Score 1	1.8(0.7-4.8)	0.222		Score 1	1.9(0.7-5.2)	0.183		Score 1	2.4(0.8-7.6)	0.135
Red	Score 0	Reference		Red	Score 0	Reference		Red	Score 0	Reference	
	Score 1	0.9(0.4-2.3)	0.821		Score 1	0.9(0.4-2.3)	0.844		Score 1	0.7(0.2-2.3)	0.533

Nodular; nodular lesion (nodular sclerosis), Exudative; exudative lesion, MesLy; mesangiolysis/microaneurysm

Model 1: Adjusted for age, gender. Model 2: Adjusted for the covariates in model 1, body mass index, systolic blood pressure, HbA1c, total cholesterol.

Supplementary Table 4

HRs of nodular lesion, exudative lesion and mesangiolysis for the all cause mortality

Univariate

Nodular

		HR 95% CI	p
All	Score 0	Reference	
	Score 1	2.4(1.5-3.9)	<0.01
G & Y	Score 0	Reference	
	Score 1	3.9(0.7-22.3)	0.127
Orange	Score 0	Reference	
	Score 1	2.8(1.0-8.1)	0.054
Red	Score 0	Reference	
	Score 1	1.5(0.8-2.7)	0.173

Exudative

		HR 95% CI	p
All	Score 0	Reference	
	Score 1	2.1(1.3-3.4)	<0.01
G & Y	Score 0	Reference	
	Score 1	1.9(0.2-16.4)	0.542

Model 1

		HR 95% CI	p
All	Score 0	Reference	
	Score 1	2.1(1.3-3.4)	<0.01
G & Y	Score 0	Reference	
	Score 1	1.7(0.2-12.6)	0.586
Orange	Score 0	Reference	
	Score 1	2.3(0.8-6.8)	0.130
Red	Score 0	Reference	
	Score 1	1.6(0.9-2.9)	0.142

		HR 95% CI	p
All	Score 0	Reference	
	Score 1	2.0(1.2-3.2)	<0.01
G & Y	Score 0	Reference	
	Score 1	1.4(0.1-12.6)	0.782

Model 2

		HR 95% CI	p
All	Score 0	Reference	
	Score 1	1.8(1.0-3.1)	<0.05
G & Y	Score 0	Reference	
	Score 1	1.0(0.1-10.6)	0.979
Orange	Score 0	Reference	
	Score 1	0.9(0.8-10.6)	0.103
Red	Score 0	Reference	
	Score 1	1.4(0.7-2.7)	0.367

		HR 95% CI	p
All	Score 0	Reference	
	Score 1	2.0(1.2-3.5)	<0.05
G & Y	Score 0	Reference	
	Score 1	2.3(0.1-42.6)	0.569

Orange	Score 0	Reference		Orange	Score 0	Reference		Orange	Score 0	Reference	
	Score 1	1.7(0.6-5.0)	0.312		Score 1	2.2(0.7-6.4)	0.163		Score 1	1.6(0.4-6.1)	0.510
Red	Score 0	Reference		Red	Score 0	Reference		Red	Score 0	Reference	
	Score 1	1.4(0.8-2.4)	0.304		Score 1	1.4(0.8-2.6)	0.222		Score 1	1.8(0.9-3.5)	0.101

MesLy

		HR 95% CI	p			HR 95% CI	p			HR 95% CI	p
All	Score 0	Reference		All	Score 0	Reference		All	Score 0	Reference	
	Score 1	2.3(1.4-3.7)	<0.01		Score 1	2.9(1.7-4.8)	<0.01		Score 1	2.3(1.3-4.2)	<0.01
G & Y	Score 0	Reference		G & Y	Score 0	Reference		G & Y	Score 0	Reference	
	Score 1	2.4(0.3-22.1)	0.439		Score 1	2.1(0.2-20.7)	0.520		Score 1	3.5(0.2-66.3)	0.398
Orange	Score 0	Reference		Orange	Score 0	Reference		Orange	Score 0	Reference	
	Score 1	2.4(0.8-7.1)	0.119		Score 1	4.3(1.2-15.8)	0.025		Score 1	3.2(0.8-13.4)	0.114
Red	Score 0	Reference		Red	Score 0	Reference		Red	Score 0	Reference	
	Score 1	1.6(0.9-2.9)	0.123		Score 1	2.2(1.2-4.1)	0.015		Score 1	2.0(1.0-4.2)	0.054

Nodular; nodular lesion (nodular sclerosis), Exudative; exudative lesion, MesLy; mesangiolysis/microaneurysm

Model 1: Adjusted for age, gender. Model 2: Adjusted for the covariates in model 1, body mass index, systolic blood pressure, HbA1c, total cholesterol.

Supplementary Table 5　Clinical background of all cases between positive and negative cases of each particular pathological findings

Nodular	Age	Gender	BMI	SysBP	DiaBP	Hb	HbA1c	Tcho	eGFR	UAlb
	Mean±SD (Median, IQR)	%	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)	Mean±SD (Median, IQR)
+	59.4±10.3 (60, 53-67)	68	23.6±3.7 (23.1, 21.2 -25.5)	148.8±23.1 (147, 134-163)	79.1±13.0 (80, 70-89)	11.2±2.1 (11.2, 9.5-12.7)	7.4±2.0 (7.0, 6.0-8.4)	227.4±77.9 (211, 177-256)	44.9±22.5 (42.6, 27.8 -59.6)	2.59±2.33 (2.08, 0.78-3.84)
-	56.4±12.6 (58, 49-65)	65	23.8±4.2 (23.3, 21.5 -25.8)	141.5±20.1 (140, 128-154)	78.7±12.3 (80, 70-88)	12.6±2.2 (12.7, 11.1-14.3)	7.7±2.1 (7.2, 6.2-8.9)	210.0±67.0 (202, 172-235)	59.7±28.6 (56.1, 39.5 -75.6)	1.18±1.50 (0.63, 0.12-1.70)
p	<0.01 #	0.42	0.60	<0.01 #	0.75	<0.01 #	0.15	0.01 #	<0.01 #	<0.01 #

Exudative	Age	Gender	BMI	SysBP	DiaBP	Hb	HbA1c	Tcho	eGFR	UAlb
+	58.3±10.9 (59, 52-66)	72	23.7±3.9 (23.5, 21.2 -25.6)	148.1±21.7 (148, 135-162)	79.0±12.9 (80, 70-88)	11.4±2.2 (11.4, 9.8-12.9)	7.5±2.0 (7.0, 6.0-8.4)	223.5±76.2 (208, 176-250)	45.4±22.4 (43.4, 30.1 -58.4)	2.45±2.16 (2.01, 0.89-3.19)
-	57.2±12.4 (59, 49-65)	62	23.8±4.1 (23.0, 21.5 -25.9)	141.4±21.2 (140, 128-153)	78.9±12.3 (80, 70-88)	12.6±2.3 (12.7, 11.1-14.2)	7.7±2.0 (7.3, 6.2-8.9)	211.3±67.5 (205, 171-235)	60.3±28.9 (57.3, 40.6 -76.1)	1.18±1.66 (0.49, 0.1-1.48)
p	0.24	0.01 #	0.69	<0.01 #	0.93	<0.01 #	0.15	<0.05 #	<0.01 #	<0.01 #

MesLy	Age	Gender	BMI	SysBP	DiaBP	Hb	HbA1c	Tcho	eGFR	Ualb

+	57.2±11.0 (58, 51-66)	69	23.7±3.8 (23.3, 21.4-25.8)	151.7±22.8 (150, 138-167)	80.7±13.1 (80, 72-88)	11.2±2.1 (11.2, 9.5-12.6)	7.5±2.0 (7.0, 6.0-8.8)	232.0±78.0 (211, 178-260)	46.1±22.8 (43.0, 30.1 -59.9)	2.72±2.36 (2.26, 1.05-3.94)
-	57.8±12.2 (60, 50-66)	65	23.7±4.2 (23.1, 21.2 -25.6)	139.9±19.8 (140, 128-150)	77.8±12.1 (78, 70-86)	12.5±2.3 (12.6, 11-14.2)	7.6±2.0 (7.2, 6.1-8.8)	208.6±65.6 (202, 171-233)	58.0±28.6 (55.5, 38.3 -73.4)	1.19±1.51 (0.61, 0.12-1.7)
p	0.53	0.43	0.99	<0.01 #	<0.05 #	<0.01 #	0.51	<0.01 #	<0.01 #	<0.01 #

Nodular; nodular lesion (nodular sclerosis), Exudative; exudative lesion, MesLy; mesangiolysis/microaneurysm

BMI; body mass index, sysBP; systolic blood pressure, diaBP; diastolic blood pressure, Hb; hemoglobin, Tcho; total choresterol, RAS; rate of renin angiotensin system inhibitor treatment, IQR; interquartile range

 # indicates statistical significance by paired t test.